INDIGENOUS FUNGI FROM MARIBAYA-BREBES-BUMIAYU: POTENTIAL FOR BATIK DYE WASTE BIODEGRADATION

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Abstract. Batik dye waste is a source of pollution that is difficult to degrade naturally and requires an effective biological approach. This study aims to evaluate the ability of four indigenous fungal isolates from Maribaya–Brebes–Bumiayu (*Fusarium* sp., *Aspergillus* sp., *Trichoderma* sp., and *Rhizopus* sp.,) to grow on PDA media mixed with batik waste. Each isolate was tested at three waste concentration treatments, namely 0%, 5%, and 20%. The results showed that all isolates were able to grow stably at all treatments, indicating a good level of tolerance to toxic components in batik waste. *Aspergillus* sp. showed the strongest and most consistent growth at all waste concentrations, making it the leading candidate for bioremediation applications. Meanwhile, Fusarium sp., Trichoderma sp., and Rhizopus sp. still showed good adaptation potential and are relevant for further study. These findings confirm that indigenous fungi from Maribaya have great potential as agents for the biodegradation of batik waste and can support the development of waste treatment technology based on local resources.

Keywords: Indigenous fungi, batik dye waste, biodegradation, Maribaya Brebes, fungal tolerance

1. Introduction

Batik dye waste is a type of textile industry liquid waste that contains complex aromatic compounds, is dark in color, toxic, and difficult to degrade naturally. Its main contents include synthetic dyes (azo dyes), surfactants, heavy metals, and additional chemicals that are persistent in the environment. If disposed of without treatment, this waste can cause eutrophication, toxicity to aquatic biota, health problems, and the accumulation of hazardous residues in soil and surface water [1][2]. Therefore, innovation in effective, sustainable, and locally-based batik waste treatment technology is urgently needed.

Biological approaches using fungi (mycoflora) are gaining attention due to their high enzymatic capacity, particularly ligninolytic fungi that can produce enzymes such as laccase, manganese peroxidase, and lignin peroxidase, which are effective in degrading complex compounds including batik dyes [3]. Indigenous (local) fungi have the advantage of better ecological adaptation to extreme environmental conditions, making them more stable in

bioremediation applications. A number of recent studies report that local fungal isolates from dry land, agroforestry, and nutrient-poor soils show high ability in the decolorization of textile waste [4][5].

Maribaya Hamlet in Kalinusu Village, Bumiayu Subdistrict, Brebes Regency, is one of the areas with unique natural conditions that have the potential to become a source of local microbial biodiversity. This 232-hectare area is located in the geological formation of the Java mountain range, has a rolling topography with a slope of 8–15%, latosol soil that tends to be unstable and a soil pH of 6.36–7.79 (neutral–neutral base), and annual rainfall reaching 3,488 mm [6]. The diversity of flora such as Artocarpus, Calophyllum, Dendrocalamus, Tectona grandis, Vitex pubescens, and Albizia procera, as well as the diversity of bird, reptile, and mammal fauna, indicate that this area has high ecological carrying capacity [6]. Environmental conditions such as dusty clay soil, high rainfall, and steep topography make Maribaya a challenging habitat and enable the presence of microorganisms—including indigenous fungi—with a high level of adaptation to environmental stress.

These ecological characteristics are relevant to the search for local fungal isolates that have the potential to be used in industrial waste processing, including batik dye waste. Fungi from regions with extreme conditions generally have more flexible metabolisms and are tolerant to fluctuations in pH, temperature, and toxic compounds. Thus, exploring indigenous fungi from Maribaya, Brebes, could be a strategic step in providing local bioremediation agents that are inexpensive, effective, and sustainable.

In line with the increasing need for environmentally friendly solutions for the national batik industry, this study aims to examine the potential of indigenous fungi from Bumiayu particularly Maribaya Hamlet in degrading the initial phase of batik dye waste. This approach is expected to contribute scientifically to efforts to conserve and utilize local biodiversity while supporting the development of bioremediation technology that is adaptive and contextual to conditions in Indonesia.

2. Methods

2.1. Experimental design

The experiment was designed as follows: 4 fungal isolates × 3 media treatments (control PDA 0% waste, PDA + 5% waste, PDA + 20% waste) with 3 biological replicates per combination. Total Petri dishes = 4 isolates × 3 treatments × 3 replicates = 36 dishes. Observation time: 0 and 4 days post-inoculation.

2.2. Materials and Equipment

The main materials used in this study included four indigenous fungal isolates, namely *Fusarium* sp., *Aspergillus* sp., *Trichoderma* sp., and *Rhizopus* sp. All isolates were local collections that had previously been isolated from the local environment and purified through repeated subculture techniques on PDA media. Prior to the experiment, each isolate was rejuvenated and prepared again in the form of pure culture using the parallel transfer or monosporic isolation method to ensure the purity and homogeneity of the inoculum.

The basic medium used for fungal growth was Potato Dextrose Agar (PDA). This medium can be a commercial product in the form of ready-to-use powder, or prepared independently with a standard formulation consisting of 200 g potato extract, 20 g dextrose, and 15 g agar per liter of distilled water. All ingredients were mixed and heated until homogeneous before being sterilized in an autoclave. PDA was chosen because it is a universal medium that supports the optimal growth of various types of saprophytic fungi and is often used in bioremediation studies.

The pollutant source tested in this study was batik dye wastewater obtained directly from a local batik industry in Sokaraja District, Banyumas Regency. The wastewater was collected in

sterile containers and immediately stored at 4°C to maintain the stability of its chemical components until processing and use in the experiment. The batik dye waste was used as an additional component in the medium to assess the growth response and decolorization ability of each indigenous fungal isolate.

2.3. Preparation of Batik Dye Waste

The collected liquid batik waste samples were homogenized, filtered to remove large solids, and then sterilized using an autoclave (121°C, 15 minutes). This sterilization procedure followed the APHA standard for handling liquid waste to be used in microbiological experiments [9]. After sterilization, the waste is stored at 4°C until use.

2.4 Preparation of PDA + Batik Waste Media (5% and 20%)

The PDA base medium was prepared according to standard procedures, then cooled to a temperature of ±50–55°C before mixing with the waste. The treatment media were arranged into three types: control PDA without waste (0%), PDA + 5% (v/v) waste, and PDA + 20% (v/v) waste. Concentrations of 5% and 20% were chosen to represent light and moderate-heavy waste conditions, as used in several studies testing fungal tolerance to textile waste [10]. After homogenization, 20 mL of the medium was poured into sterile Petri dishes, allowed to solidify, and then stored upside down until inoculation.

2.5. Preparation of Fungal Inoculum

Each fungal isolate was rejuvenated for 5–7 days on PDA before use. For isolates that produce abundant spores, such as Aspergillus and Fusarium, the inoculum was prepared in the form of a spore suspension with the addition of physiological NaCl solution + Tween 80 (0.01%) to aid spore release. The suspension is then adjusted to a concentration of $\pm 1 \times 10^6$ spores/mL using a hemocytometer. Meanwhile, isolates such as Trichoderma and Rhizopus are inoculated in the form of 5 mm diameter pieces of mycelium taken from the edge of an active colony. The use of this dual inoculum method is commonly applied in multispecies fungal physiology studies [11].

2.6. Inoculation and Incubation of Cultures

Each Petri dish containing PDA + waste medium was inoculated in the center using one mycelium disc or 10 µL of spore suspension. The dishes were then incubated at 28 ± 2°C in inverted conditions to avoid water condensation that could interfere with colony growth. This temperature was chosen because it is within the optimum growth range for most tropical saprophytic fungi [12].

2.7. Observation of Colony Growth

The growth rate of fungi was observed by measuring the diameter of the colony periodically on days 3, 5, 7, 10, 14, and 21 of incubation. The diameter was measured at two perpendicular lines intersecting at the center of the colony, then averaged. Measurements were made using a caliper or sterile transparent ruler. The growth rate was calculated based on the change in diameter per day following the linear growth approach commonly used in waste toxicity studies [13]. All colonies were photographed for visual documentation of color changes, morphology, and indications of decolorizatio

3. Results And Discussion

The Four indigenous fungal isolates—Fusarium sp., Aspergillus sp., Trichoderma sp., and Rhizopus sp.—exhibited different growth patterns at three media concentrations, namely PDA without waste (0%), PDA + 5% batik waste, and PDA + 20% batik waste. Table 1 shows that Aspergillus sp. had the most stable and highest growth (+++) in all treatments. Meanwhile, Fusarium sp., Trichoderma sp., and Rhizopus sp. showed relatively moderate growth (++) and did not experience a decline even though the waste concentration increased to 20%.

Table 1. Growth Response of Four Indigenous Fungal Isolates to Variations in Batik Waste Concentration

Fungal Isolate	PDA 0% Waste	PDA + 5% Waste	PDA + 20% Waste
Fusarium sp.	++	++	++
Aspergillus sp.	+++	+++	+++
Trichoderma sp.	++	++	++
Rhizopus sp.	++	++	++

Description
+ = low growth
++ = moderate growth
+++ = excellent growth

The results showed that all four indigenous fungal isolates had good tolerance to batik waste up to a concentration of 20%. The absence of growth inhibition in Fusarium, Trichoderma, and Rhizopus indicated that these three species had physiological resistance to complex compounds commonly found in batik waste, such as azo compounds, phenolics, and heavy metals in low concentrations [7].

The highest growth was shown by *Aspergillus* sp., which remained at a value of +++ in all treatments. This is in line with many studies that mention that Aspergillus is a genus that has high metabolic resistance, is able to grow under chemical stress conditions, and is known to produce various oxidative enzymes such as laccase and peroxidase that support its use in the biodegradation of textile waste [8]. The stability of Aspergillus sp. growth in 5–20% waste indicates that this isolate has the potential to be a prime candidate for fungus-based bioremediation processes.

On the other hand, *Fusarium* sp., *Trichoderma* sp., and *Rhizopus* sp. isolates showed consistent growth (++), although not as strong as Aspergillus sp. The ability of these three isolates to continue growing on batik waste media indicates that they are able to tolerate the presence of toxic chemical components. *Trichoderma* sp. has been reported to have a high adaptability to environments containing lignocellulose and aromatic compounds, and to produce ligninolytic enzymes such as laccase and MnP that can support color degradation [9]. Similarly, *Fusarium* sp. is known to be able to grow on contaminated media and produce oxidative enzymes that play a role in the decolorization process [10]. *Rhizopus* sp., although known to be more sensitive, still shows stable growth, possibly due to its flexible fermentative metabolic system and its ability to produce organic acids that can help modify the dye structure [11].

The absence of significant differences between the 0%, 5%, and 20% treatments in all three isolates (++ in all treatments) indicates that the concentration of batik waste used has not reached a toxicity level that can inhibit growth. This is consistent with reports that many fungi are able to adapt to azo compounds at low to moderate concentrations, before toxic accumulation or enzymatic inhibition occurs [12].

Overall, these results reinforce that indigenous fungi from local environments, especially areas with extreme natural conditions, have great potential for application in bioremediation processes due to their good ecological adaptation. The Aspergillus sp. isolate is the strongest candidate of the four isolates based on its growth ability in the batik waste environment.

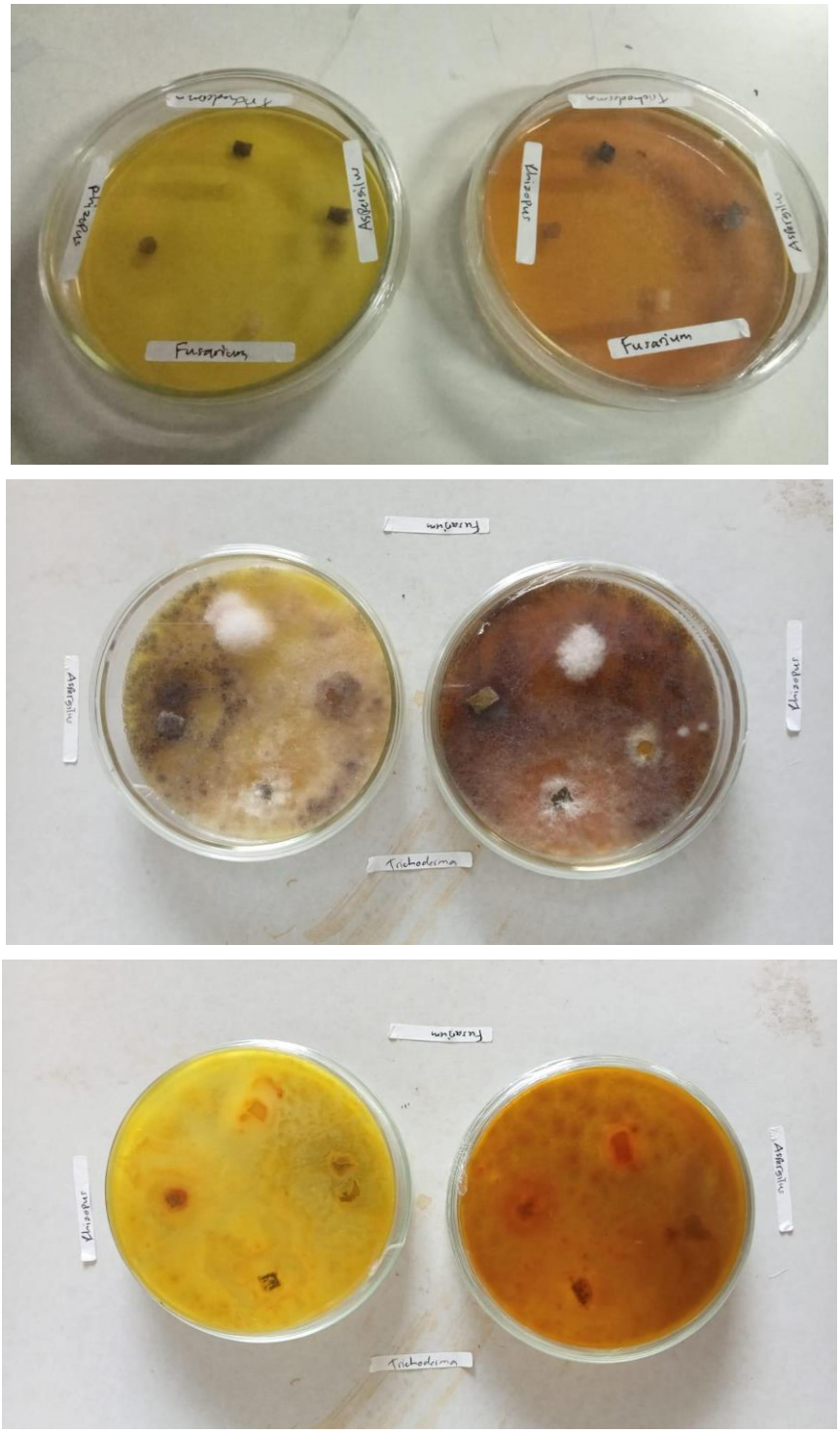

Figure 1. Fungal Growth on PDA Medium + Waste

The figures shown in this study illustrate the growth patterns of the four indigenous fungal isolates on PDA media mixed with batik waste. Visually, the growth of colonies in all treatments shows that fungi are able to adapt to the presence of dye waste, even at a concentration of 20%. The consistent color of the colonies and the absence of inhibition zones around the inoculation points indicate that the toxic compounds in the waste do not significantly inhibit mycelium development.

In *Aspergillus* sp., the figure shows dense, compact, and radial mycelium growth with clear colony edges, confirming the quantitative results that show the best growth (+++) in all

treatments. The characteristic colony color was maintained even though the medium contained waste, indicating high enzymatic and physiological tolerance. This is consistent with the characteristics of Aspergillus, which is known to have a highly active oxidative enzyme system, especially laccase and peroxidase enzymes, enabling it to adapt to media rich in aromatic compounds such as batik waste [13].

In *Fusarium* sp., *Trichoderma* sp., and *Rhizopus* sp. isolates, the figures show more moderate but stable growth in all treatments. The mycelium developed evenly without morphological changes indicating stress, such as colony edge distortion or hyphae color changes. The absence of transparent zones or color degradation around the colonies in the early phase suggests that decolorization is likely to occur more predominantly in the subsequent metabolic phase or in liquid culture, as reported in several other studies [14].

The figure also shows that the density and distribution pattern of the mycelium of the four isolates did not undergo significant changes when the waste concentration increased from 5% to 20%. This phenomenon indicates that the batik waste components at these concentrations have not reached a level of toxicity that can inhibit mycelium biosynthesis. This finding is in line with reports that many soil fungi, especially those from environments with high ecological pressure, have a natural tolerance to azo and phenolic compounds commonly found in textile waste [15].

4. Conclusion

The conclusions from this research are:

1. The four indigenous fungal isolates (*Fusarium* sp., *Aspergillus* sp., *Trichoderma* sp., and Rhizopus sp.) were able to grow stably on PDA media containing batik waste at concentrations of up to 20%, demonstrating good tolerance and adaptation to dye compounds.
2. *Aspergillus* sp. is the most promising isolate for bioremediation applications because it showed the strongest growth in all treatments, while the other isolates remain suitable for further research.

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