Mycoremediation Potential of Synthetic Textile Dyes by *Aspergillus niger* via Biosorption and Enzymatic Degradation

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ABSTRACT

Textile dyes that persist in the environment are highly resistant to the natural degradation processes that occur in the environment. Therefore, the present study isolated, identified, and optimized textile dye decolorization by fungi and elucidated the dye decolorization pathway to develop a low-cost biotechnological approach for decolorization and detoxification of textile dyes. Within 36 hours of incubation at temperatures ranging from 28 to 40°C, pH 7, and shaking at 100 rpm, *Aspergillus niger* MN990895, which was selected from a total of 77 fungal isolates, completely decolorized the model dye CI Direct Blue 201 (DB 201). *A. niger* biosorbed 8.4±1.2% of the dye used where live biomass showed complete dye removal. It was found that extracellular crude enzymes were more involved in DB 201 dye decolorization (72.7±3.3%) than intracellular crude enzymes. The enzymatic studies suggested that the primary enzyme involved in DB 201 textile dye decolorization was lacccase, which was further confirmed by the presence of distinct protein bands around 75-100 kDa on the SDS-PAGE. The FTIR spectra and seed germination assays confirmed that *A. niger* proved successful in DB 201 textile dye degradation and detoxification. The present study suggests that *A. niger* may have promising implications in the future for the development of an enzyme-based textile wastewater treatment system.

1. INTRODUCTION

Water is the ultimate receiver of the most pollutants and therefore, the hydrosphere is being polluted at an alarming level compared to the lithosphere and geosphere (Gadallah and Sayed, 2014). The chemical composition of water changes mainly due to wastewater generated by manufacturing or chemical processing industries such as leather, textile, and distilleries (Mahagamage and Manage, 2014; Xing et al., 2010). When it comes to these industries, the textile industry is the most significant polluter of surface and groundwater because it uses a large amount of water during the dyeing and finishing processes and generates large amounts of dye-containing wastewater (Bankole et al., 2018; Goud et al., 2020).

When chemical dyes adhere to compatible surfaces, they form complexes or covalent bonds with the metals, imparting a permanent or temporary color to the substrate (Ekanayake and Manage, 2017). When it relates to the annual production of synthetic dyes, azo dyes account for more than half of the total output (71×10^5 tons) (Fernando et al., 2012; Gupta et al., 2016). These synthetic textile dyes showed significant resistance to natural degradation processes, as well as a long half-life in laboratory tests (Brillas and Martínez-Huitle, 2015). For example, the half-life of the hydrolyzed Reactive Blue 19 at pH 7 and 25°C is approximately 46 years (Hao et al., 2000). It has been founded that the dyes themselves, as well as some of their breakdown daughter compounds, are toxic, carcinogenic, and mutagenic to plants and animals, including humans (Almeida and Corso, 2014; Kagalkar et al., 2009; Vairavela and Murtyb, 2020, Khan and Malik, 2014). Apart from the health implications, dye contamination in receiving environments, particularly inland water bodies or the ocean, has resulted in widespread public outcry and protest (Kalyani et al., 2008).

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To address the issue, different wastewater treatment guidelines have been established to reduce the toxicity of the textile dye effluent generated (Abdelgalil et al., 2018; Fernando et al., 2012). Although the overwhelming amount of the world's leading apparel suppliers are still facing environmental pollution issues as a result of the substantial use of textile dyes in the apparel industry, they are being forced to investigate alternative novel treatment methods to obtain the necessary wastewater consent limits and to expand their manufacturing operations (Khan and Malik, 2014). When compared to physicochemical methods such as coagulation, flocculation, and ion exchange, which are currently in use across the world (Gupta et al., 2016; Módenes et al., 2019), the use of fungal strains for textile wastewater treatment via adsorption or degradation is a promising tool for textile wastewater treatment (Bankole et al., 2018). The majority of fungi, once established, thrive and reproduce well even in harsh environments, and they can adapt their metabolism to utilize a variety of carbon and nitrogen sources in their quest for survival (Almeida and Corso, 2014; Bankole et al., 2018).

Most studies have focused on using fungi as bio-sorption agents to bind the surface of biomass, which has resulted in the bioaccumulation of toxins (Bhattacharjee et al., 2020; Mishra et al., 2021). Therefore, the use of a live biomass and their enzymes for bioremediation is a growing concern. Recent studies have documented the use of laccase-like enzymes in bioremediation processes in the presence of various chemical mediators (Shah et al., 2021). Therefore, research into the use of fungal enzymes without additional chemicals is a timely requirement. As a result, the present study is intended to isolate textile dye decolorizing fungi from water and soil in textile wastewater effluent sites and determine the mechanism of dye decolorization using the CI Direct Blue 201 (DB 201) textile dye as the model.

2. METHODOLOGY

2.1 Textile dyes and chemicals

Considering the textile dye usage in Sri Lanka, five structurally different textile dyes were selected for primary screening. These included two direct dyes: DB 201 ($C_{30}H_{16}C_{l2}N_4Na_2O_8S_2$), and Moxilon Blue GRL (MBG) ($C_{30}H_{16}C_{l2}N_4Na_2O_8S_2$), two Vat dyes: Cibanone Gold Yellow RK (CGY) ($C_{24}H_{10}Br_2O_2$), and Vat Green FFB (VG) ($C_{36}H_{20}O_4$), and a Reactive dye: Cibacron Blue FR (CB FR) ($C_{29}H_{20}ClN_7O_{11}S_3$). All of the dyes were sourced from small-scale textile dyeing operations in Sri Lanka. Considering the complexity of its chemical group, structure, and application in Sri Lanka's textile industries, the DB 201 textile dye was selected as the model dye for the study (Ekanayake and Manage, 2020b). The textile dyes used in the study were of industrial grade with 98% purity, and all of the other chemicals used in the study were of high purity and of the analytical and molecular grade.

2.2 Isolation and identification of fungi and appropriate culture conditions

Samples of water and soil were collected from seven textile wastewater effluent sites located in Western Province, Sri Lanka, and enriched for 14 days using modified Kirk's medium (pH 7.0; medium in g/L; glucose: 2, potassium: 0.20, magnesium: 0.05, calcium: 0.01, copper: 0.08, manganese: 0.05, zinc: 0.033, iron: 0.05 (Placido et al., 2016)) supplemented with a mixture of all five dyes (100 rpm). Isolated on Potato Dextrose Agar (PDA) plates following the standard spread plate method, fungi with varying morphological characteristics were identified after 14 days of incubation. Pure cultures were obtained after several inoculations.

2.3 Decolorization of textile dyes

In this experiment, the dye decolorization process was conducted in two stages. First, a solid medium screening procedure was carried out by placing a fungal mycelium disc (5 mm) on a PDA medium overlaid with 5 mL of PDA containing 0.01% (w/v) textile dye mixture and incubating for seven days at 28°C (Rani et al., 2014). The fungal isolates with the highest dye Decolorization Zone (DZ) on the overlaid textile dye mixture (DZ>5 cm) were subjected to the liquid medium screening. Following the liquid medium screening, four discs of (5 mm) fungal mycelium (0.5±1.0 g of wet weight) were introduced into 50 mg/L of each textile dye; DB 201, CGY, CB FR, MBG, and VG which dissolved in modified Kirk's medium (Ekanayake and Manage, 2020b), and the changes of the absorbance were recorded at maximum wavelengths of 570, 420, 605, 620, and 690 nm, respectively. According to Bankole et al. (2018), the percentage of dye decolorization in each experimental set-up was calculated using the equation below. In each experimental set-up, A0 and A1 denote the initial and final absorbance of dye, respectively.

$$DP\ (\%) = [(A0 - A1)/A0] \times 100$$

Following the findings, the most efficient fungal isolate was selected for further optimization studies using DB 201 textile dye as the model dye. The ITS region was used for fungal identification, and the sequence data was deposited on the GENE Bank.

2.4 The optimization of selected external parameters in the fungal decolorization of DB 201 textile dye

Following the methods described in Bankole et al. (2017), Bankole et al. (2018), Ekanayake and Manage (2017), Ekanayake and Manage (2020a), and El-Rahim et al. (2009) temperatures (24, 28, 32, 36, and 40°C), pH (5, 6, 7, 8, 9, and 10), initial dye concentrations (25, 50, 75, 100, 150, and 200 mg/L) as well as agitation conditions (0, 25, 50, 75, 100, and 125 rpm) were scaled up to determine the dye decolorization efficiency. There were three replicates of each experiment, and no fungal inoculation was used to maintain control conditions. The repeated batch decolorization experiment was carried out by repeatedly adding 50 mg/L DB 201 textile dye to the experimental setup without supplement of nutrients or fungal biomass further (Cui et al., 2014; Jadhav et al., 2012). To determine the long-term applicability of isolated fungi for textile dye decolorization, a number of cycles were carried out several times.

2.5 Biosorption assay

Using triplicates, the activity of live and dead biomasses of a fungal isolate on the dye decolorization process was evaluated, and controls were maintained without the addition of fungi to the dye. Biosorption tests were carried out using 5-day-old live, and autoclaved fungal cultures (5.0±0.5 cm diameter) that were introduced into 50 mL of dye DB 201 at a final concentration of 50 mg/L and incubated at 28°C under shaking at 100 rpm until complete dye decolorization was achieved (Erdem and Cihangir, 2018).

2.6 Enzymes that are involved in the decolorization of DB 201 textile dye

To obtain the crude extracellular source of enzymes, the decolorized dye solution was filtered through 0.22 µm filter paper. The filtrate was lyophilized and used as an extracellular source of enzymes. The fungi that remained on the filter paper were washed with sterilized distilled water. The crude intracellular enzymes were extracted using a Proteo Prep-Universal Protein Extraction Kit (Sigma Aldrich, USA), following the manufacturer's instructions. DB 201 textile dye (pH 7.0) was treated with crude extracts from each enzyme source (5% v/v), which was incubated at 28°C for 24 h. At 6-hour intervals, the absorbance was measured to see how it changed. The controls were made up of crude extracts that had been heat-inactivated.

The activity of laccase, azoreductase, lignin Peroxidase (LP), manganese peroxidase (MnP), and tyrosinase enzymes were determined spectrophotometrically, using protocols optimized for each enzyme (Kalyani et al., 2008; Placido et al., 2016; Saratale et al., 2010; Watharkar et al., 2013). In this study, total protein concentration was determined using the Bicinchoninic Acid Protein Assay kit (BCA1 AND B9643; Sigma-Aldrich) and Bovine Serum Albumin as the protein standard (Sigma Aldrich, Germany). One unit of enzyme activity was defined as a change in absorbance unit per minute per mg protein of the enzyme (both from Sigma Aldrich, Germany). All enzyme assays were performed in triplicates, and the average rates of enzyme activity were calculated for each sample.

The most promising enzymes that were found to be involved in the decolorization of DB 201 textile dye solution were studied in greater depth. The dye decolorization experiments were carried out in bulk (1 L) for selected biological agents, and the decolorized dye solutions were lyophilized and re-suspended in 10 mL of potassium phosphate buffer (50 mM, pH 7.4) for use as an extracellular source of the enzymes in the experiments (Wijesekara et al., 2011). The supernatant of each enzyme extract was partially purified with $(NH_4)_2SO_4$ precipitation, dialysis in the potassium phosphate buffer, and ultrafiltration (Amicon, USA) with a 10 kDa cut-off membrane (Arabaci and Usluoglu, 2014; Bagewadi et al., 2017; Chen et al., 2005). The concentrated enzyme solution was further purified by DEAE-anion exchange column and Sephadex G-100 column chromatography using 50 mM of phosphate buffer (pH 7.4) and sodium acetate buffer (pH 5.6) as mobile phase, respectively (Telke et al., 2010; Wijesekara et al., 2011). The putative laccase enzyme was determined through SDS-PAGE.

2.7 Toxicity evaluation of the decolorized dye solutions

Seed germination assay was employed to assess the decolorized dye solutions' toxicity after the myco-remediation process. To summarize, 5 mL of the original DB 201 dye and 5 mL of the decolorized dye solutions were sprayed on thirty seeds of two

agricultural crop seeds, *Oryza sativa* (monocot) and *Vigna radiate* (dicot), once a day for five days. After five days, the length of the shoot (plumule), the length of the root (radicle), and the percentage of seeds that germinated were measured (Ekanayake and Manage, 2020a; Kalyani et al., 2008). The experiments were carried out in triplicate.

2.8 Analysis of the decolorized DB 201 textile dye

According to the method described by Kalyani et al. (2008) the color changes were measured using an ultraviolet-visible (UV-Vis) spectrophotometer and Fourier Transform Infrared Spectroscopy (FTIR) was used to study the changes in the dye before and after treatment.

2.9 Data analyses

One-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparison test, two-sample T-test, and Balanced ANOVA tests were used. When the p-value was less than 0.05, the results were considered significant.

3. RESULTS

3.1 Isolation and identification of textile dye decolorizing fungi

In the present study, out of 77 fungal isolates with different morphological features, only six isolates showed highest dye decolorization when tested on solid medium. *Aspergillus niger* MN990895 indicated by the reference number FBWB/06/S, was the most efficient fungal species for complete decolorization of all five dyes tested in liquid medium compared to the other five isolates (Table 1). The dye decolorization pathway of *A. niger* was evaluated in detail using DB 201 dye as the model dye (Figure 1).

Table 1. Decolorization of five textile dyes by most efficient fungi selected from solid medium screening

Reference No.	Dye decolorization percentages (%) and time (days)				
	DB 201	MB GRL	CBY RK	VG FFB	CB FR
Control	0.1 (14)	0.2 (14)	0.1 (14)	0.1 (14)	0.3 (14)
FPWA/07/S	CD (03)	CD (06)	CD (08)	CD (08)	CD (10)
FPWA/09/S	CD (03)	CD (04)	CD (12)	CD (07)	CD (12)
FPWB/22/S	98.65 (14)	CD (08)	88.2 (14)	95.4 (14)	CD (07)
FBWB/06/S	CD (02)	CD (02)	CD (02)	CD (04)	CD (05)
FBWB/07/S	CD (03)	CD (03)	CD (08)	CD (12)	CD (08)
FBWC/26/S	CD (03)	CD (05)	CD (07)	CD (14)	CD (09)

CD: Complete decolorization (>99%)

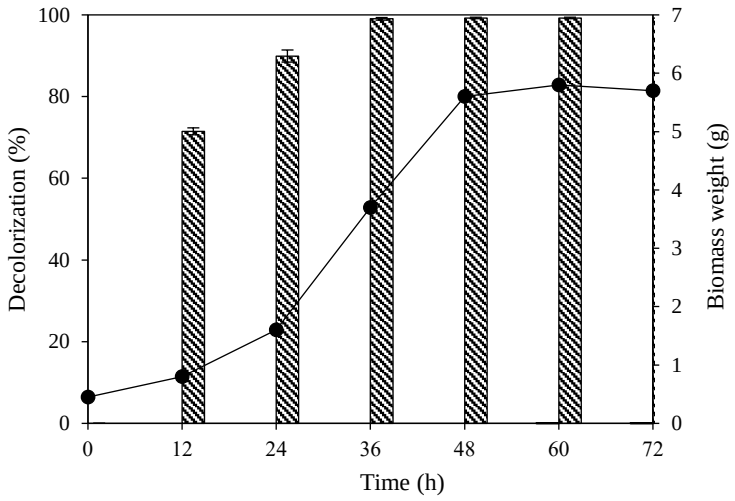

Figure 1. Decolorization of DB 201 dye by *A. niger* and growth of cell biomass (When error bars are not shown, the standard deviation was less than the width of the symbol)

3.2 Optimization of DB 201 dye decolorization by *A. niger*

When incubated for 36 h at temperatures ranging from 28 to 40°C, *A. niger* evidenced 99% dye decolorization (visually complete dye decolorization), with no statistically significant difference ($p>0.05$). The dye decolorization was slightly reduced at 24°C (Figure 2). DB 201 dye decolorization was found to be most effective under neutral pH (7) and vigorous agitation at 100 rpm (Figure 2). A descending order of dye decolorization was discovered as a result of the increasing initial dye concentrations. Throughout the study, the dye decolorization of the controls were less than 1% under all conditions tested. The repeated addition of dye into the same initial biomass resulted in over 99% dye removal up to the 5th cycle, after which the dye removal was recorded in descending order (Table 2).

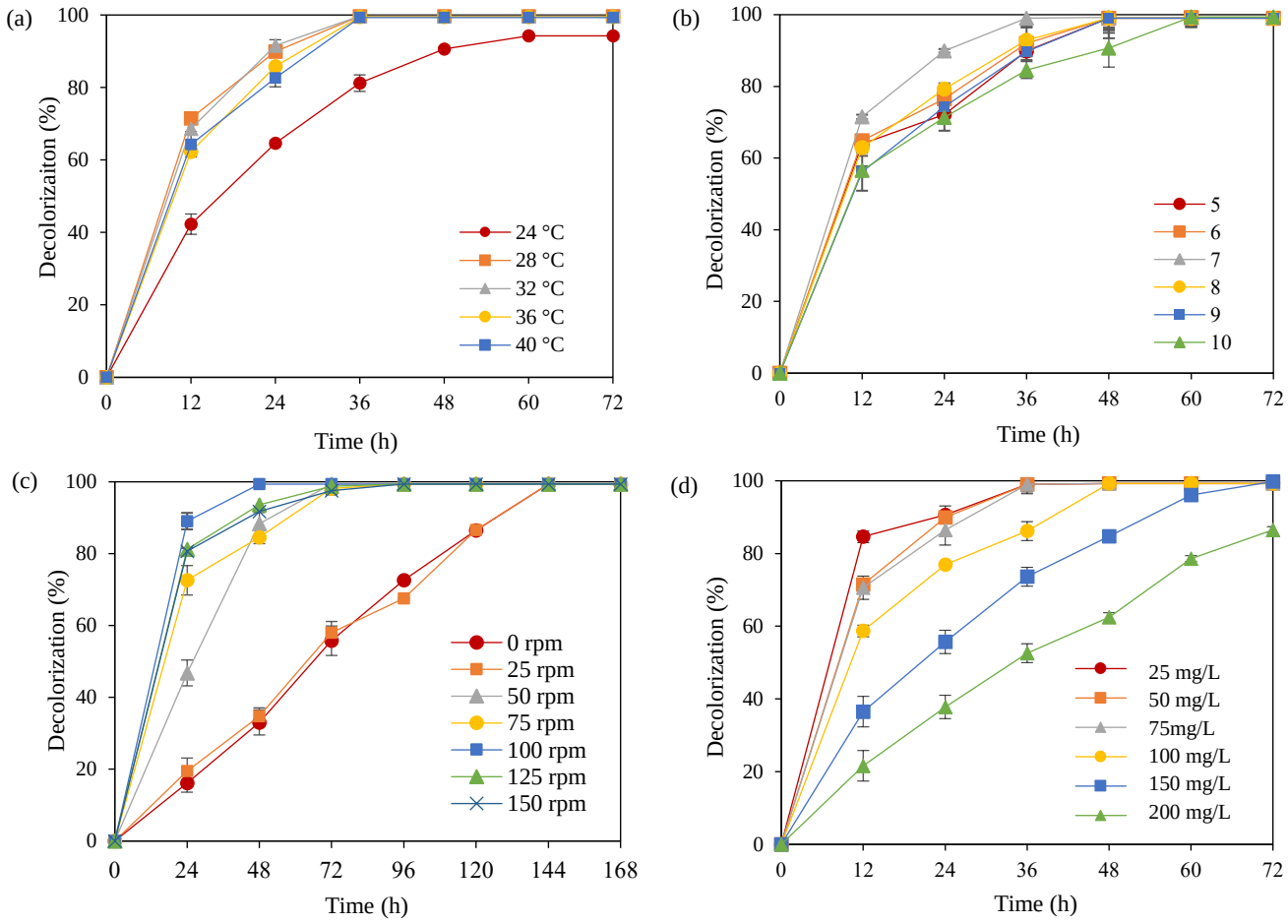

Figure 2. Effect of (a) temperature, (b) pH, (c) agitation, (d) initial dye concentrations on decolorization of DB 201 dye by *A. niger* (When error bars are not shown, the standard deviation was less than the width of the symbol)

Table 2. Effect of repetitive addition of DB 201 textile dye into the same *A. niger* biomass

Biological agent	Decolorization percentages (%) at the end of each cycle and time						
	1st cycle	2nd cycle	3rd cycle	4th cycle	5th cycle	6th cycle	7th cycle
A. niger	CD (36 h)	CD (12 h)	CD (12 h)	CD (12 h)	CD (12 h)	92.5±1.3 (12 h)	88.1±1.7 (12 h)

CD: Complete decolorization (>99%)

3.3 Evaluation of the DB 201 dye decolorization pathway by of *A. niger*

In the present study, the dead biomass of *A. niger* showed 8.4±1.2% biosorption of the DB 201 dye after 12 h of incubation whereas the live biomass showed complete removal of the dye. The amount of DB 201 textile dye decolorized by extracellular crude enzymes (72.7±3.3%) was higher than the amount of DB 201 textile dye decolorized by the crude extract of intracellular enzymes (Figure 3).

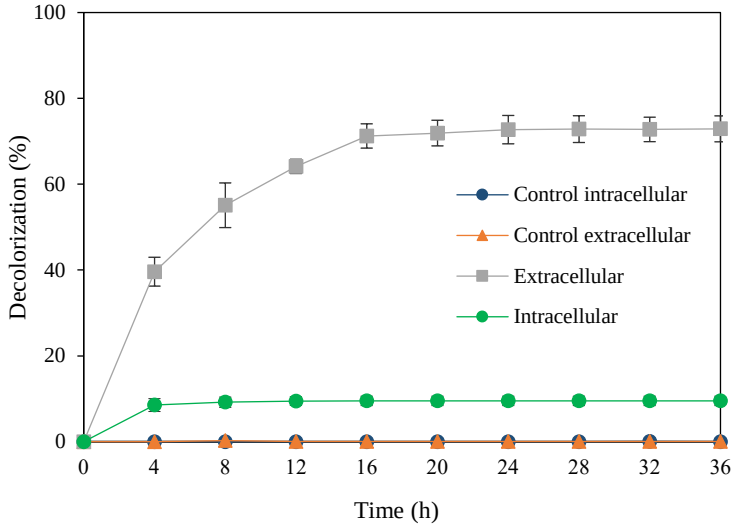

Figure 3. Effect of fungal enzymes on decolorization of DB 201 dye (When error bars are not shown, the standard deviation was less than the width of the symbol)

When the involvement of five different enzymes produced by *A. niger* was evaluated at the end of the dye decolorization process (Table 3), LP and laccase were found to be significantly enhanced ($p \leq 0.05$). Based on the preliminary studies and the literature, laccase was selected for further studies and subjected to further purification and confirmations. Table 4 illustrates the yield and the purification levels of putative laccase enzyme secreted by *A. niger* with the presence of DB 201 textile dye. The putative laccase enzyme obtained after purification was comprised of several distinct proteins (bands around 75-100 kDa) which indicated the presence of laccase enzyme (Figure 4). However, some minor bands were also detected in purified enzyme extract suggesting the importance of further optimizations to remove all the other unwanted enzymes.

Spectral comparison of original DB 201 textile dye solution and those subjected to myco-degradation by *A. niger* is depicted in Figure 5. Changes of transmittance in original dye and formation of new peaks at 3,389.64, 1,734.81, 1,653.53, 1,449.59 which may be responsible for O-H stretching, N=H vibration, N=N stretching, and N-O stretching, respectively, were recorded. Further, the changes in transmittance and peak patterns in the fingerprint region: 1,449.59, 1,400.34, 1,288.71, 1,190.28, 1,122.33, and 1,021.66 suggested formation of new bonds, especially C-O single bonds, after the myco-remediation process.

Table 3. Activity of the major enzymes recorded during the dye decolorization process

Enzyme	Enzyme activity (Umin/mg/protein)	
	Control	Decolorized solution
LP	1.153±0.128	LP
MnP	0.005±0.001	MnP
Tyrosinase	0.002±0.001	Tyrosinase
Laccase	0.090±0.014	Laccase
Azoreductase	0.140±0.026	Azoreductase

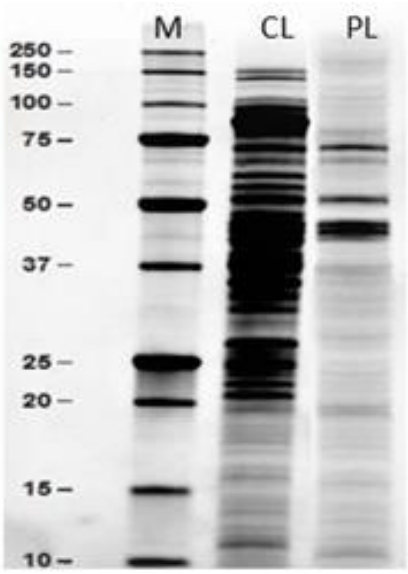

Figure 4. Molecular mass estimation of crude and putative laccase enzymes after dialysis via anion exchange chromatography (M: Marker, CL: Crude laccase, PL: putative laccase)

Table 4. Protein yield and enzyme activity of the purified laccase enzyme

Enzyme source	Purification step	Total protein (mg)	Enzyme activity (U)	Specific activity (U/mg)	Yield (%)	Purification
A. niger laccase	Crude	23.44	17.65	0.77	100.00	1.00
	Dialysis	10.52	10.65	1.02	60.33	1.32
	Ultra filtration	7.47	8.63	1.16	48.89	1.51
	DEAE	1.53	4.40	2.93	24.93	3.82
	Sephadex	1.01	3.20	3.17	18.13	4.13

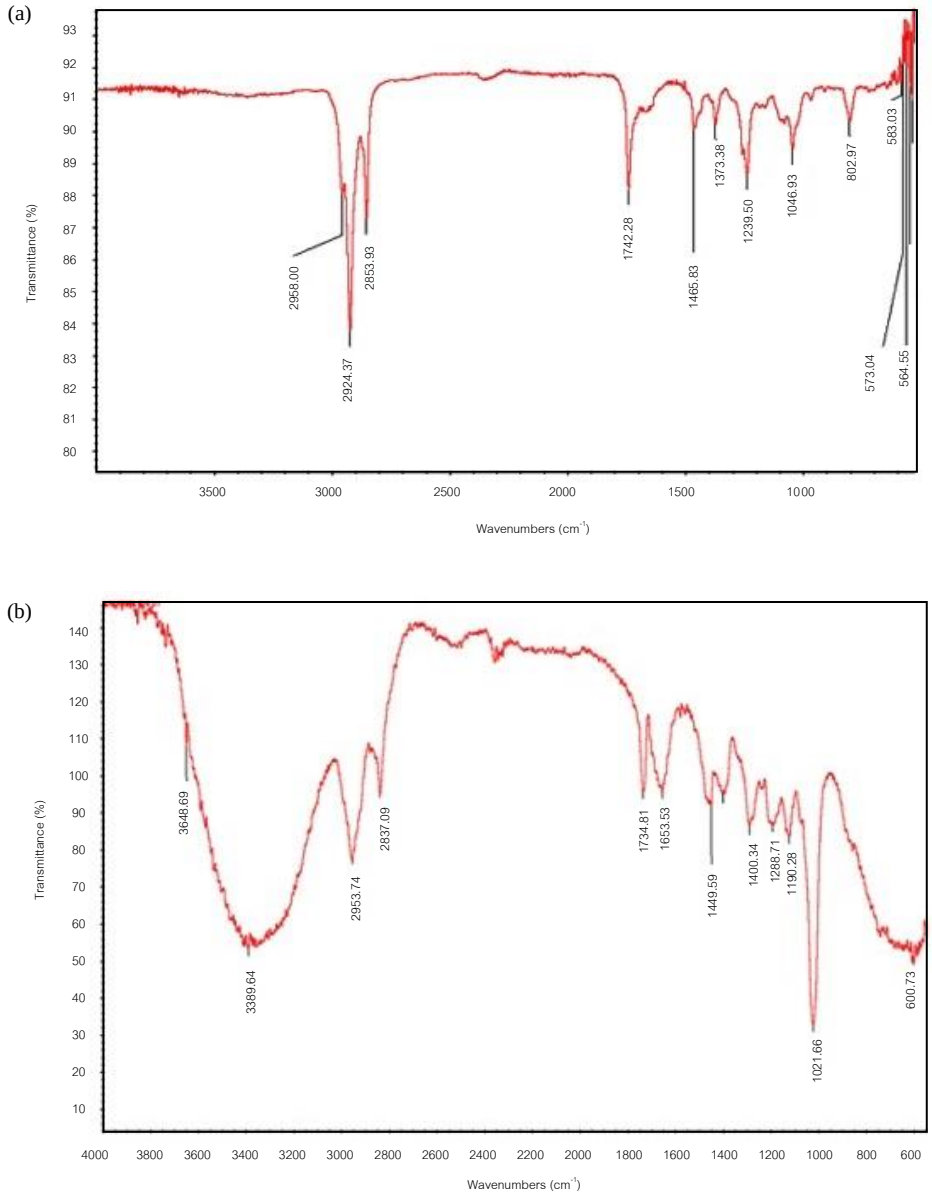

Figure 5. FTIR spectrum for (a) original DB 201 textile dye, decolorized DB 201 textile dye by (b) *A. niger*

The toxicity of the original DB 201 dye and treated dye by *A. niger* was evaluated to confirm whether the treated effluent has detoxified or not (Table 5). The results revealed that both *O. sativa* and *V. radiate* seeds treated with decolorized dye solution had significantly more seed germination, plumule and radicle growth, compared to the seeds treated with the original DB 201 textile dye ($p \leq 0.05$).

Table 5. Assessment of the toxicity of DB 201 textile dye and decolorized products

Parameter	*O. sativa*			*V. radiate*		
	Control	Dye (50 ppm)	Decolorized dye solution	Control	Dye (50 ppm)	Decolorized dye solution
Germination (%)	98.8±0.0	22.2±5.0	100±0	100±0	10.0±3.3	100±0
Plumule (cm)	2.6±0.2	0.6±0.2	2.3±0.1	1.4±0.2	0.3±0.3	1.2±0.3
Radicle (cm)	1.5±0.3	0.3±0.2	1.4±0.1	0.7±0.1	0.2±0.1	0.5±0.1

4. DISCUSSION

The textile dye DB 201, a di-azo direct dye, was selected as the model dye for the study as it had not been previously investigated in relation to decolorization by *A. niger*. DB 201 textile dye (834 g/mol) is complex, with two azo bonds and three sulfur groups on seven aromatic rings (Chemical Book, 2017) and highly resistant to photolysis degradation in the natural environment (Ekanayake and Manage, 2016). Moreover, the breakdown of aromatic components by biological degradation processes is slowed dramatically by textile dyes that contain azo and sulfo groups on the aromatic components (Saratale et al., 2010).

4.1 Isolation and identification of textile dye decolorizing fungi

Isolation of textile dye decolorizing fungi from the natural environment is highly challenging. Out of 77 total isolates studied in the present study, only one isolate, *A. niger*, was able to complete decolorization of all five selected textile dyes efficiently. A few studies have recorded the potential applicability of *A. niger* on decolorization of some other textile dyes; 99% of Rubine Toner 12 (Dhanjal et al., 2013), 92% of Direct Violet dye (El-Rahim et al., 2009), 88% of Synazol dye (Khalaf, 2008) and most of these studies discussed the application of *A. niger* as a bio-sorption agent instead using the organism as its live biochemical activities. The study by Seyis and Subasioglu (2008) recorded 45% and 38% decolorization of Methyl Orange dye by live and dead fungal biomass of *A. niger*, respectively. Fu and Viraraghavan (2002) reported the decolorization of Congo red by *A. niger* with 14.16 mg/g of adsorption capacity into fungal biomass while Rani et al. (2014) recorded the decolorization of malachite green by the fungi; *A. niger* via incorporation activities of the extracellular enzyme, absorption and adsorption. Therefore, comparison of dye decolorization potential of one species to the other is highly intricate as the dye decolorization by the same microorganism may fall under various mechanisms. Further, application of microorganisms used in one country for decolorization of textile dyes being used in their industry may not be applicable for another (Bankole et al., 2017; Seyis and Subasioglu, 2008). Consequently, the isolation and identification of potential microorganisms from the native environment where it will be used is critical for the experiment's success.

4.2 Optimization of textile dye decolorization by *A. niger*

Many external parameters, including temperature, pH and agitation conditions, as well as additional nutrients and the initial dye concentration, are known to have an impact on dye decolorization by biological agents, particularly for the growth, viability and enzymatic activities of the agents (Ekanayake and Manage, 2020b; Jadhav et al., 2012; Kalyani et al., 2008; Saratale et al., 2010). Thus, the present study explored the effect of such factors on the decolorization of DB 201 textile dye by *A. niger*. The results revealed that *A. niger* demonstrated efficient decolorization of DB 201 textile dye under shaking conditions in the temperature range of 28 to 40°C and pH 7.

The temperature range chosen was more or less similar to the optimum temperature for fungal growth (25°C to 35°C) found by Fu and Viraraghavan (2002). As a result, it is possible that *A. niger*'s dye decolorization will not be affected by temperature changes and will continue to function normally within the selected temperature range of 28 to 40°C. At the same time, Bankole et al. (2018) observed that the rate of decolorization of Scarlet R dye by *Peyronellaea prosopidis* was greatly decreased at both lower (15-25°C) and higher temperatures (>45°C), suggesting that this may be due to the reduction of lygnolytic enzymes combined with negligible cell apoptosis at high temperature. El-Rahim et al. (2009) found that pH 2, 3, 8, and 9 were the most effective pH values for dye removal by *A. niger*, and that these pH values were also the ones where the fungi produced the most biomass yield. However, in the present study, the

highest dye decolorization by *A. niger* was observed at a neutral pH value of 7. Many researchers, including Asad et al. (2007), Bankole et al. (2018), and Shah et al. (2012), have highlighted the relationship between oxygen and cell growth as well as the decolorization of textile dyes, identifying it as one of the most important factors that must be optimized. When Rani et al. (2014) investigated the decolorization of dyes by fungi under differing agitation conditions, they revealed that *A. niger* prefers static conditions for the rapid decolorization of Nigrosin and Malachite Green dyes. According to Ali et al. (2008), the *A. niger* preferred shaking conditions for its rapid growth and decolorization of Acid Red 151 and Sulfer Black dyes, implying the effect of agitation conditions on dye decolorization may be dependent on the type of textile dye used in the treatment process. As per the results of this study, *A. niger* was more efficient in dye decolorization under shaking conditions than in under static conditions ($p \leq 0.05$). The results of the present study were in agreement with Przystas et al. (2015) and Yesilada et al. (2003) for a consortium of *Pleurotus ostreatus*, *Gloeophyllum odoratum* and *Fusarium oxysporum* on decolorization of Brilliant Green dye and *Funalia trogii* on decolorization of Astrazone Blue dye. Bankole et al. (2018) hypothesized that the preference for shaking conditions could be attributed to the aerophilic nature of most fungi for enzyme activities and that the opposite results could be due to the involvement of azoreductase-like enzymes, which are inhibited by the presence of oxygen, as previously reported.

Jadhav et al. (2012) hypothesized that the reduction in dye decolorization may be due to the negative impacts occurred into the enzyme system that is involved in the dye decolorization process and the statement was confirmed by showing that the activities of laccase, vertryl alcohol, NADH-DCIP reductase were reduced at higher initial dye concentrations (250 mg/L), compared to the low concentrations (5 mg/L). Similar to the present study, Yesilada et al. (2003) recorded that decolorization of Astrazone Blue dye by *F. trogii* was successful up to five cycles and then declined sharply. Decreasing of dye decolorization after a few cycles may depend on the reduction of cell viability due to the depletion of essential minerals in the medium or entry of cells into the stationary phase followed by death phase, which results in a reduction in metabolic and enzymatic activities that are related to the dye decolorization (Jadhav et al., 2012).

4.3 Evaluation of the DB 201 dye decolorization pathway by *A. niger*

Fungal dye decolorization may occur via two different pathways: bio-sorption or biodegradation (Dhaneshwar, 2016). Polman and Breckenridge (1996) put forward that the interaction of dyes to biomass depends on the chemistry of the particular dye and the specific chemistry of the microbial biomass. Therefore, decolorization rate and behavior may vary from one species to the other as well as from the type of dye employed. Further, Modak and Natarajan (1995) recorded that most biological agents showed bio-sorption of dyes where the environmental conditions are not favorable for their growth, especially when the textile effluent is toxic.

According to the findings of the present study, both bio-sorption and biodegradation appear to be important components of the fungal dye decolorization mechanism, and this may be dependent on the complex structural nature of the dye as well as the heavy weight of biomass and the surface area of the fungi (Fu and Viraraghavan, 2002). Furthermore, Sumathi and Phatak (1999) reported complete decolorization of Remazole Red, Remazole Dark Blue, and Brilliant Orange dyes by *Aspergillus foetidus* within two days of incubation, while Fu and Viharaghavan (2002) reported complete decolorization of Basic Blue and Acid Blue dyes by dead *A. niger* biomass taking 2-30 days to complete. As per the findings of these studies, the dye decolorization behavior of the same species may differ depending on the textile dye used.

According to the present study's results, crude extracellular enzymes from *A. niger* were significantly more involved in DB 201 dye decolorization than the intracellular enzyme extract tested. As far as the authors are aware, the present study is the first study that a crude extracellular enzyme extract secreted by fungi during the previous dye decolorization cycle has been used to evaluate textile dye decolorization. Laccase enzyme, secreted by *A. niger*, was the most prominent enzyme involved in the decolorization of textile dye DB 201, as indicated by bands between 50 and 75 kDa on the SDS-PAGE. Bankole et al. (2018) and Vasdev et al. (1995) have found lignin modifying enzymes such as laccase, lignin peroxidase, and manganese peroxidase, among others, are involved in the biodegradation of textile dyes by live fungi. Bagewadi et al. (2017) recorded that the laccase enzyme produced by *Trichoderma harzianum* was

engaged in the decolorization of Methylene Blue and Congo Red dyes, with 90% of Methylene Blue and 60% of Congo Red dyes decolorized. According to the authors, 163 U/mg of specific laccase activity and a 25-fold purification after two anion exchange chromatographies (DEAE Sepharose and Sephadex G-50) were observed with protein bands around 56 kDa being responsible for the laccase enzyme being identified. In the present study, decolorized dye solution was obtained as the crude enzyme source to measure the enzyme activity, while other studies which recorded so far have used Solid State Fermented (SSF) fungi as the source of crude extract. This may be the reason for detection of low enzyme activity in the present study, compared to the previous studies. The FTIR spectra for original dye and decolorized dye solution by *A. niger* showed significant changes in peak patterns and the transmittance which confirm the myco-degradation potential of the dye.

Altogether, results of the present study suggested that decolorization of DB 201 textile dye was mainly taking place by biodegradation through extracellular activity of *A. niger* laccase enzyme with minor contribution of bio-sorption. Similar mechanism is suggested by Placido et al. (2016) for decolorization of Novacron Red and Remazol Black dyes by activity of laccase enzyme produced by *Leptosphaerulina* sp. and bio-sorption on to the cellular biomass. Laccase, benzenediol: oxygen oxidoreductase (EC 1.10.3.2), is a well-known enzyme with great advantages of being extracellular, inducible, low substrate specificity and an inexpensive cofactor (oxygen) (Placido et al., 2016; Zouari-Mechichi et al., 2006). Hence, isolation and identification of such enzyme producing fungi from native environment of Sri Lanka, with special reference to the textile dye decolorization, appear to be a green light for future biotechnological applications on textile dye decolorization.

Colored textile dyes are associated with a number of environmental hazards. The majority of these effluents eventually find their way into natural water bodies. In some cases, the secondary byproducts of some synthetic chemicals are more toxic than the original forms of the substances in question (Almeida and Corso, 2014; Guruge et al., 2007). Almeida and Corso (2014) discovered that the formation of toxic metabolites by *Aspergillus terreus* following the decolorization of Procion Red MX-5B dye was more harmful than the dye in its original form, which was due to the incomplete degradation. The formation of these metabolites during microbiological treatments is highly undesirable due to the increase in toxicity, which can have a detrimental effect on the surrounding environment. As a result of the present study, *A. niger* demonstrated complete detoxification of the dye, highlighting the potential applicability of the selected species for green and environmentally safe approaches. To design a bioreactor, the isolate can be used as live biomass, and the optimized factors can be used to simulate the process at an industrial scale. Further investigation into the role of the laccase enzyme in textile wastewater treatment may lead to the development of an enzyme-based treatment method for the treatment of textile wastewater as an intelligent approach in textile wastewater treatment.

5. CONCLUSION

Out of the 77 different fungal strains tested, *A. niger* demonstrated the most significant potential for decolorization of the selected model dye DB 201. The toxicity assay confirmed that *A. niger* could be used for textile dye decolorization without forming hazardous intermediate products. The decolorization of DB 201 textile dye occurred primarily through biodegradation, which was mediated by an extracellular enzyme: laccase. To ensure the textile wastewater treatment plant's ease of application and maintenance, additional research is required to develop an environmentally friendly, biotechnological approach to decolorize textile dyes using such enzymes rather than directly applying live biomass.

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CONFLICTS OF INTEREST

The manuscript was written collaboratively and has not been submitted simultaneously for publication elsewhere; the authors declare that they have no conflicts of interest to disclose.

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