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ORIGINAL RESEARCH

Biodegradation of diisobutyl phthalate using fungi plastisphere isolated from plastic waste in Mangrove Kadilangu area

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Abstract. Diisobutyl phthalate, an additive in plastic production belonging to the group of xenobiotic substances, has a negative impact on human health, especially on the reproductive system. In Indonesia, the presence of plastisphere fungi is often used as a degrading agent for xenobiotic substances. Plastisphere fungi show enzymatic activity to degrade xenobiotic substances but have not been widely studied. Therefore, this research aims to assess the ability of several genera of plastisphere fungi to degrade diisobutyl phthalate and evaluate their enzyme activity and their tolerance under heavy metal stress. The method for determining degradation used an UV-VIS spectrophotometer at wavelength of 362 nm, fungal identification used profile matching method, the presence of oxidoreductase enzymes in fungi was assessed using qualitative method with selective media (ABTS 200 mg/L, phenol red 50 mg/L, and methylene blue 4%), and their tolerance to heavy metals at a concentration of 50 ppm was analyzed using tolerance index method. The identification results of fungi come from the genera *Acremonium* and *Aspergillus*, and tolerance index analysis revealed that these genera have high tolerance to very tolerant (0.91-1.23) to the metals (copper, chromium, and lead) induced stress. Evaluation of oxidoreductase enzyme activity showed that *Aspergillus* from isolate LT2(1) had laccase, lignin peroxidase, and manganese peroxidase enzymes. Analysis of diisobutyl phthalate degradation using an UV-VIS spectrophotometer with wavelength of 362 nm showed that all isolates were able to degrade diisobutyl phthalate within 14 days of incubation period at room temperature (20ºC-23ºC) under copper-induced stress, with the highest isolate degrading diisobutyl phthalate is *Aspergillus* LT2(1) (75.57%), followed by *Acremonium* PK2(2) (60.65%), and *Aspergillus* HT3(2) (36.79%). Based on these findings, it can be concluded that *Aspergillus* LT2(1) shows the best agent to degrade diisobutyl phthalate because it has the ability to regulate the flow of nutrients and energy through the structure of hyphae and mycelial tissues and produces oxidoreductase enzymes that are able to degrade recalcitrant compounds.

Keywords: *Acremonium, Aspergillus, Diisobutyl phthalate degradation, Oxidoreductase enzyme, Tolerance index*

INTRODUCTION

Industrial development in the world is currently experiencing rapid development, including the plastic manufacturing industry, medical equipment, cosmetic ingredients, dispersing and emulsifying agents, and other household products. One industry that is growing rapidly is plastic production because almost all industrial product distribution activities use plastic packaging. However, the development of the plastic industry, apart from facilitating human activities, also has a negative impact on environmental pollution, namely the large amount of plastic waste and the content of plasticizer additives in the form of phthalates which pollute the environment. Phthalate is one of the dangerous materials used in plastic production [1]. Environmental pollution by phthalate is mostly caused by China and European countries whose phthalate production reaches 2.5 million tons per year [2]. Phthalates are dangerous substances for humans because they have reproductive toxicity to the endocrine glands, genotoxicity, neurotoxicity, and cytotoxicity. Apart from that, phthalates also cause several risks of human disease due to their carcinogenic properties, such as causing skin cancer, liver cancer,

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thyroid gland dysfunction, and autism spectrum disorders [3].

Biodegradation using microorganisms is one of the best solutions to remediate xenobiotic substances including phthalates. Microorganisms consist of many types including fungi, archaea, bacteria, protists, and viruses. However, the most widely used as biodegradation agents are fungi, because they are environmentally friendly, do not form chemical waste, are resistant to various environmental conditions, allow biosorbent regeneration, and are low cost. Mold which part of the fungi has enzymatic activity that can break chemical bonds or ester bonds in phthalate compounds into simpler and less dangerous compounds [4]. Previous research reported that *Aspergillus flavus* isolated from sanitary landfill soil has the ability to degrade dibutyl phthalate at a concentration of 100 mg/L reaching 99.34% for 15 days and *Fusarium culmorum* has the ability to degrade di(2-ethylhexyl) phthalate at a concentration of 1000 mg/L into butanol, hexanol, catechol, and acetic acid reaching 92% for 36 hours [5], [6]. Both research also assaying the production of esterase enzymes in degrading phthalate compounds. However, this research focuses on the degradation of other phthalate compounds, namely diisobutyl phthalate, using plastisphere mold isolated from plastic waste from mangrove area with qualitative assay of oxidoreductase enzymes (Lac, LiP, and MnP) which play an important role in the degradation of diisobutyl phthalate.

The aim of this research was to assess the ability of plastisphere fungi to degrade diisobutyl phthalate under copper-induced stress. The reason for choosing this diisobutyl phthalate degrading agent is to determine the ability of plastisphere molds to degrade diisobutyl phthalate after they adapt to environmental conditions polluted by heavy metals. It is hoped that the results of this research will provide information regarding efforts to degrade diisobutyl phthalate compounds by utilizing biological agents in the form of plastisphere fungi in areas contaminated with heavy metals.

METHODOLOGY

Isolation of Plastisphere Fungi from Plastic Waste

Plastisphere mold from plastic waste in the Kadilangu Kulon Progo Mangrove area was isolated on Potato Dextrose Agar plate medium with pH 5 supplemented with anti-bacterial chloramphenicol, then incubated at room temperature (20ºC-23ºC) for 14 days. The fungal colonies that grew on PDA plate medium were then purified on PDA slant medium with pH 5, then incubated at room temperature (20ºC-23ºC) for 7 days. The subculture obtained is used as a stock subculture used at the research stage.

Preparation of PDA and MSM Broth Media

For the preparation of PDA medium with pH 5 (Oxoid), 39 grams of PDA powder and 500 mg of chloramphenicol were dissolved in 1 L of distilled water. Meanwhile, MSM broth medium with pH 5 is made by dissolving 0.1 grams of $MgSO_4$, 1.5 grams of K_2HPO_4, 1 gram of KH_2PO_4, 1 gram of NH_4NO_3, 0.05 grams of NaCl, 0.03 grams of $FeSO_4$, 2.5 grams of glucose into 1 L of distilled water. The pH of the media is adjusted to pH 5, if the media is too alkaline then HCl is added, while if the media is too acidic then NaOH is added. Both media were then sterilized using an autoclave (Hirayama Hiclave HVE-50) at 121°C for 15 minutes.

Identification of Plastisphere Fungi

Identification of plastisphere fungi was carried out macroscopically and microscopically using the profile matching method. Macroscopic assaying was carried out with PDA plates medium with pH 5 which were incubated for 7 days at room temperature (20ºC-23ºC). Observation indicators were colony color, reverse side color, zoning, growing zone, exudate drop, radial furrow, and texture. Meanwhile, the microscopic assay was carried out using the slide culture technique, glass objects that had been dripped with lactophenol cotton blue were then covered with a cover glass made from PDA block medium with pH 5 and then observed with a microscope (Olympus CX33) at 40x magnification. Observation indicators include hyaline, hyphae, conidiophores, foot cells, vesicles, fialids, and conidia. The observation results were identified with the book "Compendium of Soil Fungi" written by Domsch, K.H., Gams, W., and Traute-Heidi Anderson as reference literature [22].

Qualitative Oxidoreductase Enzyme Activities Assay

The qualitative oxidoreductase enzyme activities assay was carried out to determine whether the plastisphere mold used had laccase (Lac), lignin peroxidase (LiP), and manganese peroxidase (MnP) enzyme activity. The laccase enzyme activity assay used a PDA plate with pH 5 supplemented with ABTS (2,2'-Azino-bis (3ethylbenzthiazoline-6-sulfonicacid) 200 mg/L with a positive indicator forming a dark green halo zone, the LiP enzyme used a PDA plate with pH 5 supplemented with methylene blue 4% with a positive indicator clear zone formed around the fungus growth, and the MnP enzyme used a PDA plate with pH 5 supplemented with phenol red 50 mg/L with a positive yellow zone indicator. All enzyme activity assay were incubated for 7 days at room temperature (20ºC-23ºC).

Plastispehere Mold Tolerance Index Assay for Heavy Metals

The tolerance index assay was carried out by cutting the plastisphere fungal culture on a PDA plate with a 5 mm corkborer, then the disc was inoculated on PDA plate medium with pH 5 supplemented with the heavy metals copper, chromium, and lead at a concentration of 50 ppm. After 7 days of incubation at room temperature (20ºC-23ºC), the tolerance index value was calculated using the following formula:

$$TI = \frac{Dt}{Du} \quad \text{..... (1)}$$

Explanation:

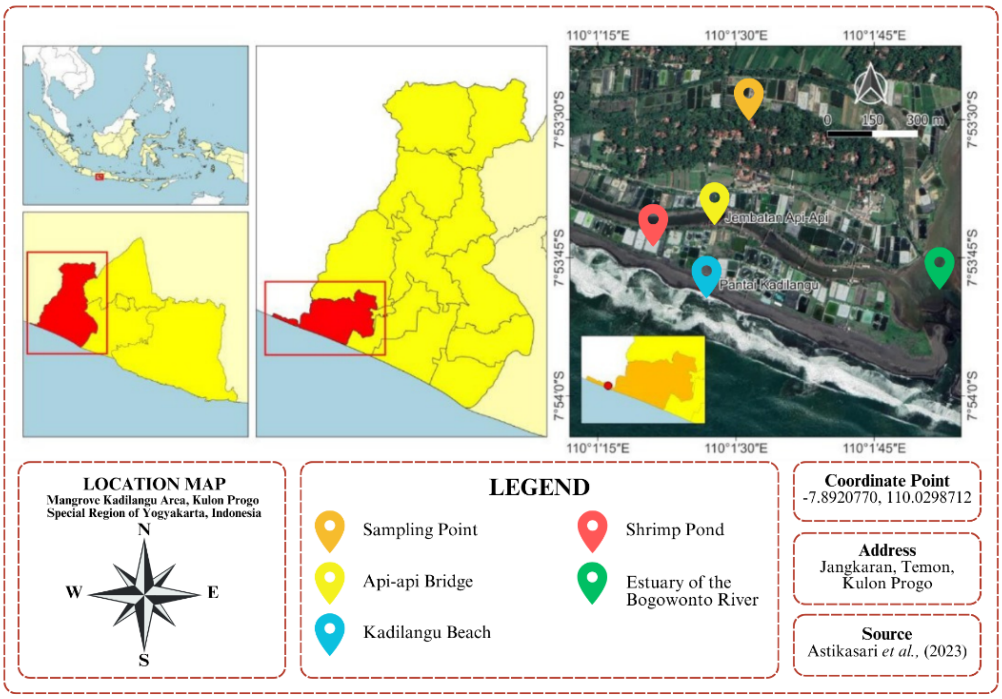


Figure 1. Map of plastic waste sampling at the Kadilangu Mangrove area, Kulon Progo, Special Region of Yogyakarta, Indonesia [23].

TI: Tolerance Index; Dt: Diameter of colonies treated with heavy metals (cm); Du: Diameter of colonies without heavy metals (cm).

Decision making on mold tolerance to heavy metals is: (a) 0 cm: non-tolerant, (b) 0.1-0.52cm: quite tolerant, (c) 0.53-1.04 cm: high tolerance, and (d) 1.05-1.56 cm: very tolerant.

Diisobutyl Phthalate Biodegradation Process

The diisobutyl phthalate biodegradation assay was carried out using a plastisphere fungal culture suspension of 10% (5 mL) of each MSM broth medium with pH 5 used, which is 50 mL of MSM broth medium per UC bottle, then incubated at room temperature (20ºC-23ºC) for 14 days dynamically using a rotary shaker (Eyela, multi shaker MMS) with a speed of 160 rev/min. Next, after 14 days of incubation period for diisobutyl phthalate degradation was completed, the plastisphere fungal colonies and the media were filtered using Whatman filter paper. The remaining MSM broth medium was taken 7 mL and put into a falcon tube to be centrifuged using a centrifuge (Kokusan H-103N) for 45 minutes at a speed of 2000 rev/min to separate the pellet and the supernatant. The absorbance from 1 mL supernatant obtained was quantified with an UV-Visible Spectrophotometer (Thermo Scientific, Genesys 10S) at a wavelength of 362 nm. After the biodegradation assay, the degradation efficiency of diisobutyl phthalate was calculated using the following formula:

$$\% \, Deg = \frac{Ao - At}{Ao} \, x \, 100\% \quad \ldots\ldots\ldots\ldots (2)$$

Explanation: % Deg: Degradation percentage (%); Ao: Initial absorbance (AU); At: Absorbance after degradation (AU)

RESULTS AND DISCUSSION

Identification and Characterization of Plastisphere Fungi

Selected plastisphere fungus isolates from the isolation and purification results from plastic waste were coded PK2(2), HT3(2), and LT2(1), then the results of identification and characterization using the profile matching method referred to the book "Compendium of Soil Fungi". The identification results show that the isolate with code PK2(2) is of the genus *Acremonium*, while the isolates with codes LT2(1) and HT3(2) are of the genus *Aspergillus*. The *Acremonium* genus is a group of fungi that are saprophytic and parasitic on vascular plants including mangroves so they can be found in the Mangrove Kadilangu Kulon Progo area. Meanwhile, the *Aspergillus* genus is a cosmopolitan group of fungi so they can be found in the wide habitat. The *Aspergillus* fungus cultured from the Wonorejo Mangrove is a fungus that is easy to find and is effective for degrading plastic waste [7]. The results of macroscopic identification are presented in Table 1.

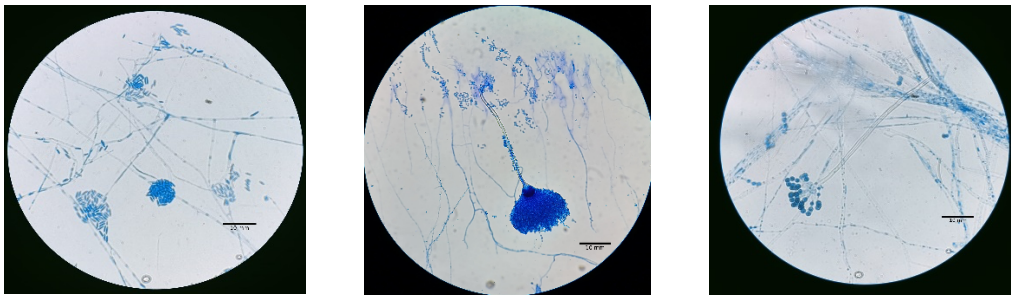
Qualitative Enzyme Activities of Oxidoreductase Assay in Plastisphere Fungi

Oxidoreductase enzymes include the enzyme laccase (Lac), lignin peroxidase (LiP), and manganese peroxidase (MnP) which are a class of enzymes that play a role in the degradation of phthalates including

Table 1. Results of identification and characterization of plastisphere fungi

Isolate	Macroscopic Characteristics						
	Color		Texture	Zoning	Growing Zone	Exudate Drop	Radial Furrow
	Top	Reverse					
Acremonium PK2(2)	White	Ivory, medium flesh	Cottony	-	+	-	-
Aspergillus HT3(2)	Permanent green olive, earth green, ivory	Light chrome yellow	Velvety	-	+	+	-
Aspergillus LT2(1)	White, cold grey III	White, ivory, medium flesh	Velvety	+	+	+	-

Note: (+) presence of each macroscopic indicator, (-) absence of each macroscopic indicator



(a) **(b)** **(c)**

Figure 2. Microscopic appearance at 40x magnification of plastisphere fungi isolates (a) *Acremonium* PK2(2) (b) *Aspergillus* HT3(2) and (c) *Aspergillus* LT2(1).

diisobutyl phthalate. In previous phthalate degradation studies, many assays were conducted on the production of esterase enzymes by molds in degrading phthalate compounds. However, this study has a difference, namely by testing the activity of the oxidoreductase enzyme. Oxidoreductase enzymes play a role in the degradation of diisobutyl phthalate because they can reduce phthalate polymer molecules and convert them into various products such as β-ketoadipic acid, 6-oxohexanoic acid, acetic acid, and carboxylic acid which can be converted into acetyl-CoA in a reaction in the cytosol involving acetyl-CoA-C-acyltransferase and converted into ATP (adenosine triphosphate) [8].

The laccase enzyme screening process was carried out using PDA and ABTS media because ABTS has specific substrate properties for laccase and is the first synthetic molecule to act as a laccase enzyme mediator which has the ability to change the color of magenta and purple crystals to green [9]. Based on Table 2, it can be observed that *Acremonium* PK2(2) and *Aspergillus* LT2(1) are able to produce laccase enzymes which are characterized by the formation of a dark green halo zone. The formation of a green zone around the growth of mold colonies is caused by the hydrolysis process of various organic and inorganic compounds including phenolic groups, non-phenolic groups, hydroxyl groups, and aromatic amine groups by the laccase enzyme by reducing molecular oxygen to water with a one-electron oxidation mechanism [10]. The laccase enzyme plays a role in the oxidation process of polycyclic aromatic hydrocarbons, phthalates, and other dangerous compounds by producing hydroxyl radicals by donating one electron, which oxidizes the aromatic hydrocarbon ring and leads to the formation of quinones and acids, which ultimately decompose into carbon dioxide and water through hydrogenation, dehydration, and etc. The former research reported that the optimal class of enzymes for the detoxification of polycyclic aromatic hydrocarbons and phthalates are oxidoreductases including laccases enzyme, which catalyze reactions with a wide range of aromatic impurities [11].

The formation of a clear zone in the lignin peroxidase enzyme activity assay shows that plastisphere fungi have the ability to absorb carbon and break down the Cα-Cβ bonds of the non-phenolic propyl side chains of aromatic ring components into radical cations in the methylene blue compound supplemented in the growth medium. Based on Table 2, it can be observed that only *Aspergillus* LT2(1) is capable of producing the LiP enzyme which is characterized by the formation of a decolorization zone. The LiP enzyme is needed in the diisobutyl phthalate degradation process to catalyze the dissolution of phthalate into simpler compounds by breaking the ester bonds in the phthalate and oxidizing the aromatic core through the release of one electron to produce cation and phenoxy radicals [4].

Based on Table 2, it can be observed that all plastisphere fungal isolates have the ability to oxidize phenol red and form a yellow zone using the MnP enzyme. The MnP enzyme produced by fungi requires Mn2+ as its reduction substrate. Then the MnP enzyme will oxidize

Table 3. Plastisphere fungi tolerance index assay results for heavy metals

Isolate	Type of Heavy Metals	Tolerance Index Value	Category
Acremonium PK2(2)	Copper	1.00	High tolerance
	Chromium	0.94	High tolerance
	Lead	1.02	High tolerance
Aspergillus HT3(2)	Copper	1.01	High tolerance
	Chromium	0.92	High tolerance
	Lead	1.00	High tolerance
Aspergillus LT2(1)	Copper	1.23	Very tolerant
	Chromium	0.91	High tolerance
	Lead	0.94	High tolerance

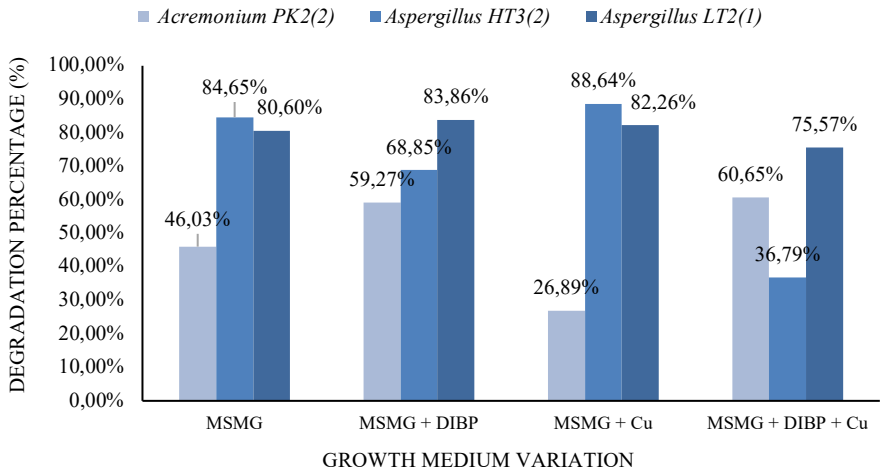


Figure 3. Effect of 50 ppm copper metal supplementation on diisobutyl phthalate degradation.

the Mn (II) compound to Mn (III) to oxidize the phenolic structure into phenoxy radicals. The Mn3+ that is formed is very reactive and forms a complex with chelating organic acids, causing color changes in the growth medium due to changes in the degree of acidity (pH) during 7 days of incubation. Previous research reported that only a few fungal isolates produced the LnP enzyme, but almost all fungal isolates produced the MnP enzyme. This indicates that the productivity of the oxidoreductase enzyme (Lac, LnP, and MnP) in each isolate varies greatly, this variation in enzymatic activity is influenced by differences in genetic variation and the substrate used [12].

Plastisphere Fungi Tolerance Index Assay for Heavy Metals

Assaying of the tolerance index of plastisphere fungi to the heavy metals copper, chromium, and lead at a concentration of 50 ppm was carried out to measure the tolerance ability of plastisphere fungi to heavy metals based on the diameter of their colonies within 7 days of incubation period. The three isolates had high to very tolerant tolerance to all heavy metals at a concentration of 50 ppm. Based on Table 3. *Acremonium* and *Aspergillus* plastisphere fungus isolates have the ability to withstand heavy metals so as not to affect their growth. This strategy is carried out using intracellular and extracellular mechanisms. Intracellular mechanisms are used to reduce the accumulation of heavy metals in the cytosol which is carried out through efflux pumps, vacuole compartmentalization, production of phytochelatin proteins, and metallothionein. Meanwhile, the extracellular mechanism is carried out by adsorption and chelation of metal ions on the cell surface [13]. However, this research still has limitations regarding the tolerance index value of plastisphere mold at heavy metal (Cu, Cr, and Pb) concentrations higher than 50 ppm, so that the tolerance ability and tolerance mechanism strategy of plastisphere mold at higher heavy metal concentrations are not yet known in depth.

The results of high to very tolerant to heavy metals indicate that the *Acremonium* and *Aspergillus* isolates have adapted to environments contaminated with copper, chromium, and lead. Microorganisms that are exposed to heavy metals for a long time have mechanisms to adapt to heavy metal contamination by means of metal detoxification, extracellular barrier secretion, intracellular and extracellular sequestration, active transport of heavy metal ions, and metal chelation production [14], [15]. The presence of inorganic pollutants including heavy metals can change the metabolism and structure of fungal communities, thereby encouraging the development of fungal strains that are more resistant to heavy metals at the sampling point. This has been reported in previous research that

gold mining in the upstream Atrato, San Juan and Baudó Rivers, Colombia causes the microorganisms in the surroundings to become tolerant to heavy metals and tolerant to hydrocarbons produced from the tailing disposal process at gold mining sites [16], [17].

Diisobutyl Phthalate Biodegradation Process by Plastisphere Fungi

The process of assaying the degradation of diisobutyl phthalate was treated with heavy metals, namely with the addition of 50 ppm copper and without the addition of 50 ppm copper. The diisobutyl phthalate degradation activity assay with or without the influence of the heavy metal copper was carried out to determine the effect of heavy metals on the ability of diisobutyl phthalate degradation by plastisphere fungi. Based on Figure 3. The results of calculating the percentage of diisobutyl phthalate degradation by plastisphere fungal isolates show that all fungal isolates have the ability to degrade diisobutyl phthalate within 14 days of incubation period.

Based on diisobutyl phthalate degradation assaying using an UV-Vis Spectrophotometer, it is known that *Aspergillus* and *Acremonium* have the ability to remove diisobutyl phthalate compounds with a percentage range of 59.27%-83.86% on medium not supplemented with copper 50 ppm and 36.79%-75.57% on medium supplemented with copper 50 ppm. These results are in accordance with previous research on phthalate degradation which reported that the *Aspergillus* genus isolated from rubbish and soil at toxic sanitary waste dump sites had the ability to degrade dibutyl phthalate up to 99.34% during 15 days of incubation period [5]. In general, the diisobutyl phthalate degradation pathway by microorganisms including fungi occurs through a series of stages, namely dealkylation, hydroxylation, and decarboxylation [18]. The diisobutyl phthalate compound that was successfully degraded by *Aspergillus* and *Acremonium* was influenced by the enzymatic activity secreted by the fungus during 14 days of incubation period. The enzymes secreted by mold include oxidoreductase enzymes (Lac, LiP, and MnP).

This research proves that in the presence or absence of the heavy metal 50 ppm copper, plastisphere fungi from the *Acremonium* and *Aspergillus* genus still have the ability to degrade diisobutyl phthalate with the highest degradation percentage of 83.86% by the isolate *Aspergillus* LT2(1). Based on Figure 3, the degradation results of diisobutyl phthalate on medium treated with 50 ppm copper decreased compared to medium without 50 ppm copper, namely the *Aspergillus* HT3(2) isolate on DIBP medium had an average degradation of 68.85%, this was higher than on DIBP supplemented Cu media which only had an average degradation of 36.79%. This degradation result is directly proportional to what occurred in the *Aspergillus* LT2(1) isolate, namely that the DIBP media had an average degradation of 83.86%, this was higher than the DIBP supplemented Cu media which had an average degradation of 75.57%. However, the results of DIBP degradation by the *Aspergillus* isolate were inversely proportional to the results of DIBP degradation carried out by the *Acremonium* isolate, because the results of DIBP degradation in media treated with 50 ppm Cu actually increased compared to media without 50 ppm Cu. The results of DIBP degradation in *Acremonium* PK2(2) isolates were that DIBP medium had an average degradation of 59.27%, this was lower than DIBP supplemented Cu media which had an average degradation of 60.65%.

These results indicate that the degradation ability of DIBP by *Aspergillus* is impaired due to the presence of the heavy metal copper. This is because the *Aspergillus* isolate experiences oxidative stress and physiological stress due to excessive production of reactive oxygen species so that the metabolism of the *Aspergillus* isolate is disturbed and this results in a decrease in the activity of the mold oxidoreductase enzyme which plays an important role in degrading DIBP. However, the degradation ability of DIBP by *Acremonium* was better if there was 50 ppm Cu supplementation. This is because the *Acremonium* genus can absorb 10-20% of the heavy metal copper and transform it in its accumulated biomass (mycelia) so that it can degrade DIBP more optimally than without the addition of Cu [19].

However, *Aspergillus* isolates, especially isolate LT2(1), still succeeded in degrading DIBP at the highest rate compared to other genus isolates, namely *Acremonium*, both on MSMG supplemented DIBP and MSMG supplemented DIBP and Cu media. Because it has the ability to regulate the flow of nutrients and energy through the structure of hyphae and mycelial tissues and produces oxidoreductase enzymes that are able to degrade recalcitrant compounds. On the other hand, these findings also reported in previous research which reported that *Aspergillus* has a good homeostatic mechanism against copper stress. Homeostasis is carried out by *Aspergillus* to maintain internal balance in the mold's body so that its body continues to function normally even though the growth environment experiences changes, such as the presence of stress from the copper [20]. This research has limitations in the form of not carrying out molecular identification of the plastisphere mold isolates, so that the advantages of the *Aspergillus* genus from isolate LT2(1) compared to *Aspergillus* HT3(2) cannot be assaying comprehensively and in more depth. However, based on the existing test results, the *Aspergillus* LT2(1) isolate is still better than other isolates to degrade diisobutyl phthalate.

The mechanism of *Aspergillus* homeostasis against Cu stress is carried out through the process of transporting copper ions from the extracellular environment to the intracellular environment. Furthermore, in the cytoplasm copper ions are bound by copper chaperones which facilitate the transfer of copper ions into the nucleus to send signals to establish homeostasis from *Aspergillus* isolates towards copper. In the cytoplasm, copper ions are again bound by copper chaperones which facilitate the transfer of copper ions into the subcellular compartment, namely the golgi apparatus and enzymes in the cytoplasm and mitochondria react for functional activation of homeostasis. If the accumulation of copper

ions in the mold cytoplasm exceeds *Aspergillus* homeostasis limit, then *Aspergillus* will use an efflux pump to detoxify the excess copper ions [20]. All stages of diisobutyl phthalate degradation occur through primary and secondary stages involving beta-oxidation, de-esterification, dealkylation, transesterification, and demethylation [21].

CONCLUSION

The genus of *Acremonium* PK2(2) and *Aspergillus* HT3(2) and LT2(1) have the ability to degrade diisobutyl phthalate assisted by oxidoreductase enzymes (Lac, LiP, and MnP). All isolates had high tolerance and were very tolerant to stress from the heavy metals at a concentration of 50 ppm. The results of diisobutyl phthalate degradation by selected isolates were 59.27%-83.86% on medium without 50 ppm cooper and 36.79%-75.57% on medium with 50 ppm cooper. *Aspergillus* LT2(1) isolate is an excellent candidate as an in-situ biodegradation agent for diisobutyl phthalate compounds in environments contaminated with diisobutyl phthalate and the heavy metal, because it has the ability to regulate the flow of nutrients and energy through the structure of hyphae and mycelial tissues and has a good homeostatic mechanism against copper stress.

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REFERENCE

[1] Ahkola, H.; Junttila, V.; Kauppi, S. 2024. Do hazardous substances in demolition waste hinder circular economy. *J. Environ. Manage.* **364** 121362. DOI: 10.1016/j.jenvman.2024.121362.

[2] Weizhen, Z.; Xiaowei, Z.; Peng, G.; Ning, W.; Zini, L.; Jian, H.; Zheng, Z. 2020. Distribution and risk assessment of phthalates in water and sediment of the Pearl River Delta. *J. Environ. Sci. Pollut. Res.* **27** (11), 12550-12565. DOI: 10.1007/s11356-019-06819-y.

[3] Dueñas-Moreno, J.; Mora, A.; Cervantes-Avilés, P.; Mahlknecht, J. 2022. Groundwater contamination pathways of phthalates and bisphenol: A origin, characteristics, transport, and fate - A review. *J. Environ. Int.* **170** 107550. DOI: 10.1016/j.envint.2022.107550.

[4] Naveen, K.V.; Sathiyaseelan, A.; Bin-Choi, H.; Zhang, X.; Wang, M.H. 2023. Biodegradation of dibutyl phthalate in liquid fermentation by endophytic *Penicillium* species and the toxicity evaluation of the by-product. *J. Sci. Total Environ.* **890** 164403. DOI: 10.1016/j.scitotenv.2023.164403.

[5] Puranik, S.; Livleen, S.; Aditi, K.; Deeba, K.; Sangeeta, P.; Govindasamy, V.; Rajna, S.; Sandeep, K.S.; Dharmendra, K.; Ajay, K. 2023. Exploring potent fungal isolates from sanitary landfill soil for in vitro degradation of dibutyl phthalate. *J. Fungi*. **9** (1), 1-15. DOI: 10.3390/jof9010125.

[6] Márquez, G.A.; Octavio, L.C.; Ericka, S.J.; Saul, T.B.; Jorge, G.D.; Gustavo, V.G.; Carmen, S. 2019. Biodegradation patterns of the endocrine disrupting pollutant di(2-ethyl hexyl) phthalate by *Fusarium culmorum*. *J. Ecotoxicol. Environ. Saf.* **170** 293-299. DOI: 10.1016/j.ecoenv.2018.11.140.

[7] Kuswytasari, N.D.; Alfia, R.K.; Aunurohim.; Nurhidayatul, A.; Enny, Z.; Maya, S.; Nimerta, K.; Arif, L. 2023. Plastic biodegradation potential of soil mangrove mold isolated from Wonorejo, Indonesia. *J. Adv. Life Sci.* **10** (2), 228-238.

[8] Okal, E.J.; Gui, H.; Ephie, A,M.; Sehroon, K.; Shixi, W.; Zhiqiang, G.; Tianfu, Z.; Peter, E.M.; Jianchu, X. 2023. Insights into the mechanisms involved in the fungal degradation of plastics. *J. Ecotoxicol. Environ. Saf.*, **262** 115202. DOI: 10.1016/j.ecoenv.2023.115202.

[9] Gałązka, A,; Jankiewicz, U.; Szczepkowski, A. 2023. Biochemical characteristics of laccases and their practical application in the removal of xenobiotics from water. *J. Appl. Sci.* **13** (7). DOI: 10.3390/app13074394.

[10] Senthivelan, T.; Kanagaraj, J.; Panda, R.C.; Narayani, T. 2019. Screening and production of a potential extracellular fungal laccase from *Penicillium chrysogenum*: Media optimization by response surface methodology (RSM) and central composite rotatable design (CCRD). *J. Biotechnol. Reports*. **23**. DOI: 10.1016/j.btre.2019.e00344.

[11] Imam, A.; Kumar, S.; Kanaujia, P.K.; Ray, A. 2022. Biological machinery for polycyclic aromatic hydrocarbons degradation: A review. *J. Bioresour. Technol.* **343** 126121. DOI: 10.1016/j.biortech.2021.126121.

[12] Ruhimat, R.; Djajakirana, G.; Antonius, S. 2022. Fungi dekomposer penghasil enzim ekstraseluler lakase, mangan peroksidase, dan lignin peroksidase dari kawasan Kebun Raya Bogor: Isolasi, seleksi, identifikasi, dan ajian aktivitas enzimnya. *J. Biol. Indones.* **18** (1), 111-119. DOI: 10.47349/jbi/18012022/111.

[13] Lotlikar, N.P. 2019. *Physiological response of fungi from marine habitats to heavy metals*. (Taleigao: Goa University).

[14] Pande, V.; Pandey, S.C.; Sati, D.; Bhatt, P.; Samant, M. 2022. Microbial Interventions in ioremediation of heavy metal contaminants in agroecosystem. *J. Front. Microbiol.* **13** 1-16. DOI: 10.3389/fmicb.2022.824084.

[15] Chau, T.P.; Bulgariu, L.; Saravanan, M.; Rajkumar, R.; Chinnathambi, A.; Salmen, S.H.; Jhanani, G. 2023. Bioremediation efficiency of free and immobilized form of *Aspergillus niger* and *Aspergillus tubigenesis* biomass on tannery effluent. *J. Environ. Res.* **231** (3), 116275. DOI: 10.1016/j.envres.2023.116275.

[16] Gutiérrez-Mosquera, H.; Marrugo-Negrete, J.; Díez, S.; Morales-Mira, G.; Montoya-Jaramillo, L.J.; Jonathan, M.P. 2020. Distribution of chemical forms of mercury in sediments from abandoned ponds created during former gold mining operations in Colombia. *J. Chemosphere*. **258** 127319. DOI: 10.1016/j.chemosphere.2020.127319.

[17] Córdoba-Tovar, L.; Marrugo-Negrete, J.; Ramos Barón, P.A.; Calao-Ramos, C.R.; Díez, S. 2023. Toxic metal(loids) levels in the aquatic environment and nuclear alterations in fish in a tropical river impacted by gold mining. *J. Environ. Res.* **224** 115517. DOI: 10.1016/j.envres.2023.115517.

[18] Wang, X.; Wei, J.; Zhang, X.; Chen, Q.; Lakshmikandan, M.; Li, M. 2024. Comparing the removal efficiency of diisobutyl phthalate by Bacillariophyta, Cyanophyta, and Chlorophyta. *J. Sci. Total Environ.* **912** 169507. DOI: 10.1016/j.scitotenv.2023.169507.

[19] Zapotoczny, S.; Jurkiewicz, A.; Tylko, G.; Anielska, T.; Turnau, K. 2007. Accumulation of copper by *Acremonium pinkertoniae*, a fungus isolated from industrial wastes. *J. Microbiol. Res.* **162** (3), 219-228. DOI: 10.1016/j.micres.2006.03.008.

[20] Song, J.; Li, R.; Jiang, J. 2019. Copper homeostasis in *Aspergillus fumigatus*: Opportunities for therapeutic development. *J. Front. Microbiol.* **10**. DOI: 10.3389/fmicb.2019.00774.

[21] Tran, H.T.; Nguyen, M.K.; Hoang, H.G.; Hutchison, J.M.; Vu, C.T. 2022. Composting and green technologies for remediation of phthalate (PAE)-contaminated soil: Current status and future perspectives. *J. Chemosphere.* **307** 135989. DOI: 10.1016/j.chemosphere.2022.135989.

[22] Domsch, K.H.; Gams, W.; Traute-Heidi, A. 1980. *Compendium of soil fungi*. (London: Academic Press).

[23] Astikasari, L.; Indriyani, S.; Muryanto, B.S.; Madani, A.R.; Muhammad, F.; Putri, A.; Andini, N.H.; Ratih, N.R.; Puji, A.K.; Muhammad, S.R.; Snada, I.T.; Sunarto,; Lia, K.; Indah, K.; Sugeng, B.; Andrie, B.F.; Setiyawan, A.D. 2023. Analysis of ecotorism development as a mangrove conservation effort in Pasir Kadilangu and Jembatan Api-api Kulon Progo, Yogyakarta, Indonesia. *J. Indo. Pac. of. Ocean. Lif.* **7** (2), 125-132.

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