JPB 11(1) (2024)

Jurnal Pembelajaran Biologi:
Kajian Biologi dan Pembelajarannya

https://jpb.ejournal.unsri.ac.id/index.php/jpb/index

Isolation and test of the ability of fungi to degredate polyethylene plastic from landfill plunges

Syarifahanum[1*], Kartika Manalu[2], Rizki Amelia Nasution[3]

[1,2,3]Program Studi Biologi, Universitas Islam Negeri Sumatera Utara, Medan, Indonesia

Article History:
Received:
03.04.2024
Accepted:
29.04.2024
Published:

Keywords:
Fungi, Biodegradation, Polyethylene, LDPE, Landfill Terjun.

Abstract: Polyethylene is one type of plastic that is widely used. The long carbon chain bonds make plastic difficult to decompose. Handling that can be done to minimize the problem of plastic waste is biodegradation. The purpose of this study was to determine the type of plastic-degrading fungi isolated from the Waterfall landfill and to determine its ability to degrade polyethylene plastic. The first method of this research was the isolation of fungi from the Waterfall landfill, then screening was carried out to obtain potential isolates and obtained 4 fungal isolates namely PME1, PME2, PME3 and PML2 based on their growth response on salt medium (MSM) agar containing LDPE powder. Based on their morphological characteristics, isolates PME1, PME2 and PME3 as the genus Aspergillus sp. and PML2 fusarium. Then tested for biodegradability. The assessment was carried out by measuring the weight loss of LDPE sheet after biodegradation treatment. Data analysis using one way Anova and Duncan test. The result is that all isolates have an effect on reducing plastic weight. The isolate with the highest degrading ability after an incubation period of 40 days was Asp1 isolate with a plastic weight loss percentage of 2.23%, Asp2 of 1.78%, Fus1 of 1.03% a Asp3 of 0.74%.

Corresponding Author:
Author Name[1]: Syarifahanum
Email*: syaarifahhanum@gmail.com

ISSN: 2355-7192
E-ISSN: 2613-9936

INTRODUCTION

Environmental problems that occur are caused by the emergence of various pressures and ecosystem interactions that have lost the ability to become a buffer to repair and restore their own conditions. Environmental problems that occur, especially in Indonesia, always increase in accordance with population growth. The increasing population will significantly increase the amount of waste production, especially household waste (Harjati and Pratamaningtyas, 2020). Waste is the remaining residue of daily human activities / natural processes in solid form (Law No.18 of 2008). The increasing growth and density of the population, the increasing volume of waste generated, not only that the diversity, types of waste characteristics also increase.

It was recorded at the end of 2017 that Indonesia's waste production reached 5.6 million tons of waste per year, which made Indonesia the second largest waste producer in the world (Aziz and Cut, 2019). According to Ermawati (2020), the increase in waste production has resulted in increasingly limited landfill space for waste. One of the items most often used by humans to fulfill their daily needs is plastic (Anah *et al,.* 2020). Medan City has TPA Terjun as the final disposal site for Medan City's waste. Terjun landfill is located in Terjun Village, Medan Marelan Subdistrict. This landfill is managed by the Medan City Cleanliness and Parks Agency, which has been operating since 1993 on an area of 137,563m* with an open dumping system and a volume of waste as large as
1,535 tons of waste per day. This causes environmental pollution problems for the surrounding community. This environmental pollution causes unpleasant odors (Fitri *et al.,* 2018).

The decomposition of plastic waste in the environment takes a very long time, namely 100 to 500 years to decompose completely (Purwaningrum, 2016). Currently, plastic waste in the community is piling up everywhere, most of the plastic waste in our environment cannot be recycled. Existing waste processing units have difficulty managing irreversible plastic waste (Elpawati, 2015). Plastic is classified as a complex polymer that has a very long degredation time due to the structure of plastic polymers which have long repeating chains, so it takes a long time to cut the chain into shorter molecules (Sari *et al.,* 2020). Plastic is one type of material that is durable and not easily damaged, the most widely used plastic material in the form of bags or also known as "crackle" (Hadiyanti, 2017). Plastic waste, especially LDPE *(low density polyethylene*) in landfills is generally only stockpiled, burned or recycled. The process has not solved all plastic waste problems. Based on this, it is very necessary to do biological control by looking for candidates for plastic polymer degredation microorganisms so that the amount of plastic waste can be minimized. Some studies mention that microorganisms such as fungi and bacteria are able to degredate plastic waste (Anah *et al.,* 2020).

Biodegradation is the process of polymer breakdown, both natural and synthetic polymers by biological agents such as fungi or bacteria (Rohmah *et al.,* 2018). Biodegradation of polymers by microorganisms is considered effective and adaptive to the environment compared to chemical and physical methods, and is considered the most suitable method for environmental sustainability and cheap for plastic waste treatment because it can reduce the molecular weight of polymers that occur naturally by microorganisms (Zuliani and Martina, 2021). Inas (2017) also stated that of the three methods it has been proven that biological methods are more effective, efficient and environmentally friendly. The biological method that is being developed is to use natural microorganisms that have the ability to degrade polymers. Microorganisms are able to utilize complex polymers in plastics as a carbon source that can be transformed into simpler units. Das & Kumar (2014) isolated fungi from plastic waste soil, obtained 5 fungal isolates, could grow on MSA medium added 3% LDPE powder and utilize the plastic powder as a carbon source. These microbes will utilize the polymer as their growth substrate (Rohmah *et al.,* 2018).

Previous research studies have shown that fungi have a degredation activity of
the highest compared to other microorganisms. Fungi are organisms that can survive in a variety of environments with different media, and obtain their food from the media in which they grow. Fungi are microorganisms capable of degrading LDPE plastic due to their ability to form hydrophobic enzyme proteins. These proteins function as surfactants that can reduce the hydrophobicity of polymers in plastics and increase the effectiveness of degradation (Bayry *et al.,* 2012). In addition, the growth of fungi in the soil is faster than bacteria, and the hyphae in fungi are able to penetrate senobiotics (Valencia, 2017). Fungi that have the ability to degrade plastics in the environment include those from the genus *Aspergillus*, *Penicillium, Rhyzopus oryzae* and *Tricoderm* (Hikmah *et al.,* 2017). singh and Gupta (2014) stated that soil fungi are able to carry out degradation of synthetic polymers under natural conditions and isolated fungal sps are also efficient in biodegradation under laboratory conditions. *Fungal* strains, *Aspergillus niger* and *A. japonicas Aspergillus flavus Fusarium sp, Penicillium sp, Mucor sp, were screened for LDPE degradation under laboratory conditions.*

Some fungi *are* known to grow in the medium treated with LDPE plastic, as done by Das & Kumar (2014) isolating fungi from plastic waste soil, getting 5 fungal isolates, after being identified 4 isolates of *Aspergillus sp* and 1 isolate of *Fusarium sp* can grow on MSMA medium added 3% LDPE powder and utilize the plastic powder as a carbon source. In addition, the types of fungi that have been studied have the potential for LDPE biodegredation including *Rhyzopus oryzae* capable of reducing LDPE by 3% of the initial weight (Awasthi *et al.,* 2017), the Aspergillus *group* namely *A. niger* showed a dry weight reduction of 5.8% and *A. japonicas* by 11.11% (Raman *et al.,* 2012). Therefore, researchers are interested in isolating fungi from landfills and seeing their ability to degredate LDPE *(low density polyethylene)* plastic waste.

METHODOLOGY

Time and Place of Research

This research was conducted at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, University of North Sumatra, Medan. Soil samples were taken from Terjun landfill in Terjun Village, Medan Marelan Sub-district.

Tools and Materials

The tools used in the LDPE biodegradation activity test and fungal isolation require Reaction Tubes, Enlemeyers, Micro Pipettes, Petri dishes, Ose needles, Bunsen, Micro Pipette Tips, Spatulas, Analytical Balance, Vortex Incubator, Autoclave, Oven and Laminar Air Flow. The materials used in this study were soil samples from Terjun landfill, black LDPE (Low Density Polyethylene) plastic bags, LDPE powder, NaCl fis, Potato Dexstrose Agar (PDA), Mineral Salt Medium (MSM), distilled water, alcohol, and Lactophenol Cotton Blue.

Sampling

Soil samples were taken from landfill sites in Terjun Village, Medan Marelan Subdistrict using purposive sampling, at 3 different points in terms of landfill conditions in the field as much as 250g. The soil samples taken were soil containing plastic that had decomposed naturally. The samples were then mixed in a sterile polybag and stirred until smooth. Next, the sample was put into an icebox and taken to the laboratory (Khan *et al.,* 2017).

Fungal Isolation

Fungi were isolated by serial dilution technique at concentrations of 10^{-4} to 10^{-6}CFU/mL with physiological solution (NaCl 0.9%). Soil samples that have been cleaned are put into erlenmeyar and given NaCl solution and then homogenized. From this dilution in a pipette as much as 1 ml is inserted into a test tube that already contains NaCl as much as 9 ml then stirred and obtained a dilution of 10^{-1}. From this dilution, 1 ml was pipetted, put into a test tube that already contained 9 ml of NaCl and obtained dilution 10^{-2}, so on until dilution 10^{-6}. From dilutions 10^{-4}, 10^{-5}, 10^{-6} each was pipetted 1 ml and poured on a Potato Dextrose Agar (PDA) media dish with the spread plate method and then incubated at temperature. The PDA medium used was modified by adding a little chloramphenicol antibiotic in 100 ml of PDA medium to inhibit the growth of contaminants such as bacteria. The growing colonies were transferred to fresh PDA medium to obtain pure isolates. Pure isolates were stored at 27°C for further use (munir *et al.,* 2018).

LDPE Powder Extraction

Five grams of LDPE seeds were dissolved in 150 mL of xylene and boiled for 30 minutes. To remove xylene from the solution, the LDPE solution was treated with alcohol. The mixture was then evaporated and oven dried at 50°C overnight. The granule lumps formed were then pulverized with a blender, then filtered to obtain the best powder.

Screening of Plastic Polymer-Degrading Fungi

MSM media was used for screening of plastic degrading fungi. For manufacturing, MSM media was made with the composition (per liter): 3 g LDPE powder, 20 g agar, 0.05 g chloramphenicol, 1 g $(NH4)H2PO4$, 1 g $KKH2PO4$, 0.2 g $NaH2PO4O$.5 g FeSO4. 0.01 g $MgSO4$, and small amounts of $ZnSO4$, $CuSO4$, and $MnSO4$ while for the control, Mineral Salt MediumAgar (MSM) did not contain LDPE powder (Hikmah, 2018). Then homogenized for 1 hour at 120 rpm and sterilized for 20 minutes. Pure isolates were taken from the edge of the colony with a diameter of 5 mm using a spatula inoculated into MSM media petriberisi dishes and the results were observed after 1week Fungi that produced clear zones were selected for degredation tests (Deepika and Jaya, 2015; Ahsan *et al.,* 2016).

Mushroom Identification

Potential fungal isolates that are able to grow on Mineral Salt Medium Agar (MSM) by utilizing LDPE *(low density polyethylene*) as a carbon source are then identified to the genus level which includes macroscopic and microphysical observations. Macroscopic observations in the form of color, surface texture, and fungal tepicolony on the media. While microscopically in the form of hyphal structures, conidia and conidiophores that refer to the book *Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Keyto Species 2th edition* (Watanabe, 2010) and *Fungi and Food Spoilage* (Pitt & Hocking, 2009) (Fa'is, 2021). Microscopic observations were made by staining the fungi. Pure fungal

culture was taken aseptically using an ose needle and placed on the surface of an object glass, then given a dye, namely *lactophenol cotton blue* to help observe the microscopic structure. After that, the preparations were covered with cover glass and observed under a microscope with a magnification of 100X (Ristiari *et al.,* 2018).

Preparation of Test Plastics

The plastic used in the form of LDPE black bags was cut into 2×2 cm sizes with the same weight. After cutting, the plastic sample was sterilized using 70% alcohol for 30 minutes. Next, the sample was rinsed using distilled water and exposed to UV light for 30 minutes. To determine the initial dry weight of the plastic, the pieces were dried in an oven at 80°C for 24 hours to obtain the pure weight of the plastic without water content. After that, the sample was weighed to determine its initial dry weight (Inas, 2017) (Singh and Gupta, 2014).

Biodegradation Test of Plastic Sheet (Low Density Polyethylene)

Biodegradation was performed on Mineral Salt Medium Agar (MSM) medium supplemented with 0.5% glucose. Sterile LDPE sheets (2×2 cm) of known weight were placed on the surface of the medium (MSM) aseptically 4 isolates of fully grown colonies were inoculated on the center side of the agar and LDPE film and incubated according to the predetermined time variation at room temperature (26 ± 2 °C). The biodegredation process was stopped by taking the LDPE sheet using tweezers, rinsing with 70% ethanol and sterile distilled water. Then dried in a 45°C oven for 24 hours, then the plastic sheet was weighed to determine the final weight (Munir *et al.,* 2018).The degredation test was carried out with an incubation period of 40 days with incubation time intervals of 20, 30 and 40 days.

Percentage Weight Loss

Each LDPE sheet was measured before and after the degradation period using an analytical balance. The percentage weight loss was determined based on the formula below (Deepika and Jaya, 2015; Hikmah, 2018). The results obtained were compared with the weight of the control LDPE.

$$\%\ Weight\ Loss = \frac{W1 - W2}{W1} \times 100$$

Note:
W1 = Initial Weight of Plastic W2 = Final Weight of Plastic

Data Analysis

The data obtained includes identification data of fungi from Terjun landfill, which includes macroscopic and microscopic characteristics of *fungi* that have the potential to degredate LDPE plastic analyzed descriptively and compared with data in the reference book Pit and Hocking (2009) *Fungi and Food* Spoil *and* Tsuneo Watanabe (2010) *Pictorial Atlas of Soil and Seed Fungi*. Meanwhile, data on the ability of fungal isolates to degredate plastic was analyzed using One-Way Anova with an error rate of 5%. If there are significant analysis results, then further tests will be carried out using the Duncan test.

RESULTS AND DISCUSSION

Fungal Isolation

The results of fungal isolation from the Terjun landfill obtained 7 fungal isolates with different morphological appearances. Purified isolates were coded PME1, PME2, PME3, PME4, PML1, PML2 and PMN1.

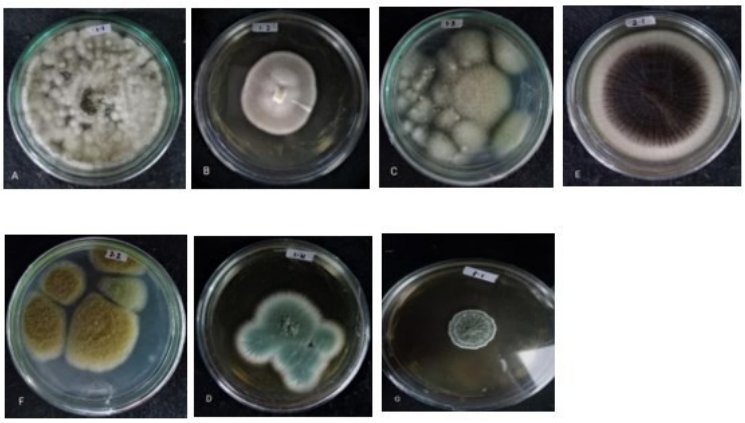

Figure 1. Appearance of purified fungal isolates on PDA media, incubation period of 7 days (A) PME1 (B) PME2 (C) PME3 (D) PME4 (E) PML1 (F) PML2 and (G) PMN1

Fungi can survive in diverse environmental conditions including landfill soil mixed with waste. Waste containing organic and inorganic compounds is difficult to degredate because it has a composite and complex polymer form and structure such as plastic. This condition is utilized by fungi as one of the favorable growing environments because fungi can utilize organic compounds as a source of nutrition. According to Darliana & Wilujeng (2020), fungi have the ability to degrade organic compounds and make them a source of nutrients for their metabolism and life. Fungi from landfill soil can decompose various types of polymers including LDPE from plastic waste, so that in laboratory treatments indigenous fungi are able to adapt when treated on media containing LDPE. Previous studies have successfully isolated various types of fungi from landfill soil including *Fusarium* and *Aspergillus flavus* (Das & Kumar, 2014), *Aspergillus oryzae* (Muhonja *et al., 2018),* 2018), *Aspergillus terreus* and *Aspergillus tubingiensis* (Khan *et al.,* 2017), *Rhyzopus orizae* NS5 and *Aspergillus clavatus* (Gajendiran *et al.,* 2016), *Aspergillus niger* (Deepika & jaya, 2015).

Fungi in the isolation stage of this study can grow optimally because they are supported by Good environment and nutrients are fulfilled. Some of the purified mushroom mycelium growth has filled the petri dish on the 7th day, although some have slow growth, especially isolate PMN1. Environmental temperature greatly affects the growth of fungi, in this study isolation was carried out with a stable room temperature of 27 ° C, an effective and stable temperature is important for the growth of microorganisms because some microorganisms can only live at certain temperatures. According to Ahmed *et al.,* (2018) the optimal temperature for fungal growth is room temperature with a range of 22-27 ° C.

Screening of Plastic Degredating Fungi

Fungal screening with clear zone appearance parameter aims to test the ability of fungal growth in degrading plastic. According to Deepika & Jaya (2015) the formation of a clear zone around the colony indicates that the fungal isolate is able to produce extracellular hydrolysis enzymes that act as catalysts for degradation reactions. The emergence of a clear zone around the fungus on media containing plastic polymers because the fungus utilizes the polymer as a carbon source to meet nutritional needs in the metabolic process (Inas, et al., 2017). Isolates are considered capable of degrading LDPE well if they show the presence of a clear zone around the colony, and have rapid colony growth and spread on MSM media (Deepika & Jaya, 2015).

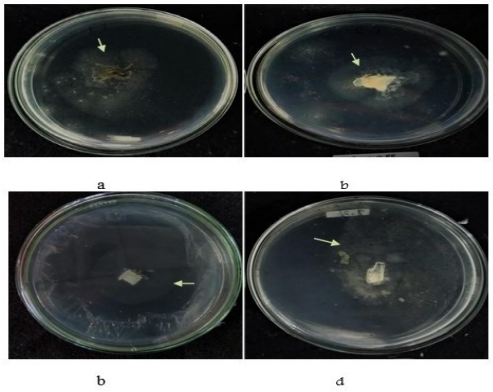

Figure 2. Clear zone growth around the colonies of MSM media with LDPE powder addition for 7 days of incubation. (a) Isolate PME1 (b) Isolate PME2 (c) Isolate PME3 (d) Isolate PML2.

The results of the selection of LDPE degredation fungal isolates are indicated by the formation of a clear zone around the colony during the 7-day incubation period. The diameter of the clear zone formed is 3-4 cm. The appearance of the clear zone produced is not very clear due to the clear MSM media. Observations on the selected isolates showed that the four fungi above had growth characteristics with a clear zone growing on the media and increasing the size of the mushroom colonies. The formation of clear zones is caused by the activity of extracellular hydrolysis enzymes excreted by microorganisms in MSM selective media with additional LDPE powder as a single carbon source (Deepika & Jaya, 2015). Clear zones were formed in all isolates, but the growth of some isolates was not optimal and even stopped. This is because MSM media is a medium with minimal nutrients, so the fungus will maximize the absorption of carbon from LDPE powder. Fungi are organisms that are able to live in extreme environments, but fungal growth in these conditions will not be optimal (Qadr and Abdullah, 2018).

Selection of isolates for the dry weight reduction test, seen from the presence of clear zones and the ability to grow isolates on minimal nutrient media plus LDPE as the only carbon source, namely isolates PME1, PME2, PME3 and PML2. Research by Depika & Jaya (2015) conducted a selection or screening process by observing clear zones that grow on selective mediums such as MSMA (mineral salt medium agar), resulting in 4 isolates that have the potential to degredate polyethylene. The isolates came from the genus *Aspergillus, Pseudomonas* sp and *Actionomycetes*. Wardani (2021) conducted screening by observing the clear zone, obtaining 5 isolates from the genus *Aspergillus, Trichoderma* and *Rhizopus.*

Identification of Potential LDPE Plastic Degredating Fungi

The identification of this fungus was carried out with the aim of knowing the genus of isolated plastic degrading fungi. Isolates of plastic degrading fungi that have been purified are then identified by making macroscopic, microscopic observations. From the observation of the morphological characteristics of fungal isolates isolated from landfill soil polluted by plastic crumbs, 3 isolates were found to have white colony edges and green centers, namely PME1, PME4, and PMN1, the surface of PME1 and PMN1 colonies had a raised surface texture (velvet) PME4 cotton (flat), while PME2 is milky white with a flat surface, PME3 is white with a cotton surface, PML1 is white around the perimeter and the colony is black and PML2 is brownish yellow with a cotton surface (powdery). Fungal growth was moderate to rapid with diameters after 7 days incubation ranging from 2-5 cm. Dineshraj and Ganesh (2016) isolated *fungi* from plastic waste in various landfills in Cuddalore city and obtained nine fungal isolates, namely *Aspergillus niger, A. flavus, A. nidulans, A. fumigatus, A. glaucus, Penicillium, Fusarium*, *Mucor,* and *Alternaria*. Williams and Hakam (2016) reported that from four disposal sites in the end, five isolates of the fungi *Aspergillum*, *Fusarium*, *Mucor, Penicillium* and *Saccharomyces* were obtained. When waste is buried in the soil, soil microorganisms including fungi and bacteria are ready to colonize the waste, which will lead to the process of biodegredation and transformation of organic materials. Microorganisms present in the soil utilize the plastic material as a carbon source for their growth and thus degrade the plastic material (Vijaya and Reddy, 2008).

Microscopic Observation (Isolate PME1)

Macroscopically, the upper surface is dark green surrounding white over time the entire surface is dark green, while the reverse surface is yellowish white, with velvety colony texture, circular shape, and spreading growth. Microscopically, fungal hyphae have septa, hyaline and smooth-walled conidiophores,

the tips of the conidiophores form vesicles, and conidia are round to semi-round. Based on the observed characters, matched with Watanabe (2002) isolate PME1 has similar characters with the genus *Aspergillus* sp.

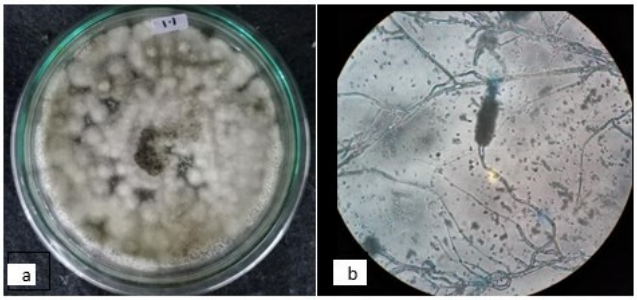

Figure 3. Macro and microscopic morphology of fungal isolate PME1. (a) Fungal colonies on PDA media. (b) Microscopic Structure of 100X magnification fungus

This is supported by the research of Noerfitryani and Hamzah (2018), who reported that the macroscopic characteristics of *Aspergillus* fungi are that the surface is bright green to dark green and black, and has a flour-like texture, while the microscopic characteristics are conidia that are round, with hyphae that are intercepted and hyaline.

Microscopic Observation (Isolate PME2)

Macroscopically, the isolate grows with a white surface color, while the reverse surface is yellowish white, flat, oval round shape, the edge of the colony is entire, has concentric circles. Microscopically, fungal hyphae have septa, hyaline conidiophores and long, round conidia. Based on the observation data matched with the pit and hocking book (2009) and watanabe (2002), this isolate is the genus *Aspergillus.*

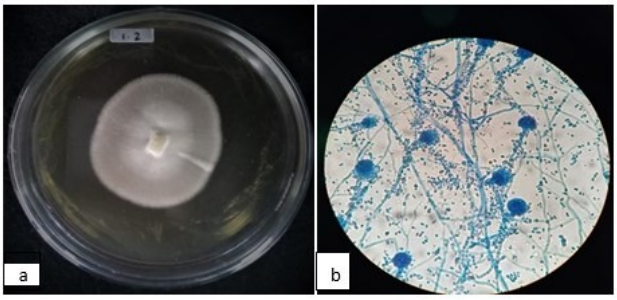

Figure 4. Macro and Microscopic Morphology of Fungal Isolate PME2 (a) Fungal colonies on PDA media. (b) Microscopic structure of the fungus at 100X magnification.

Gandjar *et al.* (2000) explained that *Aspergillus candidus* is characterized by generally thin colonies with few aerial mycelia, white conidia heads then become cream and slightly wet in fresh colonies, round to semi-round conidia.

Microscopic Observation (Isolate PMI4)

Macroscopically, the fungal isolate grew with a yellowish green surface color around the perimeter. White, while the reverse surface is yellowish white, the texture of the colony is like fine powder, circular in shape, the edge of the colony is undulate, has concentric circles. Microscopically, fungal hyphae have septa and are branched, hyaline conidiophores and on the uung part inflate to form round vesicles, round to semi-round conidia. Based on the observation data matched with the pit and hocking book (2009) and watanabe (2002), this isolate is the genus *Aspergillus sp.*

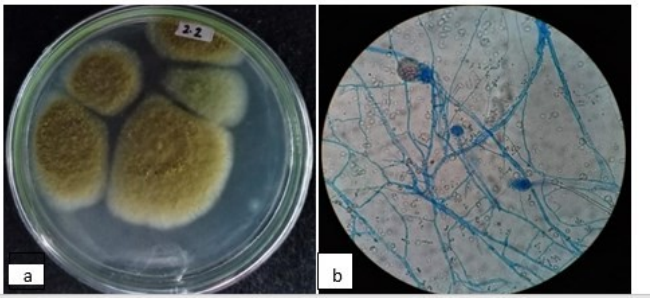

Figure 5. Macro and Microscopic Morphology of Fungal Isolate PML4 (a) Fungal colonies on PDA media. (b) Microscopic structure of the fungus magnification 100X

Gautam & Bhadauria (2012) stated that members of the genus *Aspergillus* that grew at first were white then on the fourth day turned yellowish green with white edges and the lower surface of the colony was yellowish to brown. Research by Samson *et al.*, (2010), that fungi of the genus Aspergillus have semi-round conidia with long and columnar conidiophores. Vesicles are semi-spherical.

Microscopic Observation (Isolate PME3)

Macroscopically, fungal isolates can grow with a white upper surface color, while the reverse surface is yellowish white, powdery (cotton) colony texture, circular shape, filamentous edges. Microscopically, hyaline conidiophores and slightly curved two- to four-celled macroconidia, one-two-celled microconidia. Based on the observation data matched with the pit and hocking book (2009) and watanabe (2002), this isolate is the genus *Fusarium sp.*

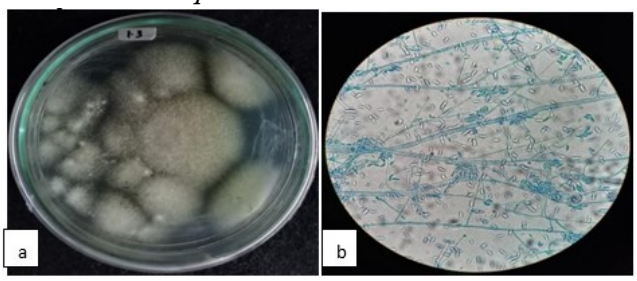

Figure 6. Macro and microscopic morphology of fungal isolate PME3. (a) Fungal colonies on PDA media. (b) Microscopic structure of the fungus at 100X magnification.

According to Wibowo, et al. (2008) most isolates of *Fusarium sp.* have white colonies. Sholihah (2019) found *Fusarium* isolates with macroscopic characteristics of macroconidia that are long, crescent-shaped with blunt ends, 1-5 septa, and abundant in number and also found the presence of long and septate hyphae. The microscopic morphology of *Fusarium* is shown from the results of microscopic observations that some isolates have oval or elliptical-shaped microconidium, non-concentrated or 1-2-concentrated, microconidium arranged at the end of a long, unbranched conidiophore,
is a single monophialid.

Biodegredation Test of Plastic Sheet (Low Density Polyethylene)

Dry Weight Reduction Percentage

Testing the ability of fungal biodegradation aims to determine the ability of each fungal isolate obtained in biodegrading polyethylene plastic. Determining the percentage of weight loss is a simple way to measure the biodegradation of LDPE polymers. Fungi that colonize the surface of the plastic and utilize the polymer will show growth characteristics that can be seen directly, while the integrity of the polymer will decrease and the biodegradability of the plastic will be reduced leads to weight loss of the LDPE sheet (Gajendiran *et al.,* 2016).

The growth of fungal isolates Asp1, Asp2, Fus1 and Asp3 on Mineral Salt Medium (MSM) Agar with carbon source is LDPE sheet is relatively slow when compared to its growth on Potato Dextrose Agar (PDA) culture media. Fungi utilize complex organic polymers and convert them into simpler molecules by secreting degrading enzymes so that they will break polymer chains and produce short chains, such as oligomers, dimers and monomers (Bhardwaj *et al.*, 2012), but the large molecular weight of LDPE, 3-dimensional structure, hydrophobic nature and lack of functional groups greatly affect the microbial attack on LDPE polymers (Esmaeli *et al.,* 2013). This causes mold growth to be very slow.

Based on the results of the calculation of the average percentage of dry weight reduction obtained plastic degredation, isolate Asp1 has the best ability after the incubation period for 20 days. At 30 days the incubation period was quite high at 1.33%. Optimal degredation of Asp1 isolate occurred after 40 days of plastic incubation with a degredation percentage of 2.23%. This shows that the Asp1 isolate has adapted well in degredating the plastic, so that the degredation shows a stable pattern. Asp2 isolate degredated by 1.78%, while Fus1 isolate was able to degredate by 1.03% greater than the Asp3 isolate which was only able to degredate by 0.74% after a 40-day incubation period. No change in weight could be detected on LDPE sheets grown in control media. Therefore, the observed percentage weight loss of polyethylene strips incubated in different fungi was not due to chemicals in the mineral salt medium but due to biological processes (Nwogu *et al.,*

2012).

The percentage reduction in plastic weight in this study is smaller when compared to Das and Kumar (2014) growing potential fungal isolates on mineral salt medium for 60 days found that Aspergillus sp. was able to reduce the weight of plastic by about 5%, while *Fusarium sp.* was able to reduce 9% of LDPE sheet weight. On LDPE black plastic seen from the effect of the type of isolate Asp1, Asp2, Fus1 and Asp3 given showed different average degredation values. This proves that with the same cell density, each isolate has a different ability to degredate plastic (Badriah and Maya, 2015).

Based on the results of statistical tests, namely the *one way anova* test on the percentage of dry weight reduction by showing significant results on Asp1 isolates with a significant level of p = 0.008 which indicates that fungi utilize polyethylene as the only carbon source (p>0.05). The results of Duncan's further test to determine the difference from each incubation period of each isolate showed that group 1 was significantly different from groups 2 and 3, group 2 was significantly different from group 1 but not significantly different from group 3, while group 3 was not significantly different from group 2 but significantly different from group 1. Asp 2 showed significant results with a significant level of p=0.004, then further tests were carried out with the Duncan test to determine the difference from each incubation period of each isolate showing that group 1 was significantly different from group 2 and different from group 3. However, based on the one way anova statistical analysis test, the types of isolates Fus1 and Asp3 showed no significant changes in the types of isolates after 20 days of incubation until 40 days of incubation, so that the anova results could not be tested further because the significant value of the treatment was higher than the significant level (p=0.05). LDPE biodegradation test results with dry weight reduction method. Based on the calculation of the percentage of dry weight loss of LDPE sheets.

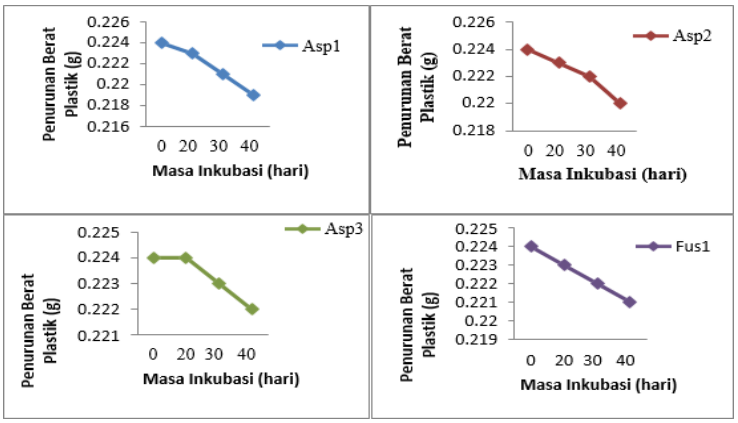

Figure 7. Weight Loss Chart of LDPE Plastic

Figure 7 above shows a decrease in the initial weight of the plastic, indicating that the fungus utilizes the plastic as a source of carbon and nutrients for its growth. Fungi growing on the polymer causes an increase in weight due to microbial adherence, while the loss of polymer integrity causes a decrease in plastic weight (Muhonja *et al.,* 2018).

According to Chinaglia *et al.,* (2018) the percentage of dry weight loss occurs due to the reaction of extracellular enzymes produced by fungi in the biodegradation process will erode the surface of the polymer. through the hydrolysis process so that the weight of the polymer will decrease and the percentage of dry weight loss can increase as the incubation period increases.

Microorganisms are not able to bind polymers directly through the outer membrane of their cells so that biochemical processes are needed that play a role in breaking down long polymer molecules. This process is called depolymerization where the polymer is depolymerized or broken first into smaller monomers before it can be absorbed and degredated in microorganism cells (JD *et al.*, 2000). The result of simple polymer breakdown will be absorbed by microorganisms as a carbon source. There are two active enzymes involved in biodegredation, namely extracellular enzymes and intracellular depolymeterases (Fadila and Maya, 2014; Tokiwa and Calabia, 2004).

Some factors that can affect the speed in biodegredation include humidity, temperature, type of microorganisms, type of polymer, pH, polymer thickness. The type of microorganism affects the biodegradation process therefore factors such as pH, temperature, minerals, nutrients, humidity and oxygen must also be appropriate for the type of microorganism. Incubation duration is a major factor in biodegradation tests because it is related to mold growth. According to Sen and Raut (2015), fungal growth can affect the reduction of dry weight more significantly, the longer the incubation period, the more fungi

that grow, so that the fungi that use LDPE polymers as a source of nutrition are also more and more.

The duration in this study was only 40 days, so the percentage of dry weight reduction percentage was also low. Some studies show a reduction in dry weight in incubation periods of more than 40 days such as the results of research by Muhonja *et al.* (2018) conducted a biodegredation test with an incubation period of 120 days to get the results of dry weight reduction by the genus *Aspergillus* sp. a total of 35.4%. In the research of Munir *et al.,* (2018) conducting biodegredation with an incubation period of 45 days reduced the dry weight of 5.13% of the genus *Trichoderma* and 6.63%. Das and Kumar (2014) grew potential fungal isolates on mineral salt medium for 60 days and found that Aspergillus sp. was able to reduce the weight of plastic by about 5%, while Fusarium sp. was able to reduce 9% of LDPE sheet weight.

The difference in dry weight reduction results is also influenced by the species and enzymes produced. The specific type of enzyme produced by each type of fungus determines the effectiveness of the biodegredation process. Based on the results obtained, the isolate with the highest degredation ability is a fungus of the genus *Aspergillus*, according to Muhonja *et al.* (2018) *Aspergillus is* able to produce two enzymes that play an important role in the LDPE degredation process, namely the enzymes lactase and esterase. Lactase enzyme can help in the oxidation of hydrocarbon bonds and catalyze the oxidation of aromatic compounds in LDPE, while esterase enzyme can catalyze esters. This is evidenced by the presence of intermediate products in the form of esters in the media after GC-MS analysis, then esters can be assimilated into microbial cells as material for the process of recapiration to produce energy (Muhonja *et al.,* 2018).

CONCLUSIONS

Based on the research that has been carried out on the isolation and testing of the ability of fungi to degredate plastics from the Terjun landfill, it can be concluded: There are four fungal isolates from Terjun landfill that have the potential to degredate polyethylene plastic type Low Density Plyethylene (LDPE), namely isolates PME1, PME2, PML2 is from the genus *Aspergillus sp.* and isolate PME3 is from the genus *Fusarium sp.* Fungal isolates Asp1, Asp2, Fus1 and Asp3 have the ability to degredate plastic, isolates with the highest degredation ability after a 40-day incubation period are Asp1 isolates with a percentage of plastic weight loss of 2.11%. Asp2 isolate was 1.40%, Fus1 isolate was 1.05% and Asp3 was 0.87%.

References

Ahmed, T., Shahid, M., Azeem, F., Rasul, I., Shah, A. A., Noman, M., & Muhammad, S. 2018. Biodegradation of Plastics: Current Scenario and Future Prospects for Environmental Safety. *Environmental Science and Pollution Research*. Vol 25. No 8.

Ahsan *et al.,* 2016. Isolasi Dan Penyaringan Jamur Pendegradasi Polietilen Daribahan Limbah Padat. *Jurbal Pertanian Dan Ilmu Dasar*. Vol 1. No. 1.

Alice, D.A., Valentine, C.A., & Sylvie, G. 2019. The Plastisphere in Marine Ecosystem Hosts Potential Specific Microbialdegraders Includingalcanivorax Borkumensisas A Key Player For The Low-Density Polyethylene Degradation. *Journal Of Hazardous Materials*. Vol 380.

Anah, I., *et al.,* 2020. Biodegredasi Plastik LDPE Menggunakan Kolom Wonogradsky. *Jurnal ENVISCIENCE*. Vol 4. No 2.

Arutchelvi, J., Sudhakar, M., Arkatkar, A., Doble, M., Bhaduri, S., & Uppara, P.V. 2008. Biodegradation of Polyethylene and Polypropylene. *IndianJournal of Biotechnology*. Vol 7. No 9.

Awasthi, S., Srivastava, N., Singh, T., Tiwary, D., & Mishra, P. K. 2017. Biodegradation of thermally treated low density polyethylene by fungus Rhizopus oryzae NS 5. *3 Biotech*. Vol 7. No 1.

Azis, I.R., *et al.,* 2019. Biodegredasi Plastik LDPE Hitam Dan Putih Pada SampahTpa Antang Dalam Kolom Winogradsky. *Jurnal Biologi*.Vol 12. No 2

Bhardwaj, H., Gupta, R. & Tiwari, A. 2012. Microbial Population Associated with Plastic Degradation. *Open Access Scientific Reports*. Vol 1. No 5.

Bayry J., Aimanianda V., Guijarro JI., Sunde M, & Latge JP. 2012. Hydrophobins-unique fungal proteins.

Plos Pathogens. Vol. 8. No 5

Chinaglia, S., Tosin, M., & Degli-Innocenti, F. 2018. Biodegradation Rate of Biodegradable Plastics at Molecular Level. *Elsevier*.

Das, M.P. and Kumar, S. 2014. Microbial Deterioration of Low Density Polyethylene by Aspergillus and Fusarium sp. *International Journal of ChemTech Research*. Vol 6. No 1.

Deacon, J. W. 2006. *Fungal Biology*. Edisi Keempat. UK: Blackwell Publishing.

Depika, S. & Jaya, M.R. 2015. Biodegredation Of Low Density Polyethylene ByMicroorganisme From Garbage Soil. *Journal Of Experimental Biology And Agricultural Sciences*. Vol 3. No 1

Dineshraj, D. & Ganesh, P. 2016. Screening and Characterization of Isolatd Fungi from Plastic Waste Dump Yard Sites. *ISJR*. Vol 5 No 1.

Esmaeli A, Pourbabaee AA, Alikhani HA, Shabani F & Esmaeili E 2013.Biodegradation of Low-Density Polyethylene (LDPE) by Mixed Cultureof Lysinibacillus xylanilyticus and Aspergillus niger in Soil. *PloS ONE.* Vol 8. No 9

Elpawati. 2015. Uji Coba Produksi Mikroorganisme Pendegredasi (Penghacur) Sampah Plastik. *Jurnal Agribisnis*. Vol. 9.No. 1

Fadilah, Fiki Rahma & Maya Shovitri. 2014. Potensi isolat bakteri bacillus dalam mendegredasi plastik dengan metode kolom winogradsky. *Jurnal teknik pomits.* Vol 3. No 2

Fa'is, A. J., Ratna, S.D ., & Ajeng, A. S. 2021. Biodegradasi Bioplastik Berbasis PatiMenggunakan Isolat Fungi Indigenous Asal Tempat Pembuangan Akhir Gunung Tugel, Kabupaten Banyumas. *BioEksakta*. Vol 3. No 4

Fitri, R., Yan, E.B., & Rahmayana. 2018. Tempat Pembuangan Akhir Sebagai Ruang Terbuka Publik (Studi Kasus : TPA Terjun Kec.Medan Marelan): *https://www.researchgate.net/publication/327393218.*

Gajendiran, A., Krishnamoorthy, S, & Abraham, J. 2016. Microbial Degradation Of LowDensity Polyethylene (LDPE) by *Aspergillus Clavatus* Strain JASK1 Isolatd From Landfill Soil. *3 Biotech.*Vol.6 No. 52.

Gandjar, I. *et al.,*2000. Pengenalan Kapang Trofik Umum. Jakarta: Yayasan Obor Indonesia.

Gautam, A.K., & Bhadauria, R. 2012. Characterization of Aspergillus Species Associated With Commercially Stored Triphala Powder. *African Journal of Biotechnology*. Vol 11. No 104

Gultom, M.S., M. Yusuf, N., & Aprida, A. 2017. Seleksi Bakteri Pendegradasi Plastik Dari Tanah. *Jurnal Generasi Kampus*. Vol 10. No 2

Harjanti, I.M., & Pratamaningtyas, A. 2020. Pengelolaan Sampah Di Tempat Pembuangan Akhir (TPA) Jatibarang, Kota Semarang. *Jurnal Planoloigi*. Vol 17. No 2.

Harmiyantu, E. 2017. Formula EM (Effective Microorganism) Hasil Isolasi Bakteri Tanah TPA Kota Madiun dengan Potensi Mendegradasi Limbah Plastik. *Prosiding Seminar Nasional Hayati V*.

Hikmah, M., *et al.,2018.* The Potential of Lignolytic Trichoderma Isolats in LDPE(Low Density Polyethylene) Plastic Biodegradation. IOP Conf. Series. 333.

Inas, R.M., Kawuri, R., & Sudirga, S.K. 2017. Potensi Bakteri *Pseudomonas Sp.*Dan *Ochrobactrum Sp*. Yang Di Isolasi Dari Berbagai Sampel TanahDalam Mendegradasi Limbah Polimer Plastik Berbahan Dasar High Density Polyethylene (Hdpe) Dan Low Density Polyethylene (LDPE). *Simbiosis Journal of Biological Sciences.*Vol 5. No 2

Indra, M & Hasrin, L. 2018. *Proses Manufaktur Plastik Dan Komposit*. Yogyakarta: Andi

Islami, A.N. 2013. Summary for Policymakers.In Intergovernmental Panel on Climate Change (Ed), Climate Change 2013 - The Physical Science Basis *Cambridge University Press*. Vol 53. No 9

JD, Gu., Ford, TE., Mitton, DB., & Mitchell, R. 2000. Microbial corrosion of metals. The Uhlig Corrosion Handbook. 2nd Edition. New York, USA : Wiley

Khan, S., Sadia, N., Zia, U.S., & Aamer, A.S. 2017. Biodegradation ofPolyester Polyurethane by *Aspergillus tubingensis*. *Elsevier*. Vol. 30.

Marjayandar, L., & Maya, S. 2015. Potensi Bakteri *Bacillus Sp*. Dalam Mendegradasi Plastik. *Jurnal Sains Dan Seni Its.* Vol. 4 No.2

Muhonja, C. N., Makonde, H., Magoma, G., & Imbuga, M. 2018. Biodegradability of Polyethylene by Bacteria and Fungi from Dandora Dumpsite Nairobi-Kenya. *PLoS One*. Vol 13. No 7

Munir. 2018. Plastic Degrading Fungi Trichoderma Viride and Aspergillus Nomius Isolated From Local Landfill Soil In Medan. *IOP Conf. Series*. 126

Nathania, H. B. 2013. Studi Potensi Isolat Kapang Wonorejo Surabaya dalam Mendegradasi Polimer Bioplastik Poly. Vol 2. No 2

Noerfitryani dan Hamzah. 2018. Inventarisasi Jenis – jenis Cendawan pada Rhizosfer Pertanaman Padi. *Jurnal Galung Tropika*. Vol 7. No 1

Nwogu, N.A., *et al.* 2021. Capability Of Selected Mushrooms to Biodegrade Polyethylene. *Mycosphere.* Vol 3. No 4

Pangestu, N. S., *et al.* 2016. Isolasi, Identifikasi 16sr Rna Dan KarakterisasiMorfologi Bakteri Pen1degradasi Plastik Polietilen (Pe). *Jurnal Biologi*. Vol 5. No 1

Pitt, j and ailsa, d. h. 2002. Fungi And Food Spoilage. Third Edition. New York. Springer Science Business Media

Prabhat, S., *et al.* 2013. Studies on Isolation and Identification of Active Microorganisms during Degradation of Polyethylene/Starch Film. *International Research Journal of Environment Sc11iences.*Vol 2. No 9

Praputri, E., dan Sari, E. 2016. Pengolahan Limbah Plastik Polypropylene Sebagai Bahan Bakar Minyak (BBM) Dengan Proses Pyrolysis.*Seminar Nasional Teknik Kimia–Teknologi Oleo Petro Kimia Indonesia Pekanbaru, Indonesia.Vol* 1. No 1

Pramila, R and Vijaya, R. 2011. Biodegradasi Polietilen Densitas Rendah (Ldpe) Oleh Jamur Yang Diisolasi Dari Air Laut– Analisis Sem. *Jurnal Penelitian Mikrobiologi Afrika* Vol. 5 No. 28

Qadr, N., & Abdullah, F. 2018. The Same Physiological And Nutrional Faktors That Effect The Grown Of Some Fungi. Zanco Journal Of Pure And Applied Sciences. Vol 3. No 5.

Raaman, N. 2012. Biodegradation Of Plastic by Aspergillus Spp. Isolated From Polythene Polluted Sites Around Chennai. *J Acad Ind Res*. Vol 1. No 6

Restrepo,F. 2014. Microbial Degrada- Tion and Deterioration of Polyethylene-A Review. *Int Biodeterior Biodeg- Radation* 88

Ristiari, N.P., *et al.* 2018. Isolasi Dan Karakteristik Bakteri Tanah Pada Tempat Pemprosesan Akhir Di Desa Bengkala Kabupaten Buleleng. *Jurnal Matematika, Sains Dan Pembelanjarannya.*Vol 12. No 1

Rohmah, U.M., *et al.* 2018. Degradasi Plastik Oleh Jamur Aspergillus terreus (LM1021) Pada pH 5 dan 6; Serta Suhu 25℃ dan 35℃. *Jurnal Sains Dan Seni ITS*. Vol 7. No 2.

Sangale, M.K., *et al.* 2012. A Review on Biodegradation of Polythene: TheMicrobial Approach. *Journal of Bioremediation & Biodegradation*.Vol 3. No 10

Salina, I., Yuli, C., and Yustika, G. 2017. Pengolahan Sampah Plastik Jenis PP (polypropylene) Sebagai Material Pada Tas Laundry. *E-Proceeding of Art & Design*. Vol 4. No 3

Sari, D.P., *et al.,* 2020. Isolasi Bakteri Dari Tempat Pembuangan Sampah TPA Kota Medium Dengan Potensi Mendegredasi Limbah Plastik Polyethylene. *Jurnal Pendidikan Dan Ilmu Kimia*.Vol 4. No 20.

Sen, S. K. and Raut, S. 2015. Microbial Degradation of Low Density Polyethylene(LDPE): A Review. *Journal of Environmental Chemical Engineering*. Vol 3: 462-473.

Shah, A.A., Farida, H., Abdul, H., and Ahmed, S. 2008. Biological Degradation ofPlastic: A Comprehensive Review. *Biotechnology Advances*. Vol 26. No 2

Sholihah, R. I. 2019. Identifikasi Jamur Fusarium solani yang Berasosiasi dengan Penyakit Busuk Batang pada Tanaman Buah Naga (Hylocereus sp.) Di Kecamatan Bangorejo, Kabupaten Banyuwangi. *E-Jurnal Agroekoteknologi Tropika*. Vol 8. No 1

Singh, J and K.C.Gupta. 2014. Screening and Identification of Low density Polyethylene (LDPE) Degrading Soil Fungi Isolated from Polythene Polluted Sites around Gwalior city (M.P.*). Int.J.Curr.Microbiol.App.Sci*. Vol 3. No 6

Sonal, G.C., *et al.* 2012. Studies on degradation of Synthetic Polymer Nylon 6 By Fungus Trametes Versicolor Ncim 1086. *International Journal OfEnvironmental Sciences* Vol 2. No 3.

Sulchan, M. dan Nur, E.W. 2007. Keamanan Pangan Kemasan Plastik dan Styrofoam. *Jurnal Maj KedoktIndon.* Vol 57. No 2.

Tasmita, K., Shubhamsingh, T. & Tejashree, S. 2015. Isolation of plastic degrading micro-organisms from soil samples collected at various locations in Mumbai, India. *International Research Journal of Environment Sciences*. Vol 4. No 3

Tokiwa, Y. *et al.,* 2009. Biodegrability of plastics. *International Journal of Molecular Sciences*. Vol 10.

Usha, R., Sangeetha, T., & Palaniswamy, M. 2011. Screening of Polyethylene Degrading Microorganisms from Garbage Soil. Libyan Agric.Res.Cen,*Journal Intl*. Vol 2. No 4.

Valencia, P. E., & Vincentia, I. M. 2017. Isolasi Dan Karakterisasi Jamur Ligninolitik Serta Perbandingan Kemampuannya Dalam Biodelignifikasi. *Scripta Biologica*. Vol 4. No 3

Watanabe, T. 2002. Pictorial Atlas of Soil and Seed Fungi. London: CRC Press

Williams, J. O., & Hakam, K. 2016. Microorganisms Associated with Dump Sites in Port Harcourt Metropolis, Nigeria. J. Ecol. Nat. Environ. Vol 8.No 2.

Zuliani, A., & Atria, M. 2021. Skrining Jamur Pendegradasi Plastik Low Density Polyethylene (LDPE).Fakultas Matematika dan Ilmu PengetahuanAlam, Universitas Riau.

Zusfahair.,*et al.* 2007. Biodegradasi Polietilena Menggunakan Bakteri Dari TPA (Tempat Pembuangan Akhir) Gunung Tugel Kabupaten Banyumas.*Molekul*.Vol. 2. No. 2.