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The Ability of Fungi Isolated from Landfill in Decolorization of Liquid Waste of Batik Industry

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Abstract. Isolation of soil fungi from landfill in Medan, North Sumatera, Indonesia was conducted. The aim was to investigate the fungal that potential to decolorize waste liquid batik home industry. Results showed that twenty-three species of filamentous fungi were successfully isolated. All of the isolates were cultured in minimum salt medium containing waste liquid batik at concentration 25, 50, and 75%. At 75% waste, only 4 fungal isolates (Ys02, Ys13, Ys14, and Ys21) showed growth response. Cultured of the fungal isolates in minimum salt medium with 25% waste + 0.5% glucose were found three fungal isolates (Ys02, Ys14, and Ys21). Whereas, medium with 25% waste with no glucose were found only two fungal isolates (Ys14, and Ys21).

1. Introduction

Batik is one of Indonesia's cultural heritage which has many types. Each of region in Indonesia has its own batik characteristic and produced traditionally for many generations [1]. Batik industry is one of the economic sectors that requires many labors. The processing consisted of pattern making, coloring and drying [2]. The waste waste is consisted of 99.9% water and 0.1 solid substances [3].

Some chemical compounds contained in batik liquid waste are dye stuffs that commonly released to the environment such as rivers, lakes etc. The undecompose substance of the coloring agent can increase chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solid (TSS) and pH [4]. [5]. Quality standards of BOD and COD levels were found in textile wastewater BOD is 60 mg/L, while for COD contained in liquid waste was 150 mg/L. [6] BOD and COD quality standard in textile wastewater are 75 mg/L and 150 mg/L. The dye molecule is an unsaturated organic compound with chromophore as a color carrier and auxochrome as a color binder with fiber. Unsaturated organic substances found in the formation of dyes are aromatic compounds such as aromatic hydrocarbons and their derivatives, phenols and their derivatives, and hydrocarbon compounds containing nitrogen.

Waste of batik boiling water is dumped directly into the river improperly. The waste contains synthetic material which is undissolve and undegradable. The waste contain synthetic dyes, starch, wax, and detergent. If the waste directly dumped into the river, it can damage the environment and lead to the death of aquatic organisms and human health [7, 8]. Turbidity liquid waste of batik industry is caused by wax deposits or mixture dyes, soda and indigo. Reducing the level of turbidity by solid substances in water environment is required to increase the water quality [9]. Many efforts conducted physically or chemically to reduce waste of batik industry. Bioremediation method particularly by fungi are potential to degrade toxic components in the waste [10].



2. Materials and Methods

2.1. Microbiological media

The medium used was potato dextrose agar (PDA) and mineral salts medium (MSM). Potato dextrose agar used for isolation fungi from soil sample, and MSM used with two conditions are solid and liquid. MSM was prepared as described by Chao *et al.* [11] with slight modifications. The medium contained (g l^{-1}): NH_4NO_3 , 0.5; K_2HPO_4 , 1.5; KH_2PO_4 , 0.5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g; FeSO_4 , 0.02 g; CaCl_2 , 0.05; and CuSO_4 , 0.02 g. Liquid medium was used for testing the decolorization activity of all fungal species.

2.2. Isolation and characterization of fungi isolates

Fungal isolates were obtained from soil sample in two landfills i.e PP Mawaridussalam and Marindal 1 village landfill, one kg each and 4 different sites for each location. The environmental condition was measured (pH 4-6 and temperature 26-29°C). Whereas, 3 L liquid batik waste was obtained from the local home batik industry in Medan. Soil samples were stored in cool temperature prior to laboratory use. Ten grams of soil was added into 90 mL NaCl 0.9 % in flask 250 mL followed by agitation 15 minutes. Serial dilution was made up to 10^{10} . One mL of sample from selected dilution was inoculated to PDA and incubated for 4 days (29°C). Fungal colonies were isolated and characterized based on their morphology.

2.3. Screening of soil fungi in wastewater containing media

Fungal isolates were screened by two steps, the first step with solid media MSM containing waste, and the second step with liquid media containing waste. Those to evaluate growth response or ability decolorization batik waste. Fungal isolates were cultured in solid minimum salt medium agar (MSMA) containing batik waste 25, 50, and 75% without glucose. Cultures were incubated for 7 days (29°C), and their growth was observed. The growth was expressed as 'growing' (+) and 'no-growth' (-).

2.4. Screening potential fungi in decolorization ability

Fungi growth in solid media 75% MSM containing batik waste, then was tested in liquid media containing 25% waste with two treatments; by adding 0.5% glucose and without glucose. The aim of those methods to know the ability of the fungus which is the most potential in degrading waste. Firstly, fungi were grown in 75% MSM media for 5 days and then grown in liquid media containing 25% waste and observed the changes of the color of waste.

3. Results and Discussion

3.1. Morphological characteristic of fungi isolates

Twenty-three different isolates based on colony morphology were identified, the color of mycelium that appears on average is pale green, white, ash and black. Characteristic fungi growth in which most of them were belongs to the genus of *Aspergillus*, *Mucor*, *Penicillium*, and *Fusarium* [12].

3.2. The ability of fungi isolates growth in wastewater containing media

Quantitative observation is shown by growing fungi on MSM (Table 1). There are 16 isolates, 10 isolates and 4 isolates which grow at concentration 25%, 50% 75% respectively. Fungi can convert waste as a carbon source to grow, absorption dyes by removing aromatic compounds and dissolved organic compounds [13]. Non-enzymatic fungi can decolorize dyes in waste by absorption with fungal cell wall [14].

Table 1. Fungal isolates in MSM medium

No	Isolates	Solid Waste		
		MSM 25%	MSM 50%	MSM 75%
1	Ys 01	+	+	-
2	Ys 02	+	+	+
3	Ys 03	+	+	-
4	Ys 04	+	+	-
5	Ys 05	+	+	-
6	Ys 06	+	-	-
7	Ys 07	+	-	-
8	Ys 08	+	+	-
9	Ys 09	-	-	-
10	Ys 10	-	-	-
11	Ys 11	+	-	-
12	Ys 12	-	-	-
13	Ys 13	+	+	+
14	Ys 14	+	+	+
15	Ys 15	+	-	-
16	Ys 16	+	-	-
17	Ys 17	-	-	-
18	Ys 18	-	-	-
19	Ys 19	-	-	-
20	Ys 20	+	+	-
21	Ys 21	+	+	+
22	Ys 22	-	-	-
23	Ys 23	+	-	-

3.3. Quantitative screening results of fungi

Growth analysis of fungal isolates (Ys02, Ys13, Ys14, and Ys21) in liquid medium containing batik waste was conducted with two treatments; with glucose addition and no-glucose. After 14 days incubation (29°C), fungal isolates that grow containing glucose were Ys02, Ys14, and Ys21, whereas only Ys14, and Ys21 were grown on medium with no glucose (Table 2). Based on visual observation, the medium with glucose has clear appearance and the presence of color deposition at the bottom of medium. Observation of liquid waste is obtained by the deposition of dyes on the basis and changes in the color density of waste. Each fungi was brightly different, Ys14 waste color was brightest, while isolates Ys02 was a second one and Ys21 isolates was the darkest (Figure 3c). Addition glucose into medium enhanced decolorization process. These results are in agreement with [15] who showed that the maximum decolorization of trypan blue by *A. niger*, *A. fumigatus* and *A. flavus* occurred with medium supplemented with 2% glucose at pH 4. The ability of isolates Ys14 and Ys02 to decolorize waste are better due to the condition which are more extreme so that is more resistant comparing to Ys21.

Table 2. Isolates fungi Growth in Liquid media MSM containing waste

No	Isolates	25% waste + 0,5% Glucose	25 % with no glucose
1	Ys02	+	-
2	Ys13	-	-
3	Ys14	+	+
4	YS21	+	+

The color change with two mechanisms which are carried out in an enzymatically or non-enzymatically. Enzymatically fungi use extracellular enzymes to decolorize lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), glucose 1 oxidase, glucose 2 oxidase, phenol oxidase, and laccase. These enzymes have nonspecific properties to a substrate so that the fungi have decolorization ability [16].

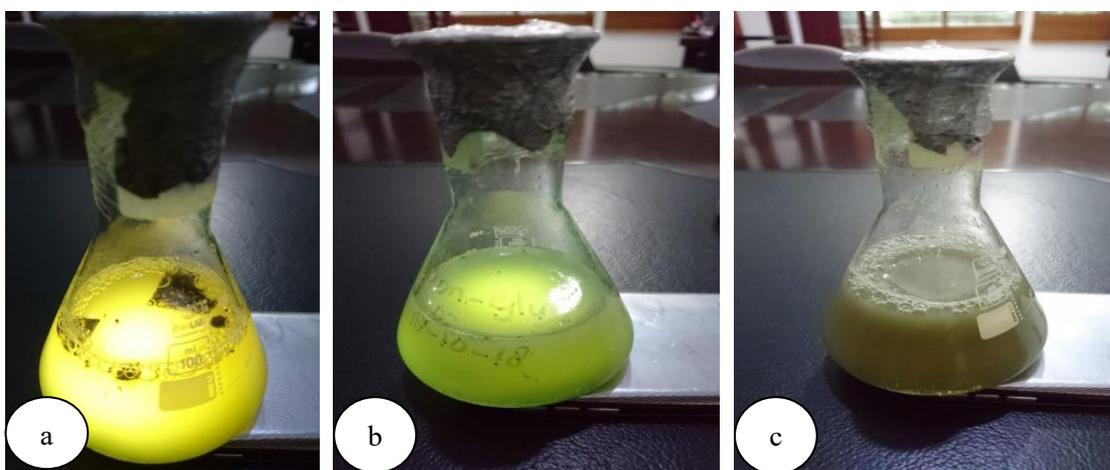


Figure 1. Fungal isolates in MSM liquid medium containing 25% batik waste

The color change of fungal mycelium from the initial color show a decolorization process caused by the mechanism of absorption dyes by fungi, which can remove aromatic compounds and dissolved organic compounds. The results is also supported by Madhuri [17] changes in dyes carried out by fungi through absorption [18]. Fungal cell wall emits a gel that functions as an adhesive, this gel is able to absorb the dyes added to the medium. Fungi mycelium is hydrophobic and the dyestuff is hydrophilic, therefore, release of the gel causes interaction between the mycelium and dyestuff which can cause absorption mechanism.

4. Conclusion

Twenty-three fungi isolated from landfill were able to decolorize batik waste liquid in laboratory scale based on growth performance and visual observations.

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