

The Potential of Lignolytic *Trichoderma* Isolates in LDPE (Low Density Polyethylene) Plastic Biodegradation

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Abstract. Plastic is experiencing buildup in the environment. Biodegradation process can be used as an alternative for LDPE plastic degradation because the process is environmentally friendly. Some fungi of the genus *Trichoderma* are known to have a role in plastic biodegradation. This study aims to find out how the potential of that lignolytic *Trichoderma* spp. isolates in LDPE biodegradation. Five isolates were screened by growing on MSMB (mineral salt medium broth) emulsified LDPE powder, with 35 days incubation at 30°C and shaking at 80 rpm. TL1, TL4, and TL5 are the three most potential isolates, indicated by the growth marked by increasing colony size on screening media. They were then tested for biodegradability by growing the isolates in MSMA (mineral salt medium agar) which then inoculated by 4 sheets of sterile LDPE 1x3 cm² above the colony surface, incubated for 5, 15, 25 and 35 days. The degradedability assessment is done by measuring the weight loss of LDPE sheets after biodegradation treatment. The obtained degradability percentage of TL1, TL4, and TL5 are 4.87%, 7.12%, and 7,51% respectively. The visual micrograph of LDPE film by SEM showed the appearance of damage and unevenness on the surface of the post-degradation film.

1. Introduction

Plastics are synthetic polymers that are difficult to degrade, because they have a high molecular mass density, resulting in a buildup in the environment [1]. Plastics are material that can potentially threatens the survival of living things on earth, because they are made of chemicals that are difficult to degrade by microbes in the environment. One of the most commonly used types of plastic is low density polyethylene or LDPE.

Biodegradation process can be used as an alternative degradation because the process is environmentally friendly and does not cause natural damage. Biodegradation is the process when microorganisms are able to degrade or break down natural polymers (such as lignin and cellulose) and synthetic polymers (such as polyethylene and polystyrene) [2]. Biodegradation can be done by microorganisms such as bacteria, fungi, yeast and algae [3]. Fungi that have the ability of degradation of plastic in the environment among others derived from the genus *Aspergillus*, *Penicillium*, and *Trichoderma* [4]. In many studies, fungus are considered capable of LDPE biodegradation because of their ability to form hydrophobic proteins that can stick to polymer surfaces [5]; ability to produce suitable degradation enzymes against insoluble LDPE [6]; the growing speed of fungi biomass in soil faster than bacteria [7]; extension of body mass and penetration into other locations through the

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distribution of hyphae; and also the fungus can survive in environments with poor nutrition availability, low pH, and low humidity [8].

Prior research which did isolation and selection of the lignolytic fungi from zalacca leaf litter, produces fungal isolates from the genus *Trichoderma*. The lignolytic activity of fungi is due to the presence of lignolytic enzymes, some of which are most laccase, manganese peroxidase, and lignin peroxidase [9]. The polyethylene degradation may be performed by the *Trichoderma harzianum* fungi and produce a high percentage of degradability [10]. From the results of the research known that the enzymes that play a role in the process are laccase and manganese peroxidase, two enzymes are also encountered in the lignolytic test before. This study aims to test the potential of *Trichoderma* spp isolates. lignolytics from previous studies to determine whether there is a capability in LDPE polyethylene biodegradation.

2. Experimental

Isolates of lignolytic *Trichoderma* spp. obtained from the stock of biological laboratory of FMIPA UNS, they are TL1, TL2, TL3, TL4 and TL5. The culture stock is isolated from leaf litter of zalacca plantation in previous research and has been tested to have lignolytic ability.

2.1. Preparation for Media

Media that is used for rejuvenation of *Trichoderma* spp. is potato dextrose agar medium (PDA). While the media used for screening and biodegradation tests is mineral salt medium (MSM), with compositions in 1000 ml of aquades containing K₂HPO₄ 1 g; KH₂PO₄ 0.2 g; NaCl 1 g; CaCl₂·2H₂O 0.002 g; (NH₄)₂SO₄ 1 g; MgSO₄·7H₂O 0.5 g; CuSO₄·5H₂O 0.001 g; ZnSO₄·7H₂O 0.001 g; MnSO₄·H₂O, 0.001 g and FeSO₄·7H₂O 0.01 g. For solid media (MSMA) agar is added for as much as 20 grams.

2.2. LDPE Powder Preparation

LDPE polyethylene crystals in amount 5 grams dissolved into 150 mL xylene and then boiled for 30 minutes. To remove xylene from the solution, the LDPE solution was given ethyl alcohol. The mixture is then evaporated and dried by oven at a temperature of 40-50°C during overnight. The rough powder that is formed is then smoothed with a blender, then filtered to obtain the best powder.

2.3. Screening

The five isolates were inoculated on MSMB medium emulsified LDPE in erlenmeyer by 1 cork borer (39.25 mm²) of inoculums. After that, it was incubated at 30°C in a shaker incubator at 80 rpm for 35 days. Isolates that exhibited growth characterized by increasing colony size or inoculum diameter in the media are then assessed as potentially biodegradable. This growth was observed visually or qualitatively, then measured the colony area with millimeter block.

2.4. LDPE Sheets Preparation

LDPE sheets with a thickness of 0.03 mm cut into 1x3 cm² pieces. The pieces are weighed until a constant dry weight is obtained. The LDPE sheet was then disinfected using 70% ethanol for 30 minutes, then transferred to sterile distilled water for 20 minutes and then dried again.

2.5. Biodegradation Test

Two pieces cork borer of 5-day-old *Trichoderma* spp. inoculum was inoculated on a petri dish containing MSMA, then incubated for 1 week at room temperature. A total of 4 pieces of sterile LDPE that have been known to have their dry weight inoculated on the surface of the growing isolates on the test medium. The culture was incubated at room temperature with a period of 5, 15, 25, and 35 days. In each incubation period, one sheet of LDPE film is taken. The LDPE film was first rinsed with alcohol and distilled several times, then dried with oven at 50°C for 6 hours. The constant dry weight

of each LDPE sheets is then weighed. The value of degradation efficiency is obtained from the weight loss of the incubation start and end time with the equation:

$$DE\% = \frac{W_0 - W_1}{W_0} \times 100\% \quad (1)$$

W_1 : the original weight of the sample
 W_2 : the weight of sample after biodegradation treatment
 DE%: percentage of degradation efficiency

2.6. Observation of LDPE Film Morphology with SEM

SEM analysis was done at the UNDIP Integrated Laboratory of Semarang

3. Result and Discussion

3.1. Growth of Low Density Polyethylene Degrading Fungi (LDPE)

Visually, the isolates appeared to be growing quite well on media that do not support the growth of fungus in general. MSMB is a medium containing only mineral salts without addition of sugar or other carbon sources as triggers and growth supporters. In the medium, the only carbon source was the LDPE powder. At screening, the material LDPE appeared to be on the surface of the media and surrounded the isolates, or partly at the bottom of the media and appeared to be trapped, clinging, and covering the mycelium. LDPE powder was attached to the fungal colony (mass of mycelium). This is a sign that the fungal isolates used LDPE as a carbon source in MSMB. The initial size of the isolates inoculated into the medium was 1 cork borer (39.25 mm²) of 1 week old isolates. From these early colonies it was possible to assess the growth of isolates after 35 days incubation, from the increasing of colony size.

At this screening, growing colonies showed irregular shapes of different sizes in liquid medium. Isolate TL1 colony looked round shaped with uneven edges, brownish green, mycelium looked dense, like there were fine hairs around the colony, a little bit of LDPE powder attached and entangled on the surface of the mycelium. In the TL4 isolate, spherical colonies appeared clumped unevenly, some of the mycelium that came out elongated like a tail and irregular, bright green colony color, only a small amount of LDPE powder attached to the surface. Whereas in the TL5 isolate, visible colonies with long and large, branched and irregular clumps, colony morphology and coloration could not be clearly seen because the colony is totally covered by LDPE powder at the base of erlenmeyer. Growth of these isolates in screening can be seen as in Figure 1.

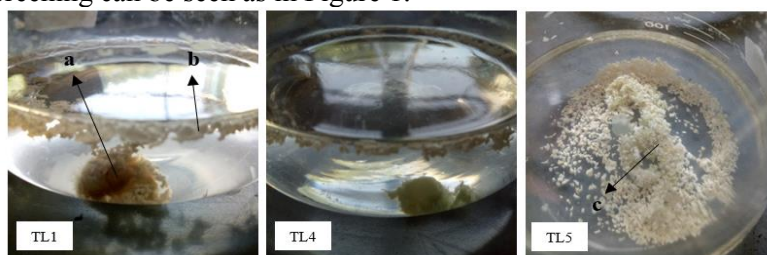


Figure 1. Growth of LDPE degrading potential isolates in screening media. (a) colony. (b) LDPE powder partially floated on the surface of the media. (c) the colony was shrouded by powder at the base of the media.

After visual observation, the measurement of colony wide area was done by using millimeter block paper placed on the base of erlenmeyer. From the five isolates, three isolates (TL1, TL4, and TL5) showed good growth as seen in Table 1 which shows colony area data, so the three isolates were taken for further biodegradation test.

Table 1. Wide Range Size of Colony by Screening

Numb.	Isolate code	Early broad (cork borer)	Wide range area by screening
1	TL1	39,25 mm ²	3.1 cm
2	TL2	39,25 mm ²	1.8 cm
3	TL3	39,25 mm ²	2.8 cm
4	TL4	39,25 mm ²	2.8 cm
5	TL5	39,25 mm ²	0 cm
6	control	-	-

3.2. Biodegradation of LDPE Plastic Sheet by Potential Isolate

Potential isolates from screening were further tested in this stage. Growth of inoculum *Trichoderma* spp. on the MSMA was not as fast as growth on the usual standard media for fungal cultures such as PDA. This MSMA was containing mineral salts only with the addition of plain agar in the absence of glucose and other carbon sources, thus less supporting growth. The colonies that grow on the medium appeared to be small to medium size but spread throughout the petri surface with the size and thickness of colony color varying each isolate. After the inoculums on MSMA are about one week old, the sterile LDPE sheets were prepared to be inoculated on the inoculum surface as seen in Figure 2.

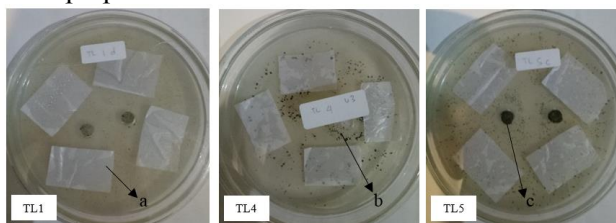


Figure 2. LDPE sheets placed on the surface *Trichoderma* spp. colonies. (a) LDPE sheet. (b) colonies of isolates (c) inoculum.

The growth of fungal isolates TL1, TL4, and TL5 in MSMA with LDPE membrane as a carbon source was relatively slow and there was no significant increase in mass of mycelium during the incubation period. The fungi utilizes complex organic polymers and converts them into simpler molecules by secreting the degrading enzymes to break the polymer chain and producing short chains such as oligomers, dimers, and monomers of a size small enough to pass through a semipermeable membrane [4]. However, the large molecular weight of LDPE, 3-dimensional structure, hydrophobic properties, and lack of functional groups strongly influence microbial attachment efforts in LDPE polymers [11]. This causes the growth of the fungus becomes very slow. During the degradation process, LDPE is first converted into its monomers, then the monomer is mineralized. LDPE is too large to pass through cellular membranes, it must first be depolymerized into smaller monomers before being adsorbed and biodegraded in microbial cells. These low molecular weight compounds will then be utilized by microbes as a source of carbon and energy.

3.3. Decreasing of Dry Weight Film by Presence of Biodegradation

Considering the mass of the polymer before and after the biodegradation process over a period of time is the simplest quantitative method to characterize the biodegradation of a polymer. Fungi that colonize plastic surfaces and utilize their polymers will exhibit visible growth traits with the naked eye, while the integrity of the polymer will decrease and lead to weight loss of LDPE sheets [12]. From the average dry weight gained, we got the graph of dry weight loss from the beginning to the end of the incubation period of each isolate and control as can be seen in Figure 3.

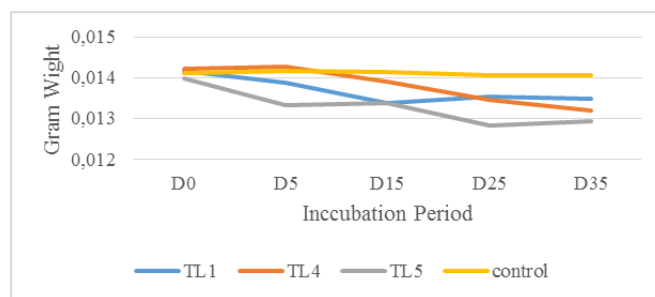


Figure 3. Film Weight Loss by Biodegradation

3.4. Percentage of Isolate Degradability

After 35 days of biodegradation test mass, control LDPE did not show any significant weight change, meanwhile LDPE sheet weight treatment was reduced by TL 1 fungal isolate by 4.87%, TL4 isolate by 7.12%, TL5 isolate of 7.51 %, and the control yielded a 0.28%. The controls showed no significant difference in each period. The control shows that there is a number indicating the percentage value of degradation, so it can be assumed that there is a decrease of plastic biomass due to the physical factor due to shake and rotary shaker during the incubation period. Comparison of percentage value of degradability of each isolate and control can be seen in Figure 4.

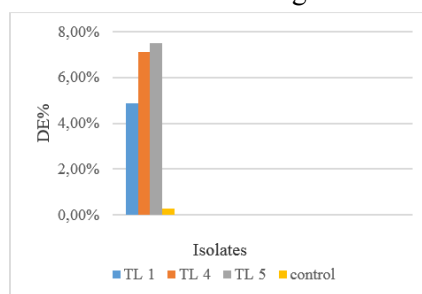


Figure 4. Percentage of Degradation Efficiency (DE%) of Isolates

Das and Kumar cultivated potential fungal isolates in MSM incubated at 33.3°C and shaken on a rotary shaker at 130 rpm for 60 days, with LDPE sheets being the only carbon source. After 60 days incubation it was found that *Aspergillus* sp. capable of reducing about 5% -8%, while *Fusarium* sp. capable of reducing 9% of LDPE sheet weight [13]. Singh and Gupta reported changes in LDPE weight were degraded by *Aspergillus flavus* (30%), *Aspergillus niger* (20%), *Aspergillus japonicus* (36%), *Mucor* sp. (16%), *Penicillium* sp. (24%), and *Fusarium* sp. (32%) after a 30-day inactivity period on synthetic medium (without yeast extract) and LDPE sheet as the only carbon source [14].

Harefa [15] conducted the research by isolating the fungus from TPA Namo Bintang. Two fungal isolates capable of degrading potential LDPE plastics from the study were isolates of the *Aspergillus* genus, with the percentage of degrees are 5.31% and 6.63% respectively after 45 days of biodegradation testing. If the results of this study were compared with those three studies, then the percentage of LDPE weight loss treated by the ligolytic *Trichoderma* spp isolates TL1, TL4, and TL5 were higher compared to the percentage of boot loss obtained by Das and Kumar [13] and Harefa [15], but still much lower with the degradability values obtained by Singh and Gupta [14].

3.5. Observation of Film Morphology After Biodegradation Test

The biodegradation process can cause changes in surface morphology. The result of LDPE sheet micrograph representing the three isolates along with the control at 3000X magnification can be seen in Figure 5. The analysis using SEM (Figure 5) shows that the LDPE surface control is incubated and after 35 days of biodegradation is flat, without cracks and holes, and no particles are attached to the surface. LDPE treatment with isolated TL1 isolates shows that the LDPE surface becomes more rough, forming curves, leading to cracks in LDPE materials. Similarly, in LDPE treated TL4 and TL5

isolates, they also suffered damage, produced pores on the surface, and even visible spore particles left behind during rinsing of LDPE membranes.

The natural properties of LDPE that are resistant to biological attacks are caused by hydrophobic carbon bonds and have high molecular weight polymers [16]. However, once the organism is attached to the polymer surface, it will begin to grow by utilizing the polymer as a carbon source. The filamentary fungi are generally capable of attaching to the hydrophobic surface by forming hydrophobic proteins [17]. The extracellular enzymes are too large for deeper penetration into the polymeric material so that they work only on the surface of the polymer alone. As a result plastic biodegradation is generally only a process of surface erosion.

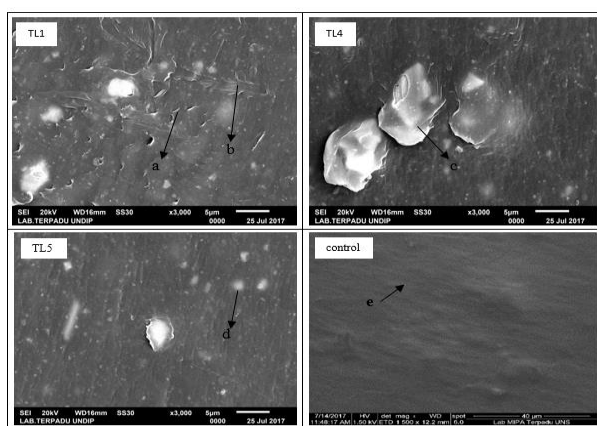


Figure 5. Appearance of LDPE Film Morphology at 3000X magnification. (a) pores or holes. (b) damage and cracking on the surface of the film. (c) SEM electrons reflect the particle on the sample due to uneven surface of the sample. (d) spore particles left on the surface of the film. (e) the control film surface looks flat and clean

4. Conclusion

From this research it can be concluded that 3 of 5 isolates of lignolytic *Trichoderma* spp. isolated from leaf litter of zalacca plantation has potential in LDPE plastic biodegradation, demonstrated by the ability of isolates to grow on the screening media emulsified LDPE powder as carbon source, and from biodegradation test which resulted in shrinkage of dry weight indicated by the degradability value of each isolate, TL 1 of 4,87%, TL4 of 7,12%, and TL5 by 7,51%. Potential degradedability is also supported by polymer surface observations with SEM indicating a membrane damage due to biodegradation by fungi.

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