Biodegradation of Polymeric Medical Waste by *Aspergillus oryzae*: A Preliminary Study

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**Abstract.** Hospital solid medical waste is a type of solid waste that is difficult to decompose. It can be concluded as pathogenic waste, which will be dangerous for the environment, if not handled properly. On the other hand, *Aspergillus oryzae* was reported to be able to degrade pollutants by its enzymatic mechanism. Hence, this study examined *A. oryzae* fungi as a degrading agent for this solid medical waste in the form of plastic infusion vessels. The plastic infusion vessel waste (3x1 cm²) was treated by *A. oryzae* in Potato Dextrose Broth (PDB) medium and then incubated for 14, 28, 42, and 56 days. The results showed that medical waste was degraded in amounts of 0.15 mg and 0.2 mg in 14 and 28 days, respectively. At 42 and 56 days of incubation, biomass weighing stagnated and there was no difference, which means that degradation had ended. Furthermore, this medical waste that had been degraded was also analyzed by using SEM and FTIR characterization. SEM images showed different morphological surfaces before and after degradation, a cavity with a random shape was found and indicated a reduction in waste samples. Meanwhile, the FTIR result exhibited the functional groups of infusion vessels. Both of the analyzed treatment samples before and after showed alkene peaks. Therefore, this study demonstrates that *A. oryzae* can be used as a degrading agent for solid medical waste. In addition, using *A. oryzae* as a degradation agent also has the advantage of handling waste in an eco-friendly way.

# Introduction

Indonesia has a larger number of hospitals, considering the community's need for medical care facilities, which is important. One of the biggest hospitals is Dr. Soetomo Hospital in Surabaya. According to a research study, this hospital produced about 40 tons of solid medical waste between 2019 and 2022 [1]. Solid medical waste is defined as solid waste from medical operations and diagnostic activities that may be dangerous due to the presence of radioactive elements, sharp items, toxic chemicals, or infectious organisms [2]. Syringes, used gauze, gloves, IV bottles, and bandages that have come into contact with body fluids are a few examples of solid medical waste [2]. Since this medical waste is regarded as a dangerous and poisonous substance, it needs to be eliminated with special attention [3]. In addition, there is a worry that careless people could reuse it if it is not destroyed completely, while the majority of health services equipment is single-use [4].

Medical solid waste treatment systems generally use physical methods [2]. This waste was burned at a high temperature of about 1,200 °C to decompose [5]. However, not all hospitals can use this method because of its high technology and the high cost of the equipment. In another hospital, such as General Hospital of Haji Surabaya, it uses a furnace called the incinerator, which is operated at a maximum temperature of 800 ºC. As a result, the combustion process will not complete and will still produce residues [5]. Meanwhile, the other hospitals that don’t have incinerators will have to send their waste to hospitals that have this furnace.

Based on this concern, a proper handling treatment is needed. Another waste reduction method is the biological method using microbes (e.g., bacteria, fungi, yeast, etc.). [6]. Compared to physical methods, using microbiological agents as a waste handling process has some advantages [7, 8.] Biological methods are more environmentally friendly but are still rarely used in solid waste because microbes need a carbon source and energy to survive [9, 10]. A journal article states that one type of plastic, namely low-density polyethylene (LDPE) polymers, can be degraded by *Bacillus krulwichiae, Bacillus pseudofirmus, Prolinoborus fasciculus*, and *Bacillus* sp. approximately 9.9%, 8.3%, 5.1%, and 6.3%, respectively [11]. LDPE is composed of ethylene monomers, a type of thermoplastic polymer, and has a highly branched molecular structure, making it difficult to degrade [12]. In addition, some species of fungi from the *Aspergillus* genus, such as *A. oryzea, A. flavus, A. fumigatus, A. niger, A. awamori, A. tubingensis*, and *A. carbonarius,* have also been reported to be able to degrade plastic [13]. Furthermore, *Aspergillus oryzae* was also able to degrade LDPE up to 36.5% [14].

*A. oryzae* is one of the fungi that is often used in daily life [15]. This fungus is known as a mold fungus to saccharify rice in the making of alcohol [15]. This fungus produces many extracellular enzymes that degrade carbohydrates, polypeptides, and nucleic acids [16]. In addition to plastic waste, *A. oryzae* is also reported to be able to degrade many pollutants, including dye, chlorophenol, and benzoic acid [17, 18]. Compared to bacteria, fungi have the ability to degrade pollutants by mineralizing pollutants into CO₂ and H₂O [19]. *A. oryzae* is able to secrete enzymes from the oxidoreductase class, such as laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP) [20]. Laccase is able to oxidize aromatic compounds into simple forms [16]. Several studies have used these enzymes to degrade pollutants such as dyes [20–22]. In addition, MnP and LiP are also able to degrade complex aromatic compounds [23]. Furthermore, this fungus also secretes cellulase and xylanase, which can degrade cellulosic organic matter in solid waste [24]. Therefore, based on those abilities, this research studied *A. oryzae*'s involvement in degrading polymeric medical waste.

# Materials and MethodS

## Collection of Sample

The sample for this study was collected from a hospital in Surabaya, East Java, Indonesia. The specific solid medical waste was an infusion vessel. This solid medical waste used for this research was cut into small pieces and sterilized using 70% ethanol [5].

## *A. oryzae* Culture Condition

The culture stocks of *A. oryzae* were kept on potato dextrose agar (PDA) plates and incubated at 30 °C. After the mycelia growth, it was mixed with 25 mL of sterile water and then homogenized for 1 minute. About 1 mL of this mixture was inoculated into 9 mL of Potato Dextrose Broth (PDB). These cultures were incubated for 7 days at 30 °C [25].

## Biodegradation of Solid Medical Waste

The pre-incubated cultures of *A. oryzae* were used for the degradation process. As controls, some cultures were killed by autoclaving (121 °C, 15 minutes) [26, 27]. Furthermore, solid medical waste of 1 x 3 cm was placed in the bottom of the mycelium. The samples were degraded for 2-8 weeks.

## Determination of Mass Loss Biodegradation

After each variation of degradation times (14, 28, 42, and 56 days), the solid medical wastes were recovered. Then it was rinsed using distilled water. The difference between initial and final weights were counted by using a digital weighing balance and is represented as mass loss due to the biodegradation process [13, 28].

## Determination of Dry Biomass

The mycelium of fungus was filtered after those various incubation times. Then, it was dried by using an oven at 70 °C for 10 hours [13]. After that, the mycelium was counted using a digital weighing balance.

## Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The presence of functional groups of solid medical waste was analyzed using Fourier Transform Infra-red (FTIR) spectroscopy. The solid medical waste before and after degradation was characterized by FTIR analysis using the Shimadzu 8400S spectrometer at wavenumbers between 4000-400 cm-1 [29].

## Scanning Electron Microscopy (SEM) Spectroscopy Analysis

The medical solid wastes were also analyzed using a scanning electron microscopy (SEM-EDX, JEOL 6360 LA, Japan) instrument [30]. This process can confirm the degradation process in the sample by reduction of the morphological image [31]. The samples were sputtered with Au first and conducted at 5,000 x magnification.

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# Results and Discussions

## Biodegradation of Solid Medical Waste by *A. oryzae*

After the degradation process, solid medical waste was filtered and washed using distilled water. The mass weight from each solid medical waste and mycelium was calculated. The determination of the analysis results of biodegradation by *A. oryzae* is shown in Table 1. On the 14th day, *A. oryzae* degraded medical waste by 0.15 mg. While on the 28th day, 0.2 mg of solid medical waste was degraded. However, the control only showed approximately 0.05 mg of degradation. This number is still the same amount on the 42nd day and 56th day. It means that the optimum incubation period of *A. oryzae* degrading solid medical waste is 28 days. On the next incubation time, the fungus cannot degrade continuously. This could be influenced by the material of the infusion vessel, which was made of LDPE or polyvinyl chloride (PVC) [12,32]. These materials are highly resistant to biodegradation due to their hydrophobic nature and high molecular weight [33]. The graphic of mass degradation also can be seen in Figure 1. It exhibited clearly that degradation efficiency remained relatively low, and no further degradation was observed beyond 28 days.

**TABLE 1.** Degradation of solid medical waste using *A. oryzae* fungus.

|  |  |  |
| --- | --- | --- |
| **Incubation period** | **Mass Loss** | |
| **(day)** | **Treatment (mg)** | **Control (mg)** |
| 14 | 0.15± 0.071 | 0.05 ± 0.07 |
| 28 | 0.2± 0.14 | 0.05 ± 0.07 |
| 42 | 0.2± 0.14 | 0.05 ± 0.07 |
| 56 | 0.2± 0.14 | 0.05 ± 0.07 |

**FIGURE 1.** Graphic of degradation mass of solid medical waste

A previous study explained that accumulation of toxic intermediates (e.g., microplastics or oxidized fragments) could inhibit fungal enzymes [34]. In addition, after prolonged incubation, fungal biofilms may have limited direct contact between hyphae and the plastic surface, reducing degradation efficiency [35]. Moreover, the biomass of fungal mycelium was also carried out. In Figure 2, it was seen that the *A. oryzae* mycelium did not undergo significant changes. On the 14th day, the mycelium was 64.25 mg. The next variation time, the mycelium weight was 61.9 mg and 60.2 mg on the 28th and 42nd days, respectively.

Compared with other studies, it exhibited that *A. oryzae* had lower degradation efficiency than *Aspergillus clavatus* (up to 58% weight loss in LDPE after 90 days) [14]. This difference could stem from strain-specific enzyme production or experimental conditions. While bacterial consortia (e.g., *Pseudomonas* spp. + *Bacillus* spp.) have achieved higher degradation rates (up to 20% in 60 days) due to synergistic enzymatic action [13].

**FIGURE 2.** Graphic of mycelium biomass

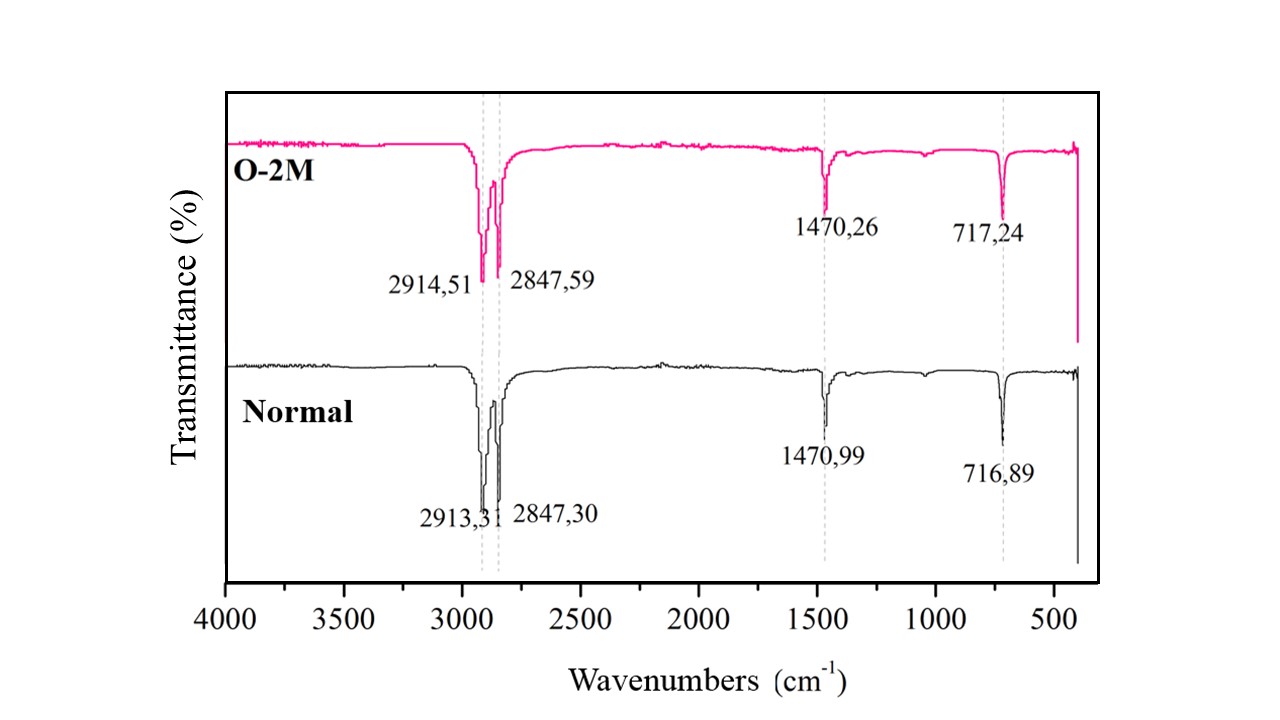
The biodegradation of solid medical waste (primarily polyolefin-based plastics like LDPE/PVC) by *A. oryzae* involves a complex interplay of biochemical and physical processes. While the study demonstrated measurable degradation, the underlying mechanisms warrant deeper exploration to understand the limitations and potential optimization strategies. *A. oryzae* employs a suite of extracellular enzymes to initiate plastic breakdown, including oxidative and hydrolytic enzymes. Laccases and peroxidases catalyze the oxidation of C-H and C=C bonds, introducing hydroxyl and carbonyl groups [16, 36]. MnP may generate free radicals that cleave polymer chains, though their role in synthetic polymer degradation is less documented than in lignin breakdown [21]. Furthermore, cutinases and esterases could target ester linkages in plastic additives (phthalates in PVC), but their efficacy against pure polyolefins is limited [37].

Hence, this study declares that degradation occurred during the experiment, but the extent was low (~0.2 mg loss). This concern is due to the high resistance of the infusion vessel material. However, given the large volume of waste in hospitals, further research is certainly needed. Various variations are possible, such as the concentration of added nutrients, the mass of biomass used, and the length of incubation time.

## *Fourier Transform Infrared* (FTIR) Analysis

FTIR data showed the functional groups in solid medical waste (Figure 3). This sample has a C=C functional group (716 cm⁻¹), -CH₂ stretching(1470 cm⁻¹), and alkanes (2913 cm⁻¹). C=C stretching (716 cm⁻¹): the presence of this peak in the untreated sample indicates unsaturated carbon bonds, typical of polyolefins like LDPE or PVC. A reduction in intensity or shift in this peak post-degradation suggests chain scission or oxidation of double bonds, a common initial step in microbial polymer breakdown [12]. CH₂ Stretching (1470 cm⁻¹) and Alkanes (2913 cm⁻¹): these peaks, characteristic of hydrocarbon backbones in plastics, showed altered intensities after degradation. A decrease in CH₂ symmetry (1470 cm⁻¹) implies disruption of crystalline regions, while changes at 2913 cm⁻¹ (asymmetric CH₂ stretching) indicate oxidative cleavage of alkyl chains [38].

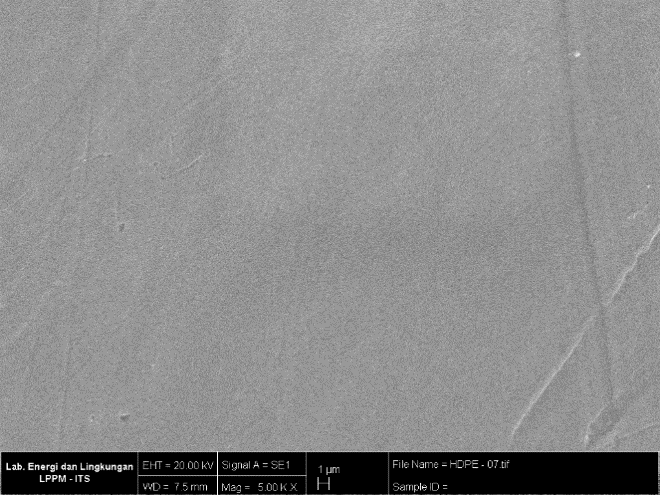
As seen in Figure 3, there is no change in the position of wavelength and absorption intensities between normal and sample after degrading for about 14 days. This could have happened because medical plastic waste was difficult to break down into its intermediate product degradation [12]. Moreover, LDPE is a long polymer in plastic that is composed of ethylene monomers, so that if the structure is broken down, the functional groups contained also consist of alkanes and alkyl chains [12]. A previous study that conducted plastic waste biodegradation only shows remaining plastic weight data [13].



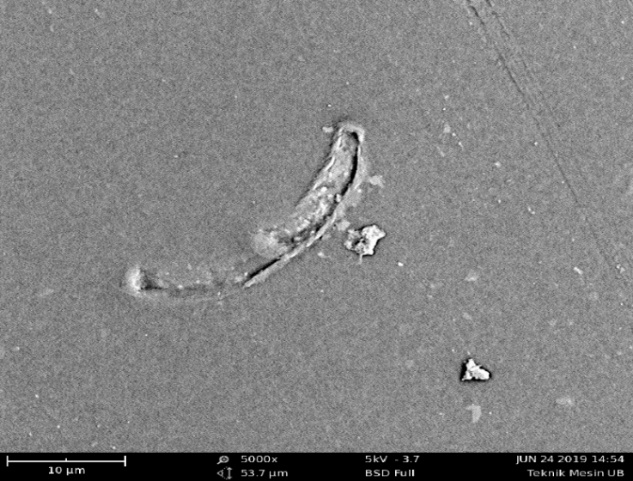
**FIGURE 3.** FTIR analysis of solid medical waste

## *Scanning Electron Microscopy* (SEM) Analysis

Analysis of solid medical waste using the Scanning Electron Microscopy (SEM) instrument aims to determine the surface morphology. The SEM analysis provided visual evidence of structural modifications to the solid medical waste following biodegradation by *A. oryzae*. Figure 4 showed the surface morphology of the solid medical waste sample before degradation, which showed no cavity on the surface area of the sample. The smooth, homogeneous surface with no cavities is characteristic of intact polyolefin-based medical plastics. This aligns with the material's inherent resistance to environmental degradation due to high hydrophobicity and crystallinity [39]. In addition, Figure 5 showed a cavity with a random shape, which indicates a reduction in waste samples on certain sides. This result means that the solid medical waste was degraded by *A. oryzae.* Furthermore, the appearance of irregular cavities and surface erosion indicates localized fungal attack. Similar pitting patterns were reported for *Aspergillus flavus* on LDPE after 60 days, but with more pronounced fissures [14].



**FIGURE 4.** SEM analysis of solid medical waste before degradation (magnification of 5000x)



**FIGURE 5.** SEM analysis of solid medical waste after 14 days (magnification of 5000x)

# Conclusion

In this study, *A. oryzae* fungus can be used as a degrading agent for solid medical waste using the bottom mycelium method. The best incubation period for *A. oryzae* degrading solid medical waste is 28 days, and the maximum degradation achieved was 0.2mg. In addition, further research experiments are still needed due to the massive solid waste that is produced by hospitals in this world.

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