Biological Treatment of Naphthol Yellow S and Batik Effluent using *Aspergillus tamarii* and *Aspergillus sclerotiorum*

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Abstract

Wastes released from the textile dyeing industry contain different hazardous compounds that are difficult to degrade such as azo dyes which are the main source of environmental pollution. Biological treatment processes are a great solution to prevent the environmental pollution caused by wastes because of their environmental friendliness, low cost and minor sludge-giving properties.

Fungi are known as mycoremediation in which dyes are eliminated by fungi through biosorption, detoxification and biodegradation. Two fungal isolates Aspergillus tamarii and A. sclerotiorum were isolated from dye-contaminated soil. The maximum decolourization efficiency against 250 ppm dye concentration of Naphthol Yellow S was 87.62% and 89.99% for A. tamarii and A. sclerotiorum respectively at room temperature after 5 days in shaking conditions. The physicochemical characteristics of batik effluent such as BOD, COD, total dissolved solids and total suspended solids had removal percentages of 42.10%, 37.13%, 36.10% and 21.20% for A. tamarii and 37.44%, 42%, 42% and 31.3% for A. sclerotiorum. The removal percentage indicated the potential application of both isolates for biological treatment.

Keywords: Decolourization, fungi, Naphthol Yellow S.

Introduction

Industrial wastewaters are a significant contributor to water pollution by polluting rivers, lakes and oceans[21]. The effluents released from the textile industry are considered high liquid pollutants. About 280,000 tons of textile dyes are discharged as wastes in textile effluents every year[23]. Wastes released from the textile dyeing industry contain different hazardous compounds that are difficult to degrade such as azo dyes which are the main source of environmental pollution[22]. They are characterized by the presence of one or more azo groups -N=N- which are responsible for their colouration and recalcitrant nature and hence are less biodegradable. Reactive dyes are commonly used in textile industries because they have favourable characteristics such as being bright and water resistant. They are also easy to apply with low energy consumption[26].

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Azo dyes are commonly used by the textile industry and they are classified as reactive dyes including naphthol. Naphthol dyes provide strong colours and react quickly with fabrics making them difficult to dissolve in air[3].

The structures of naphthol which are not easily degraded naturally, have been studied by various methods to degrade this dye, for example electrochemical methods[11]. This method is effective in degrading different types of soluble and insoluble dyes by reducing COD but this method consumes high electricity costs and produces sludge and pollution from chlorinated organics and heavy metals because of indirect oxidation[15]. At present, effective biological treatment processes are important because of their environmental friendliness, low cost and minor sludge-giving properties[24].

Nowadays research has indicated that biosorption is one of the most promising technologies and the removal of dyes by different kinds of biosorbent materials has been receiving more attention. Biological wastewater treatment is often the most economical and eco-friendly alternative relative to other physical and chemical processes. Microbial decolourization methods such as bioremoval by growing culture in a medium and biosorption by microbial biomass are commonly applied to the treatment of textile industry effluents because various microorganisms such as bacteria, yeasts, algae and fungi are able to remove different classes of dyes[10,12,26].

Fungi are known for their superior abilities to produce a well-built variety of extracellular proteins and other organic compounds[25]. Moreover, degradation by fungi is known as mycoremediation in which dyes are eliminated by fungi through biosorption, detoxification and biodegradation.

The main objective of this research was to examine the decolourization of synthetic reactive dye (Naphthol Yellow S) which has a wide range of utilization areas in the textile sector and exists in aquatic environments using living biomass of *Aspergillus tamarii* and *A. sclerotiorum* isolated from the effluent of textile waste dyeing industry under a variety of concentration of Naphthol Yellow S dye. This study also extends to the advanced treatment of real batik effluents. The efficiency of wastewater decolourization depends on the removal of organic colourants such as pH, biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and total suspended solids (TSS).

Material and Methods

Characterization and identification of fungal isolates: Colonies of *Aspergillus tamarii* and *A. sclerotiorum*, isolated from contaminated soil samples grown on each medium were distinguished on the basis of their surface characteristics such as texture, colour, zonation, sporulation and diameters[16]. The microscopic characteristics were carried out by slide culture methods with a drop of lactophenol cotton blue covered with a cover slip and examined under a microscope Optical Laboratory using x40 objective lens. The fungal isolates were identified by DNA sequencing according to standard protocols. The contigs (formed from forward and reverse sequences) obtained were analyzed using BioEdit 7.2.5 software and aligned using Clustal W of MEGA 7.0 software. The fungal isolates were assigned species names after comparison with representative sequences available in NCBI (National Center for Biotechnology Information)[8].

Microbial source and inoculant preparation: Fungal isolates, namely *Aspergillus tamarii* and *A. sclerotiorum* isolated from contaminated soil samples from previous studies were used in this research. The batik dye used in this study was Naphthol Yellow S and batik effluents. *Aspergillus tamarii* and *A. sclerotiorum* were grown on potato dextrose agar in Petri dishes which were previously sterilized by autoclaving at 121°C and 2 atmospheric pressure at room temperature for 5 days[6,7].

Decolourization assay of *Aspergillus tamarii* and *A. sclerotiorum* based on variation concentrations of Naphthol Yellow S and incubation time: A disc (6 mm) cutting from the edge of the cultivated medium of *Aspergillus tamarii* and *A. sclerotiorum* was aseptically inoculated onto a 250 mL Erlenmeyer flask containing 50 mL of potato dextrose broth (PDB) supplemented with different concentrations of Remazol black B (RBB) dye (250, 500, 1000, 1500 mg/L) as a sole carbon source. The flasks were incubated using a shaker at 75 rpm and 28°C ± 2°C for 5 days. Triplicate sets of flasks were used for each fungus isolate and dye concentration. The supernatant was analyzed for decolourization percentage by measuring the absorbance of the supernatant by UV–Vis spectrophotometry. The percentage decolourization was calculated using the following formula[5]:

% Decolourization: (first absorbance − last absorbance)/first absorbance × 100%

Analysis of batik effluent before and after treatment: Physicochemical characteristics of batik effluent including pH, biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and total suspended solids (TSS), were estimated before and after treatment for analysis.

Statistical analysis: Decolourization was analyzed using Analysis of Variance and Duncan's test of data to complete the significant difference test in SPSS 16 at a 0.05 significance level. All experiments were conducted in triplicate and the results were expressed as mean ± standard error.

Results and Discussion

Morphology characteristics of fungal isolates: Two fungal isolates consisting of *Aspergillus* genera were obtained in this study (Table 1). The results in table 1[4] also revealed the macroscopic characteristics of the isolates in terms of colour, surface characteristics, reverse, edge and exudate. The microscopic features of the isolates presented in table 2 showed the vesicles, conidiophores, conidia and hypha. *Aspergillus* species had septate hyphae, hyaline conidiophores and radial conidial heads bearing the spores. The genus *Aspergillus* is one of the most well-researched fungi genera with over 200 officially recognized species[19].

Table 1 presents the growth characteristics of the *Aspergillus* genera. *Aspergillus* sp.1 could be distinguished due to the colour of the colonies. *Aspergillus* sp.1 had a dark brownish mycelium while *Aspergillus* sp.2 had a yellow mycelium. This isolate was morphologically different from *Aspergillus* sp.2 not only from the colour of the colonies but also from their capability to produce exudate. *Aspergillus* sp.2 produced exudate on the surface colonies. Other differences between these two isolates were the zonation on the colonies surface of *Aspergillus* sp.2 which varies from white to yellow, while *Aspergillus* sp.1 showed no zonation on their colonies' surface.

Gene sequences of fungal isolates: Identifications based on morphology characteristics were confirmed by sequence analysis of the isolates. Basic Logical Alignment Search Tool (BLAST) results of ITS region sequences of this study in the National Centre for Biotechnology Information (NCBI) provided relationships and similarities with reference sequences in GenBank (Table 2). The results in table 2 revealed that most isolates had above 99% similar identity to reference sequences of GenBank[4].

Decolourization assay of *Aspergillus tamarii* and *Aspergillus sclerotiorum* based on variation concentration of Naphthol Yellow S and incubation time: The results presented in table 3 indicated that *Aspergillus tamarii* and *A. sclerotiorum* reached the maximum decolourization of 87.62% and 89.99% respectively[4] after 120 h of incubation at an initial dye concentration of 250 mg/L. The increasing decolourization percentage of the two potential isolates to Naphthol Yellow S is consistent with the research[15] which showed that *Lenzites elegans* had an increasing decolourization percentage to Naphthol Green B dye within 4 days of incubation.

The percentage of *L. elegans* decolourization reached 97% on the fourth day. During decolourization, incubation for 120 h showed a change in the pH of Naphthol Yellow S by using *Aspergillus tamarii* and *A. sclerotiorum* (Table 1)[4]. In

this study, the decolourization percentage of Naphthol Yellow S using *A. tamarii* (87.62%) and *A. sclerotiorum* (89.99%) was high when compared with the research[7], indicating that *Aspergillus* sp. 3 could decolourize naphthol black dye up to 56.67%.

During decolourization, incubation for 120 h showed a change in the pH of *A. tamarii* and *A. sclerotiorum* with different concentrations of Naphthol Yellow S (Table 4)[4]. At 250 ppm of Naphthol Yellow S, the pH of *A. tamarii* and *A. sclerotiorum* at the beginning of incubation was 10.6 and 9.82 respectively. During decolourization which lasted up to 120 h, the incubation time changed the pH to 7.33 and 7.01. At 500 ppm of Naphthol Yellow S, the pH of *A. tamarii* and *A. sclerotiorum* was 11.2 and 10.36 respectively. In addition, the pH tends to decrease to 7.54 and 7.79 at 120 h of incubation.

At 1000 ppm of Naphthol Yellow S, the pH of *A. tamarii* and *A. sclerotiorum* was 11.87 and 10.59 respectively. The pH tends to decrease to 8.22 and 8.18 at 120 h incubation. At 1500 ppm of Naphthol Yellow S, the pH of *A. tamarii* and *A. sclerotiorum* was 11.81 and 12.14 respectively. In general, the pH at each concentration changes to 7–9.

According to Koivusaari et al[14], substrate decomposition decreases the pH because of the metabolic process of fungi. In this case, Naphthol Yellow S is a substrate that is metabolized by fungi through decolourization resulting in a decrease in pH at each concentration of Naphthol Yellow S.

Physicochemical characterization of batik effluent: The physicochemical parameters of batik effluent before and after fungal treatment were investigated (Figure 1)[4]. The results showed that the batik effluent before any treatment with the two fungi had a BOD of 3205.39 mg/L, pH of 10.7, COD of 541.72 mg/L, TDS of 5230 mg/L and TSS of 22.2 mg/L.

Table 1
Morphology characteristics of fungal isolates from contaminated soil

Macroscopic characteristics	**Isolates Code**	
	***Aspergillus* sp.1**	***Aspergillus* sp.2**
Surface characteristics	Granular	Granular
Colour	Dark-green	Whitish yellow
Exudate	No	Yes
Reverse colour	Cream	White
Edge	White, irregular	Yellow, circular
Microscopic characteristics		
Vesicles: Shape Diameter (µm)	 Radiate 25-50	 Radiate 20-50
Conidiophore: Length (µm) Diameter (µm) Colour	 365.84 – 945 2.27 – 6.5 Hyaline	 501.14 – 859.86 5-6.9 Hyaline
Conidia: Shape Diameter (µm) Colour	 Radial (globose) 3 – 6.5 Dark brownish	 Radial (globose) 2 – 6.5 Yellow
Hyphae: Shape Colour	 Septate Hyaline	 Septate Hyaline

Table 2
Results of similarities in fungal isolates according to Basic Logical Alignment Search Tool (BLAST)

Isolate	**Accession Number**	**Identity (%)**	**Fungi**
Aspergillus sp.1	MH345894.1	100.00%	*Aspergillus tamarii* isolate 54
	MT340979.1	100.00%	*Aspergillus tamarii* strain L1
	MT065769.1	100.00%	*Aspergillus tamarii* isolate PT-7
Aspergillus sp.2	KT581403.1	100.00%	*Aspergillus sclerotiorum* strain WSMT12
	KY963136.1	100.00%	*Aspergillus sclerotiorum* isolate ANDEF08
	KX712456.1	100.00%	*Aspergillus sclerotiorum* strain ASP054

Table 3
Effect of various concentrations of Naphthol Yellow S and incubation time on the decolourization percentage of *Aspergillus tamarii* and *A. sclerotiorum* grown on PDB at 28°C for 5 days. Data are expressed as mean values of triplicate ± SE of the mean

Conc. (mg/L)	Incubation time	Decolourization (%)	
		Aspergillus tamarii*	***Aspergillus sclerotiorum****
250	24	60.02 ± 0.11^{fg}	45.07 ± 0.05^{fghi}
	48	70.20 ± 0.09^{ef}	51.31 ± 0.06^{efg}
	72	79.37 ± 0.06^{cd}	74.43 ± 0.04^{cd}
	96	84.28 ± 0.05^{b}	82.22 ± 0.05^{bc}
	120	87.62 ± 0.03^{a}	89.99 ± 0.00^{a}
500	24	51.22 ± 0.14^{ij}	33.76 ± 0.04^{ij}
	48	63.31 ± 0.11^{fg}	49.44 ± 0.07^{fgh}
	72	69.22 ± 0.09^{f}	71.90 ± 0.02^{cd}
	96	76.79 ± 0.05^{de}	80.54 ± 0.01^{bc}
	120	83.52 ± 0.03^{bc}	85.02 ± 0.03^{ab}
1000	24	32.44 ± 0.14^{j}	26.16 ± 0.02^{jk}
	48	43.21 ± 0.13^{hij}	39.71 ± 0.07^{hij}
	72	56.12 ± 0.11^{h}	51.67 ± 0.02^{efg}
	96	59.69 ± 0.12^{gh}	62.94 ± 0.05^{de}
	120	65.24 ± 0.08^{f}	74.23 ± 0.04^{cd}
1500	24	33.30 ± 0.15^{j}	18.11 ± 0.04^{k}
	48	42.29 ± 0.14^{hij}	38.40 ± 0.02^{ij}
	72	50.02 ± 0.12^{ghij}	45.15 ± 0.01^{fghi}
	96	50.60 ± 0.12^{ghij}	55.60 ± 0.02^{ef}
	120	58.40 ± 0.06^{gh}	63.17 ± 0.02^{de}

*Different letters show a significant difference at 5% probability based on DMRT

Table 4
Changes in pH during decolourization process in various concentrations of Naphthol Yellow S dye by *Aspergillus tamarii* and *Aspergillus sclerotiorum*

250 mg/L			500 mg/L		
Incubation time	***Aspergillus tamarii***	***Aspergillus sclerotiorum***	**Incubation time**	***Aspergillus tamarii***	***Aspergillus sclerotiorum***
24	10.6	9.82	24	11.2	10.36
48	9.13	10.01	48	11.67	9.8
72	8.56	9.21	72	10.63	8.62
96	7.7	8.65	96	8.71	8.42
120	7.33	7.01	120	7.54	7.78
1000 mg/L			1500 mg/L		
Incubation time	***Aspergillus tamarii***	***Aspergillus sclerotiorum***	**Incubation time**	***Aspergillus tamarii***	***Aspergillus sclerotiorum***
24	11.87	10.59	24	11.81	12.14
48	11.34	10.4	48	12.2	11.07
72	10.5	9.32	72	11.76	10.62
96	9.7	8.76	96	10.12	9.54
120	8.22	8.18	120	9.23	8.2

In addition, the BOD, COD, TSS and TDS of batik effluent decreased with removal percentages of 42.10%, 37.13%, 36.1% and 21.2% respectively because of the decolourization activity of *A. tamarii*. Meanwhile, the BOD, COD, TSS and TDS of batik effluent decreased with removal percentages of 37.44%, 42%, 42% and 31.3% respectively because of the decolourization activity of *A. sclerotiorum*. After decolourization, the pH of batik effluent decreased in the range of 7.1–8.7 at different concentrations of *A. tamarii* and *A. sclerotiorum*.

The comparison between the results of previous studies with the results of this study shows that the decrease in BOD and COD levels using *Aspergillus tamarii* and *A. sclerotiorum* tends to be low (<50%). The BOD levels remain quite far compared with the quality standards. However, the decrease in BOD and COD levels indicates that the amount of organic matter contained in the batik effluent had been partially decomposed and that the complex compounds of dyes have been degraded into simpler aromatic compounds (short chain). The biodegradation of complex dyes into simple aromatic compounds can be observed on the basis of colour removal when compared with that before biodegradation.

In this case, an aerobic process occurs where simple aromatic compounds will be degraded through a hydroxylation opening of the chromophore ring to increase mineralization in the dye[27]. The high value of TDS in this study can interfere with the diffusion of sunlight into the water, thereby disrupting photosynthesis by aquatic flora. Consequently, dissolved oxygen levels in the water were reduced[17].

In this study, the pH of batik waste is quite high which is 10.3. High pH plays an important role in photocatalysis because of its effect on OH production[13]. In a study conducted by Khalik et al[13], the efficiency of batik waste degradation at pH 10 was lower (67.6%) when compared with that of batik waste degradation treatment when the pH was adjusted to 3 (88.2%). Therefore, the high pH level of batik waste could cause a less optimal degradation of the two potential fungi.

This finding can be attributed to the effect of pH which affects the enzymatic activity of fungi and their ability to metabolize heavy metals in batik waste. The adsorption of heavy metals in batik waste by fungi will also be disrupted. Apart from adsorption, pH also affects the mobility of heavy metal compounds in soil[9].

After the decolourization of wastewater using *Aspergillus tamarii* and *A. sclerotiorum* isolates, the pH decreased to 8.7 and 7.1 after incubation for 5 days. Therefore, when the decolourization treatment time is longer, the pH of both isolates is becoming neutral. According to Ningsih[18], wastewater treatment can reduce the pH of the treated water.

The decrease in BOD, COD, TSS and TDS levels in batik waste after being treated with *Aspergillus tamarii* and *A. sclerotiorum* indicates the reduction of organic and inorganic compounds in batik waste. In addition, according to Dewi et al[7], the decrease in BOD, COD, TSS and TDS levels after being treated with fungal isolates indicates the use of compounds in batik waste as a source of nutrients for fungal isolates grown in it.

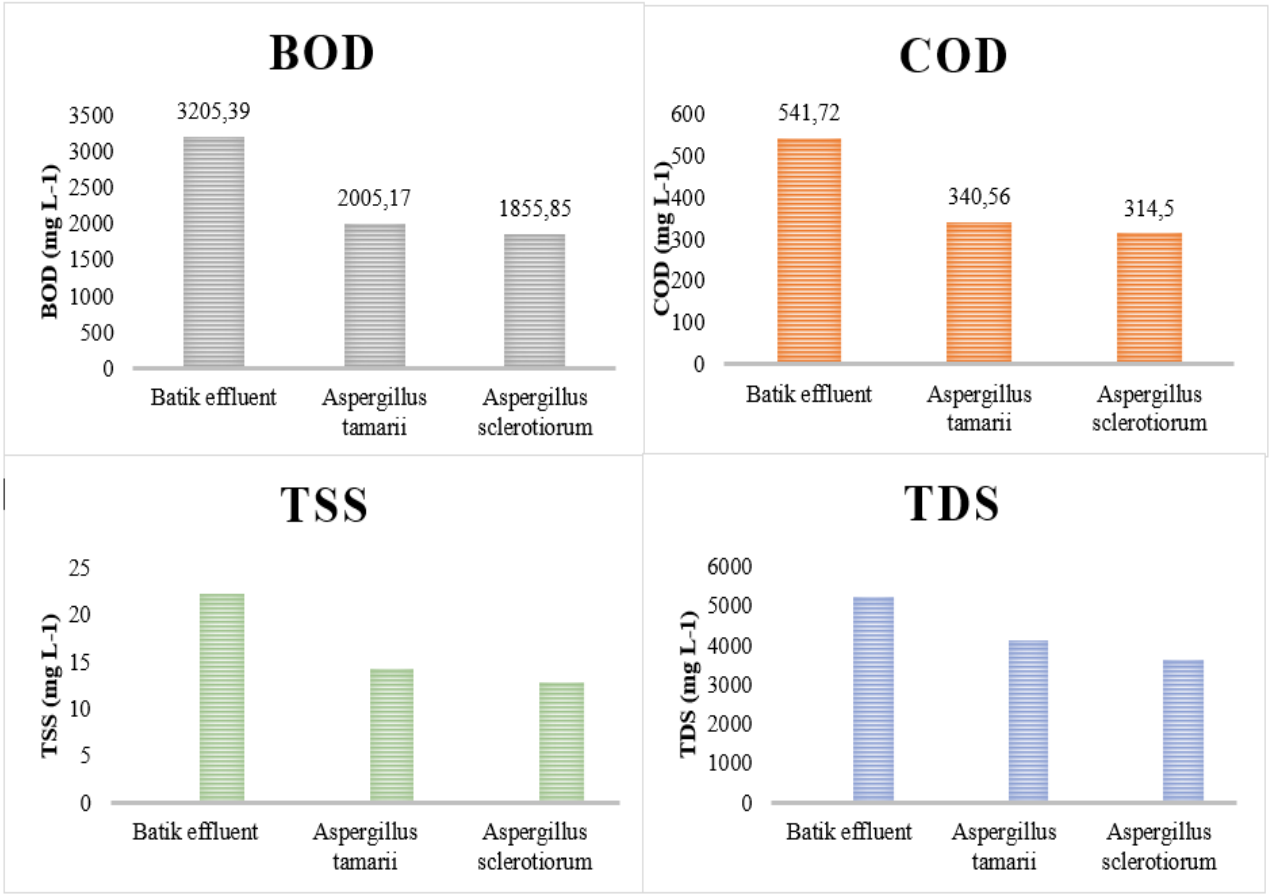

Figure 1: Physicochemical characteristics of the batik effluent after treatment with *Aspergillus tamarii* and *A. sclerotiorum*

Therefore, fungal isolates grown in batik waste result from cultures previously grown on PDB which is used as the initial nutrition of the fungi until mycelium pellets are formed before the medium is separated from the pellets to be grown on batik waste. This method ensures that the use of organic and inorganic compounds in batik waste is the only source of nutrients used by fungal isolates[6,7].

Conclusion

This study showed that *Aspergillus tamarii* and *A. sclerotiorum* could decolourize Naphthol Yellow S with a high percentage of 87.62% and 89.99% respectively after 120 h of incubation. The removal percentage of the physicochemical characteristics of batik effluent by using *Aspergillus tamarii* and *A. sclerotiorum* showed the potential application of both isolates for biological treatment.

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