1. INTRODUCTION

1.1. Research Background

Plastic is made of few derivative compounds from petrochemical that is synthetically making by using monomer and adding few chemical agents to shaping it into long polymer chains (Shimao, 2001). Plastic is suitable for many products and has so many functions in daily life because it has suitable characteristics such as light-weight, cheap, reliable, and durable. Plastic is also suitable for industrial production because it is fluid, moldable, easy to print, and heat sealable. It can be integrated into production processes from the molding package, filled, and sealed all in the same production line (Marsh & Bugusu, 2007). Food packaging has a crucial function to preserve, protect, promotion media, and food distribution process. Food packaging comes in two main shapes according to their purpose: rigid packaging (wood boxes, glass, plastic bottles, cans, tins) and flexible packaging (plastic films, papers, and foil) (Raheem, 2012).

According to data from World Count (2020), the total amount of plastic waste entered the ocean is around 8.798.208 tons. The floating plastic waste in the ocean already makes a "floating plastic island," which covered up to 2,531,819,106 km^2. This data keeps growing up every second, meaning the plastic pollution in the environment is getting worse every second.

There are three current methods to manage plastic waste, i.e. incineration, dumping in landfills, and recycling. However, these methods have not been effective in managing the actual amount of plastic waste that is increasing due to the higher demand in various sectors, especially in the food industry. Due to this waste problems is why we need to get rid of the existing plastic waste with natural, eco-friendly methods, and rapidly.

Scientists are trying to maximize the ability of indigenous microorganisms that are adapted in the chemical or modified environment to optimize degrading plastic material. Using this indigenous microorganism means the resource to find them is easy, so we can isolate them to get pure culture (Montazer *et al*., 2018; Singh *et al*., 2016; Gajendiran *et*

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al., 2016; Asmita *et al.*, 2015; Konduri *et al.*, 2011; Pramila & Ramesh, 2011; Usha *et al.*, 2011). The research from Singh *et al.*, (2016) able to isolate *Staphylococcus* sp., *Pseudomonas* sp., and *Bacillus* sp., from soil, which is able to degrade polyethylene material. There are some fungi species that able to degrade plastic material; Pramila & Ramesh (2011) found that *Aspergillus flavus* and *Mucor cicinelloides* can degrade the LDPE film.

Most of the scientist already found the microorganism species that can degrade plastic material by using a laboratory method to analyze them. The previous review study focused on the finding of microorganism species and plastic types that able to degrade by microorganisms (Moharir & Kumar, 2019; Gu, 2003; Koshti *et al.*, 2018; Pathak & Navneet, 2017; Sharma *et al.*, 2015; Sangale, 2012; Leja & Lewandowicz, 2010); types of degradation method, and factors that affect biodegradation (Moharir & Kumar, 2019; Sivan, 2011; Singh & Sharma, 2008). According to previous research, there are lots of research study about the polyethylene biodegradation, factors that affect the biodegradation, and assessment method. This review aims to collect the current research and looking for the current theory and techniques on polyethylene biodegradation for the better result of polyethylene biodegradation.

1.2. Literature Review

1.2.1. Food Packaging

Food packaging has few essential roles in keeping the food products safe from biological, physical, and chemical damage from the outside, food containment, preventing food loss, and providing the consumers with the nutritional information, expiry date, and ingredients. The function of food packaging is mainly to retard product deterioration, keep the beneficial effect of food and processing, extend shelf life, food marketing media, food traceability, and maintain and increase consumer convenience, food safety, and quality (Raheem, 2012; Marsh & Bugusu, 2007).

Plastic is a polymer made of a petrochemical derivative compound that is synthetically made of monomer by adding some chemical agents during the process to transform the

monomer into a long-chain polymer (Shimao, 2001). Plastic is the newest material for packaging in comparison with others because the plastic was discovered in the 19th century. Plastic is usually used as packaging for many goods such as agricultural products and is mostly used as food packaging. Almost 30% of our plastic that we used in the world most are polyethylene (LDPE, HDPE, MDPE, LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), polybutylene terephthalate (PBT), and nylon (Jumaah, 2017).

These are the six types of recycled plastic resin that are commonly used for food packaging.

1. Polyethylene Terephthalate (PET): mouthwash bottles, beverages bottles, boil in bag pouches.
2. High-Density Polyethylene (HDPE): milk jug, plastic bag, bottles
3. Polyvinylchloride (PVC): cooking oil bottles, packaging around meat
4. Low-Density Polyethylene (LDPE): grocery bags, food wrap, squeezable bottle
5. Polypropylene (PP): reusable food containers, straws, yogurt containers
6. Polystyrene (PS): hot beverage cups, single-use food containers, egg cartons, meat tray

PVC is a type of plastic that is usually challenging to recycle because plasticizers such as adipates may immigrate to food, and it cannot be burned in incineration because of its chlorine (Raheem, 2012).

Plastic material for food packaging usually uses new material instead of using recycled plastic material. The reason is to prevent the chemical contaminants in recycled plastic that may include the recycling process and migrate into the food. Also, there are some risks, such as microbial contamination and structural integrity of recycled plastic, which are considered essential to maintain food quality and safety (Raheem, 2012).

Food plastic packaging can contain more than one kind of plastic. Usually, this combination of plastic types can enhance the food shelf life because of the advantages of each plastic-type and reduces the total amount of material required for production. There are two methods for combining plastics: lamination and co-extrusion. Lamination is usually used to combine plastic into another material like paper or aluminum. This

process requires water, solvent, or solid bases adhesive as the bonding intermediator, then both plastic and other material are passed between the roller to pressure and bond them together. Another method of lamination, such as laser, has been used for the thermoplastic material. The co-extrusion method is combining two or more layers of molten plastics during the film manufacture; in comparison with the lamination method that requires multiple steps, co-extrusion only requires one step. Combining plastic material makes the recycling method is more complicated (Raheem, 2012).

1.2.2. Polyethylene for Food Plastic Packaging

The term plastic has come from the Greek word "plastikos," that is means that the material can be molded into different shapes (R. Pramila, 2011). Kumar *et al*. (2011) give an estimation that 41% of plastics are used in packaging, and almost half of it is used for food packaging. Most of food plastic packaging are xenobiotics component in nature, so it is hard to degrade naturally. This plastic compounds are foreign material and not easily recognized by the microorganism enzymes and make it resistant material (Nwachukwu *et al*., 2010).

There are three categories for synthetic polymers: degradable, slowly degradable, and resistant (Gu, 2003). Most of the food plastic packaging are thermoplastics type. The characteristic of thermoplastics is a linear chain macromolecule that linked end-to-end into a long carbon chain (Singh & Sharma, 2008). Then, some types of plastic for food packagings such as PE, PP, and PS are hydrophobic polymers that do not contain any of hydrolyzable bonds and expected to be the most stable polymer (Singh & Sharma, 2008).

Polyethylene plastic is made of cheap petrochemical derivatives that extract from oil and gas industries through the efficient catalytic polymerization of ethylene monomers (R. Pramila, 2011). Polyethylene plastic shares 64% of plastic waste between the other types of plastic that are produced as bottles, carry bags, trash bags, margarine tubs, disposable articles, and water pipes (Sangale, 2012). Another application of polyethylene in food packaging is the plastic film that is used to wrapping food, fruits, and vegetables (Nwachukwu *et al*., 2010). Most polyethylene wastes are generated from the consumption

of food that is packed in polyethylene sachets, bags, and other forms of food containers (Nwachukwu *et al*., 2010). Polyethylene is the most commonly used plastic that is categorized as non-degradable plastic because of the long carbon chains of ethylene monomers (C_2H_4) (R. Pramila, 2011).

Polyethylene graded in two categories, which branched (low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE), very low-density polyethylene (VLDPE), and ultralow-density polyethylene (ULDPE)) or unbranched (high-density polyethylene (HDPE) and ultrahigh-density polyethylene (UHMWPE)). Figure 3 shows the general structure of several PE types. The linear PE is the primary form of polyethylene, and it has a high level of crystallinity, high melting point, and also high-density material, so it is commonly known as high-density polyethylene (HDPE). Another example of this linear PE is ultrahigh molecular weight polyethylene (UHMWPE), which has over 5 million Daltons of molecular weight and forms a unique fiber that serves as one of the most durable materials (Zohuri *et al*., 2012).
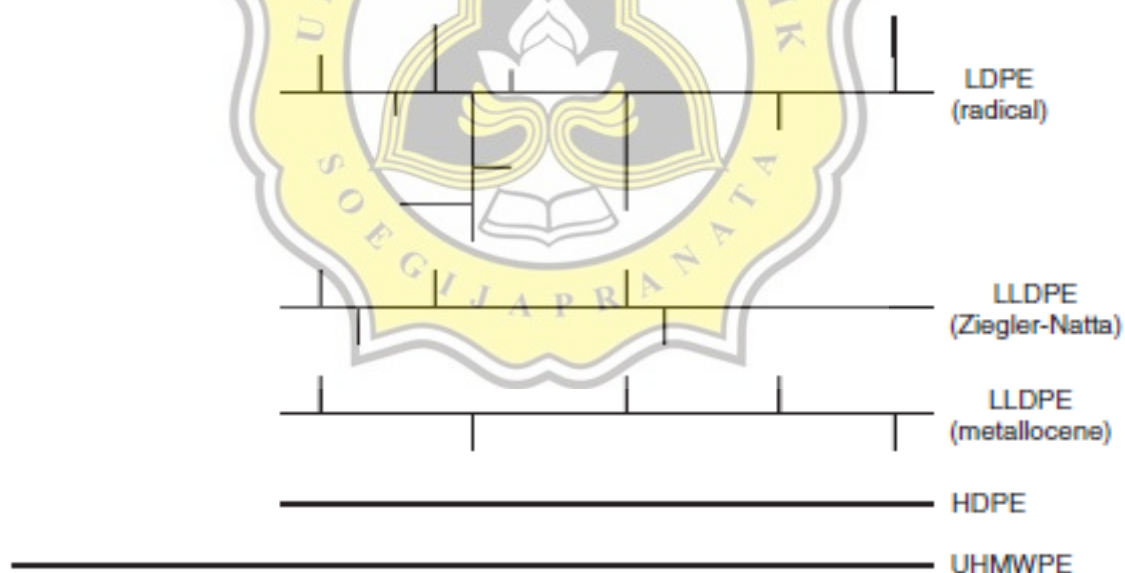

Figure 1. The general structure of several polyethylene types (Zohuri *et al*., 2012)

Table 1 provides some properties for each type of polyethylene according to their density, molar mass, crystallinity, and melting temperature, while table 2 presents the structure of each type of polyethylene. Branched PE comes in two general forms as LDPE and LLDPE. LDPE contains both long and short irregular branches, and it is made by using a

free-radical process, while LLDPE only contains short branches (Zohuri et al., 2012). There are some characteristics of LDPE plastic: able to survive in 80^0C continuously and 95^0C for a short time, it is slightly flexible and tough but breakable, it has more branching around 2% of the carbon atoms, it has weak intermolecular forces, low tensile strength, high resilience, and the chain molecules are less tightly packed and less crystalline because of the side branches (R. Pramila, 2011).

Table 1. General properties of polyethylenes

Grade	Density (g/cm^{-3})	Molar Mass (g/mol^{-1})	Crystallinity (%)	T_m (^{o}C)	Structure
HDPE	0.940–0.965	10^3–10^7	55–95	125–145	Linear structure (linear carbon chain)
LDPE	0.915–0.930	8.9×10^4 to 4.7×10^5	30–55	104–120	Random branching contains both of short and long branches
LLDPE	0.90–0.94	5.0×10^4 to 5.0×10^5	40–60	120–125	non-uniformly distributed short-branched copolymer
VLDPE	0.89–0.91	5.8×10^4 to 1.2×10^5	25–40	92–123	High co-monomer content; short branches; lower density and T_m
ULDPE	~ 0.86		15		Highly monomer content; short branches; very low T_m and crystallinity (soluble at the ambient temperature)
UHMWPE	0.93	$> 10^6$	≥ 50	≥ 132	Linear structure; very high M_n

Source: (Zohuri et al., 2012)

LDPE is hard to be degraded by the biological attack because of the hydrophobicity and high molecular weight properties, also because of the low amount of functional groups that recognized by microbial enzyme systems (R. Pramila, 2011). R. Pramila, (2011) have been doing some preliminary research on LDPE biodegradation and proved that LDPE is able for biodegradation by the use of suitable microorganisms. The hydrophobic

properties of LDPE film are suitable as a substrate for some microorganisms that able to perform a biofilm on the LDPE surface; *Aspergillus sp* and *Aspergillus versicolor* can grow in the minimum media that use LDPE powder as a sole carbon source, even without any nitrogen source; and found that LDPE can be decomposed into CO_2.

The first comparative research of biodegradability between paraffin and polyethylene from Jen-hou and Schwartz (1961) in Sangale (2012) found that only low molecular weight polyethylene (MW 4800) that able to be degraded by microbes. Then in nineteen years later, the research from Albertsson & Bánhidi (1980) found that microbe also able to degrade high-density polyethylene (HDPE) film (MW 93000). The main degraded component in the HDPE film is the short-chain oligomer. The short-chain element as a branched structure will help to increase the biodegradation rate by reducing crystallinity, molecular weight, melting point, and density, also increase the amorphous area.

1.2.3. Food Plastic Packaging Disposal Method

Nowadays, the use of plastics for packaging material has increased worldwide around 280 metric tons. The packaging industry is the largest user of plastic. The packaging is divided into two types, *i.e.,* flexible and rigid packaging, and almost 90% of flexible packaging made from plastics, then only 17% of rigid packaging made of plastics (Raheem, 2012). There are so much overflowing of food packaging waste that almost two-thirds of the total packaging waste by volume because food is the only product that is consumed three times per day per capita (Marsh & Bugusu, 2007). This food packaging waste disposal is a new challenge for solid waste management, and its already a significant source of pollution (Pathak & Navneet, 2017).

There are three current methods to get rid of the plastic waste material by recycling, incineration, and dumping in landfills. The standard recycling method is mechanical recycling that is costly, needs a large amount of energy, and time-consuming because it needs presorting plastic, removal of the organic residue by washing them, then followed by shredding, melting, and remolding. However, using 1 ton of recycled plastic material to make a new packaging could save energy up to 130 million kJ compared to use the

new raw material to make the packaging. There are few limitations in the recycling method because of all types of plastic have their characteristics (chemical, mechanical, and thermal properties). So, even a small amount of contamination from the other plastic-type could change the properties and potentially failed to reuse the recycled material for making a new packaging. Consequently to this limitation, only two types of plastic are recycled using this mechanical recycling: PET (polyethylene terephthalate) and polyethylene, which only represent 9% and 37% of the plastic production per annum respectively (J.M Garcia & M.L. Robertson, 2017).

(i) **Incineration Method**

The incineration process is a method of Waste to Energy project currently used in many countries such as Denmark, Sweden, Switzerland, Netherland, Norway, Japan, South Korea, the United States, China, and other developing countries. This method is considered an effective method to treat municipal solid waste (MSW) in a very delightful amount by reducing the weight and volume of MSW at 80% and 90%, respectively. The fantastic achievement that possible to control the overflowing waste in MSW in a short time because it does not need to separate and to group the waste categories. This method still has a side effect that could be considering as an environmental threat for an extended period because the waste that comes along with the process contains dioxin, heavy metal, and other carcinogenic substances (Song *et al*., 2017). Dioxin has strong lipophilicity and stability; it can be transmitted into human bodies through food and water. Dioxin is a part of persistent organic pollutants (POPs), which are the group of organic substances that endangered human health and environmental safety. Because of their toxic characteristics such as persistent, bio-accumulate, prone to long-range transboundary atmospheric transport and deposition, it is possible to cause significant adverse effects on human health and environmental safety near to and distant from their resource (Jumaah, 2017; Song et al., 2017; Harmens et al., 2013). Although the emission from incineration is already achieving its minimum standard for the pollution emission, it still possible to have an accumulation of the toxic in the environment and endangered human health.

(ii) Landfills Dumping Method

The most desirable method in many countries to get rid of the MSW is by throwing them away in the landfill because it requires less investment and appropriate for all types of MSW (Song *et al*., 2017). Indonesia is one of the countries that use this method to deal with the MSW, but few problems came up with this method, such as the landfill's volume limit, environmental pollution, and human health. Appropriate landfill system such as sanitary landfill should be applied to prevent environmental pollution and adverse human health. The rain that falls around the landfills will infiltrate through the MSW and dissolving waste decomposition products in the form of toxic leachate water that could contaminate the soil water (Hutabarat & Pujiindiyati, 2010). The research of Hutabarat & Pujiindiyati (2010) looks for the heavy metals pollutant in leachate water, the resident's water system, and the river surrounding the Bantar Gebang landfill, Bekasi. This research found that Zn, Fe, Cr, and Hg concentration beyond the dangerous level in every location, and Co concentration is beyond the limit for few areas. This heavy metal is hazardous for human health that lives surrounding this area because they also consume the water for daily use. Another environmental issue is methane (CH_4), and carbon dioxide (CO_2) emission is difficult to calculate the magnitude because they are categories as a natural source that comes from landfills (Rajagukguk & Siagian, 2017). These gasses appear as the results of the bio-decomposition of waste in the Jatibarang landfill, Semarang, which 62% is the organic waste, and the rest are inorganic. Landfills usually produce 0.235 m^3 methane gas that equal to 0.5 tons of compost. Methane and carbon dioxide are part of greenhouse gasses that could have a global warming effect in the long term (Dias, 2009).

(iii) Biodegradation Method

The biodegradation process can be running in the aerobic or anaerobic condition and producing different by-products. The biodegradation process that runs in the aerobic environment will produce CO_2 and H_2O. In contrast, the process that runs in the anaerobic environment will produce CO_2, H_2O, and CH_4 as waste products from the

metabolism (Jumaah, 2017; Gu, 2003). The biodegradation will be more efficient by using an aerobic environment because O_2 that includes in this metabolism will have a better electron receptor property compared to CO_2 and SO_4^{2-}. Aerobic and anaerobic conditions depend on the microorganisms; the fungal biodegradation process will be useful in the aerobic condition, while bacterial degradation can do in both conditions (Pathak & Navneet, 2017).

Extensive research on plastic biodegradation has been going on for these last three decades, and this biodegradation method is fully compatible with other waste management methods (Shimao, 2001). Environmental pollution issues can be reduced by using biodegradation, such as microbial degradation, which can be enhanced by using photodegradation, ozone degradation, and mechanochemical degradation. This biodegradation is a natural process that is believed to avoid the toxic compound from the degradation of plastic to spread in the environment (Pramila *et al*., 2012; Singh & Sharma, 2008). The American Society for Testing of Materials (ASTM) defined biodegradation in their ASTM standard D-5488-94d. The process which is capable of decomposition of material into carbon dioxide, methane, water, inorganic compounds, or biomass in which the predominant mechanism is the enzymatic action of microorganisms that can be measured by a standard test, in a specified period, reflecting available disposal condition (Singh & Sharma, 2008).

The action of UV light undergoes photodegradation, and whether it comes from the sunlight or UV bulb, this process will attack polymers bond photochemically and turn it into small pieces. Then, the photodegradation process is followed by microbial degradation to turn the small pieces of polymers into an end product naturally found in the environment (Jumaah, 2017; Asmita *et al*., 2015; Kumar *et al*., 2011; Singh & Sharma, 2008). The degradation process can be microbial or enzyme-based, but both of them are using microorganisms adheres to the plastic surface. Microbial degradation will take the plastic surface as their place for colonization, whereas the enzymatic degradation will proceed with hydrolysis and followed by attachment of microorganisms. The enzymes will react with the polymer surface and break down the hydrolytic bonds of the polymer and convert it into a simpler form such as a

monomer, a dimer, or a trimer. These monomers will be used as metabolism and a viable source of nutrition for the microorganisms (Jumaah, 2017; Asmita *et al*., 2015; Gu, 2003). This polymer degradation will produce physical and chemical changes in the properties, such as a reduction in tensile strength, discoloration, shape (cracking, erosion, separation, and delamination), chemical transformation, and a new functional group. This process is often termed "aging." (Jumaah, 2017; Kumar *et al*., 2011; Singh & Sharma, 2008).

1.2.4. The Ability of Microorganisms in Degrading Plastic Material

The biodegradation method for a polymer degradation is a natural process that is believed to avoid the toxic compound from the degradation of plastic to spread in the environment (Pramila *et al*., 2012; Singh & Sharma, 2008). The American Society for Testing of Materials (ASTM) defined biodegradation in their ASTM standard D-5488-94d. "Process which is capable of decomposition of material into carbon dioxide, methane, water, inorganic compounds, or biomass in which the predominant mechanism is the enzymatic action of microorganisms, that can be measured by a standard test, in a specified period, reflecting available disposal condition" (Singh & Sharma, 2008). The degradability properties of a material are the ability of a material to the simpler explanation for the biodegradability is the ability of a material to decompose in their constituent molecules by natural degradation process (Singh & Sharma, 2008).

Some treatments can enhance the microbial ability to degrade petrochemical-based plastic or commonly used plastic such as additives and bio-surfactant that aim to increase the hydrophilicity of the polymer. Petro-based plastics tend to be hydrophobic so that it could interfere with the formation of microbial biofilm on the polymer surfaces (Talkad *et al*., 2014). Microorganisms need to create biofilm by using the "microfouling" process, which is the process that was establishing the complex community on the surface material. Biofilms contain microorganisms and their extracellular polysaccharides that highly diverse and variable according to their space and time. This process is a prerequisite for the deterioration process of the underlying materials (Gu, 2003). There are two types of biodegradation process, it can be microbial or enzyme-based, but both of them are using

microorganisms adheres to the plastic surface. Microbial biodegradation will colonize the plastic surfaces; however, the enzymatic degradation will proceeds with hydrolysis and followed by adheres of microorganisms (Asmita *et al*., 2015).

Microorganisms such as bacteria, fungi, and algae have potential to degrade a polymer material because it contains organic compounds that are useful for microbial metabolisms such as monosaccharides and amino acids. Two major enzymes are actively taking part in the polymer biodegradation: extracellular and intracellular depolymerase. The extracellular enzymes will cut off the complex polymers by disrupting the secondary and tertiary structures that are stabilized by Van der Waals force and hydrogen bonds into short chains such as monomers, dimers, and oligomers and small enough to pass the outer semipermeable membrane of bacteria. These monomers will be used as metabolism and a viable source of nutrition for the microorganisms. The intracellular enzymes are responsible for the depolymerization process that utilized the shorter chain of a polymer as carbon and energy sources. (Jumaah, 2017; Asmita *et al*., 2015; Gu, 2003; Singh & Sharma, 2008).

The structure of the polymer also gives a different reaction to the biodegradation rate; the more similar of the polymeric structure to a natural molecule, the more comfortable to be degraded and mineralized (Gu, 2003). The output of the biodegradation process is an inorganic material like CO_2, H_2O, or CH_4; this process is called the mineralization process. The degradation process will undergo due to the environment condition; in the high O_2 condition, most of the aerobic microorganisms will take part in biodegradation and produce CO_2 and H_2O as the end products. When the biodegradation occurs in the anoxic condition, the anaerobic microorganisms are responsible for biodegradation and produce CO_2, H_2O, and CH_4 under methanogenic condition or CO_2, H_2O, and H_2S under sulfidogenic conditions (Jumaah, 2017; Asmita *et al*., 2015; Gu, 2003; Usha *et al*., 2011; Sangale, 2012; Arutchelvi *et al*., 2008; Singh & Sharma, 2008). There will be more efficient biodegradation in aerobic conditions because it produces much more energy and able to support the more significant population of microorganisms than the anaerobic process. The reason is that O_2 is more efficient for an electron acceptor comparing with SO_4^{2-} and CO_2 (Gu, 2003).

Aerobic and anaerobic conditions depend on the microorganisms; the fungal biodegradation process will be useful in the aerobic condition, while bacterial degradation can do in both conditions (Pathak & Navneet, 2017). The biodeterioration and biodegradation of a polymer material usually hard to reach 100% because of the small portion of the polymer that will be fused as microbial biomass, humus, and other natural products (Gu, 2003). According to Singh & Sharma (2008), the microbial degradation going through in sequential steps, there are:

(i)   bio-deterioration (modifying the chemical and physical characteristic of the polymer);

(ii) depolymerization (polymer fragmentation into a simpler form by enzymatic cleavage);

(iii) assimilation (absorbing the molecules by microorganism);

(iv) mineralization (forming by-products as oxidized metabolites such as CO_2, H_2O, CH_4).

The microbial ability on attacking polymer depends on few factors such as the enzyme availability, the presence of a site in the polymer for the enzyme attack, the specified enzyme for the polymer, and the existence of the coenzyme (Singh & Sharma, 2008). Few arguments of "in vivo" degradation might not be the same as "biodegradation." Biodegradation means active participation in biological entities (microbes and enzymes) along with the biodegradation process. Although, it is hard to define what is the role of microbes in the in vivo degradation because both hydrolytic and enzymatic processes may contribute to the degradation of the polymer. The degradation process may be started by hydrolysis, but along with the polymer breaks, then surface area, and the accessibility increases, the enzymatic process will take control of the rest of the biodegradation process (Singh & Sharma, 2008). The full mechanisms of polymer biodegradation are still not fully known.

Most researchers assume that the biodegradation of polymer might be undergone by the extracellular enzyme that secreted by microbes and slowly breaks the complex chain structure of polymer (Kathiresan, 2003a). Another report from Grima *et al*., (2000)

finding that the mechanism of polymer degradation mainly is biological hydrolysis and biological oxidation. The biodegradation mechanism depends on the ability of the microorganism to create a biofilm and attach it to the polymer surface. When the microbes can attach to the polymer surface, it starts growing by utilizing the polymer as a carbon source. In the initial growth, microorganisms will start to degrade the low molecular weight chains (oligomers, dimers, and monomers) (Usha et al., 2011a).

Current research aims to find what bacterial and fungal species can degrade types of plastic material. These are a few bacterial genera that can degrade plastic material: *Bacillus*, *Pseudomonas*, *Actinomycetes*, *Klebsiella*, *Micrococcus*, *Nocardia*, *Microspora*, *Streptomyces*, *Escherichia*, *Proteus*, *Staphylococcus*, *Mycobacterium*, *Rhodococcus*, *Flavobacterium*, *Comamonas*, *Azotobacter*, *Alcaligenes*, and *Thermoactinomycetes*.

Also, some fungal genera participating in the biodegradation process: *Aspergillus*, *Penicillium*, *Phanerochaete*, *Sporotrichum*, *Phanerochaete*, *Gano derma*, *Talaromyces*, *Thielavia*, *Thermoascus*, *Paecilomyces*, *Cladosporium*, *Geotrichu m*, *Phlebia*, *Candida*, *Trametes*, *Chaetomium*, and *Aerobasidium* (Sharma et al., 2015; Sangale, 2012).

1.2.5. Factor Affecting Biodegradation Process

Few factors that are affecting the biodegradation rate, such as the use of specific microorganisms, physical and chemical properties of the material, crystallinity, molecular weight, environmental condition, material composition, additives, and surfactant (Gu, 2003). The polymer structure contained two-part: the amorphous area and the crystalline area. The amorphous area has been reported as a labile area in the polymer chains that are susceptible to the thermal oxidation compared to the crystalline area. The oxidation is happening because of the high permeability properties of the oxygen molecule. The presence of heteroatom such as oxygen, will make the polymeric chains are labile to the thermal degradation and biodegradation (Singh & Sharma, 2008). Biodegradation rates are affected by some factors such as irradiation with UV light and chemicals, surface area,

antioxidant, pro-oxidant, additives, and molecular weight of a polymer (Kyaw *et al.*, 2012).

A. Chemical Composition and Structure of the Polymer

The ability of microorganisms to degrade plastic material depends on its chemical composition, whether it can support the growth or not by providing the nutrient sources (Pathak & Navneet, 2017). The material composition will be an essential factor affecting the biodegradation rate (Singh & Sharma, 2008; Gu, 2003). The absence of short chains carbon and only long chains carbon that presence in the polymer will make it hard to be degraded by microorganisms. The presence of non-polymeric impurities such as residues of the polymerization catalyst, and additives, coloring agents, and filler will affect the polymeric resistance to the degradation process (Singh & Sharma, 2008).

The chemical bonding structure will affect the degradation rate of a polymer. The head-to-head or tail-to-tail addition of monomer units will produce a weak point in the carbon linkage and increase the rate of plastic degradability. However, the crosslinking chain structure will decrease the photo degradability of plastic by locking the polymeric structure and preventing lamellar unfolding. This crosslinking structure prevents the segregation of photo-produced radicals and creates the radical-radical structure (Singh & Sharma, 2008). The longer and linear molecular chains of polyethylene will make it recalcitrant to microbial attack. The role of the abiotic (such as UV light) and enzymatic factor to initiate the oxidation of polyethylene chains and create similar characters between polyethylene and olefins that responsible for hydrocarbon degradation. The length of the carbon chains in polyethylene needs to be reduced from 10 to 15 carbons molecule chains to make it suitable for the enzymatic reaction (Talkad *et al.*, 2014).

The chemical additive is commonly reacted as a catalyst for the degradation process of the polyethylene, and it works when the degradation process is starting (Kumar *et al.*, 2011). The addition of specific additives will enhance microbial degradation because it helps disconnect the continuity of C–C bonds (Singh & Sharma, 2008). The additives such as antioxidants, starch, coloring agents, plasticizers, and sensitizers may enhance the biodegradability of a polymer. Polyethylene pretreatment by UV light enhanced the

polymer degradation because of the photosensitizer will increase the degradation rate for 2-4% (Gu, 2003).

The addition of pro-oxidant additives will make polyolefins are able to degrade by oxidative degradation and make it hydrophilic and catalyze the degradation process of high molecular weight polymer into lower molecular weight (Singh & Sharma, 2008). Adding benzophenone as an additive for plastic production will produce a polymer that photodegradable. This additive can accelerate the photochemical reaction by transforming the polymer composition by increasing the number of UV absorbing groups such as carbonyl and create a new polymer with a light-sensitive component (Kumar *et al*., 2011). The specific additive will improve the carbonyl groups in a polymer that is increasing the photo degradability along with the increasing of chromophores, which able to absorb more photons and start the photodegradation reaction. Carbonyl chromophores are important to absorb UV radiation and create a radicals by the Norish type I, II, and H-abstraction processes in photochemical degradation (Singh & Sharma, 2008).

The modification of the polymeric chain by copolymerization or blending micronutrients and biodegradable fillers will enhance the biodegradability of a polymer. These micronutrients will support the growth of specific microorganisms that may induce the biodegradability and by the formation of polar functional groups that facilitate the microbial attack (Singh & Sharma, 2008). Based on Singh & Sharma (2007) studied about the biodegradation of pure PS and three types of grafted PS films ([PS-g-poly(AAc)], [PS-g-starch] and [PS-g-poly(AAc)-co-starch]. The soil burial method was chosen as the biodegradation method and found that the PS with starch molecule can degrade 37% after 160 days; however, there is no degradation occur in the PS with poly(AAc) molecule.

Some types of plastic that are grafted with starch will be able to attack by microorganisms when it touches the water of soil surfaces. The first digestive part is starch and leaving a porous and sponge-like structure in the high interfacial area and low structural strength. After the starch completely digest, microorganism start to digest the polymer matrix by an enzymatic process, and slowly reducing the weight of the polymer (Kumar *et al*.,

2011). Research from Wool et al., (2000) found that there are two models for the starch degradation: (1) microbial attack to the substrate, and (2) macromolecular enzyme diffusion that produce small molecules to the surface so that microbes can absorb it.

Although some of the additives are increasing the biodegradation process, few additives aim to hinder the degradation process. An example of an additive that may hinder the degradation process is Hindered Amine Light Stabilizers (HALS). This additive has proven to be effective in prolonging the lifetime of 200μm PE film from 3 months to more than five years (Gijsman *et al*., 1993).

B.   The Molecular Weight of The Polymer

The biodegradation of a polymer depends on its molecular weight, which determines various physical properties and chemical composition (Tokiwa *et al*., 2009). The degradability of a polymer is inversely proportional to its molecular weight. The molecular weight properties control few properties of polymer material, such as tensile strength, low-temperature brittle point, and tear resistance (Richards, 1951). The biodegradability of polymers depends on their molecular weight. High molecular weight will give a sharp decrease of the solubility, and it is unfavorable to the microorganism because bacteria require the substrate that can be absorbed through the cellular membrane for further degraded by the cellular enzyme. In contrast, the lower molecular weight of the polymer (monomer, dimer, and oligomer) will be easily degraded and mineralized by microorganisms (Singh & Sharma, 2008; Gu, 2003). The decrease of molecular size of the polymeric molecule will increase the mechanical degradation, thermal degradation, and biodegradation (Singh & Sharma, 2008)

C.   Crystallinity

Polyethylene is consists of semicrystalline material made up of crystalline phase embedded inside an amorphous matrix (Behjat *et al*., 2014). The direct measurement of polyethylene using the infra-red spectrum at the bands 731 cm^{-1} and 1301 cm^{-1} will show the proportion of the amorphous and crystalline materials (Richards, 1951). Some methods could be used to measure the degree of crystallinity of polymer, such as

measurement of density, measurements of the heat of fusion, measurement of the intensity of X-ray diffraction patterns, and by using infra-red spectrum (Richards, 1951). The mechanical properties of the polymer will be affected by the molecular weight and degree of crystallinity. Figure 12 shows the schematic diagram about the relation between the molecular weight and crystallinity into the physical properties of the polymer (Richards, 1951).

Figure 2. Molecular weight effect on the degree of crystallinity (Richards, 1951).

The effect of molecular weight on the degree of crystallinity is only matters for the polymer with a molecular weight between 1000 – 20.000; above 20.000, the degree of crystallization is almost independent of molecular weight (Richards, 1951). Another factor that affects the degree of crystallinity is the presence of branching; this graph below is showing the relation between the amounts of branching and the amount of amorphous region that will affect the crystalline region.
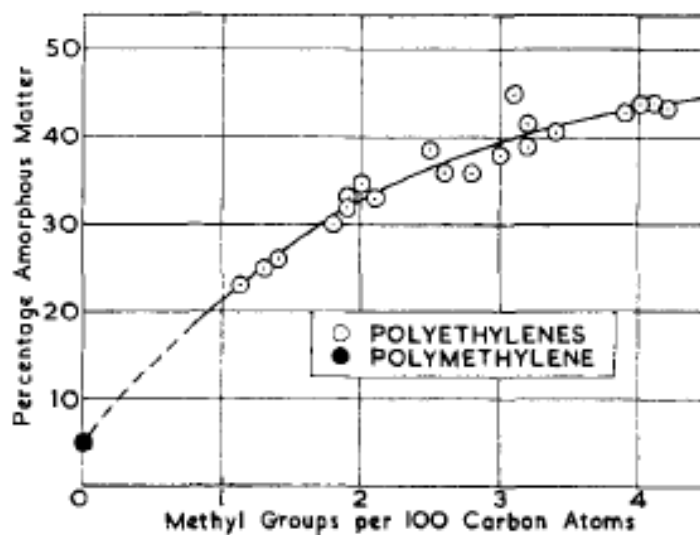
Figure 3. The relation between methyl groups to the percentage of the amorphous area (Richards, 1951).

Based on Figure 13 show that the increasing number of branching in the polymer will increase the percentage of amorphous regions. Because polyethylene has two different regions (amorphous and crystalline), the increase of the amorphous region will be impacted by the decrease of the crystalline region. As a result, it will change the polymer properties by making them more flexible and tough. The observation through FTIR found that the mechanism of the side chain to reduce the amount of crystalline region is by binding into the crystalline regions in the main chains, and it might be considered as the initiation or centers of amorphous regions (Richards, 1951).

Manzur *et al.* (2004) have been found that the amorphous area has two structures: (1) the amorphous area surrounds the crystalline particles, and (2) the amorphous area that defines the boundaries area of crystalline blocks of the crystalline mosaic. Richards (1951) observe the character of crystalline and amorphous region are arranged into spherulites.
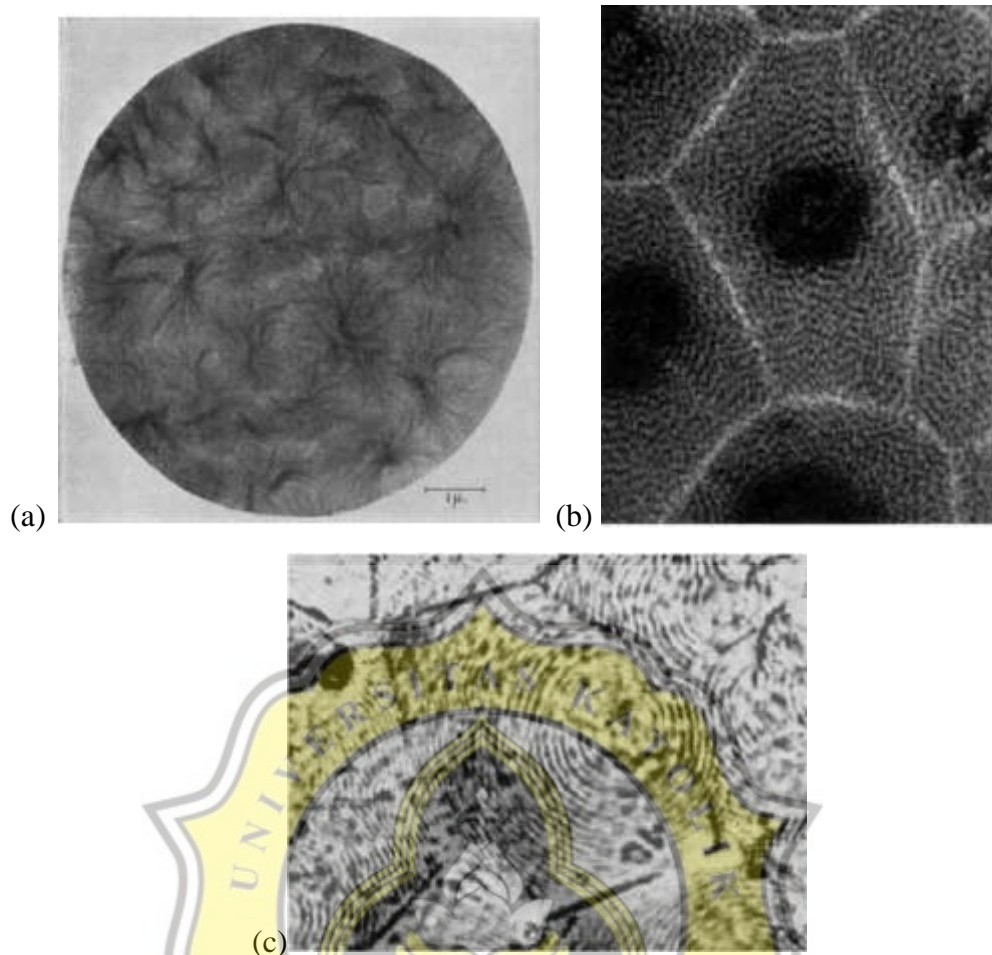
Figure 4. Spherulites observation. (a) SEM observation of polyethylene film (Richards, 1951), (b) SEM observation (1600X), and (c) Light microscope observation (480X) of fine spherulitic structure in polyethylene (Claver *et al*., 1956).

Those spherulites (Figure 14) have some evident characteristic: (1) there is a quite sharp boundary among the spherulites that divide into a spherulitic and non-spherulitic area, (2) the spherulitic form contains amorphous (lamella) and crystalline area, (c) spherulites can be observed through polarized light, the birefringent pattern, and the Maltese cross as the microscopic observation method (Claver *et al*., 1956).

Figure 15 shown detailed images of the polyethylene spherulite's shapes and growths. The spherulites build of individual lamella (dominant) that continuously made a new branching of new individual lamella (subsidiary) after the dominant structure melted. The lamellar growth starts from the core of spherulite (A) into the edge (B and C), and perform new layers. At the edge of spherulite, individual lamella grows continuously into the melt, while subsidiary lamella developed inside the interior (Bassett, 2003).
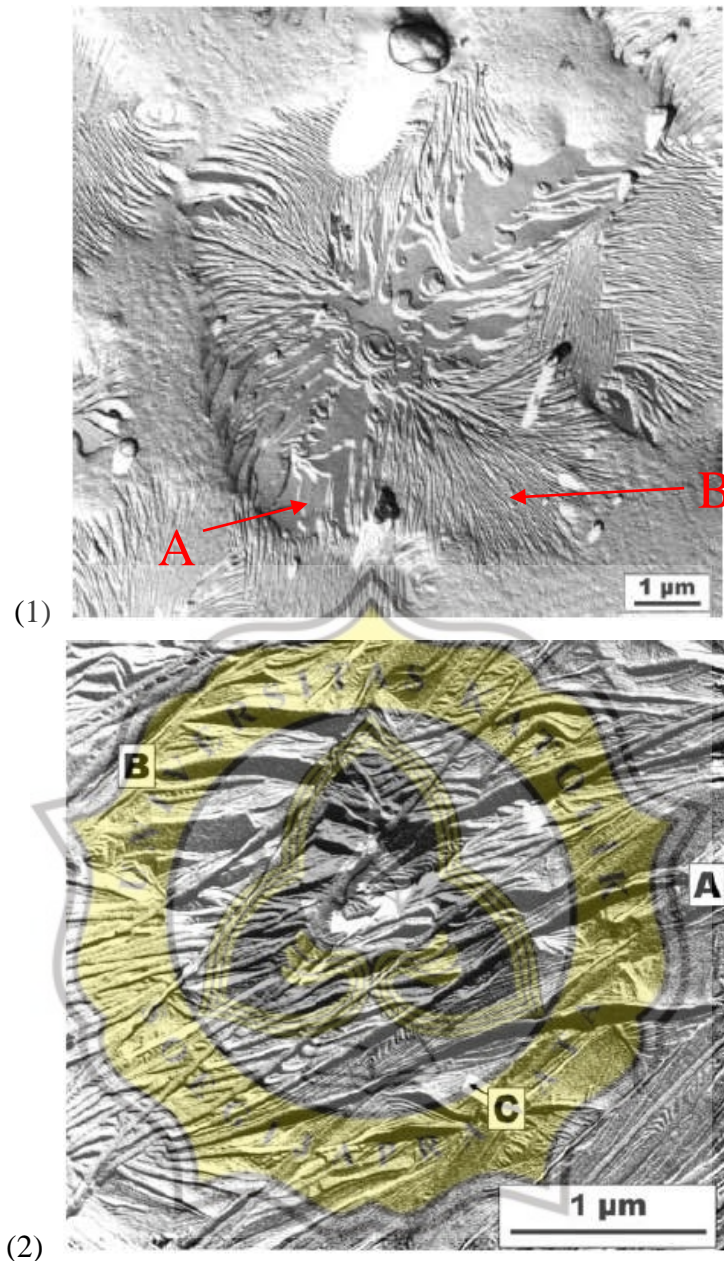
Figure 5. Lamellar structure. (1) The lamellar structure of linear low-density polyethylene (LLDPE) spherulite grown at 124°C then cooled down, (2) the inside structure of linear polyethylene spherulite grown at 128°C (A) the dominant lamella (B) the subsidiary lamella growing from dominant lamella (C) infilling lamella that might be formed on quenching (Bassett, 2003).

In general, high crystallinity will increase the susceptibility to embrittlement of the polymer. The long-range of polyethylene structure and morphology is considered to be an important factor to the biodegradation, due to the amorphous area that easily to degrade compared to the crystalline area (Talkad *et al*., 2014; Manzur *et al*., 2004). The research from Gatenholm, Ashida, & Hoffman (1997), crystallinity properties will be increased

during the first 5 min of ozone exposure to PP material and then decrease along with the increase of ozone exposure time. This increase and decrease of crystallinity happen because the degradation process starts in the amorphous phase of PP, and the chain scission will improve the crystabillity of the polymer by shortening the polymeric chains and make it easy to form a crystal. The increase and decrease of crystallinity during the biodegradation process can be explained into two stages of mechanism. This mechanism was introduced in Figure 16 by research from Manzur *et al*., (2004) when observing on the kinetics of crystallinity changes of physicochemical-treated (PCT) LDPE subjected to biological treatment. This research was comparing four samples: (thermal treatment) TT/150, TT/105, (accelerated aging treatment) AAT, and (untreated milled LDPE) U-LDPE.
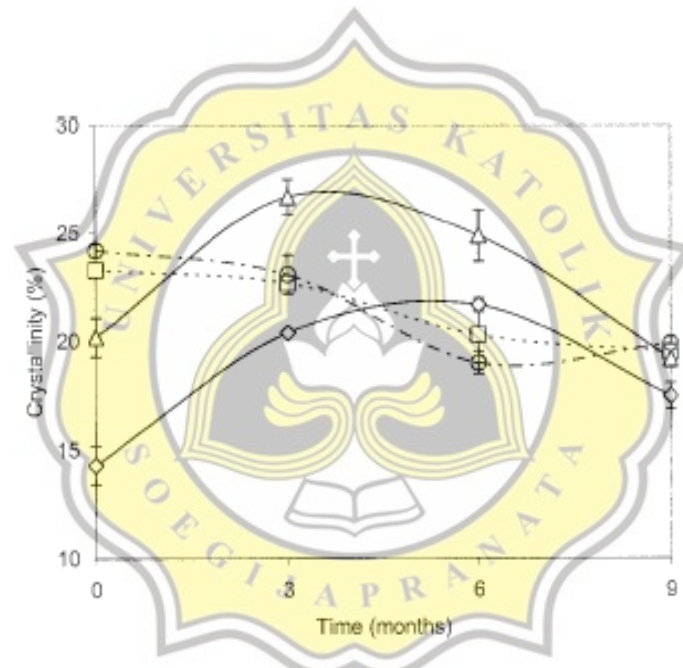

Figure 6. Degree of crystallinity changes during biodegradation. □U-LDPE, Δ TT/105, ◊ TT/150, ○ AAT (Manzur *et al*., 2004).

The thermal treatment gives the highest changes on the degree of crystallinity by a significant increase at 3 and 6 months (6.5% in sample TT/105, and 7.4% in sample TT/150), then followed by the decrease of crystallinity. First, the degree of crystallinity will increase in the first stage of biodegradation due to the microbial attack in the amorphous area. The second stage is by attacking the smaller and imperfect crystal, then reduce the degree of crystallinity during the biodegradation process. This mechanism confirmed by calculating the mean size of the crystalline particles in the polyethylene by using the following equation:

$$L_{hkl} = \frac{K\lambda}{\beta cos\,\theta}$$

This L_{hkl} represents the mean crystal normal to the corresponding *hkl* plane, *K* is the constant of the order of 1, λ is the wavelength, 2θ is the scattering angle, and the β represents the half-height width of the scattering peak. The result is the increase of the mean size of crystalline particles after nine months of biodegradation process, by this result, it can be concluded as the high microbial activity on attacking amorphous area and the smaller crystalline particles while leaving behind the bigger crystalline particles (Manzur *et al*., 2004).

Figure 17 shows the explanation from Behjat *et al*. (2014) about the brittle failure mechanisms of polyethylene in the lamella-crystalline structure in spherulites.
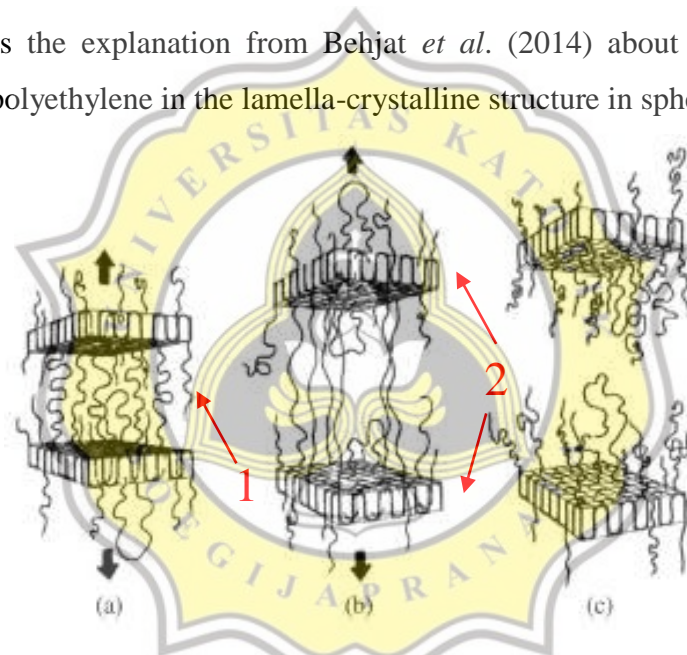

Figure 7. The brittle mechanisms of PE. (1)Lamella part, (2) crystalline part (Behjat *et al*., 2014).

These brittle mechanisms cause by stress in the inter-lamellar bond (a) lamella structure start to pull away; (b) inter-lamellar bond begins to disintegrate; (c) total break on the inter-lamellar bond. The continuous stress to the bonds will accumulate stress and break the inter-lamellar bonds into smaller crystals (Behjat *et al*., 2014). The biodegradation process by microorganism also undergoes into this mechanism by binding and destroy the lamella surfaces as the amorphous area and increasing the degree of crystallinity by the separation of crystalline blocks from the crystalline mosaic. The next mechanism is an attack on the small size crystal inside the lamellar structure and increasing the number

of amorphous areas while reducing the crystalline area and reduce the degree of crystallinity (Manzur *et al*., 2004).

D. Environmental Condition

Based on the explanation Orhan *et al*. (2004), the environmental condition including moisture, temperature, oxygen, and suitable microorganism population during the biodegradation process determines the effectivity and degradation rate of plastic material. The biodegradation rate of polymer increase in the warm condition and the relative humidity of more than 70% (Singh & Sharma, 2008). The absence of oxygen in landfills further resists its natural degradation process. There are two metabolism method in the biodegradation of plastic, aerobic and anaerobic metabolism. Carbon dioxide and water as the end product in both metabolisms, but methane is also released in anaerobic metabolism (Asmita *et al*., 2015).

Temperature plays a key role in the biodegradation process, performs a strong influence on the metabolic activities of microbial communities. The easiest way to describe the effects of temperature on the rate of the physiological, biochemical, or behavioral process is to create a thermal performance curve (TPC). This TPC's graph has three distinct part: (1) a rising phase, while the temperature increase; (2) a plateau phase, which is the thermal optimum (T_{opt}) for the process; and (3) a falling phase slope, at higher temperatures (Pischedda *et al*., 2019). The research from Pischedda *et al*. (2019) shows a great correlation between the incubation temperature and the rate of mineralization in Figure 18. This mineralization rates then process into the TPC graphs (Figure 19), which plot the specific mineralization rates K and the respective temperature (T).
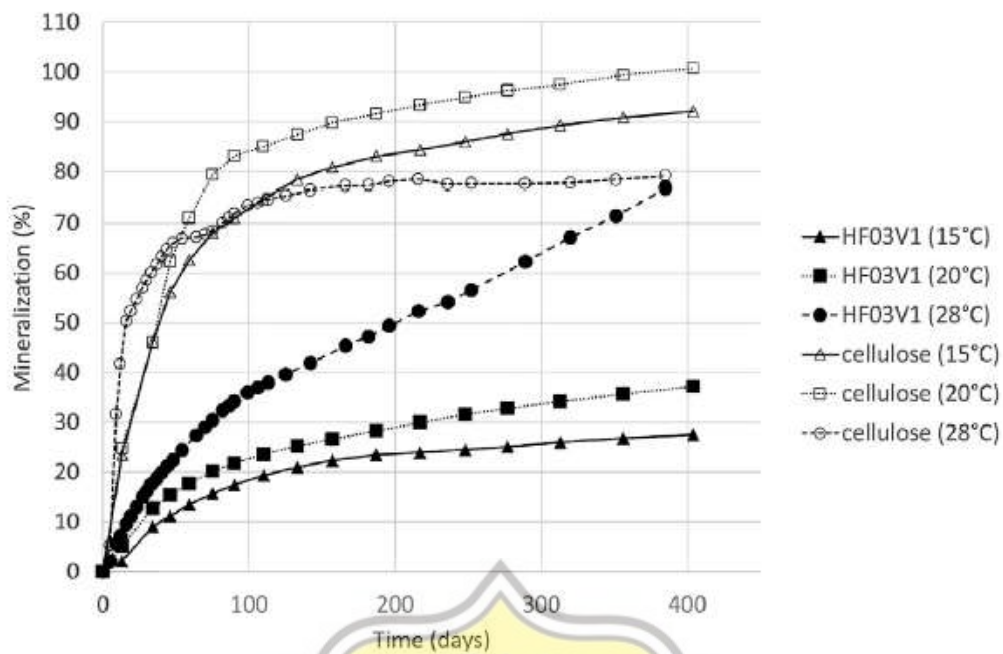
Figure 8. Mineralization curves of sample and reference material were tested at different temperatures (Pischedda *et al*., 2019).
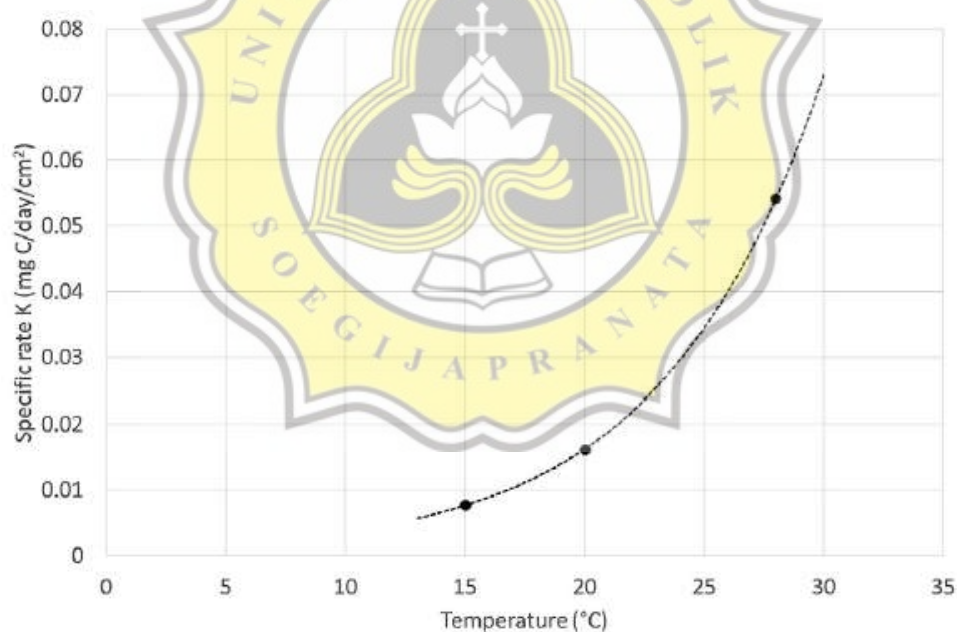

Figure 9. Thermal Performance Curve (TPC). Showing an exponential result (Pischedda *et al*., 2019)

These are some researches that support the theory of temperature effect on the biodegradation rate of the polymer. The research from Lotto *et al*., (2004) found that the biodegradation rate at 46°C was higher than at 24°C on the three types of biodegradable plastic in the compost biodegradation method. Other research from Mergaert (1992)

found that the biggest mass loss of biodegradable polymer in soil biodegradation method was at 40°C, compared to 15°C and 28°C. In contrast, the research from Nishide *et al*., (1999) reported that the highest biodegradation rate for three out of four types of the polymer was achieved at 30°C of incubation inside the soil, while at 52°C only show the high biodegradation rate for one type of polymer.

Based on Figure 18 and 19, the incubation temperature can divine the biodegradation rate or mineralization rate, in which higher temperatures will give a higher rate of biodegradation process, as long as the temperature is still in the boundary between the maximum and minimum temperatures for microbes to live (Pischedda *et al*., 2019). The research from Nishide *et al*., (1999) also supports this theory of temperature boundary, showing that the microorganisms were optimum at 30°C instead of 52°C, which far beyond the temperature boundaries of microorganisms to live. Commonly, the range of mesophilic microorganisms is at 10° – 45°C, so at the 52°C, microorganisms will difficult to live (Pischedda *et al*., 2019).

E. Surfactant

Some treatments can enhance the microbial ability to degrade petrochemical-based plastic or commonly used plastic such as bio-surfactant such as Tween 80 that aims to increase the hydrophilicity of the polymer. Petro-based plastics tend to be hydrophobic so that it could interfere with the formation of microbial biofilm on the polymer surfaces (Talkad *et al*., 2014; Hadad *et al*., 2005[a]; Yamada-Onodera *et al*., 2001). The more hydrophobic and high water repellency properties, then the polyethylene materials are considered to be inert to biodegradation (R. Pramila, 2011). Bio-surfactant may enhance the solubility and biodegradation of hydrophobic hydrocarbons (Barkay *et al*., 1999).

The research from Gilan *et al*., (2004) found that the addition of mineral oil to the *Rhodococcus ruber* strain C208 was able to increase both colonization and biodegradation of polyethylene, while the addition of nonionic surfactants such as Tween 60 and Tween 80 did not affect. This theory is the opposite of Talkad *et al*. (2014) that say the addition of surfactants such as Tween 80 will increase the hydrophilic properties of the polymer so that microbes can attach with the polymer surface. According to the

bacterial adhesion to the hydrocarbon (BATH) test, the species of *Rhodococcus ruber* strain C208 is tend to be more hydrophobic. This characteristic works well with the hydrophobic properties of the polymer, and the addition of mineral oil increase the hydrophobicity of bacteria cells, so it was successfully increasing the ability of biofilm formation on the polymer surface (Gilan *et al*., 2004).

In contrast, the research from Gilan *et al*. (2004) did not show an increase in biofilm formation of microorganisms after the addition of nonionic surfactant, while the research from Albertsson *et al*., (1993) showed that nonionic surfactant (Tween 80) increased the biofilm formation and biodegradation of polyethylene by *Pseudomonas aeruginosa*. Other research from Yamada-Onodera *et al*. (2001) showed a nonionic surfactant (Triton X-100) supports the growth of *Penicillium simplicissimum* in medium contains polyethylene.

According to previous researches from (Talkad *et al*., 2014; Gilan *et al*., 2004; Yamada-Onodera *et al*., 2001; Albertsson *et al*., 1993), the addition of surfactant will improve the growth of microorganisms and their ability to perform a biofilm on the polyethylene surface. Most bacteria are hydrophilic, but some of them are hydrophobic, so the types of surfactants are depended on the hydrophobic properties of microorganisms' cells. Too much addition of surfactant will possibly decrease the biodegradation rate because the microbes will utilize the surfactant instead of the polyethylene (Gilan *et al*., 2004).

F. Biofilm Formation

The ability of microbes to perform biofilm on the polymer surfaces depends on the cell surface hydrophobicity properties of the microorganism, and biofilms formation is considered to be an important factor in enhancing the biodegradation process (Usha et al., 2011a; Vijaya & Mallikarjuna Reddy, 2008). Microorganisms need to create biofilm by using the "microfouling" process, which is the process that is establishing the complex community on the surface material. Biofilms contain microorganisms and their extracellular polysaccharides that highly diverse and variable according to their space and time. This process is a prerequisite for the deterioration process of the underlying materials. Biofilms can be established on any surfaces in both terrestrial and aquatic

environments such as metals, inorganic minerals, and organic polymer under the suitable humidity. On the molecular level, the development of the biofilm on surfaces is controlled by chemical signaling between bacteria. These are examples of the specific chemical molecules that relevant to biofilm formation: *N-(3-oxododecanoyl)-L-acylhomoserine* in *Pseudomonas aeruginosa*, and ɣ-butyrolactone in *Streptomyces* spp (Gu, 2003).

The biofilm formation usually starts at the end of the microorganisms lag phase due to the acclimatization with the growth media and carbon source as nutrients from the polymer degradation. The research from Kyaw *et al*. (2012) found that *Pseudomonas* spp., able to form biofilms along with the planktonic cell growth. However, in the first rapid planktonic cell growth and proliferation shows the constant growth of biofilm formation, while in the less constant of planktonic cell growth, it will show a steady growth of biofilm formation. Another research from (Vijaya & Mallikarjuna Reddy, 2008) found that the weight loss of polymer was detected after four months of the composting process and assume a lag period of four months was because of the late formation of biofilm by microorganisms. Imam *et al*. (1999) observed that a significant biodegradation rate would be achieved only after the colonization or biofilm of microorganisms on the plastic surface, which depends on the resident microbial population. Biofilm formation and growth are able to quantify by using conventional TPC tests (Manijeh *et al*., 2008).

Biofilm formation could be a factor for the biodegradation rate, but it cannot be used as the only one measuring method for the biodegradation rate. The research from Pramila *et al*., (2012ᵃ) and Hadad *et al*., (2005) found that even the result of quantification of biofilm was low, the bacteria are still able to biodegrade the polymer material due to the decrease of dry weight loss and molecular weight loss. Also, Pramila *et al*., (2012ᵃ) found that one of the isolates shows a low ability for biofilm formation, but it has the highest doubling time compared to other isolates that were used. This founding from Pramila *et al*., (2012ᵃ) and Hadad *et al*., (2005) was leading into a hypothesis that microorganisms can biodegrade polymeric component even though it was not performing a biofilm on the polymer surface.

G. Substrate and Microorganism Pre-treatment

Polymer degradation can be done as a pretreatment using abiotic degradation, such as photo-oxidation and physical disruption to the polymer, which may enhance the biodegradation process. There are three types of pre-treatment for plastic before the biodegradation process, *i.e.,* physical or mechanical treatment, chemical treatment, and physicochemical treatment.

This pretreatment will increase the surface area for the microbial colonization and also reducing the molecular weight (Singh & Sharma, 2008). Physicochemical treatment to a polymer will initiate the oxidation and further decay by supporting microorganisms to easily adhere to the polymer surface by changing the hydrophobicity characteristic of the polymer surface, which is a prerequisite for the biodegradation process (Talkad *et al*., 2014). The research from Manzur *et al*. (2004) found that the thermal treatment (TT) will more affect the crystallinity by the change of crystallinity properties than forming carbonyl groups on the polyethylene bonds; this is reverse to the accelerated aging treatment (AAT) result. These are the example of treatment into the substrate or the microorganisms:

(i) Induce Mutation to Microorganism

The induced mutation method can be effective in improving the ability of microorganisms on the biodegradation process. The research from (Talkad *et al*., 2014) reported that the mutation of *Pseudomonas putida* by using UV light (254 nm) and Ethyl methanesulfonate (EMS) treatment successfully increases the ability to convert biomass material, especially plastic (LDPE) into sugar, compared to the result from the non-mutated bacteria.

(ii) UV Light Exposure to Polymer

Photooxidation of plastic by UV radiation and followed by microbial metabolism for the natural biodegradation process (Asmita *et al*., 2015). UV light pretreatment to plastic will enhance the degradation rate of the polymer, and this process is commonly known as photo-oxidative degradation. The UV wavelength damaging intensity is

different for every plastic-type because it depends on the bonds present in the plastic structure; for PE, it is around 300 nm and around 370 nm for PP. The most damaging effect of this photo-oxidative degradation is the changes in the visual appearance (yellowing), the loss of the mechanical properties, and changes in the molecular weight. The physical properties of PE and PP films will be decreased when exposed to UV radiation, the loss of extensibility, mechanical properties, and strength, along with the decrease of the molecular weight average. The combination of light and oxygen (oxo-biodegradation) induce the reaction in polymers that able to change the mechanical properties and discoloration (Gijsman *et al*., 1993). The presence of chain-breaking antioxidants in the polymer will prevent the thermo-oxidative condition during the oxo-biodegradation process (Singh & Sharma, 2008).

(iii) Ozone Exposure to Polymer

Some of the previous research has been shown the result of O_3 (ozone) treatment to modify the polymer surfaces to control the bacterial cell adhesion to the polymer surfaces (Ozen *et al*., 2002). The presence of ozone in the degradation process of the polymer, even in the small concentration, will speed up the aging process of the polymer (Singh & Sharma, 2008). The reaction between ozone and polymers will generate the formation of oxygen-containing functional groups and the degradation process of a polymer chain. This reaction will change the polymer properties, such as polarity and surface tension (Ozen *et al*., 2002). The reaction between ozone and the polymer will occur in the main chains containing the C=C bonds, aromatic rings, or saturated hydrocarbon rings. The output from this reaction is the unstable intermediates like the bipolar ion or peroxy radicals that able to degrade or isomerize and causing the degradation of macromolecules in the polymer (Singh & Sharma, 2008). The result from ozone treatment to PE, PP, and PET are increasing of the polymer surface tension and hydrophilicity, also give an improvement in the adhesion characteristic (Ozen *et al*., 2002).

Ozen *et al*. (2002) have been researched the ozone oxidation process to PE, PP, and polybutylene resulting in changes in their physical properties such as a reduction in

melting point, intrinsic viscosity, and increase of solubility. The ozone exposure to PS and PE films also showing changes in the color of PS powder after exposure, and the result of PS film made by using this PS powder becomes brittle and opaque. PE films that being exposed to ozone for 100 h resulted in degradation of their macromolecules, and for the longer exposure, up to 300 h resulted in increases of crystallinity and elastic modulus of the PE films (Ozen *et al*., 2002).

Ozen *et al*., (2002) analyzes the tensile strength (TS) and elongation at break (EB) in linear low-density polyethylene (LLDPE) film that treated with three variation concentration of ozone, time, relative humidity, and temperature. This research showed a sharp decrease at TS and EB, along with the increase of the ozone concentration and temperature. The increasing time in ozone exposure will decrease the oxygen permeability properties due to the increase of molecular polarity in polymer and decrease the permeability of the polymer. The result of 24 hours treatment of polymer with the ozone exposure is the oxygen permeability decrease up to 40-50% (Ozen *et al*., 2002).

The ozone exposure to microporous polypropylene (PP) in 1-2 h will degrade the PP material, showed by the SEM observation in Figure 20 as larger pores and brittle characteristics.
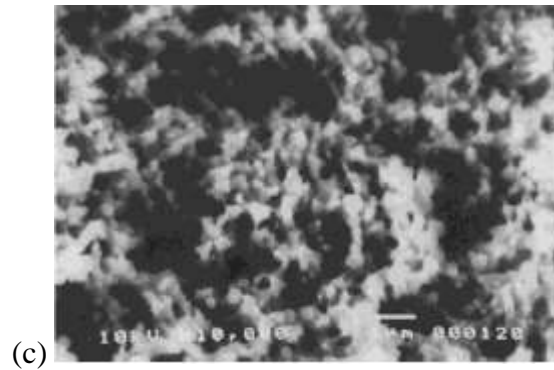

(a)   (b)

(c)
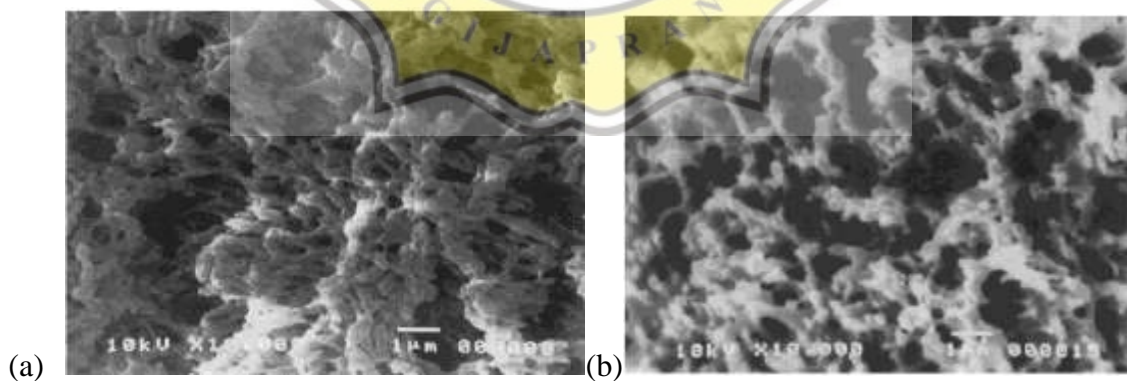
Figure 10. SEM observation on PP sample. (a) Cross-section of the virgin membrane, (b) cross-section of membrane ozone-treated for 30 min, (c) cross-section of membrane ozone-treated for 60 min (Gatenholm *et al.*, 1997).

The molecular weight of PP was reduced significantly in the first 5 minutes of ozonation, and the longer ozone exposure time will continuously decrease the molecular mass (Gatenholm *et al.*, 1997). Prolonged exposure to the ozone will further reduce the molecular weight and gradual modification of the polypropylene chemical composition because of the various polar molecules from the degradation process change the backbone structure. The chain scission during the ozonation process will be taken place on the material surfaces and in the intercrystalline amorphous region (Gatenholm *et al.*, 1997).

Figure 21 shows that the mechanisms of ozone-induced degradation are undergone in three principal steps. First is the formation of ozone-olefin bonds or known as "primary ozonide," this unstable bonds because it contains two very weak O–O bonds. The second step is the degradation of the primary ozonide into carbonyl compounds and carbonyl oxide. This carbonyl oxide is considered to be a key intermediate in the ozonolysis mechanism of C=C bonds. The third step is the fate of the carbonyl oxide in the environment (Singh & Sharma, 2008).
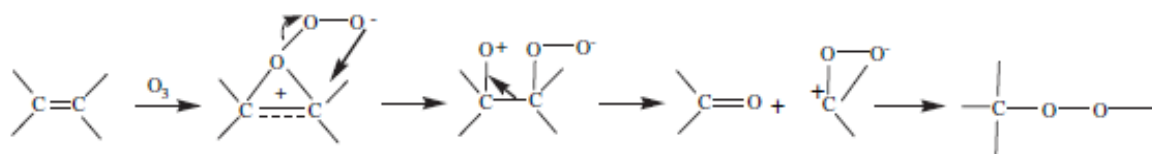

Figure 11. Mechanism of ozone degradation to polyethylene material (Singh & Sharma, 2008)

1.2.6. Standard Testing and Analytical Assessment for Biodegradation

This polymer degradation will change the physical and chemical properties, such as a loss of weight, reduction in tensile strength, discoloration, shape (cracking, erosion, separation, and delamination), chemical transformation, loss of molecular weight, and a new functional group. This process is often termed "aging." (Jumaah, 2017; Kumar *et al*., 2011; B. Singh & Sharma, 2008).

The correlation between weight loss and tensile strength of the plastic is indicating a strong positive correlation to the biodegradation result. This correlation means when the microorganisms are able to metabolize the plastic material as a nutrient for their growth, the plastic weight will be reduced and resulting in a decrease of the tensile strength (Vijaya & Mallikarjuna Reddy, 2008). The American Society for Testing of Materials (ASTM) and the International Standards Organization (ISO) define the degradable plastics are the plastic which undergoes a significant change in chemical structure in specific environmental condition. They provide the analytical protocol for analyzing plastic degradation due to their fundamental alteration in chemical structure and loss of physical properties. There are four types of degradable plastic: photodegradable, oxidatively degradable, hydrolytically degradable, and biodegradable plastic that able to be composted (Kumar *et al*., 2011).

A. Visual Observation

The biodegradation result usually performs visible changes in plastic such as cracks, de-fragmentation, change in color, and biofilm formation. This visual observation can be done by using several tests or molecular observation such as Scanning Electron Microscope (SEM) or Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Colorimetry (DSC), nuclear magnetic resonance spectroscopy (NMR), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), contact angle measurements and water uptake (Mahalakshmi, 2014).

(i) Fourier Transform Infrared Spectroscopy (FTIR)

The spectroscopic analysis usually uses the FTIR (Fourier Transform Infrared Spectroscopy). Watanabe *et al*. (2009) used this method to analyze the result of LDPE degradation. The main carbon chain of –C=C– was detected around 1640 cm^{-1}, and the result was the presence of –OH starching vibration around the 1080 cm^{-1} derived from alcohol and the –OH stretching vibration around 3400 cm^{-1} were responsible for the increasing of whitened parts compared to the transparent parts (Figure 22).
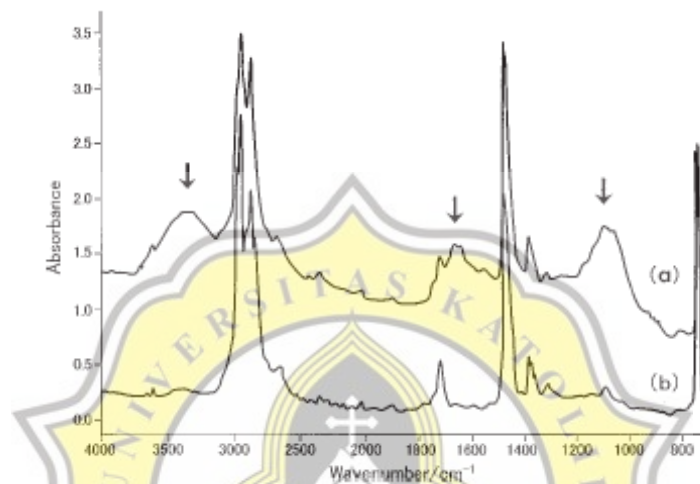

Figure 12. FTIR microscope spectra of the LDPE mulch film. (a) Whitened part. (b) Clear part (Watanabe et al., 2009a).

Another research from Ohtake *et al*. (1998) shown that the yakult package films and LDPE mulch film are able to be degraded by soil microorganisms; the assessment test was using an FTIR microscope (Figure 22). This research made an index of particular absorption bands of C=C (1640^{-1})[56] to indicate the biodegradability. Then, another absorption bands that important was carbonyl groups C–O (1715 cm^{-1}), hydroperoxide –OH (3600 cm^{-1}) as the output of normal deterioration, and –C–O– (1100 cm^{-1}) from the alcohol. The biodegraded Yakult package film found that absorption near 1640 cm^{-1}, which indicates the biodegradation process. The absorption also found near 1715 cm^{-1} that indicating the synergistic effect of photo-degradation and biodegradation.

The mulch film was divided into two areas, which were fully buried in the soil (whitened part) and partially buried in the soil. However, the result from mulch film shows that a little absorption of ester-carbonyl groups is detected near 1740 cm^{-1} at

a clear part-1. The clear part-1 was not showing any degradation, and almost similar to the undegraded LDPE. Compared to the clear part-2 that detected the absorption near 1715 cm^{-1} and 1640 cm^{-1}, which indicate the photo-degradation process in the absorption near 1715 cm^{-1}. This photo-degradation only gives a minimal impact because the –C–O– vibration near 1100 cm^{-1} caused by alcohol and hydroperoxide vibration around 3600 cm^{-1} were undetected. The contrast result from the whitened part that indicating a positive result of the biodegradation process. The absorption in 1715 cm^{-1} is low, but absorption near 1640, 1100, 3600, and 3400 cm^{-1} showing a characteristic of the biodegraded sample remarks on changes in the chemical bonds.
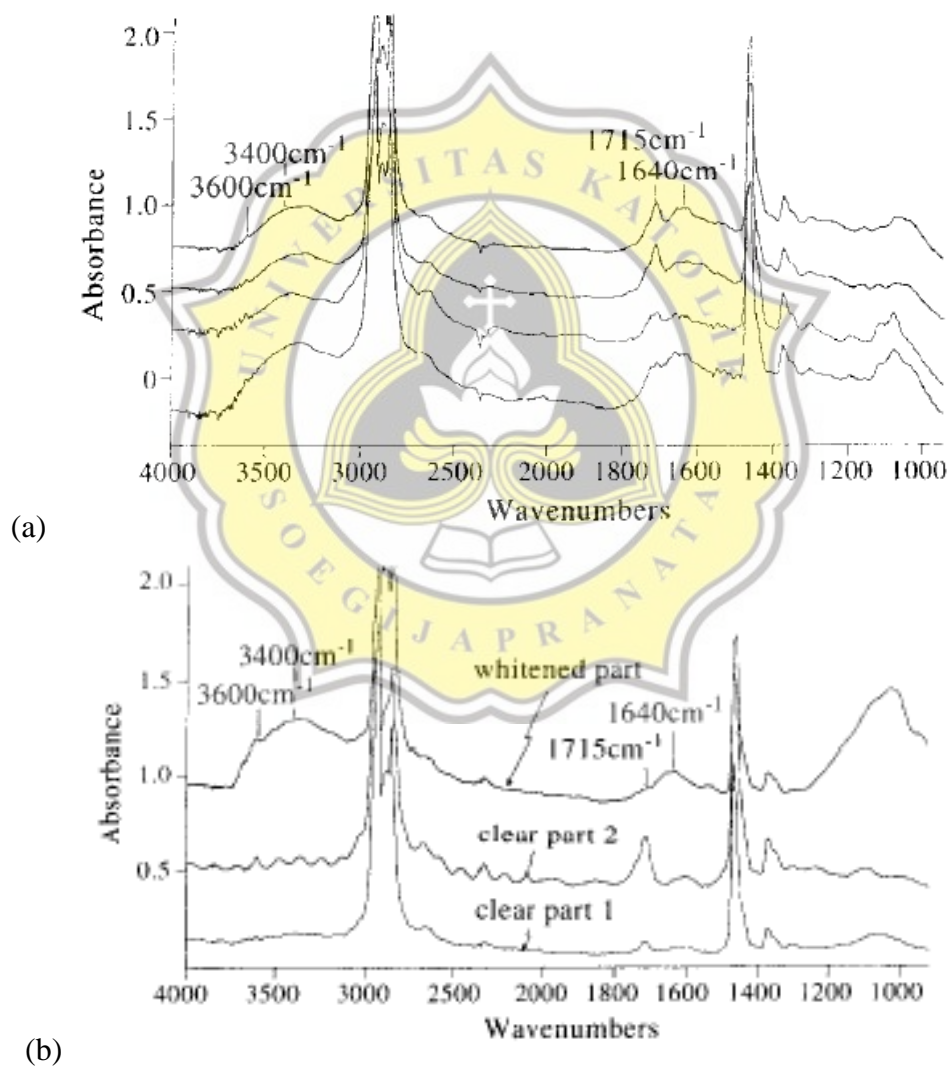

(a)

(b)

Figure 13. FTIR result of biodegraded sample. (a) FTIR result of biodegraded Yakult package films. (b) FTIR result of mulch films (Watanabe et al., 2009a)

This method aims to find the changes in the polymeric bonds due to the biodegradation process by using an FTIR-ATR spectrophotometer. The analysis was obtained by calculating the carbonyl index (CI) as a valid method to measure the degree of biodegradation. CI method is obtained by the formula (Kyaw *et al.*, 2012):

$$\text{Carbonyl Index (CI)} = \frac{Absorption\ at\ 1740\ cm^{-1}\ (the\ maximum\ of\ carbonyl\ peak)}{Absorption\ at\ 1460\ cm^{-1}\ (the\ maximum\ of\ carbonyl\ peak)}$$

(ii) Clear Zone Formation

Microorganisms can create a clear zone as the parameter for the successful degradation process. Augusta, Müller, & Widdecke (1993) observed that the extracellular hydrolyzing enzymes from microorganisms able to hydrolyze the suspended polyesters inside of the turbid agar medium and convert it into water-soluble products, then it showed a clear zone around the colony. This method used agar plate to inoculate the polymer with the microorganism inside, and after the inoculation process, there will be a formation of a clear halo around the colony. This halo formation indicates that the microbes can degrade the polymer, and also it can be used as semi-quantitative results by analyzing the growth of clear zones (Mahalakshmi, 2014).

There are several requirements to achieve the biodegradation process and clear zone formation: the microorganisms must be able to secrete the exo-enzymes, the microorganisms able to diffuse through the surrounding medium, and the interaction between the enzymes and the polymer. Figure 24 shows an example of a clear zone in the biodegradation process of PHB-co-HV by bacteria culture.
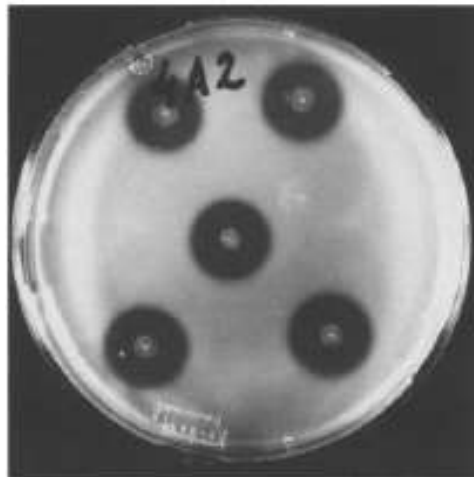
Figure 14. Clear Zone observation of a plate-puncture clear zone test (Augusta *et al*., 1993).

B. Bacterial Adhesion to Hydrocarbon (BATH) Test

This test can be an important prerequisite before giving a surfactant for the biodegradation test. Bacterial adhesion to hydrocarbon (BATH) test can be used as a general, quantitative assay for measuring cell-surface hydrophobicity. This test aims to estimate the bacterial cell hydrophobicity to a hydrocarbon material that can be related to the ability of bacteria to form a biofilm on the hydrophobic surfaces. In some cases, the BATH test can be used to isolate the non-hydrophobic strain (Rosenberg, 1984).

According to Rosenberg (1984), the mechanisms for the BATH test is easy to understanding. The mechanisms can be summarized as follows: a suspension of washed bacteria cells is mixed by vortexing in the addition of a test liquid hydrocarbon under controlled conditions. Then, after the mixing, the two phases will be separate and perform an oil-water layer. In the case of adherence, bacteria cells from a turbid aqueous phase will be bound with the hydrocarbon droplets and rise to the surface following the mixing to form an upper 'cream' layer consisting of cell-coated oil droplets. The non-adherent bacteria cell will be creating two phases separate after the mixing, with the cells remaining in the turbid aqueous suspension.

The percentage of adherent cells counted by a decrease in absorbance of the aqueous phase after the assay compared to the absorbance of the original bacteria suspension

before the assay. The higher percentage of adherent cells shows the cells are more hydrophobic than non-adherent cells, and it will be easier to attach on the polymer surface and perform a biofilm formation. The hydrophobic bacteria will grow better in the biodegradation of polyethylene with the addition of mineral oil as a surfactant, while the hydrophilic bacteria will grow better with a nonionic surfactant such as Tween 80, Tween 60, and Triton X-100 (Gilan *et al*., 2004; Yamada-Onodera *et al*., 2001; Albertsson *et al*., 1993).

Based on Rosenberg (1984), found that there are several major aspects of the assay that are important:

(i) It is important to make sure that bacterial suspensions are free of surfactants, which can inhibit the assay. Also, the test tubes must be clean and preferably acid-washed to prevent the residues.

(ii) Both the lower aqueous phase and the upper phase should be tested microscopically to ensure that the cells adhere to the hydrocarbon. So, the water interface and the decrease of turbidity is not because of other factors like clumping or lysis.

(iii) The mixture of cell and hydrocarbon can be 'overloaded' if the initial bacterial suspension is too concentrated. Bacteria cell concentration should be assayed before the BATH test.

(iv) The short duration or insufficient mixing will be affecting the low ability of bacterial adherence. This problem can be fixed by increasing the duration of the vortexing procedure.

(v) The ionic strength and composition of the buffer may disrupt the assay and affecting the results. Some of the previous research found the decrease of adherence because of lowering the ionic strength of the suspending buffer.

(vi) The adherence of a bacteria strain largely depend on the growth conditions, so the adherence should be checked at various growth phases, different growth temperatures, and different growth media before proclaiming a strain to be non-adherent.

C. Quantification of Biofilm and Bacterial Biomass Colonizing the Polymer

This test is aimed to check the amount of colonizing microorganisms on the polymer surface, first is by counting the biofilm formation, and the second is by calculating bacterial biomass. The biofilm formation is counting the amount of bacteria cells by using an absorbance test (O'Toole et al., 2000). Then, the bacterial biomass test is aimed to calculate the population density of the bacteria colony by calculated the extractable protein compound on the polymer surface (Hadad et al., 2005b).

This bacterial biomass test can be achieved by using the standard method to calculate the protein concentration such as Lowry assay (LOWRY et al., 1951), Bradford assay (Bradford, 1976), Smith assay (Smith *et al*., 1985), and Sedmak assay (Sedmak & Grossberg, 1977). The mechanism for this method was to boil the polymer sample using 5 ml of NaOH 0.5 mol 1^{-1} to extract the protein, and the suspension was centrifuged at 10.000 rpm for 15 minutes, then followed by the protein assay procedures (Hadad et al., 2005b).

D. Sturm Test

This method is detecting CO_2 as the output of aerobic biodegradation because it easy to detect the presence of CO_2 in the biodegradation process (R. Pramila, 2011). The research from (Pramila & Ramesh, 2011; R. Pramila, 2011) demonstrate the modified Strum test by using gravimetrically and volumetrically Sturm test (Figure 25).
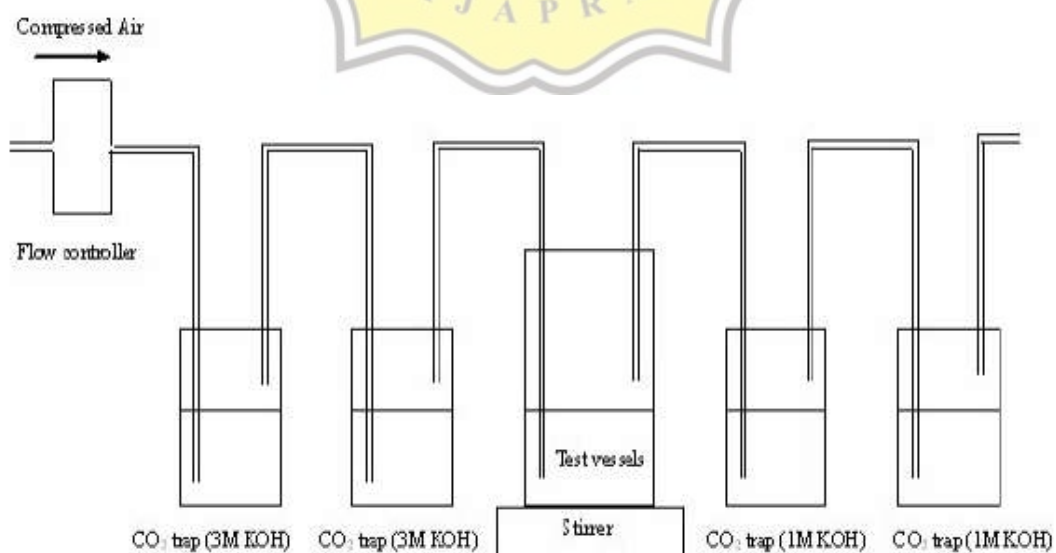

Figure 15. Sturm test mechanism (R. Pramila, 2011)

The microorganism was put inside the test vessel, and sterile air was allowed to flow through the 3 M KOH as the air purifier from CO_2. Then, the air will be utilized by microorganisms and release CO_2 as the output, and the air goes onto the next bottle with 1 M KOH to trap the CO_2. The next step is to measure the CO_2 amount in the bottle by adding 0.1 M $BaCl_2$ to create $BaCO_3$ (barium carbonate) in the bottom of the bottle.

This Sturm test is known as the gravimetric method by measuring the weight of the precipitate form of barium carbonate. The volumetric method is designed to measure the dissolved CO_2 that present in the growth medium by using the titration method. This titration was started by taking 25 ml of medium sample to the conical flask, 0.05 ml of 0.1 N Thiosulphate solution was added, and two drops of methyl orange indicator. Then, the titration was undergone by 0.02 M Sodium Hydroxide solution. The color change notes the end of titration from orange-red to yellow. The next step is the addition of two drops of phenolphthalein indicator and continues the titration until the color change into pink. The total volumes of titrant used in titration were noted and use for the calculation of CO_2 by using the formula:

$$\text{Total CO}_2 \text{ (g/L)}: \frac{A \, x \, B \, x \, 50 \, x \, 1000}{V}$$

A = mL of NaOH titrant
B = normality of NaOH
V = mL of the sample

Sturm test usually used for the evaluation of the biodegradability of polymer materials, and the modified Sturm test aims to measure the CO_2 both in the gaseous and dissolved form in the media (Pramila & Ramesh, 2011).

E. Weight Loss Presentation

Weight loss presentation can be used as an indicator of successful polymer biodegradation (Vijaya & Mallikarjuna Reddy, 2008). The weight loss percentage of a polymer can be calculated by the following formula (Kyaw *et al*., 2012):

$$\text{Weight loss (\%)} = \frac{initial \; wight - final \; weight}{initial \; weight} \; x \; 100$$

F.   Observation on the Mechanical Properties

Vijaya & Mallikarjuna Reddy (2008) found that the biodegradation period by composting of a polymer will significantly affect the decrease in the tensile strength of the polymer, besides the plastic types. The degradation process will affect the properties changes of the polymer: tensile strength, color, and shape. These changes in plastic properties, usually called aging. (Kumar *et al*., 2011). This test can be observed by using Tensile strength (TS), and extension at break (EAB) test.

G.   Gas Chromatography Coupled with Mass Spectroscopy (GC-MS) Analysis

Biodegradation of a polymer will produce a wide variety of volatile and semi-volatile compounds that can be detected by gas chromatography (Kyaw *et al*., 2012). Some chemical compounds were detected as the result of LDPE biodegradation by using *Pseudomonas aeruginosa* PAO1 such as long-chain fatty acids, hydrocarbons, esters, oxygenated chemical compounds (aldehydes, ketones, esters, and ether groups, unsaturated fatty acids, and few unknown compounds. The first detected compounds in the initial stage of the test are low molecular weight compounds such as benzene, trichloroethylene, alkanes (Octadecane, Tetracosane, Pentacosane, and Hexacosane), and fatty acid compound (Hexadecanoic acid, and Octanoic acid). Then the last detected compound is the high molecular weight compound such as palmitic acid and other unsaturated fatty acids (Kyaw *et al*., 2012).

1.3. Objectives

The objective of this study is to collect research data about the biodegradation of polyethylene material or polyethylene food packaging. Then identify the important factors that affect the biodegradation process, and suggesting the better method to increase a biodegradation result.