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Bioremediation of Reactive Dyes by Fungal Species

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ABSTRACT

Reactive dyes are one of the most common dyes used in fabric re-dyeing; as such, their indiscriminate discharge into the environment is causing serious pollution in urban Kano, Nigeria. This research was aimed at assessing the potential of fungal species isolated from one of the major dyeing sites in Kano: Kofar Na'isa dyeing pit for the remediation of reactive dyes. The fungal species (*Aspergillus striatus* NEF4, *Candida tetrigidarum* NRRL Y-48142 1, *Fusarium equiseti* SPF466, and *F. oxysporum* FusCic45B) were isolated and identified from the dye-contaminated soil using dilution plating, pour plate, streak culture techniques, and DNA analysis. The isolated organisms were used to assess their bioremediation potential through biosorption and biodecolourisation of dye wastewater. The highest dye removal efficiency through biomass biosorption and enzymatic action was recorded after 48 hours, at pH 11.3 and a temperature of 37 °C. The dye removal by biosorption and biodecolourisation was within the ranges of 19.7 – 86.9% and 58.9 – 71.4% for *A. straitus*, 23.9 – 84.4% and 50.6 – 80.8% for *C. tetrigidarum*, 18.3 - 97.9% and 47.7 - 86.7% for *F. equiseti,* respectively. However, *F. oxysporum* displayed a negative biosorption but achieved 53.6 – 90.2% colour removal by enzymatic action. Dye removal increased with an increase in contact time due to gradual mycelial absorption. The isolated fungal species have proven to be effective in the remediation of reactive dyes, and thus, can be employed in regulating environmental contamination caused by dyes.

INTRODUCTION

The fungal breakdown of organic compounds such as dyes stems from their efficient use of biosorption, bioaccumulation, and biodegradation [1]. Among the various remediation strategies, fungi predominantly utilize biodegradation, which is attributed to their ability to secrete a broad spectrum of intra- and extracellular enzymes (like azoreductases, lignin peroxidases, manganese peroxidases, and laccases) that catalyze the mineralisation of different organic substrates [1,2].

In fungi, biosorption takes place through the adherence of dye molecules to specific functional groups present in the cell wall, a process that can be facilitated by either viable or non-viable biomass [3]. The low cost and high efficiency displayed by filamentous fungi make them an attractive alternative for dye removal, particularly given their potential to achieve complete mineralisation [4]. Fungal adsorption is pH-sensitive, displaying increased efficiency at a pH of 2–3, likely due to electrostatic interactions between the charged dye molecules and the oppositely charged fungal cell surface. At elevated temperatures, dye removal tends to decline, possibly due to thermal deactivation of the adsorbent or the loss of functional active sites [5]. Additionally, adsorption efficiency improves with rising dye concentrations, which suggests a positive correlation between dye load and fungal uptake capacity [6].

Singh and Sable [7] reported that native fungal strains hold a potential for treating dye-rich textile wastewater/effluents. This has gained global attention due to the metabolic versatility of white-rot fungi. *Phanerochaete chrysosporium*, one of the most researched species in this context, produces oxidative enzymes such as lignin peroxidases (LiP) and manganese-dependent peroxidases (MnP), enabling it to break down structurally complex compounds like lignin, dioxins, polychlorinated biphenyls (PCBs), azo dyes, and various chloro-organic pollutants [4,8,9,10]. Azo dyes, though resistant to degradation by most microorganisms, are susceptible to enzymatic attack by *P. chrysosporium* [11].

In addition to well-studied species, other fungi such as *Hirschioporus laricinus, Inonotus hispidus, Phlebia tremellosa,* and *Coriolus versicolor* have been reported to possess dye-decolourizing capabilities [1,3]. *P. chrysoporium*, in particular, is capable of mineralising recalcitrant aromatic pollutants through its robust ligninolytic enzyme system [1,11]. While fungal remediation proves to be very effective, its practical use is constrained by operational challenges like the need for acidic conditions, prolonged hydraulic retention period, and the possibility of suppressing coexisting microbial communities, which makes the approach less suitable for balanced microbial ecosystems [1,5,8,12].

MATERIALS AND METHODS

Sampling methods

Sterilised sampling containers were used to collect wastewater samples containing specific reactive dyes (reactive red 198 (RR198), reactive yellow 176 (RY176), reactive green 19 (RG19), reactive orange 122 (RO122), reactive red 195 (RR195) and reactive violet 1 (RV1)) from a local fabric re-dyeing pit situated at Kofar Na'isa, Kano, Nigeria.

Fungal isolation and identification

Fungal species were isolated from dye-contaminated soil collected from the dyeing site using the dilution plating and direct isolation techniques as outlined by Al-Mohanna [13]. Species identification was performed via DNA extraction followed by sequencing. Amplification of the 18S rRNA gene using specific primers (Fungi ITS-F (5′ - ATATGCTTAAGTTCAGCGGGT) and Fungi ITS-R (3′ - GTTCCGTAGGTGAACCTGC)) resulted in sequences (FASTA formats) that were submitted to the NCBI - BLAST database (USA) for taxonomic identification [14,15].
Pure cultures of the species were incubated on potato dextrose agar and broth at 37 ± 2 °C for five days to produce mycelia and enzymes required for the assays [16]. Following incubation, the harvested mycelial mats were transferred into sterile labelled test tubes, while the liquid phase was centrifuged (Centrifuge 80-2) at 10,000 rpm for 15 minutes to obtain the enzyme-rich supernatant for the biodecolourisation experiment.

Biosorption experiments

The biosorption process was initiated by introducing 0.4 g of fungal mycelia into separate test tubes containing 1.0 mL of wastewater (separate for each dye – RR198, RY176, RG19, RO122, RR195, and RV1) and 5.0 mL of sterile saline solution. Initial optical density was measured before incubating the samples at 37 °C. Spectrophotometric readings (spectrophotometer - Model 722) at the dye's wavelength of maximum absorption (RR198 = 518 nm, RY176 = 429 nm, RG19 = 636 nm, RO122 = 493 nm, RR195 = 542nm, and RV1 = 545 nm) were taken periodically over a 48-hour period. Subsequently, the concentration of dye per gram of mycelia and corresponding biosorption percentage were computed using the following formulas [17]:

$$Biosorption\ (\%) = \frac{(A-B)}{A} \times 100$$

$$Qe = A - B \times \frac{V}{M}$$

Where,
Q_e = concentration of dye at equilibrium
A = Initial concentration of dye in solution
B = Final concentration of dye in solution
V = volume of solution in millilitres, and
M = quantity of biomass.

Biodecolourisation was assessed using the cell-free supernatant obtained after mycelial removal. In sterilised test tubes, 9.0 mL of the supernatant was mixed with 1.0 mL of dye wastewater and stirred. The initial absorbance was measured at each dye's wavelength of maximum absorption, which was then followed by incubation at 37 °C. Absorbance readings were taken at 24-hour intervals for 48 hours. Enzyme-mediated dye removal was quantified using the equation presented below [16,17]:

$$Biodecolourisation\ (\%) = \frac{(A-B)}{A} \times 100$$

Where,
A = Initial concentration of the dye in solution
B = Final concentration of dye in solution after enzyme activity

All experimental trials were conducted in three replicates, and the data obtained were expressed as the mean with corresponding standard errors and analysed using the IBM SPSS statistical package (version 26) to determine statistical significance.

RESULTS

A total of four distinct fungal species were isolated from the dye-contaminated soil. Morphological differentiation observed through both macroscopic colony features and microscopic hyphal structures indicated variability among the species (**Fig. 1**).

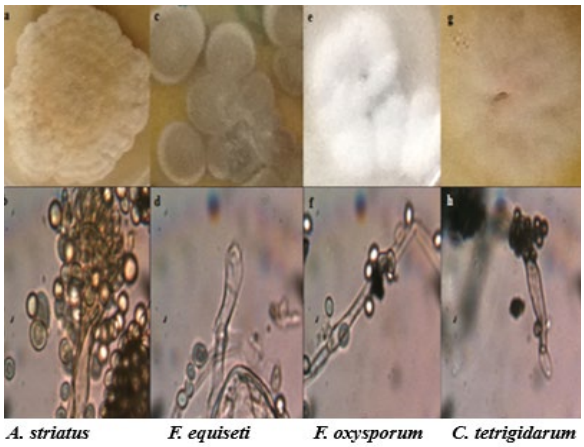

Fig. 1. Colony and microscopic views of fungal species isolated from dye-contaminated soil of Kofar Na'isa Dye Pit, Kano, Nigeria (mag. ×1/3 for colonies and mag. ×100 for microscopy).

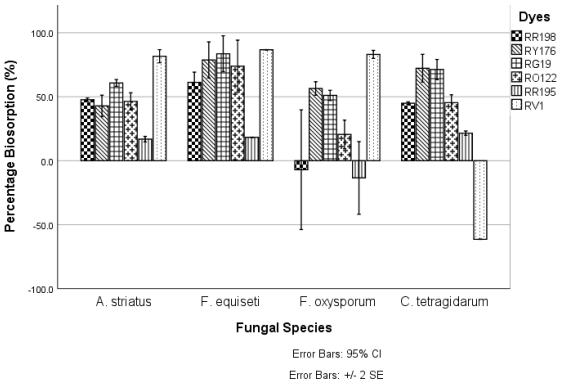

Fig. 2. Percentage biosorption of dyes by fungal species within 48 Hours.

In the fungal biosorption, the highest dye absorption by the species was recorded after 48 hours of inoculation, except for F. oxysporum, which attained saturation after 24 hours (**Fig. 2**). Statistically, there was no statistical difference in dye absorption by individual species within 0-48 hours. However, there was a statistical difference in dye absorption among the four species (0.037) at the 0.05 level. In the decolourisation assay by the fungal species, the maximum decolourisation of the dyes was observed after 24 hours of incubation; however, a negative decolourisation was observed in some of the dyes after 48 hours. Statistically, there was no statistical difference in dye removal by individual species within 0-48 hours. However, there was a statistical difference in dye removal among the four species (0.013) at the 0.05 level (**Fig. 3**).

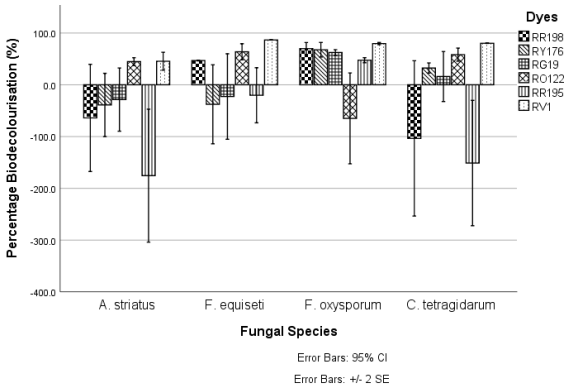

Fig. 3. Percentage biodecolourisation of dyes by fungal Species within 48 hours.

DISCUSSION

Numerous studies have demonstrated that certain soil-dwelling microfungi produce ligninolytic enzymes capable of breaking down lignocellulosic substrates and detoxifying xenobiotic substances like industrial dyes [1-5]. Microfungi can biosorb dyes through multiple processes, including complex formation, physical adsorption, precipitation, entrapment within mycelial matrices, surface ionisation driven by ion exchange and hydrogen bonding [1,6,10]. In the present study, the four fungal species isolated from the dye-contaminated soils were *A. striatus, C. tetrigidarum, F. equiseti* and *F. oxysporum* (**Fig. 1**).

All the fungal species had the ability to biosorb and bleach the dyes at varying levels (Figures 2 and 3). Fungi have been found to be very effective in the decolourisation and degradation of textile wastewater because of the presence of various non-selective enzymatic systems, which can act upon a wide range of substrates, enabling them to survive under harsh conditions [7,8,11]. The secretion of laccase, lignin peroxidases, and manganese peroxidase helps them in degrading the recalcitrant components of the wastewater [7].

Research is still ongoing on the remediation of synthetic dyes by members of the genus *Aspergillus*. To date, there are no published articles on remediation by *Aspergillus straitus*, though many related species from the same genus have proven to be excellent bioremediators of synthetic dyes and other organic compounds. Mathur et al. [18] reported *A. lentulus* and *A. fumigatus* in the remediation of reactive dyes via bioaccumulation and biosorption. Tisma et al. [19] used *Aspergillus ochraceus* for the treatment of wastes from the food industry. They also observed the production of various exo-enzymes that transformed different dyes.

Non-ligninolytic fungi such as *Aspergillus niger* attain biosorption through production of dead biomass, which acts as an adsorbent [1,11]. Dead biomass of *Aspergillus niger* has been effectively utilized as a biosorbent, with an optimum pH of 5.0 [2,4]. Previously, it was reported that *Aspergillus* species removed colour by 62.6%, 69.8%, and 87.0% from reactive black, reactive red, and remazol black B dyes solutions, respectively [9,20]. Akar et al. [21] reported *A. paraciticus* to biosorb RR198 dye within 50 minutes at a maximum dye biosorption capacity of $1.03x10^{-4}$ mol g^{-1}. Congo red was completely remediated by *A. niger*; acid red 151 and orange II by *A. flavus* [1]. *A. niger* was also reported to have remediated reactive brilliant red K-2BP to 94.7% in 120 hrs [11]. *A. bombycis* has shown better dye decolourisation of RR31 (94.7%) in a short period of time (12 hrs) as compared to other reported fungal cultures [22]. Singh et al. [23] reported the percentage decolourisation of reactive yellow 17 by *Aspergillus tamari* to be 58.8%. Biosorption of reactive green by *A. niger* was also observed by Kumari and Abraham [24].

During remediation tests for RO122, it was observed that all the species had the potential to biosorb as well as reduce colour intensity of the dyes. Four fungal strains (*A. flavus*, *A. niger*, *A. oryzae*, *A. terreus*) and their consortia showed effective decolourisation of reactive dye orange M2R [12]. Under optimized conditions, degradation by *A. niger* was found to be 93.0% and 80.0% for reactive red 195 and reactive green 11, respectively [2]. Abd El-Rahim et al. [25] observed that *Aspergillus* species have the ability to remediate a wide variety of azo dyes. They also observed that direct violet had a percentage decolourisation range of 71.1-93.3%. It was also reported by Gajera et al. [26] that *A. niger* discoloured reactive violet 5 to 58 % within 12 days. The fungal culture efficiency for dye removal could be affected by several operational conditions such as pH, temperature, concentration, and structure of the dyes, and the oxygen transfer rate [7]. Visvanathan et al. [10] stated that fungal decolourisation is usually accomplished either through adsorption or enzymatic degradation.

The genus *Fusarium* comprises mostly pathogenic species that are capable of producing a wide variety of secondary metabolites; as such, members may display different methods of organic and inorganic matter remediation [27]. Al-Tohamy et al. [28] reported the effective degradation of toxic contaminants from wastewater by yeasts isolated from termite gut, which also revealed the complete removal of reactive blue 5 (RB5) dye within 24 hours due to its unique enzymatic system.
Fusarium oxysporum is a soil-borne pathogenic ascomycete that causes *Fusarium* wilt in plants [29]. It has the ability to break down several organic compounds due to the possession of cellulases; as such, the species may thrive in various industrial effluents and wastewater [9]. Many studies have reported the use of *F. oxysporum* for the remediation of synthetic dyes. For instance, Porri et al. [30] reported 100.0% degradation and detoxification of a glycoconjugate azo dye (GAD). The ability of *F. oxysporum* to decolourise various dyes is well documented in the literature [1,4,31,32,33].

Fusarium equiseti is a known pathogenic microfungus causing a number of deformities in plants, such as crown and root rot, foliar necrosis, yellowing and wilting of leaves, etc. [34]. Most reports in the literature on *F. equiseti* are centered on its mycotoxin metabolism and toxicity [35] as well as the pathogenic effects of the species on plants [36]. *F. equiseti* was found to have a high potential for bioremediating reactive dyes in the present study; previously, the species had not been reported to have such a bioremediative potential.

Candida is a genus that comprises the popularly known yeast species, which have been reported to have great potential in removing various organic contaminants, such as dyes from industrial effluents [37]. Though the species (*Candida tetrigidarum*) used in this study has not been previously reported to have any remediation potential, many other species belonging to the same genus have been reported with such potential. Effective remediation of various synthetic dyes by *Candida* species is well documented in previous literature [38-40]. Fungal species with excellent bioremediation potential for synthetic dyes were isolated from the textile re-dyeing site. All the isolated species displayed effective remediation of the dyes and were identified as: *Aspergillus striatus*, *Candida tetragidarum, Fusarium oxysporum,* and *F. equiseti*.

ABBREVIATIONS

DNA: Deoxyribonucleic acid
PCBs: Polychlorinated biphenyls
LiP: Lignin peroxidase
MnP: Manganese peroxidase
RR198: Reactive red 198
RY176: Reactive yellow 176
RG19: Reactive green 19
RO122: Reactive orange 122
RR195: Reactive red 195
RV1: Reactive violet 1
FASTA: Federal Assets Sale Transfer Act
BLAST: Basic Local Alignment Search Tool
NCBI: National Centre for Biotechnology Information
USA: United States of America
ANOVA: Analysis of variance
mag: magnification
RB5: Reactive blue 5
GAD: Glycoconjugate azo dye.

CONFLICT OF INTERESTS

The authors declare no conflict of interests

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