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Research Article

Marine-derived *Penicillium oxalicum* M6A as a halotolerant biocatalyst for enzymatic biodegradation and detoxification of the azo dye Direct Red 75

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Abstract

The structural complexity and synthetic origin of azo dyes such as Direct Red 75 (DR75) make them environmentally persistent and challenging to remove from industrial effluents. In this study, *Penicillium oxalicum* M6A, a halotolerant marine-derived fungus isolated from the Nigerian coastline, was evaluated for its capacity to biodegrade and detoxify DR75. The influence of pH, temperature, salt, and dye concentration on degradation efficiency was assessed, along with enzymatic activity. Fourier-transform infrared (FTIR) spectroscopy and gas chromatography–mass spectrometry (GC–MS) analyses were employed to identify degradation products and predict metabolic pathways. Toxicity was determined using three bacterial strains and two crop plant seeds. Optimal degradation was achieved at a pH of 5 (90.37%), a temperature of 35°C (66.82%), a dye concentration of 50 mg/L (91.53%), and a NaCl concentration of 4% (67.08%). Enzymatic assays revealed significant upregulation of laccase (24.17 U/mL), azoreductase (13.14 U/mL), and lignin peroxidase (12.54 U/mL), indicating their involvement in dye breakdown**.** FTIR analysis confirmed the disappearance of characteristic azo and sulfonic peaks, while GC–MS identified key metabolites such as 2,3-dihydrobenzofuran, m-hydroquinone, and 6-ethoxy-6-methyl-2-cyclohexenone. Microtoxicity and phytotoxicity assessments revealed that the degradation products of DR75 by *P. oxalicum* M6A did not inhibit the bacterial strains and exhibited low toxicity to the seeds of the crop plants used. These findings establish that *P. oxalicum* M6A can effectively degrade and detoxify DR75, converting it into less toxic metabolites. These observations highlight

Özet

Direct Red 75 (DR75) gibi azo boyaların yapısal karmaşıklığı ve sentetik kökenleri, bunları çevreye kalıcı hale getirir ve endüstriyel atıklardan uzaklaştırılmasını zorlaştırır. Bu çalışmada, Nijerya kıyılarından izole edilen halotolerant deniz kaynaklı bir mantar olan *Penicillium oxalicum* M6A, DR75'i biyolojik olarak parçalama ve detoksifikasyon kapasitesi açısından değerlendirilmiştir. Enzimatik aktivite ile birlikte pH, sıcaklık, tuz ve boya konsantrasyonunun bozunma verimliliği üzerindeki etkisi değerlendirilmiştir. FTIR ve GC-MS analizleri, bozunma ürünlerini tanımlamak ve metabolik yolakları tahmin etmek için kullanılırken, toksisite üç bakteri suşu ve iki mahsul bitkisi tohumu kullanılarak değerlendirilmiştir. Optimum bozunma pH 5 (90,37%), 35°C (66,82%), 50 mg/L boya konsantrasyonu (91,53%) ve %4 NaCl (67,08%) değerlerinde elde edilmiştir. Enzimatik analizler, laccaz (24,17 U/mL), azoredüktaz (13,14 U/mL) ve lignin peroksidaz (12,54 U/mL) enzimlerinin önemli ölçüde yukarı regülasyonunu ortaya çıkarmış ve bunların boya parçalanmasında rol oynadığını göstermiştir**.** FTIR analizi, karakteristik azo ve sülfonik piklerin kaybolduğunu doğrularken, GC-MS, 2,3-dihidrobenzofuran, m-hidrokinon ve 6-etoksi-6-metil-2-sikloheksenon gibi önemli metabolitleri tanımladı. Mikrotoksisite ve fitotoksisite değerlendirmeleri, *P. oxalicum* M6A tarafından DR75'in bozunma ürünlerinin bakteri suşları üzerinde herhangi bir inhibisyon bölgesi göstermediğini ve kullanılan mahsul bitkilerinin tohumları için daha az toksik olduğunu göstermiştir. Bu sonuçlar, *P. oxalicum* M6A'nın DR75'i etkili bir şekilde bozunabileceğini ve detoksifiye edebileceğini, onu daha az toksik metabolitlere

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the biocatalytic potential of *P. oxalicum* M6A for treating dye-contaminated saline wastewater and encourage further scale-up for environmental applications.

dönüştürebileceğini göstermektedir. Bu bulgular, boya ile kirlenmiş tuzlu atık suların arıtılmasında *P. oxalicum* M6A'nın biyokatalitik potansiyelini vurgulamakta ve çevresel uygulamalar için daha fazla ölçeklendirilmesini teşvik etmektedir.

Keywords: *Penicillium oxalicum M6A*, laccase, recalcitrant pollutants, peroxidase, synthetic dyes, ecotoxicity assessment

Introduction

Azo dyes are the largest group of dyes widely used in industries, including textiles, cosmetics, printing, paper, and pharmaceuticals (Vinodhkumar et al., 2013). In the textile industry, dyeing processes generate dye-rich wastewaters containing various functional groups. These dyes interfere with ecological processes such as photosynthesis, posing further environmental and health concerns owing to their carcinogenic and mutagenic properties (Gavril & Hodson, 2007; Basu & Kumar, 2014; Ben Mbarek et al., 2023). Azo dyes are synthetic dyes, and their complex intrinsic structural properties make them challenging to decolorize, especially in industries that utilize sewage systems to treat dyes. Their water solubility and colored, acidic, and reactive nature make these dyes highly resistant to degradation, and they remain unaffected during conventional treatment (Willmott et al., 1998). Biodegradation processes, which employ microbial enzyme transformation and the detoxification of pollutants, have been the subject of numerous investigations (Couto, 2007). Fungi are considered to be more resilient than bacteria and are able to withstand high concentrations of recalcitrant pollutants (Ellouze & Sayadi, 2016). Fungi utilize economical and environmentally benign processes to generate vast quantities of extracellular enzymes with significant industrial potential (Ellouze & Sayadi, 2016; Ozojiofor et al.; 2025). The majority of fungi investigated have been isolated from terrestrial environments such as forests and marshlands, and the diversity of marine fungi has not been extensively studied (Ben-Ali et al., 2020). However, various fungal species are present in marine environments, which are incredibly complex (Amend et al., 2019).

Fungi belonging to the phylum Ascomycota are recognized for their significant role in degrading aromatics and other recalcitrant organic pollutants (Singh et al., 2015). Of these, *Penicillium* spp. are particularly well known for their metabolic versatility, enabling them to colonize a wide range of habitats. This versatility allows them to thrive in some of the most extreme environments on earth, including deep-sea sediments, the poles, highly acidic habitats, and those with extreme temperatures (Frisvad & Samson, 2004). Though typically thought to be decomposers, *Penicillium* spp. possess high hydrocarbon-assimilating capacities with low cosubstrate demands and produce an enormous amount of bioactive metabolites (Houbraken et al., 2011). Their ability to utilize numerous carbon sources reflects their metabolic flexibility and the potential to break down a wide range of substrates. Several reports are available on the potential of *Penicillium oxalicum* to degrade complex compounds, such as textile dyes, food residues, lignocellulosic residues, and hydrocarbons (Singh et al., 2015), as well as detoxify xenobiotic chemicals into less mutagenic or toxic products (Zhang et al., 2013). Due to its halotolerance, *P. oxalicum* presents promising possibilities for treating textile industry wastewaters with high salt concentrations. Saroj et al. (2014) isolated SAR-3 *P. oxalicum*, which was able to degrade a broad spectrum of azo dyes, including Acid Red 183, Direct Blue 15, and Direct Red 75 (DR75).

This study aimed to characterize and detoxify an azo dye, DR75, using a halotolerant *P. oxalicum* M6A isolated from the marine habitat and to determine the degradation products and the toxicity of the dye and its products.

Materials and Methods

DR75, the textile dye used, as shown in Figure 1, was obtained from Glentham Life Science. Veratraldehyde, catechol quinone, 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potato dextrose agar (PDA), Mueller–Hinton agar, and malt extract agar were purchased from Sigma–Aldrich. A 10,000 mg/L stock solution of the dye was prepared and sterilized via filtration through a 0.22 µm filter. The solution was then appropriately diluted to the desired concentration before use.

Isolation of Fungi

The fungal strain *P. oxalicum* M6A employed in this study was previously isolated from samples (woods immersed in seawater, seaweeds, marine plants, pieces of nets, and sands from sea shores) from the Lagos Atlantic Ocean shores (coordinates: 6° 27′ 55.5192″ N and 3° 24′ 23.2128″ E) by the authors Ozojiofor

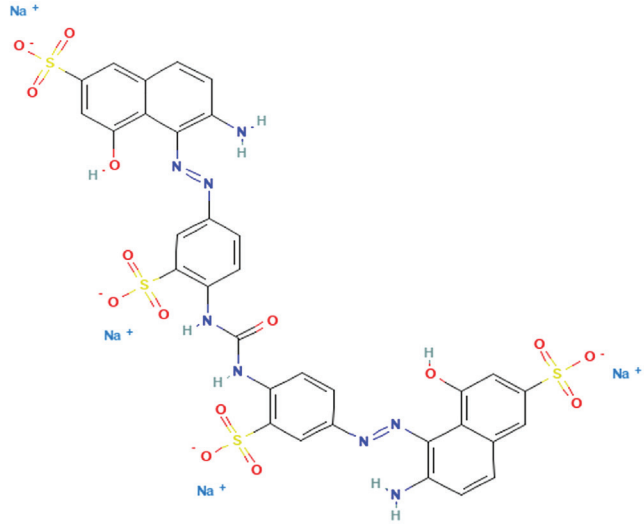

Figure 1. Structure of DR75. DR75 = Direct Red 75.

et al. (2025). The fungus was purified and maintained on 3.9% (w/v) PDA and 1.8% (w/v) malt extract supplemented with 3.4% (w/v) NaCl (Zouari-Mechichi et al., 2016). The gene sequence of *P. oxalicum* M6A can be found in the NCBI GenBank under the accession number PP077349.

Decolorization on Solid and Liquid Media

PDA plates supplemented with chloramphenicol (30 µg/mL) and 200 mg/L of DR75 were inoculated with mycelia placed in the center of each plate (Fernández-Remacha et al., 2022). The plates were incubated for 7 days at room temperature and then examined daily for the presence of halos corresponding to the areas in which the dye was degraded.

For further investigation in the liquid medium potato dextrose broth (PDB), the fungus was inoculated into 10 mL of preautoclaved medium supplemented with 200 mg/L of the dye at 25°C. After centrifuging the liquid medium containing the fungal mycelia for 10 min at 5,000 rpm, aliquots of the supernatant were transferred to fresh tubes and diluted as needed to obtain the desired absorbance. An untreated liquid medium was used as a control for each dye.

The standard curve of each dye was generated at its maximum absorbance wavelength, with concentrations ranging from 0 to 200 mg/L. The percentage of dye in the samples was calculated at different time intervals of 24 h for 5 days using the formula $D = \left(\frac{(AC\text{-}AT)}{AC}\right)$ x 100, where D represents the decolorization rate (%) and A_C and A_T represent the absorbance measured for the control and treated samples, respectively (Rosales et al., 2011).

Enzyme Assay

The decolorized DR75 sample pellet obtained via centrifugation was used to perform enzyme activity assays selected to substantiate the proposed dye degradation pathway. Azo-reductase activity was determined by assaying the decrease in color absorbance of methyl red at 430 nm, and veratryl alcohol oxidase activity by assaying the reduction in color absorbance of veratraldehyde at 310 nm (Phugare et al., 2011). Furthermore, tyrosinase activity was determined by observing the development of catechol quinone at 495 nm (Parshetti et al., 2007). The observation of an increase in absorbance at 420 nm, resulting from the oxidation of the ABTS compound, was used to detect laccase activity. Lignin peroxidase activity was assayed at 310 nm using veratryl alcohol as the substrate (Parshetti et al., 2007). Aldehyde dehydrogenase activity was assayed by monitoring the changes in absorbance owing to the formation of NADH at 340 nm (Murshid & Dhakshinamoorthy, 2021). Enzyme activity was measured spectrophotometrically at 25°C. One unit (U) of enzyme activity was defined as the amount of enzyme required to produce one micromole of the product per minute.

Growth Study of the Isolate at Different Dye Concentrations

The growth of P. oxalicum M6A at different concentrations of DR75 (0.4 g/L, 0.6 g/L, 0.8 g/L, and 1.0 g/L) in PDB supplemented with the textile dye was measured at 600 nm at intervals of 24 h during incubation for 5 days (Al-Tohamy et al., 2023).

Effects of Various Conditions on Dye Degradation by the Selected Isolates

The factors affecting dye decolorization were studied via single-factor optimization to investigate the dye decolorization capability of P. oxalicum M6A. The effects of dye concentrations, pH, salt, and temperature on the degradation of the dye by the fungus were examined. The ability of P. oxalicum M6A to decolorize different concentrations of the dye was analyzed using various initial dye concentrations (50, 100, 200, 300, and 400 mg/L). The dye-containing media were adjusted to different pH levels between 3 and 11 using either 0.1 M NaOH or 0.1 M HCl.

The effects of different temperatures (15–45°C, with an interval of 10°C) were tested in a thermostatic incubator (GEMTOP, ZSH-250 F, China). A range of salt concentrations, from 0% to 5% NaCl, was tested to assess the effects of salinity.

The decolorization efficiency of DR75 was studied, and during the investigation of a particular factor, all other variables were held constant as follows: 200 mg/L DR75, pH 7, 1% salinity, and a temperature of 25°C. The culture underwent static incubation for 120 h. Samples were withdrawn from the flasks at 24 h intervals. All the experiments were conducted in triplicate (Al-Tohamy et al., 2023).

Identification of the Metabolites of Dye Degradation

Extraction and chromatographic investigation of the metabolites were performed to fully understand the degradation pattern and metabolites from the decolorization of the dye. Multiple analytical methods were used, such as gas chromatography–mass spectrometry (GC–MS), Fourier-transform infrared (FTIR) spectroscopy, and ultraviolet–visible (UV–Vis) spectroscopy.

FTIR analysis was conducted at the National Research Institute for Chemical Technology (NARICT), Zaria, Kaduna, Nigeria, using an Agilent Technologies Cary 630 FTIR spectrophotometer to monitor the structural and distinctive changes in the dye.

Dye samples were subjected to FTIR analysis before (without the fungus) and after degradation (with the fungus). The fungus was incubated at 28°C in 25 mL of solution containing the dye and was grown for 7 days. The samples were carefully filtered after 7 days, and the resulting residues were washed three times with distilled water. To remove extra moisture from the test sample, it was held in a hot air oven set at 110°C for 30 min with the introduction of potassium bromide. For analysis, the materials were further ground at a 5:95 (w/w) ratio (Al-Tohamy et al., 2023). For a total of 16 scans, the sample data were recorded in the midinfrared range of 650–4,000 cm^{-1}.

GC–MS was employed to separate and identify the possible active constituents from the dye metabolites. GC–MS was performed at NARICT, Zaria, Kaduna State, using a Shimadzu GC–MS-QP2010 PLUS (Japan) machine.

Lyophilized samples were dissolved in GC-grade methanol and filtered through a syringe filter before being injected into the GC–MS column. One microliter of sample was taken for injection via a standard operating procedure based on the manufacturer's instructions (SHIMADZU GC–MS) (Klatte et al., 2017). Helium was used as the carrier gas. A comparison of the retention times, mass spectra obtained with GC–MS solution software, and fragmentation patterns was performed to identify the metabolites. The fragmentation method was used to determine the structures of the identified compounds, and mass spectrometry analysis provided the m/z values (Al-Tohamy et al., 2023).

Microtoxicity and Phytotoxicity Studies of Dyes and Their Degradation Products

The microtoxicity of the original dye and its degradation product, as determined using GC–MS analysis at a concentration of 200 mg/L, was assessed. The dye was extracted with dichloromethane and then dissolved in sterilized water, and it was tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Bacillus cereus.* The inhibition study was performed on Mueller–Hinton agar plates using the agar well diffusion method. Wells of 0.9 cm in diameter were carefully punched into the media and filled with 200 µL of control (sterilized water) and treated and untreated dye. The experimental study was conducted in triplicate, and the petri dishes were sealed with parafilm and incubated for 24 h at 30°C.

The zone of inhibition was calculated by subtracting the well diameter from the total zone of inhibition, and the results were recorded (Bagewadi et al., 2017; Yang et al., 2016).

The phytotoxicity of the original dye at a concentration of 200 mg/L and the products of dye degradation by the fungus were extracted with dichloromethane and then dissolved in sterilized water. These extracts were evaluated on the seeds of *Zea mays* (maize) and *Vigna unguiculata* (cowpea). The experiments were conducted at room temperature by placing 10 seeds of each crop on a bedded filter paper in a petri dish for germination and irrigating them daily with 10 mL solutions of the respective samples (original dye or treated dye) for 7 days. The control plants were irrigated with distilled water. The toxicity was assessed in terms of percentage germination (%), plumule length (cm), and radicle length (cm) (Bagewadi et al., 2017).

Statistical Analysis

The results were presented as the mean ± standard deviation of three determinations, which were subjected to one-way analysis of variance to compare the means of three or more groups and determine if at least one group was significantly different from the others. The Duncan multiple range test was used to determine which specific groups differed from each other, with a significance level of $p < 0.05$.

Results

The effects of DR75 concentration on the growth of *P. oxalicum* M6A were studied, as shown in Figure 2. The time course study of the impact of different concentrations (0.4, 0.6, 0.8, and 1.0 g/L) of DR75 on the growth of *P. oxalicum* M6A is presented in Figure 2. The maximum growth of *P. oxalicum* M6A was observed at a concentration of 0.8 g/L in DR75 after 5 days of incubation with the dye. The maximum absorption wavelength for DR75 was 520 nm.

The fungus *P. oxalicum* M6A degraded the dye optimally at a pH of 5, with > 70% degradation after 120 h (5 days). The degradation ability of the fungus was time dependent, and it degraded the dye better after 72–120 h (3–5 days) at pH 5–7, with a degradation percentage of > 80%, but degraded poorly at pH 3 and 11. The time course of dye degradation by *P. oxalicum* M6A at different pH values is presented in Figure 3.

P. oxalicum M6A degraded > 60% of DR75 at 35°C after 120 h (5 days) of incubation. The time course of the degradation of the dye by *P. oxalicum* M6A at different temperatures is shown in Figure 4.

The decolorization percentage of DR75 at various dye concentrations (ranging from 50 mg/L to 400 mg/L), as presented in Figure 5, showed that *P. oxalicum* M6A degraded > 90% of DR75 at 50 mg/L after 5 days of incubation, and the degradation percentage decreased with increasing dye concentration.

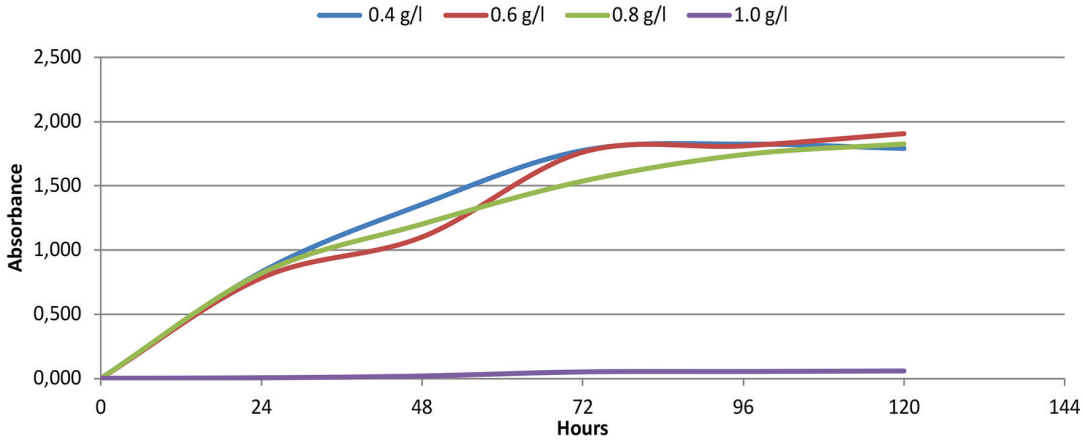

Figure 2. Effect of different concentrations of DR75 on the growth of *Penicillium oxalicum* M6A. DR75 = Direct Red 75.

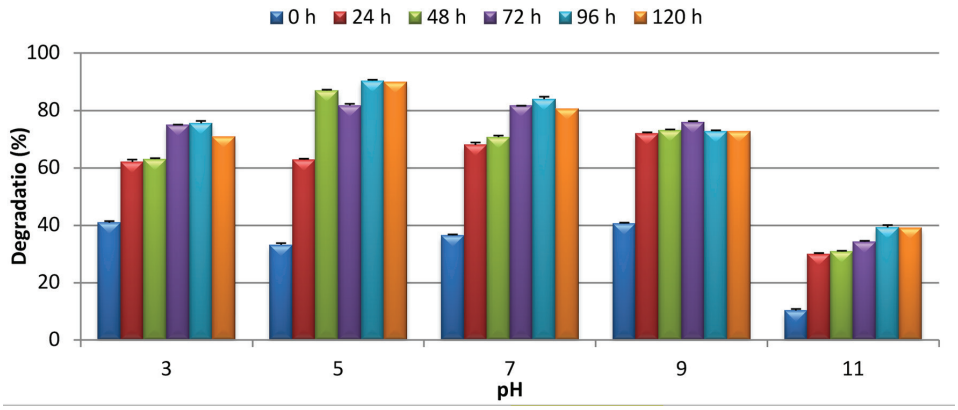

Figure 3. Effect of different pH values on the degradation of DR75 by *Penicillium oxalicum* M6A. Error bars represent the standard deviation of the degradation (%) at a given pH and time. DR75 = Direct Red 75.

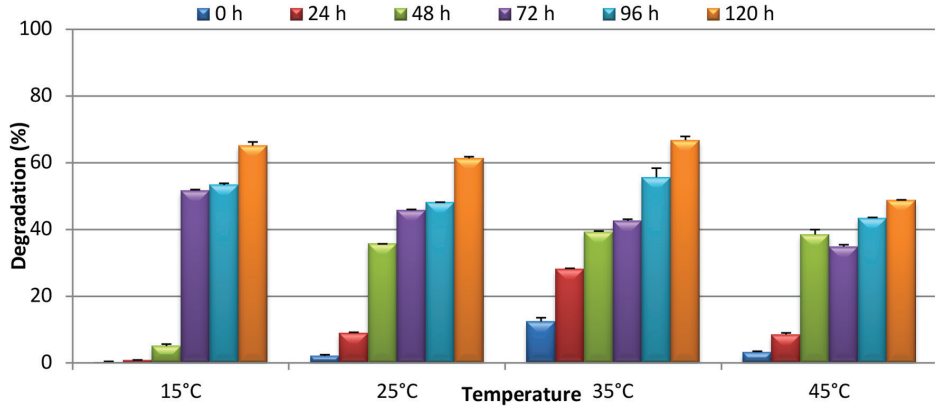

Figure 4. Effect of different temperatures on the degradation of DR75 by *Penicillium oxalicum* M6A. Error bars represent the standard deviation of the degradation (%) at a given temperature and time. DR75 = Direct Red 75.

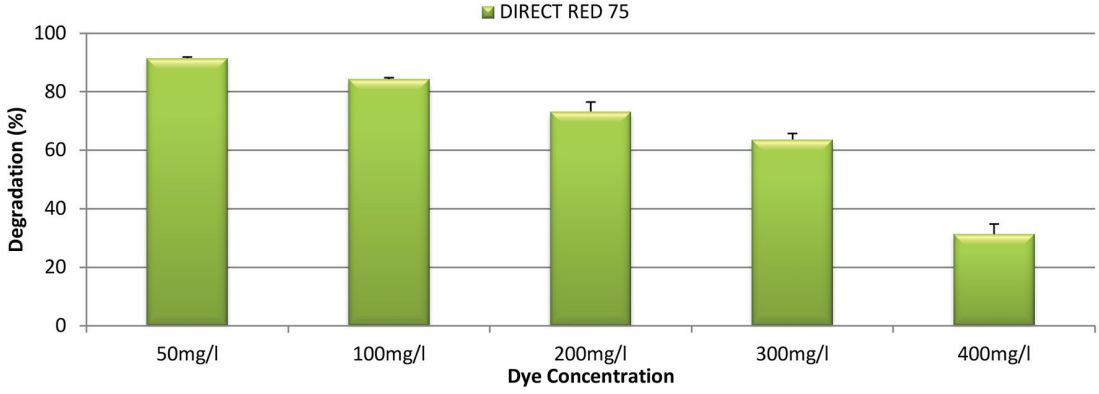

Figure 5. Effect of different dye concentrations on the degradation of DR75 by *Penicillium oxalicum* M6A. Error bars represent the standard deviation of the degradation (%) at a given dye concentration and time. DR75 = Direct Red 75.

The decolorization percentage of DR75 at various salt concentrations (1–5%) is presented in Figure 6. The maximum decolorization rate of *P. oxalicum* M6A for DR75 was 67% at a 4% salt concentration on Day 4 of incubation. The optimum salt concentration for the degradation of DR75 was 1% on Days 2–5.

The activities of different enzymes before and after degradation showed higher activities for laccase (24.17 ± 0.83), azo reductase (13.14 ± 0.87), and lignin peroxidase (12.54 ± 0.33), as presented in Table 1.

FTIR spectral peaks for the degraded and nondegraded DR75 are presented in Figure 7a and b. FTIR spectra of the untreated dye (Figure 7a) exhibited significant peaks corresponding to azo linkages (–N=N–), C=C aromatic stretching, and vibrations of sulfonic groups, indicative of the complicated structure of the azo dye.

The GC–MS revealed 17 peaks and 10 possible metabolites, namely, 2,3-dihydrobenzofuran, m-hydroquinone, n-phenylacetic acid, 1-pentadecanecarboxylic acid, cis-9-hexadecenal, 13-octadecenal,

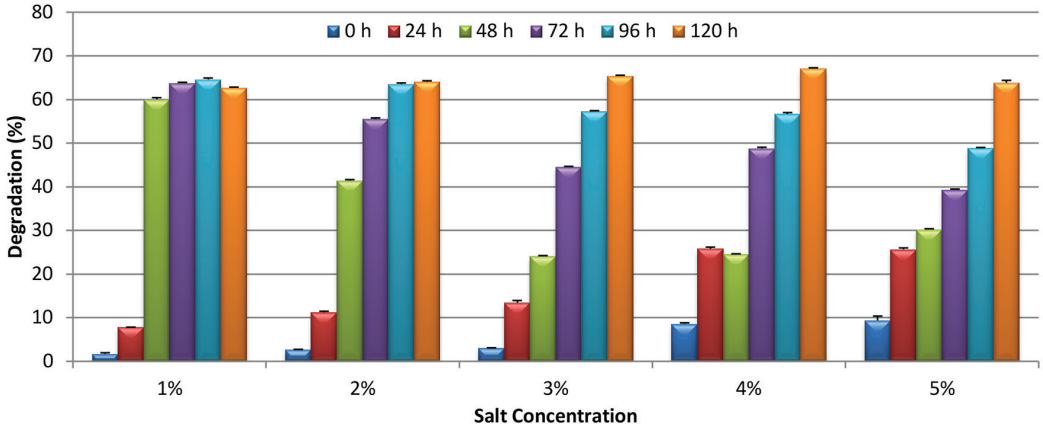

Figure 6. Effect of different salt concentrations on the degradation of DR75 by *Penicillium oxalicum* M6A. Error bars represent the standard deviation of the degradation (%) at a given salt concentration and time. DR75 = Direct Red 75.

Table 1. Enzyme activities before and after degradation of 50 mg/L of DR75 by *Penicillium oxalicum* M6A.

Enzyme	Activity before degradation (U/mL)	Activity after degradation (U/mL)	Negative control (U/mL)
Laccase	3.67 ± 0.18	24.17 ± 0.83	5.43 ± 0.22
Azo reductase	5.65 ± 0.67	13.14 ± 0.87	6.12 ± 0.38
Lignin peroxidase	2.34 ± 0.77	12.54 ± 0.33	3.56 ± 0.27
Veratryl alcohol oxidase	1.67 ± 0.06	2.43 ± 0.92	2.11 ± 0.23
Tyrosinase	1.34 ± 0.44	3.54 ± 0.61	2.50 ± 0.54
Aldehyde dehydrogenase	ND	3.20 ± 0.66	2.55 ± 0.17

DR = Direct Red 75, ND = not detected.

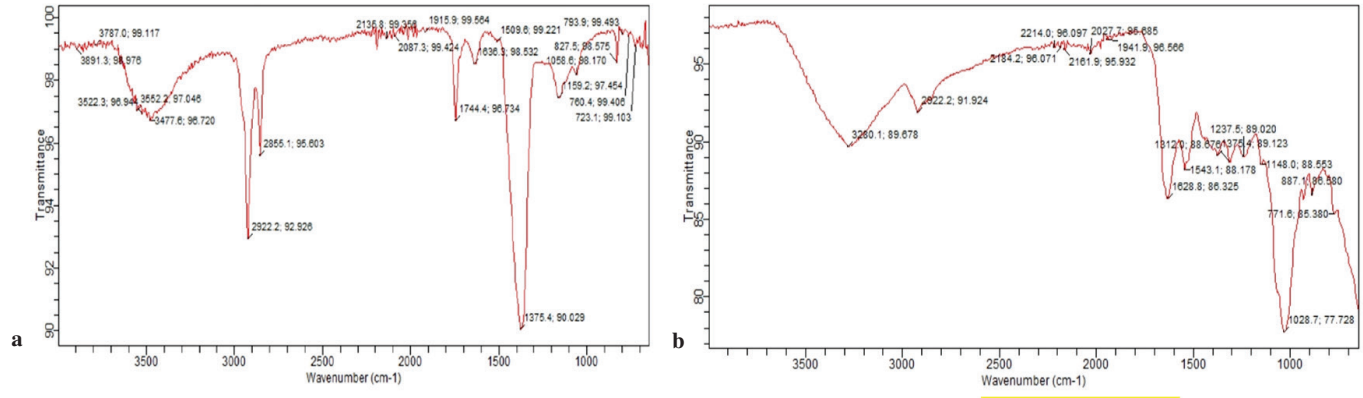

Figure 7. (a) FTIR spectrum of nondegraded DR75 (control); (b) FTIR spectrum of DR75 degraded by *Penicillium oxalicum* M6A. FTIR = Fourier-transform infrared spectroscopy; DR75 = Direct Red 75.

14-octadecenoic acid methyl ester, 9,17-octadecadienal, 13-docosenamide, and 6-ethoxy-6-methyl-2-cyclohexenone. The GC–MS peaks and spectra of the key metabolites of degraded DR75 are presented in Figures 8 and 9. The FTIR spectrum (Figure 7a and 7b) and GC–MS chromatogram (Figures 8 and 9) jointly validated the effective biodegradation of DR75 by *P. oxalicum* M6A. The degraded sample (Figure 7b) exhibited a loss of peaks corresponding to azo and sulfonic groups, along with the emergence of additional bands indicative of carbonyl (C=O) stretching (approximately 1,700 cm^{-1}), hydroxyl (–OH), and aliphatic C–H stretches, which signified dye degradation and the generation of simpler metabolites.

The microtoxicity results of the dye and its degradation products on *S. aureus*, *P. aeruginosa*, and *B. cereus* are presented in Table 2.

The results showed that the dye inhibited the growth of the bacteria used in this study more effectively than its degradation products by *P. oxalicum* M6A. The degradation products of DR75 by the fungus showed no zone of inhibition on the three bacterial strains. The phytotoxicity results of the dye and its degradation products on the seeds of *Z. mays* (Maize) and *V. unguiculata* (Cowpea) are presented in Table 3.

Discussion

Dye degradation results in the formation of new peaks, as Al-Tohamy et al. (2023) reported the appearance of new peaks during the decolorization of the reactive azo dye Reactive Black 5 (RB5) using the novel halotolerant yeast consortium HYCA, which comprised *Meyerozyma guilliermondii* (*M. guilliermondii)* SSA-

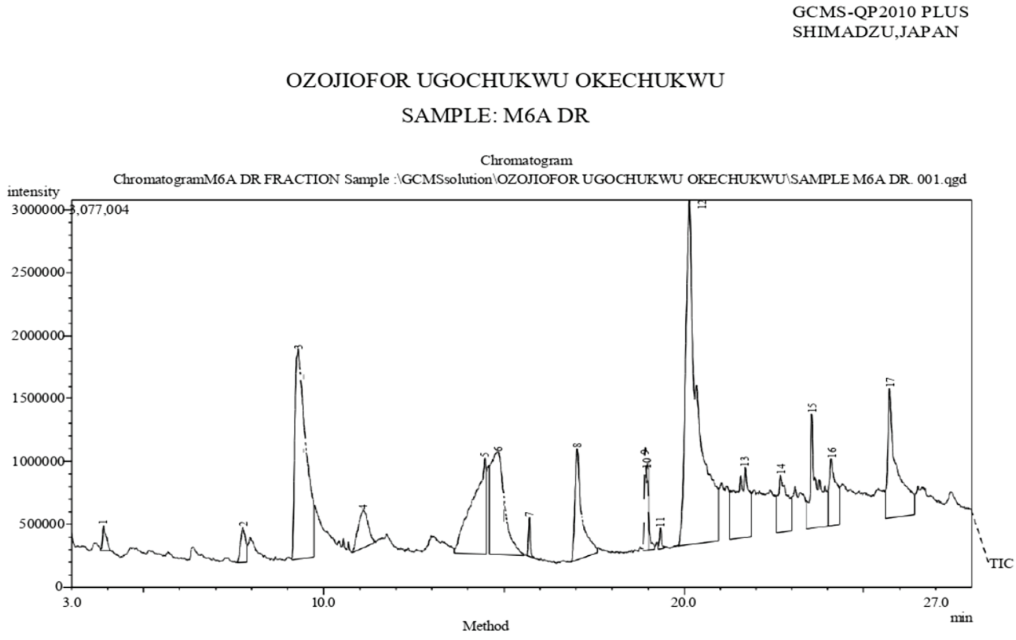

Figure 8. GC–MS chromatogram of DR75 degraded by *Penicillium oxalicum* M6A. GC–MS = gas chromatography–mass spectrometry.

Table 2. Zone of inhibition (cm) of DR75 and its products of degradation by *Penicillium oxalicum M6A* on certain bacterial strains.

Bacterial strain	Control	DR	DR-P
Staphylococcus aureus	-	0.6 ± 0.2	-
Pseudomonas aeruginosa	-	0.23 ± 0.1	-
Bacillus cereus	-	0.7 ± 0.3	-

DR = Direct Red 75; DR-P = Direct Red Product.

Table 3. Phytotoxicity assessment of DR75 and its products of degradation by *Penicillium oxalicum M6A* on *Vigna unguiculata* and *Zea mays.*

Parameters	*Vigna unguiculata*			*Zea mays*		
	Control	DR	Products	Control	DR	Products
Germination (%)	90 ± 16^{b}	43.3 ± 5.8^{a}	70.0 ± 0^{b}	100 ± 0^{c}	66.7 ± 5.8^{a}	90.0 ± 10^{b}
Plumule (cm)	4.4 ± 0.9^{b}	2.4 ± 1.0^{a}	4.4 ± 1.0^{b}	6.4 ± 0.9^{c}	2.0 ± 0.6^{a}	3.2 ± 0.8^{b}
Radicle (cm)	2.9 ± 0.7^{b}	1.2 ± 0.3^{a}	2.5 ± 0.9^{b}	2.5 ± 0.3^{c}	1.0 ± 0.3^{a}	1.5 ± 0.5^{b}

Superscript (a, b, c…) indicates significant difference across the rows at a 95% confidence interval. DR = Direct Red 75; DR-P = Direct Red Product.

1547 and *Sterigmatomyces halophilus* SSA-1575. Furthermore, Gharieb et al. (2020) documented the appearance of new peaks for DR75 (552–240 nm) and Reactive Red (534–244 nm), which were degraded by *M. guilliermondii* and *Naganishia diffluens,* respectively. The most common technique employed for assessing dye degradation is UV–Vis spectroscopy. There was an obvious association between dye degradation and the observed absorption peaks. If a dye's absorption peak disappears and a new peak appears in the UV–Vis range, it is indicative of dye degradation.

Fungal growth in media supplemented with dyes is strongly affected by the structure and concentration of the dye used. At low dye concentrations, some fungi may exhibit better growth because the enzymes involved in dye degradation can break down the dye more efficiently. However, at high concentrations, the dye becomes toxic to the fungi, inhibiting their growth in media supplemented with the dye by blocking the active sites of enzymes involved in the detoxification process (Li et al., 2019).

In a study on the growth of *Aspergillus niger* in Congo Red, maximum growth was observed at a concentration of 0.25 g/L. As the initial dye concentration increased to 1 g/L, the growth rate decreased significantly owing to exacerbated toxicity (Asses et al., 2018).

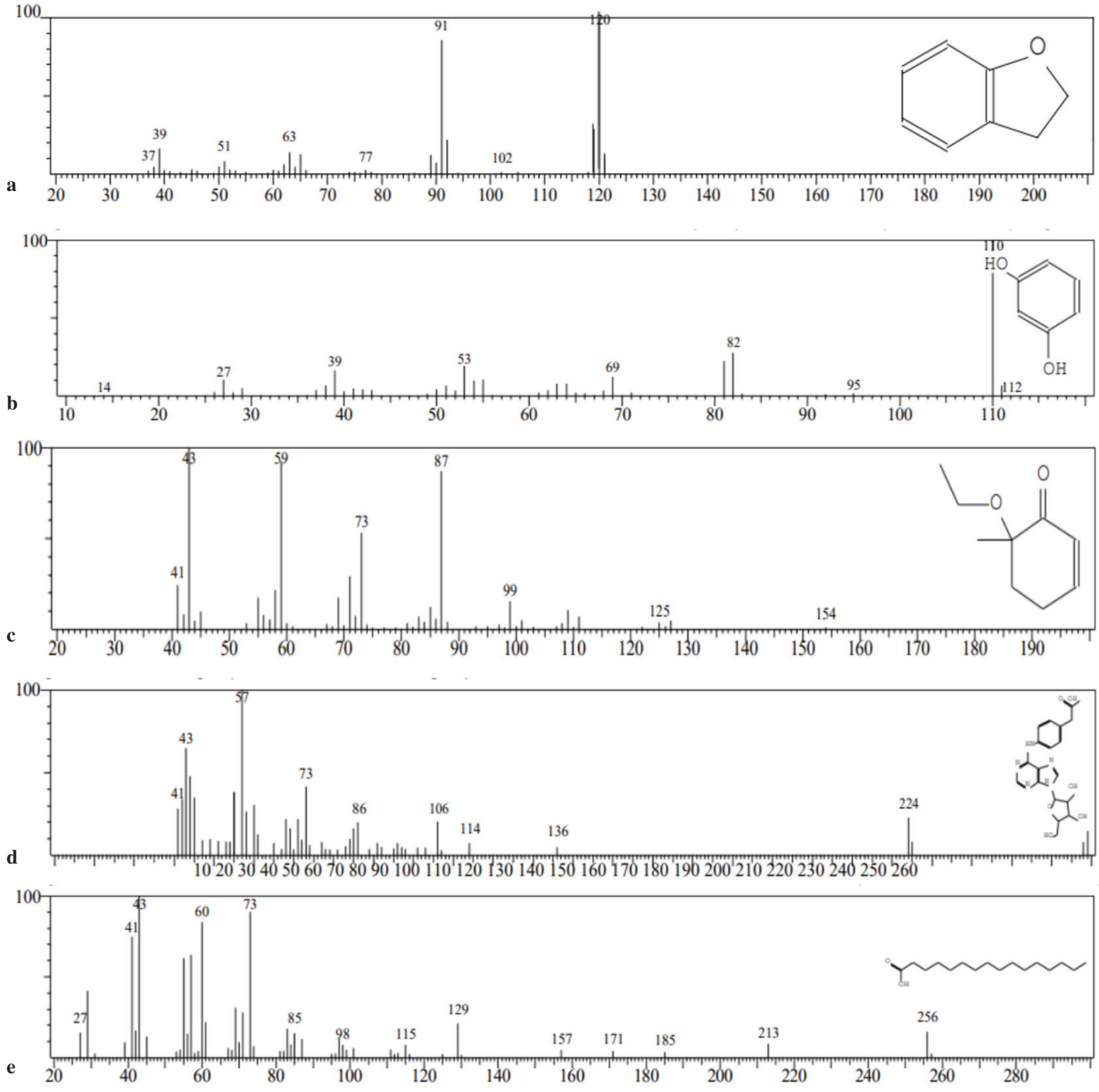

Figure 9. Identification of metabolites of DR75 via GC–MS. (a) 2,3-dihydrobenzofuran, mass peak (m/z) 120; (b) m-hydroquinone, mass peak (m/z) 110; (c) 6-ethoxy-6-methyl-2-cyclohexenone, mass peak (m/z) 154; (d) n-phenylacetic acid, mass peak (m/z) 401; (e) 1-pentadecanecarboxylic acid, mass peak (m/z) 256. DR75 = Direct Red 75; GC–MS = gas chromatography–mass spectrometry.

In a study by Wang et al. (2017) on the decolorization of Congo Red by *Ceriporia lacerate,* the authors reported maximum growth rate at a dye concentration of 0.5 g/L. These published works agree with the findings of this study. The observed growth rate decreased with increased dye concentration. Furthermore, as the number of dye molecules per unit volume increased, fungal growth declined because of increased stress, and the enzyme degradation ability decreased owing to a reduction in the amount of the enzyme (Cheng et al*.,* 2012; Yang et al*.,* 2012).

Erum and Ahmed (2011) reported maximum growth for *A. niger* SA1, *A. flavus* SA2, and *A. terreus* SA3 in media supplemented with Acid Red 151 at a dye concentration of 50 mg/L. Akdogan et al. (2014) obtained maximum growth for two fungi, *Coprinus plicatilis* and *Lentinus crinitus*, when incubated in media supplemented with Remazol Brilliant Blue R at concentrations of 0.05 g/L and 0.25 g/L, respectively. These reports are not in agreement with the present study, and this discrepancy could be attributed to the fact that the fungi used in the previous study were not derived from marine environments and could not grow at high dye concentrations.

pH is a crucial factor in the degradation of dyes as different enzymes work optimally at specific pH levels. The degradation of DR75 by *P. oxalicum* M6A was time dependent and decreased with increasing pH. Gharieb et al. (2020) documented the maximum decolorization percentage (80.3%) for DR75 degraded by *M. guilliermondii* at pH 5 and that of Reactive Red degraded by *N. diffluens* (70.8%) at pH 7, and their work agrees with the present study. In this investigation, the decolorization percentage for DR75 was the lowest at the two extremes of pH 3 and 11. In the decolorization activity of Direct Violet by *Aspergillus fumigatus*, the extremes of decolorization were observed from 94% at pH 5.5 to 47% at pH 3 (El-Sayeh, 2010). The extremes of pH observed in this study, at 3 and 11, may not favor the enzymes involved in dye degradation.

According to Trevizani et al. (2018), a slightly acidic pH has an impact on growth, resulting in a small increase in degradation activity, as observed in this study. This finding can be explained by the nature of the azo dye used in this research. Decolorization is slower at extreme pH values as dye cations are less able to interact owing to the massive accumulation of H^+ and OH^- ions. At slightly elevated pH values, the electrostatic attraction force between the negatively charged membrane surface of fungi and positively charged dye cations is strong, which enhances the transition of dye molecules through the cell membrane, thereby augmenting decolorization (Martorell et al., 2017). For DR75, the enzymatic activity peaked at a pH of 5, which might have facilitated improved binding of the enzyme's active site to the dye surface, leading to decolorization (Cheng et al*.,* 2012). The dye decolorization efficiency is largely influenced by pH, with the normal range of color removal typically between pH 6 and 10 (Kilic et al*.,* 2007).

The DR75 used in this study is an azo dye. The biological reduction of azo bonds has been reported to cause an increase in pH as microorganisms form aromatic amine metabolites, which are more alkaline than the original azo compound (Ikram et al., 2022), explaining the inability of *P. oxalicum* M6A employed in this study to degrade DR75 effectively at alkaline pH.

Temperature is a crucial factor that influences microbial growth, survival, and metabolism. Temperature considerably affects all microbial survival processes, including those involved in water and soil remediation. In this study, the optimum temperature for the degradation of DR75 by *P. oxalicum* M6A was 35°C. Al-Tohamy et al. (2023) observed that the optimum decolorization efficiency of the halotolerant consortium HYC in terms of temperature was 35°C (87%). Furthermore, Gharieb et al. (2020) stated that *M. guilliermondii* and *N. diffluens* decolorized DR75 and Reactive Red most effectively at 30°C, with 85.3% and 80.8% color reduction, respectively. Both of these studies agree with our work.

Feng et al. (2023) opined that the optimal temperature for the degradation of azo dye by the marine-derived fungus *M. guilliermondii* A4 was 35°C. In addition, Ren and Osborne (2020) demonstrated that *P. oxalicum* RJJ-2 degraded 84.88% of erythromycin after 96 h of incubation at 35°C, although this study focused on pharmaceuticals rather than dyes.

Based on the experimental findings, when the fungus was exposed to 50 mg/L of DR75, a degradation rate of 91% was achieved. During the same period, the degradation efficiency of the fungus decreased when the dye concentration was > 50 mg/L. At a dye concentration of 400 mg/L, the degradation rate was found to be the lowest at 31%.

The degradation rate of RB5 by a yeast consortium of *S. halophilus* and *M. guilliermondii* decreased with increasing dye concentration from 50 mg/L to 400 mg/L (Al-Tohamy et al., 2023). The observed decrease in degradation efficiency could be attributed to the toxicity of the dye, which might have inhibited the enzyme activities of the fungus by blocking the active sites of the enzymes involved in dye degradation (Ameenudeen et al., 2021). A study conducted by Kalyani et al. (2009) found that azo dyes at high concentrations significantly inhibited microbial growth owing to the presence of sulfonic acid (SO_3H) groups on the aromatic rings of the reactive groups.

In another study, Gharieb et al. (2020) identified that the biodegradation of DR75 by *M. guilliermondii* and *N. diffluens* decreased significantly with increasing dye concentration (up to 1,500 mg/L) and that increasing the dye concentration to > 50 mg/L resulted in a marked decline in biodegradation. Fetyan et al. (2016) reported that *Saccharomyces cerevisiae* was highly effective at decolorizing Direct Blue 71, reaching a maximum activity of 100%. The decolorization ability decreased at concentrations > 200 mg/L, which may be due to the toxicity of high dye concentrations on the yeast. Both studies agree with this work, and this observation could also be explained by the insufficient biomass concentration for the uptake of the dye by the organisms at higher concentrations (Sumathi & Manju, 2020).

The consortium of *S. halophilus* and *M. guilliermondii* HYC showed degradation rates for RB5 ranging from 88.1% to 9.2% at

salinity levels of 0–5% (Al-Tohamy et al., 2023). An increase in the salt level resulted in a considerable decrease in enzyme activity, eventually leading to total inactivation (Sarkar et al., 2020), as hypersalinity can cause plasmolysis and cellular inactivity (He et al., 2017). The decolorization of DR75 and Reactive Red decreased from 77.9% and 53.5% to 20.87% and 7.99%, respectively, when the concentration of NaCl was increased from 0.0% to 10.0% for the yeasts *M. guilliermondii* and *N. diffluens*, (Gharieb et al., 2020). This difference may be the result of elevated external osmotic pressure, as suggested by Jafari et al. (2014), and these studies agree with this work.

During the degradation of DR75 by *P. oxalicum* M6A, a considerable increase was noted in the activities of enzymes such as laccase, azoreductase, and lignin peroxidase, indicating their prominent roles in the cleavage of azo bonds and aromatic rings. Minimal increases in tyrosinase and veratryl alcohol oxidase activity were observed, suggesting their involvement in hydroxylation and other oxidation reactions. Aldehyde dehydrogenase, which was previously undetectable owing to degradation, became active after degradation, facilitating the detoxification of aldehyde intermediates. Such enzymatic activity supports the proposed degradation pathway and underscores the robust bioremediation capacity of *P. oxalicum* M6A.

FTIR analysis served as a powerful tool to confirm the structural alterations in DR75 before and after biodegradation by *P. oxalicum* M6A. In this study, a spectral comparison of the control (Figure 7a) and the degraded dye (Figure 7b) revealed certain key changes in functional groups, indicating chemical alterations upon fungal treatment. In the FTIR spectrum of the nondegraded DR75, bands were observed at approximately 1,620–1,640 cm^{-1}, which could be attributed to C=C stretching vibrations of the aromatic rings. Moreover, the peaks at 1,500–1,530 cm^{-1} correspond to azo (–N=N–) stretching, which is characteristic of azo dyes and agrees with the work of Saratale et al. (2011). The maxima at the region 1,020–1,080 cm^{-1} results from S=O stretching of the sulfonic acid groups, accounting for the solubility of the dyes (Saratale et al., 2009). In addition, the broad band at 3,400 cm^{-1} indicates O–H or N–H stretching from phenolic or amine groups. Upon the degradation of *P. oxalicum* M6A, the FTIR spectrum demonstrated notable depletion or disappearance of the azo bond peak (approximately 1,500 cm^{-1}), suggesting successful cleavage of the bond, which is often the initial step of azo dye decolorization, as reported by Rai et al. (2005). Attenuation of the C=C aromatic peaks and the appearance of new peaks at 1,700–1,740 cm^{-1} owing to C=O stretching suggests oxidative cleavage of the aromatic ring and the formation of carboxylic acid or aldehyde functional groups (Kalyani et al., 2009; Parshetti et al., 2007). This finding supports the activity of ligninolytic enzymes, such as laccases and lignin peroxidases, which have been documented to catalyze aromatic ring degradation and oxidative decoupling (Pointing, 2001).

In addition, the reduction in S=O stretching peaks at approximately 1,050 cm^{-1} of the degraded sample implies desulfonation, which is a necessary step to alleviate the toxicity of the dye and enhance its biodegradability (Ali et al., 2009). The broadening of the band at 3,400 cm^{-1} may also justify the formation of hydroxylated or aminated intermediates by microbial degradation (Singh et al., 2015).

These shifts observed via FTIR spectroscopy agree with the identification of numerous metabolites via GC–MS. 2,3-dihydrobenzofuran and m-hydroquinone are oxygenated aromatic derivatives, indicating oxidative cleavage of the aromatic rings of the dye. These metabolites possess hydroxyl groups in their structures and conform to the FTIR-determined –OH and aromatic C–O stretching bands (1,220–1,260 cm^{-1}), confirming partial ring opening and functionalization (Singh et al., 2015). For 6-ethoxy-6-methyl-2-cyclohexenone, the presence of ketone (C=O) and ether (C–O–C) functional groups in the compound is evidenced by FTIR bands at approximately 1,710 cm^{-1} and 1,050–1,150 cm^{-1}, reflecting carbonyl group formation upon dye degradation. N-phenylacetic acid, a carboxylic acid derivative with an aromatic ring, follows aromatic cleavage after oxidation, consistent with FTIR peaks for carboxylic functionality, and is indicative of oxidative process–mediated degradation. Long-chain fatty acid derivatives, such as 1-pentadecanecarboxylic acid, cis-9-hexadecenal, 13-octadecenal, and 14-octadecenoic acid methyl ester, signify the formation of aliphatic products, reflected in FTIR spectroscopy as broad C–H stretches (2,850–2,950 cm^{-1}) and C=O stretches of esters and aldehydes (1,700–1,740 cm^{-1}). These metabolites suggest extensive structural fragmentation and probable catabolism of fungal lipids during dye degradation (Rai et al., 2005; Kaushik & Malik, 2009). 9,17-Octadecadienal and 13-docosenamide are aliphatic amides and aldehydes containing unsaturation, and their presence indicates the cleavage of complex dye molecules into long-chain intermediates, as inferred from FTIR peaks for C=N and C=O bonds. m-Hydroquinone and 2,3-dihydrobenzofuran are of immense significance as they are known to be low-toxicity aromatic intermediates obtained via microbial azo bond cleavage and aromatic amines, which agrees with the findings of Telke et al. (2010).

The spectral alterations and GC–MS results confirm that *P. oxalicum* M6A decolorized DR75 via azo bond cleavage, aromatic ring biodegradation, and modification of side-chain substituents, thereby validating the detoxification potential of the fungus. These findings concur with earlier observations on fungal dye degradation (Revankar & Lele, 2007; Kaushik & Malik, 2009).

In this study, a possible degradation pathway was proposed based on the GC–MS metabolites and enzymes identified, as presented in Figure 10.

The results of the microtoxicity assessment showed that the degradation products of DR5 had no inhibitory effect on any of the three bacterial strains tested. The nondegraded dye exhibited the smallest zone of inhibition, measuring 0.23 cm, against *P. aeruginosa*. This finding indicates that the fungus was able to break down the dye into less toxic products. An apparent zone was not seen outside the wells containing the degraded dye or the control as the test microorganisms were able to withstand the reduced toxicity of the dye products. Zubbair et al. (2020) reported the

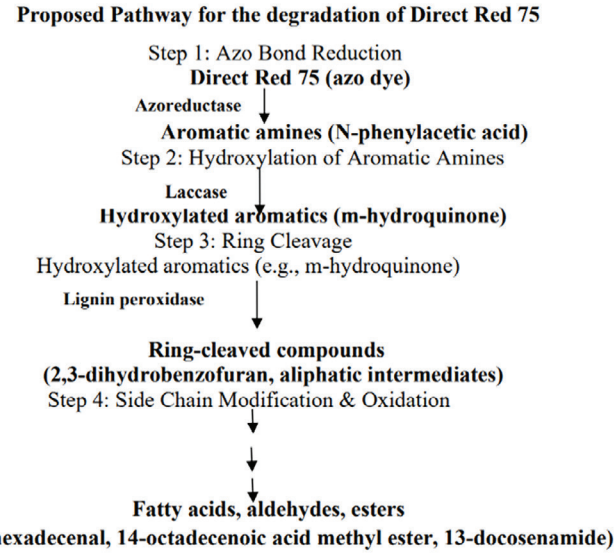

Figure 10. Proposed pathway for the degradation of DR75 (azo dye). DR75 = Direct Red 75.

microtoxicity of reactive black to *P. aeruginosa, Escherichia coli*, and *Klebsiella pneumoniae*. The results showed that *P. aeruginosa* and *E. coli* grew within clear zones measuring 6 mm and 3 mm, respectively; in contrast, *K. pneumoniae* demonstrated resistance to dye toxicity.

Phytotoxicity evaluation revealed that the percentage germination of the plant seeds of the two crops was highest for seeds irrigated with the control (sterilized water) (100%), followed by the degradation products of the dye by the fungus. In contrast, the percentage of germinated seeds was lowest for the dye. The rate of germination was highest for the seeds of *Z. mays* irrigated with the degradation product of DR75 (90 ± 10). The plumule and radicle lengths of the plant seeds of *Z. mays* and *V. unguiculata* irrigated with the degradation products of the dye were statistically significant and higher than those of the seeds irrigated with the nondegraded dye.

These findings demonstrate that the dye was detoxified and that the phytotoxicity of industrial effluents containing these dyes can be reduced by treating them with the fungus. In the study by Bagewadi et al. (2017), a phytotoxicity assessment of MG, Congo Red, Methylene Blue, and their degradation products revealed that the original dye significantly inhibited the germination of *P. mungo* seeds compared with its degradation products and the control treatment (sterilized water). This finding is consistent with the results of this research. In this investigation, seeds irrigated with the products of dye degradation exhibited better germination than those irrigated with the original dye. As azo dye is complex and robust, it exerted a greater inhibitory effect on *V. unguiculata*, as demonstrated by the greater toxicity of DR75. When Reactive Black dye was broken down by *Ganoderma tsugae,* the untreated dye prevented maize and bean seeds from sprouting. In contrast, the degraded dye did not, due to its reduced toxicity (Zubbair et al., 2020).

Conclusion

This study confirms the potent biocatalytic capacity of the marine-derived, halotolerant fungus *P. oxalicum* M6A in degrading and detoxifying the recalcitrant azo dye DR75. The fungus effectively reduced the dye concentration under saline conditions, driven by elevated activities of key oxidative enzymes, particularly of laccase, azoreductase, and lignin peroxidase, which resulted in azo bond cleavage and disruption of aromatic structure. Confirmation via spectroscopic (FTIR) and chromatographic (GC–MS) analyses revealed extensive structural modification of the dye, resulting in the formation of low-toxicity intermediates. Ecotoxicological assessments further established the environmental compatibility of this bioprocess. These results position *P. oxalicum* M6A as a promising candidate for the bioremediation of dye-laden saline wastewater. Future work should focus on pilot-scale validation with wastewater systems, genome-level elucidation of enzyme pathways, and optimization of continuous flow systems for industrial applications.

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Ethics

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Data Sharing Statement: All data are available within the study.

Footnotes

Author Contributions: Conceptualization: U.O.O., and M.S.A.; Design/methodology: U.O.O., and N.E.E.; Execution/investigation: U.O.O., N.E.E., and A.A.H.; Resources/materials: U.O.O.; Data acquisition: U.O.O., and N.E.E.; Data analysis/interpretation: U.O.O., and M.S.A.; Writing – original draft: U.O.O., and M.S.A.; Writing – review & editing/critical revision: M.S.A., N.E.E., and A.A.H.

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