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DEGRADATION OF LOW DENSITY POLYETHYLENE PLASTIC WASTE BY INDIGENOUS MICROBIAL CONSORTIUM AND FUNGI

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ABSTRACT

Aim: The aims of this research is to determine the ability of an indigenous microbial consortium to degrade Low Density Polyethylene plastics. The plastic was cut mechanically into 1 x 1 cm^2 pieces because the smaller the size of the plastic, the larger the surface area. The samples are input in an Erlenmeyer flask containing indigenous microbial consortium and fungi (20% v/v) and Stone Mineral Salt solution media (80% v/v). This research lasted 10 days with a pH of 7 controlled and temperature variations of 25, 30, 35°C. As preliminary research, the sensitivity test seeks to demonstrate that the indigenous microbial consortium and fungi are resistant or insensitive to LDPE. The degradation of LDPE plastic was analyzed using gravimetric methods, Fourier Transform Infrared, and a scanning electron microscope. **Methodology and results:** According to the results of gravimetric and FTIR analysis, the highest removal value was at a temperature variation of 30°C. The gravimetric analysis revealed that the weight loss in LDPE plastic was 0.0082 gr to 0,0074 gr or 9.76 %, while the FTIR analysis revealed that the intensity removal result was 6,27 %. **Conclusion, significance, and impact of study:** Scanning Electron Microscope (SEM) analysis revealed morphological changes on the surface of LDPE plastic samples, confirming these findings. Several factors influence the changes that occur in this study's LDPE plastic samples.

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- Degradation
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- Microbial consortium
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1. INTRODUCTION

Recently, there has been an increase in the emergence of problems in the aquatic environment, both in river waters and in the oceans, caused by plastic waste pollution. The vast majority of plastic waste that pollutes rivers ends up in the ocean (Lebreton *et al.,* 2017). Over the last 60 years, global plastic production has increased dramatically, and it is now recognized as a serious threat to the marine environment (Avio *et al.,* 2017). Plastic waste in these waters is a major issue around the world, particularly in Indonesia (Sahwan *et al.,* 2005). Plastics are widely used in a variety of daily activities in society, including at home, in industry, and in trade, due to their low cost and lightweight.

According to Verschoor (2015), plastic waste, which is typically only used once, has become a global environmental problem. Plastic waste in nature degrades into microplastics. The presence of microplastics in the environment is a problem because plastics are persistent, often contain potentially toxic and carcinogenic chemicals, and because they are consumed by organisms, they will affect aquatic life. Furthermore, plastic waste has been shown to pollute the oceans, poison marine biota, damage coral reefs, and disrupt the balance of marine ecosystems. This microplastic waste can enter the food chain and have an impact on both human and environmental health.

Furthermore, Caruso (2015) stated that microplastic contamination of the aquatic environment caused by wastewater, industrial raw materials, and pellet mills is a priority for future research because it has been identified as an emerging global threat with a variety of implications for social and environmental conditions. One of the interesting strategies and approaches to controlling microplastic pollution is to use a bioremediation technology approach (Caruso, 2015; Alshehrei, 2017; Wei-Min *et al.,* 2017), which takes advantage of the potential of indigenous microbes or bacteria grown in an environment exposed to media.

Several researchers have researched plastic bioremediation. This research examines the findings of research related to bioremediation technology by utilizing the potential of indigenous microbes or bacteria using descriptive qualitative methods, i.e. methods to investigate objects that cannot be measured by numbers or other precise measurements and that tend to use analytical methods. The approach is deductive. Thus, the goals of this research are to 1) determine the ability of microbes or bacteria to reduce plastics in plastic-exposed

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media on a laboratory scale, and 2) use Scanning Electro Microscopy (SEM) to determine changes in bacterial morphology after the plastic reduction process.

Plastic bioremediation has been studied by several scientists. This research examines the findings of research on bioremediation technology by utilizing the potential of indigenous microbes or bacteria using descriptive qualitative methods, i.e. methods to investigate objects that cannot be measured by numbers or other precise measurements and that tend to use analytical methods. Deductive reasoning is used. Thus, the objectives of this research are to 1) determine the ability of microbes or bacteria to reduce plastics in plastic-exposed media on a laboratory scale, and 2) use Scanning Electro Microscopy (SEM) to determine changes in bacterial morphology after the plastic reduction process.

Plastic degradation can be defined as a change in material properties such as mechanical properties, bond changes, and the formation of new functional groups (Arutchlevi *et al.,* 2008). The synthesis of oxidizing enzymes encoded by genes on chromosomes or plasmids, as well as the ability to produce emulsifiers, is the mechanism by which microbes degrade hydrocarbons from petroleum (Nugroho, 2006). In contrast to petroleum hydrocarbons, the molecular weight of plastic is very large, so the plastic degradation process involves two stages: abiotic degradation followed by biodegradation. The abiotic degradation reaction of alkane compounds is the depolymerization stage of macromolecules to form shorter chains that occur outside the bacterial cell (Lucas *et al*., 2008; Leja and Lewandowicz, 2009, Ibiene *et al*., 2013).

Biodegradation is the process of breaking down a compound caused by enzymatic activity released by microbes before the chemical decomposition process (Arkatka *et al.,* 2009). Luckachan and Pilaii (2011) define biodegradation as a process involving the activities of living things that allows the complete loss of polymers or degradation products from the environment, whereas Nanda and Sahu (2010) define plastic degradation through microbial assistance as a process caused by enzymatic activity that allows for polymer cutting, which perhaps metabolized by cells.

Microbes are very adaptive to the environment by releasing endoenzymes and exoenzymes to degrade substrates into simpler components. These components are used as a source of carbon and energy (O'Brine and Thompson, 2010). There are at least five functional groups that support the chemistry of life, namely hydroxyl, carbonyl, carboxyl, sulfhydryl, and phosphate (Campbell and Reece, 2005). Polymers having one of the five functional groups can enter the

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cell and be further processed enzymatically. Plastic biodegradation can take place in four stages: 1) microbe attachment to the plastic surface; 2) microbial growth using plastic as a carbon source; 3) primary degradation, and 4) final degradation. Biofilms will form on the polymer surface as a result of polymer degradation (Gu, 2003).

According to Fachrul and Rinanti (2018), one of the interesting strategies and approaches to controlling microplastic pollution can be done by utilizing the potential of indigenous microbes or bacteria and fungi or fungi that are grown in a controlled environment exposed to microplastics. Bacteria and fungi can absorb macromolecules as a nutrient source and use them as an energy source via metabolic processes. Microorganisms such as fungi and bacteria, which are key components of the biosphere, play an important role in the breakdown of organic compounds and environmental cycles.

Several studies have shown that several bacteria, including *Pseudomonas, Bacillus, Clostridium, Streptomyces* (Usha *et al.,* 2011), and *Rhodococcus*, as well as fungi, including *Aspergillus niger, Phanerochaete chrysosporium, Penicillium* sp, *Mucor rouxii*, and *Fusarium redolens*, have (Kanaly *et al.,* Harayama, 2000). *Acinetobacter* sp. is a soil bacteria capable of degrading polyethylene. The genus *Brevibacillus, Pseudomonas*, and *Rhodococcus* spp. were able to degrade polyethylene through several treatments with dry weight percentages of 37.5%, 40.5%, and 40.5%, respectively (Nanda, and Sahu, 2010).

Two consortiums of microorganisms from the genera *Sphingomonas* and *Pseudomonas* degraded polyethylene at a high rate of up to 42.8 % dry weight reduction (Gu, 2003). Plastic can be degraded by *Pseudomonas* spp. using serine hydrolase and esterase enzymes (Bhardwaj, Gupta and and Tiwari, 2012). *Brevibacillus borstelensis* has the ability to reduce the molecular weight of polyethylene by 11-30%. (Hadad, 2005). In addition to bacteria, yeast can degrade plastic polymers using its enzymes. Some yeasts can degrade plastic polymers; for example, *Candida rugosa* has esterase and urease enzymes that allow it to degrade polyurethane (Bhardwaj, Gupta and and Tiwari, 2012).

2. RESEARCH METHODOLOGY

2.1 Bacteria Cultivation and Preparation of Plastic

The Indigenous Microbial Consortium and fungi were obtained from the collection of Universitas Trisakti's Microbiology Laboratory of Environmental Engineering. Batch culture of

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indigenous microbial consortium and fungi was carried out aerobically in Erlenmeyer flasks containing Stone Mineral Salt solution as a growth media. Every day, the indigenous microbial consortium and fungi growth was observed. The indigenous microbial consortium and fungi were ready to be tested after reaching the exponential phase. LDPE samples were mechanically cut into 1x1 cm^2 squares; the smaller the plastic, the greater the surface area, and thus the greater the chance of effective contact with degradation bacteria.

2.2 Sensitivity Test

Bacterial sensitivity testing is a method for determining bacteria's susceptibility to antibacterial substances or antibiotics, as well as the effectiveness of an antibiotic in killing bacteria (Waluyo, 2009). Antibiotics contain a toxic chemical compound produced by microorganisms or synthesized synthetically. The compounds formed can kill or inhibit the growth of bacteria and other organisms that come into contact with these bacteria (Schmidt *et al.,* 2015).

Before the main research, a sensitivity test was performed to determine the vulnerability of the indigenous microbial consortium to LDPE as a xenobiotic substance that can inhibit or kill bacteria in certain concentrations (Eunike *et al.,* 2018). The LDPE sample was placed in the center of a petri dish filled with Nutrient Agar (NA) containing an indigenous microbial consortium and fungi. The formation of an inhibition zone around a plastic sample inhibited culture growth, indicating that it cannot be used as a biodegradator and vice versa.

2.3 Research Design

In this research, the dependent variables were low-density polyethylene (LDPE) plastic samples and a pH of 7, while the independent variables were temperatures (oC) of 25, 30, and 35. The LDPE samples were placed in an Erlenmeyer flask with Stone Mineral Salt solution (70% v/v) and indigenous microbial consortium and fungi (20% v/v). This ten-day experiment was repeated three times. Gravimetry, Fourier Transform Infrared (FTIR), and Scanning Electron Microscope were used to analyze the data (SEM).

2.4 Gravimetric Analysis

The percentage of LDPE plastic sample weight loss was calculated using gravimetry. Gravimetry is a quantitative chemical analysis that is based on the principle of measuring plastic samples

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before and after degradation. As a result, the percentage of degraded plastic weight loss can be calculated. The percentage of degraded plastic weight loss can be calculated using the following formula:

$$\%\ weight\ loss = \frac{W(a)-W(b)}{W(a)}\ x\ 100\% \quad (1)$$

W(a) : plastic weight before degradation

W(b) : plastic weight after degradation

2.5 Fourier Transform Infra-red (FTIR) Analysis

To determine changes in functional groups of LDPE samples before and after contact with indigenous microbial consortium and fungi, plastic content analysis using Fourier Transform Infrared (FTIR) based on the infrared spectrum was performed. The principle of spectroscopy is used in FTIR, which measures the amount of infrared radiation absorbed or emitted by a sample as a function of wavelength. The obtained spectrum results are then plotted as wavelength (m) or wavenumber (cm-1). Chemical structure measurements were performed using FTIR in the wavelength range 400-4000 cm-1. To determine or indicate changes in physical chemistry, Fourier Transform Infrared (FTIR) characterization is required. The emergence of new groups indicates chemical changes, whereas decreasing and increasing wavenumbers (cm-1) indicate physical changes (Bunaciu *et al.,* 2011). Within the 2500-3000 cm-1 wavenumber, which is the typical peak of PE substance, LDPE biodegradation can be confirmed.

2.6 Scanning Electron Microscope (SEM) Analysis

The purpose of this analysis was to determine the morphological structure and shape of the LDPE sample surface before and after incubation with an indigenous microbial consortium. SEM was used to examine the morphology of an LDPE sample that had completed the biodegradation process by bacterial activity at 5000 magnification, 4-0.4 mm depth of field, and 1-10 nm resolution.

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3. RESULT AND DISCUSSION

3.1 Bacterial Sensitivity Test

The ability of bacteria to grow against toxic plastic compounds was tested by performing a bacterial sensitivity test which is a method to determine the level of susceptibility of bacteria to plastic compounds with an incubation time of 48 hours. The diameter formed in the inhibition zone can be an indication of the susceptibility of bacteria to antibacterial agents (Fraga, 2016). The inhibition of the growth of microorganisms is seen in the area around the plastic overgrown by microorganisms. The results of the bacterial sensitivity test for 48 hours showed a response to bacterial growth with the formation of biofilms on the surface of plastics.

Biofilm is a physical barrier formed by an assemblage of microorganism cells, particularly bacteria, attached to a surface and covered by carbohydrate adhesive released by bacteria, where these cells remain physiologically dormant. As a result, biofilms are highly resistant to physical, chemical, and biological stresses. Biofilms are highly resistant to environmental stresses and have evolved into the most resilient forms of microbial life.

Furthermore, according to Flemming (1998), biofilm is a slimy layer in which bacterial cells can wrap themselves in a hydrated matrix of polysaccharides and proteins consisting of water (80-95 %), extracellular polymeric substances (EPS) that contribute 85-98 % of trapped organic matter, microorganisms, organic and inorganic particles. Bacterial growth is inhibited or inhibited in the area around the bacteria-overgrown plastic. If an inhibition zone forms around the bacteria that have been exposed to various doses of plastic. This indicates that the bacteria are susceptible to plastic compounds. If there is no zone of inhibition around the paper disc, the bacteria are resistant to the plastic compound content. The aims of microorganism sensitivity testing are to determine the susceptibility of microorganisms to antibiotics. Several methods were used to assess microorganism sensitivity to antibiotics, including disk diffusion (Kirby Bauer test, Stokes test), broth dilution (MIC determination), diffusion, and dilution (E-test).

The disk diffusion method evaluates sensitivity in a petri dish containing solid nutrient agar with microbes evenly distributed on its surface. Nichols (2004) states that when testing antibiotic sensitivity, the filter disk is immersed in antibiotic liquid, whereas in plastic cases, the plastic disk can be placed directly on the surface of the petri dish. After a 24-48-hour incubation period, the nature of the microorganism can be determined using an antibiotic disk or a plastic

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disk. If the microorganism is susceptible, an inhibition zone will form around the antibiotic disk or plastic disk. CLSI (2008). Meanwhile, if the microorganism is resistant or insensitive, the zone of inhibition around the sample disk does not form (Nijs *et al.,* 2003). Figure 1 depicts the indigenous bacteria consortium's sensitivity test to LDPE disks in this research.

A petri dish containing solid nutrient agar containing indigenous bacteria was evenly distributed with an LDPE disk and incubated for 48 hours, as shown in Figure 1. There was no inhibition zone formed around the LDPE disk after the incubation period. This demonstrates that the indigenous bacteria are not sensitive to or resistant to the LDPE disk. Furthermore, because they can use LDPE as a source of nutrients, the indigenous bacteria in this research have the potential to degrade LDPE.

The presence of this film indicates that the inhibition zone did not form during the observation, indicating that the indigenous microbial consortium is quite resistant and capable of growing on plastic-contaminated media. Figure 1 shows the results of testing the sensitivity of indigenous microbial consortium and fungi to LDPE in this research; the inhibition zone did not form after 48 hours of incubation. This demonstrates that the indigenous microbial consortium and fungi can survive and grow in toxic compound environments. As a result, the indigenous microbial consortium and fungi are resistant or insensitive to LPDE, allowing them to degrade LDPE.

Color changes before and after incubation showed that xenobiotic LDPE plastic samples did not affect the growth activity of the indigenous microbial consortium and fungi. Carbon compounds (C) are used as a source of nutrients by indigenous microbial consortiums and fungi. The sample was placed in the Erlenmeyer flask and its weight was determined using an analytic balance. The LDPE sample weight decreased after 10 days (Table. 1). This shows that an indigenous microbial consortium and fungi can degrade LDPE by using compound (C) as a nutrient source.

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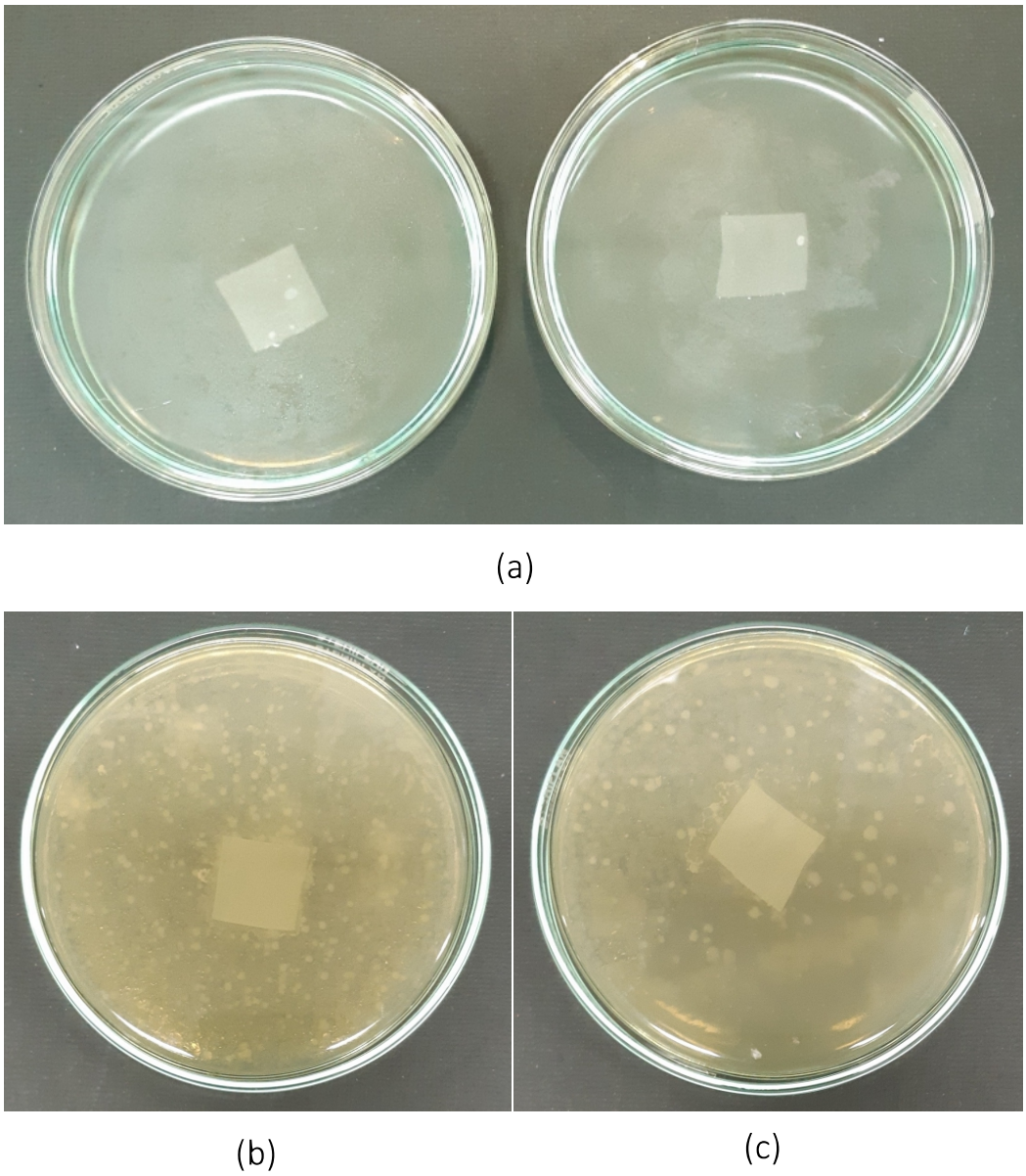

Figure 1 Sensitivity test of indigenous bacteria to LDPE
(a) t0 : 0 hours (b) and (c) t1 : 48 hours

These color changes revealed that the growth activity of indigenous bacteria was not disturbed by LDPE plastic sample with xenobiotic property. These bacteria were suspected to grow by using carbon substance (C) as a nutrient source in growth media contained in LDPE sample. The result of sensitivity test showed that there was no inhibition zone surrounding LDPE sample, thus indigenous bacteria can be considered resistant or non-sensitive to toxic

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xenobiotic substance Therefore, these mixed-cultured bacteria were suspected to have the ability to degrade or decompose LLDPE plastic.

3.2 Gravimetric Analysis

Gravimetric analysis was carried out in an Erlenmeyer flask containing indigenous microbial consortium and fungi (20% v/v) and Stone Mineral Salt solution (80% v/v). Before and after the LDPE sample was put into the Erlenmeyer, the weight of the sample was weighed using an analytic balance. After 10 days, the LDPE sample weight decreased (Table. 1). This proves that indigenous microbial consortium and fungi can degrade LDPE by utilizing compound (C) as a source of nutrients.

Table 1. Gravimetric analysis degradation of LDPE by indigenous microbial consortium and fungi

	Weight of LDPE (gr)		
t0 (0 day)	0.0091	0.0088	0.0093
t1 (10 days)	0.0083	0.0081	0.0085

Based on Table.1 indigenous microbial consortium and fungi can degrade LDPE by 8.45% within 10 days at 30°C

3.3 Bacterial Morphological Change Test

Figure 2 depicted morphological changes on an LDPE plastic surface, from smooth and non-porous to damage with fine pores. The indigenous microbial consortium and fungi appeared to use the plastic as a carbon or nutrient source for their growth, resulting in an incomplete biodegradation process. The loss of 8.45 % LDPE weight after 10 days of incubation showed that LDPE plastic decomposition or degradation occurred very slowly because morphological changes only occurred on the plastic surface.

Low-density polyethylene (LDPE) is a synthetic polymer composed of molecules with linear polyethylene with monomer ethylene long chain (C 2H4) with 0.90-0.94 g/cm3 density (Kyaw *et al.,* 2012; Rajandas *et al.,* 2012). Aside from that, plastic is a substance or substrate that is foreign to biological systems, resulting in resistance due to substrate incompatibility with

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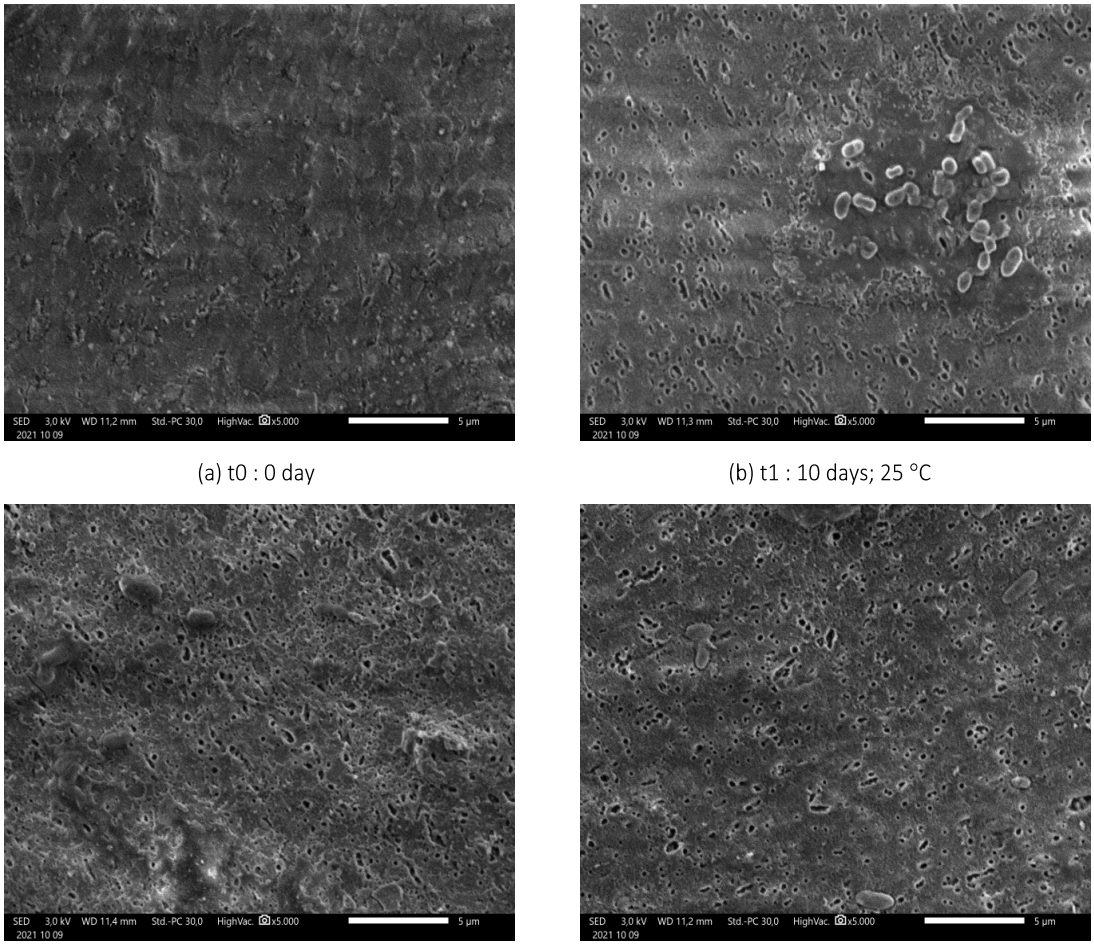
available enzymes (Maier, 2015; Godheja *et al.,* 2016). Microorganisms, 80-95 % water, inorganic particles, and 85-98 % extracellular make up the biofilm layer.

A Scanning Electron Microscope can be used to observe the degradation of LDPE by an indigenous microbial consortium and fungi (SEM). Figure 2 depicts the morphological changes of LDPE after a 10-day incubation period with temperature variations (°C) of 25, 30, and 35. According to Figure 2. The LDPE sample with a temperature variation of 30 °C suffered the most morphological damage. This shows that 30 °C is the optimum temperature for the indigenous microbial consortium and fungi to degrade LDPE.



(a) t0 : 0 day

(b) t1 : 10 days; 25 °C

(c) t1 : 10 days; 30 °C

(d) t1 : 10 days; 40 °C

Figure 2 Scanning Electron Microscope (SEM) on LDPE sampels

Research conducted by Mahalakshmi *et al.,* (2012) regarding the degradation of polyethylene by *Bacillus* sp and *Pseudomonas* sp bacteria by observing using a Scanning

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Electron Microscope (SEM) after the incubation period, showed changes in bacterial cell morphology. The results showed that there was morphological damage or changes in the structure of bacterial cells which were getting bigger with the amount of microplastic dose and erosion occurred on the surface of the PE film. Furthermore, research using SEM observations that have been carried out by Yoon *et al.,* (2012) also showed the biodegradation of Low-Molecular-Weight Polyethylene (LMWPE) by Pseudomonas sp. as shown in Figure 3.

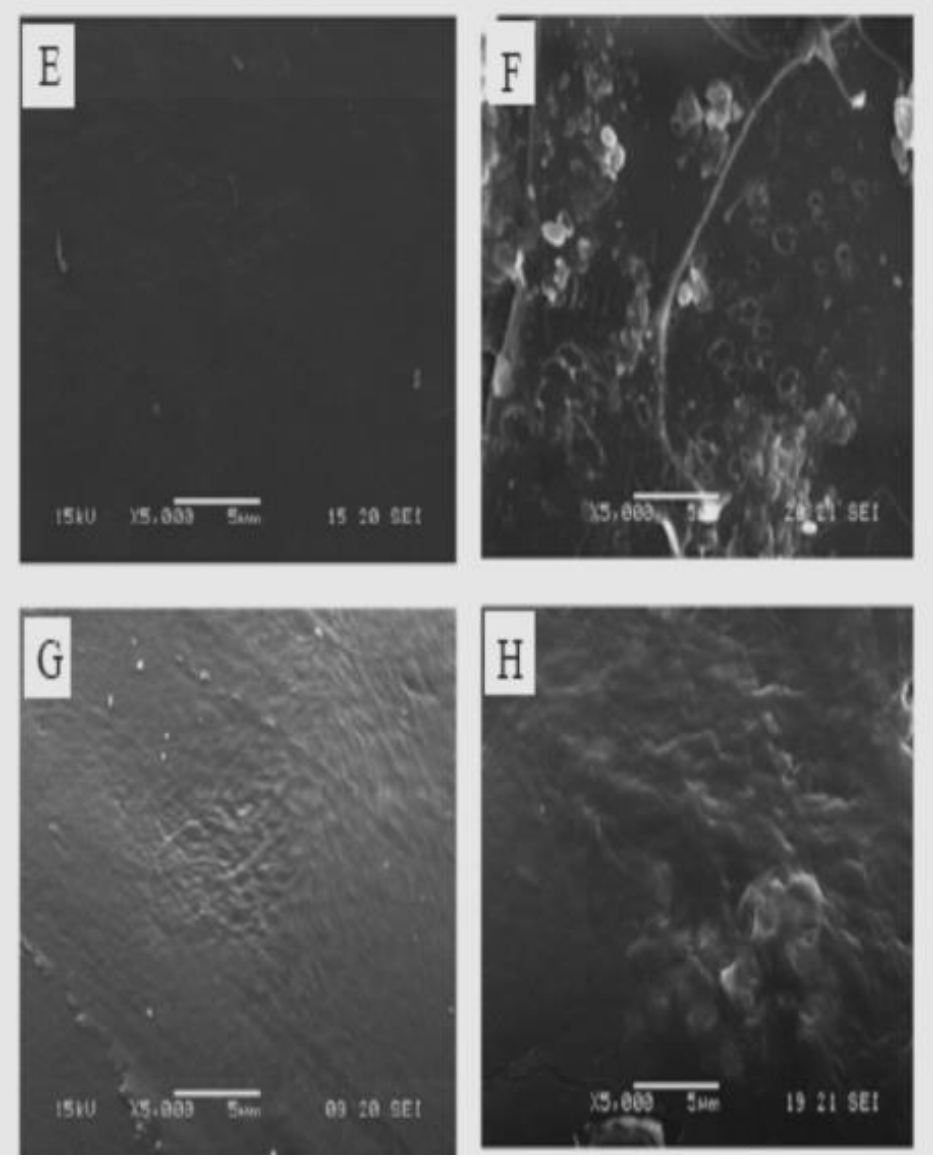

Figure 3 SEM results on LMWPE sheets after 80 days of biodegradation at 37˚C in sterile compost inoculated with Pseudomonas sp.). (E) and (G); before biodegradation of PE-1, PE-2, PE-3 and PE-4, (F) and (H); after biodegradation of PE-1, PE-2, PE-3 and PE-4. (Yoon *et al.,* 2012)

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Visible damage to bacterial cells in the form of cell shrinkage, cell elongation, the formation of bulges (blebs) on their surfaces, and the formation of ghost cells to bacterial cell lysis. The formation of biofilms on the surface of plastics observed using a scanning electron microscope (SEM) indicates a morphological change on the surface of these plastics caused by colonies of microorganisms attached to the surface to form biofilms (Gomes and Mergulhão, 2017).

3.4 Fourier Transform Infrared (FTIR)

According to Gulmine (2002), the standard curve of LDPE was 2920 cm-1, which represented CH2 asymmetrical stretching. Figure 4 shows that when the LDPE wave number was 2919.15, the % transmittance value at t0 was 87.45, but after 10 days, the % transmittance value decreased to 81.97. The decreasing value of percent transmittance indicates that LDPE is being degraded by an indigenous microbial consortium and fungi. The degradation was 6.27 % at 30°C, while temperatures of 25, 30°C and 40, 30°C showed no degradation activity by the indigenous microbial consortium and fungi.

The peaks in each spectrum seen in Figure 4 can be identified in determining the functional groups of migrating compounds. Each peak contained in a certain wavenumber (cm-1) indicates the functional group of the group of compounds that are components of the plastic sample. The functional groups are known based on the peak spectrum. There are negative peaks and positive peaks based on the shape of the FTIR spectrum in Figure 4. Negative peaks in the FTIR spectrum can indicate that the plastic constituent components have migrated or have been released into the acid solution. The negative peak of the FTIR spectra was used to identify the type of plastic constituent components that were released (migrated) into the lactic acid solution. When compared with the control, the negative peak indicates the release of certain functional groups or chemical bonds in the treated plastic samples, while the positive peaks indicate the addition of certain functional groups or chemical bonds in the sample.

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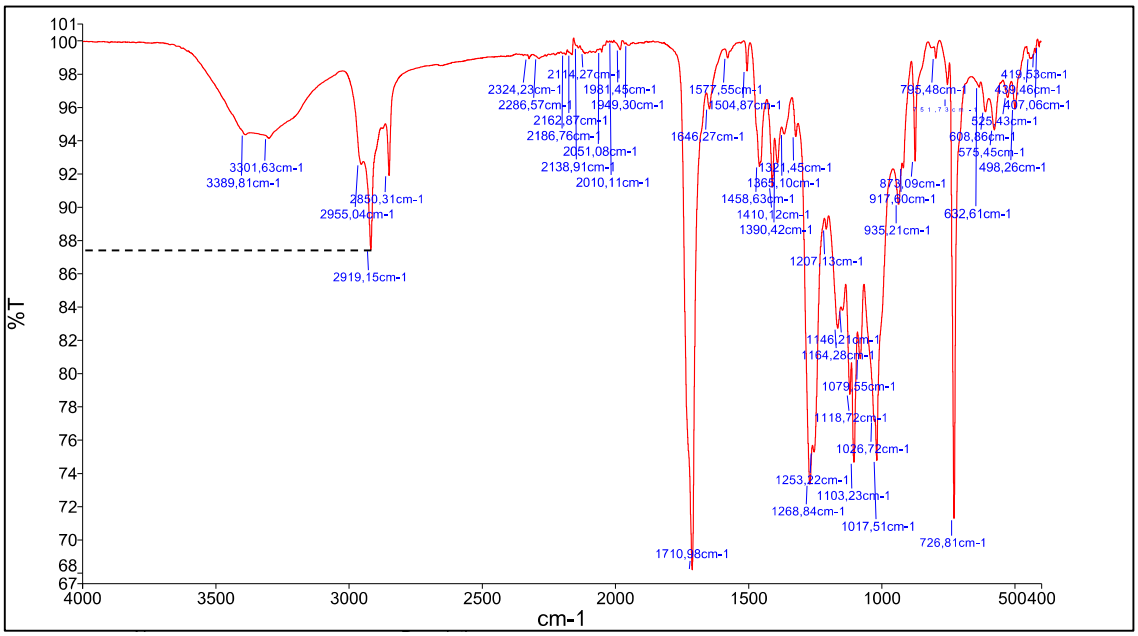
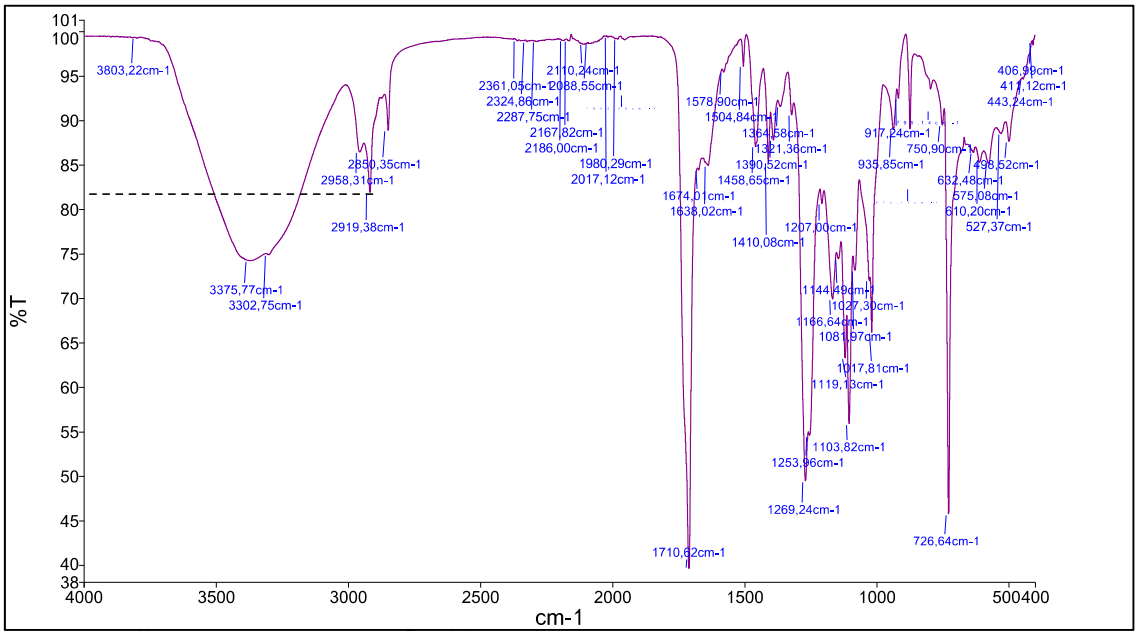


(b) t0 : 0 day



(c) t1 : 10 days; 30 °C

Figure 4 The FTIR of LDPE degradation by indigenous microbial consortium and fungi

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4. CONCLUSION

In this research, Indigenous microbial consortium and fungi are resistant or insensitive to LDPE, whereas Indigenous microbial consortium and fungi can survive and grow in LDPE-containing media. SEM was also used to observe LDPE morphological changes; after 10 days, the surface of the LDPE was severely damaged at 30°C. Furthermore, an indigenous microbial consortium and fungi were able to degrade LDPE by 8.45 % using the gravimetric method and 6.27 % using the FTIR method in 10 days. 30°C is the optimal temperature for indigenous microbial consortium and fungi to degrade LDPE.

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