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Research Article

Halotolerant Biodegradation and Detoxification of Synthetic Dyes by Marine-Derived *Penicillium oxalicum* M6A: Metabolite Profiling and Ecotoxicity Assessment

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Abstract

The complex aromatic structures of synthetic dyes in contaminated water bodies make them difficult to degrade, posing a serious environmental challenge and representing a significant emerging pollutant that warrants urgent intervention. This study evaluated the biodegradation and detoxification potential of a halotolerant, marine-derived fungal strain, *Penicillium oxalicum* M6A, isolated from the Nigerian coastline, against Indigo carmine (IC) and Remazol Brilliant Blue R (RBBR). The effects of pH, temperature, salinity, and dye concentration on degradation efficiency, alongside enzymatic activity, were assessed. FTIR and GC–MS analyses were employed to identify degradation products and predict metabolic pathways, whereas toxicity was evaluated in three bacterial strains and two crop plant seeds. *Penicillium oxalicum* M6A effectively degraded the dyes, with maximum growth observed at 1.0 g L^{-1} for the IC and 0.8 g L^{-1} for the RBBR. Optimal degradation occurred at pH 5–7, 35 °C for the RBBR, and 45 °C for the IC. The most favorable salinity levels were 1% for the RBBR and 5% for the IC. The RBBR activity significantly increased for laccase (16.14±0.34 U mL^{-1}), lignin peroxidase (10.14±0.12 U mL^{-1}), and alcohol dehydrogenase (8.20±0.46 U mL^{-1}), and the IC activity significantly increased for laccase (15.58 ±0.52 U mL^{-1}), lignin peroxidase (12.54 ± 0.33 U mL^{-1}), and alcohol dehydrogenase (9.31 ± 0.33 U mL^{-1}). FTIR spectra and GC–MS analysis revealed significant structural changes in the dyes and several potential metabolites, including 4-methylcyclopentadecanone, 9-octadecenamide, and 8-cyclohexadecen-1-one for RBBR and 4-amino-2(1H)-pyridinone, 3,7-dimethyl-1-octene, E-2-octadecen-1-ol, and 7-tetradecenal for IC. Phytotoxicity and microtoxicity tests confirmed that *P. oxalicum* M6A metabolized these toxic dyes into less toxic compounds. Its halotolerance and enzymatic versatility make it a promising biocatalyst for the treatment of dye-contaminated saline industrial effluents and other recalcitrant pollutants.

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Introduction

Industrial effluents from the textile industry are a major source of environmental pollution, with synthetic dyes such as Indigo Carmine (IC) and Remazol Brilliant Blue R (RBBR) representing persistent and potentially toxic contaminants. In an effort to evade scrutiny by environmental protection agencies, many industries discharge their effluents irresponsibly into rivers, lakes, ponds, and streams [1]. It is estimated that more than 10,000 different textile dyes and pigments are in use globally, with over 100,000 tons of organic colorants produced annually [2]. Among these pollutants, synthetic

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dyes are considered the most hazardous [3]. They exhibit varying degrees of solubility and can easily be absorbed by substrates, imparting distinct coloration [4].

Structurally, synthetic dyes contain three key components: the chromophore, which consists of atom interaction sites responsive to electromagnetic radiation; the auxochrome, which are functional groups attached to the chromophore that increase fiber affinity while reducing water solubility [5]; and aromatic units such as benzene, naphthalene, or anthracene rings [6]. Dyes are classified into various groups on the basis of their chemical structure, including anthraquinone, sulfur, azo, triarylmethane, and phthalocyanine [6]. These dyes are widely used in industries such as textiles, cosmetics, printing, paper, and pharmaceuticals [7]. However, their intricate structural complexity makes decolorization particularly difficult.

Synthetic dyes are challenging to treat via conventional wastewater treatment methods, and their decolorization remains a major environmental concern [8]. Physico-chemical treatment techniques, such as adsorption, precipitation, chemical degradation, and photodegradation, are often time-consuming, costly, inefficient, environmentally unfriendly, and methodologically complex.

To overcome these limitations, increasing research has focused on microbial enzymatic transformation and the detoxification of pollutants [9]. Fungi are generally more robust and resilient than bacteria and are capable of tolerating high concentrations of recalcitrant pollutants. They can secrete large quantities of extracellular enzymes via cost-effective and eco-friendly processes, making them highly valuable for industrial applications [10]. While most studied fungi have been isolated from terrestrial environments such as forests and marshes, marine fungal diversity remains underexplored [11]. Marine environments, however, harbor a wide array of fungal species that possess unique enzymatic properties, including halotolerance, thermostability, barophilicity, and cold activity [12].

Textile effluents are often characterized by high salinity and alkalinity. Therefore, marine-derived fungi present a biological advantage in effluent decolorization because of their natural adaptation to extreme conditions of salinity and pH. For industrial-scale applications, fungal strains that produce laccases with high redox potential, salt resistance, thermal stability, or cold adaptability are increasingly sought after. Kantharaj et al. [13] reported laccase production in fungal strains isolated from algae, seagrass, and decaying wood. For example, *Cerrena* unicolor, a marine-derived fungus, was found to produce a heat-stable, metal-tolerant laccase [14]. Similarly, *Trematosphaeria mangrovei*, a laccase-producing fungus, was isolated from a mangrove habitat by Atalla et al. [15].

Halotolerant fungi are especially suitable for treating saline wastewater contaminated with synthetic dyes. Some fungal species thrive under high-salinity conditions while maintaining relatively high metabolic activity [16–17]. Members of the phylum Ascomycota are particularly notable for their role in degrading aromatic compounds and other recalcitrant organic pollutants [18]. Within this group, *Penicillium* species are recognized for their remarkable metabolic versatility, enabling them to colonize a wide range of ecological niches, including extreme environments such as deep-sea sediments, polar regions, highly acidic areas, and habitats with extreme temperatures [19]. Although primarily known as decomposers, *Penicillium* spp. exhibit strong hydrocarbon-assimilating capacities, low cosubstrate demands, and the ability to produce a vast array of bioactive metabolites [20]. The adaptability of these materials to numerous carbon sources reflects their potential to degrade various substrates, including industrial pollutants.

Multiple studies have demonstrated the ability of *Penicillium oxalicum* to degrade complex organic compounds such as textile dyes, food waste, lignocellulosic biomass, and hydrocarbons [18]. It also plays a role in the detoxification of xenobiotic compounds, converting them into less mutagenic or toxic metabolites [21]. Owing to its tolerance to high salt concentrations, *P. oxalicum* holds promise for the bioremediation of industrial textile wastewater, which is typically saline. Saroj et al. [22] reported that a strain of *P. oxalicum* (SAR-3) exhibited broad-spectrum dye-degrading activity, effectively catabolizing various azo dyes, including Acid Red 183, Direct Blue 15, and Direct Red 75.

This study aims to evaluate the biodegradation and detoxification potential of a halotolerant, laccase-producing fungal strain, *Penicillium oxalicum* M6A, which was isolated from the Lagos Atlantic Ocean shoreline, in the degradation of two structurally complex synthetic dyes—IC and RBBR. This research investigated the effects of environmental parameters (pH, salinity, temperature, and dye concentration) on degradation efficiency, identified the generated metabolites via Fourier transform infrared spectroscopy (FTIR) and gas chromatography–mass spectrometry (GC–MS) analyses, and assessed the environmental safety of the degradation products through phytotoxicity and microtoxicity tests.

Materials and methods

1) Dyes and chemicals

The two commercial textile dyes used in this study, IC and RBBR, were obtained from Glentham Life Science. The substrates for the assays of laccase activity, 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), and the media, potato dextrose agar (PDA), Mueller-Hinton agar, and malt extract agar, were purchased from Sigma–Aldrich. A stock solution of 10,000 mg L^{-1} dyes was prepared and then sterilized by filtration via a 0.22 µm filter. It was diluted to the required concentration whenever needed.

Only analytical-grade chemicals and reagents were used in this study.

2) Isolation of fungi

The fungal strain, *Penicillium oxalicum* M6A, employed in this study was previously isolated from samples (woods immersed in seawater, seaweeds, marine plants, 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 et al. [23]. *P. oxalicum* M6A was screened for laccase-like activity following the methods of Zouari-Mechichi et al. [24]. The gene sequence of *P. oxalicum* M6A was deposited in the NCBI GenBank and assigned the accession number PP077349.

3) Enzyme assay

The decolorized DR75 sample pellet obtained by centrifugation was utilized to carry out enzyme activity assays that were chosen 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 was determined by assaying the decrease in color absorbance of veratraldehyde at 310 nm [25]. The determination of tyrosinase activity was carried out through the observation of the development of catechol quinone at a wavelength of 495 nm [26]. An increase in the absorbance at 420 nm was observed because the oxidation of the 2,2′-azino-bis(3-ethylbenzthiazoline)-6-sulfonate (ABTS) compound was employed for laccase. Aldehyde dehydrogenase enzyme activity was assayed by following changes in absorbance owing to the formation of NADH at 340 nm [27]. Lignin peroxidase (LiP) activity was assayed at 310 nm using veratryl alcohol as the substrate [26]. 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 product per minute.

4) Decolorization on solid and liquid media

To test for decolorization ability on solid media, potato dextrose agar was autoclaved and supplemented with 200 mg L^{-1} sterile synthetic dyes RBBR and IC and later plated on Petri dishes. *P. oxalicum* M6A mycelial inocula were spotted in the middle of each plate as described previously [28]. The plates were maintained at room temperature for seven days and monitored daily for halo formation, indicating dye decolorization.

For further study in liquid media, *P. oxalicum* M6A was inoculated in 10 mL of autoclaved potato dextrose broth (PDB) supplemented with 200 mg L^{-1} of each dye at 25 °C. Following centrifugation of the sample mixture (*P. oxalicum* M6A, dyes and media) for 10 minutes at 5000 rpm, aliquots of the supernatants were transferred to new tubes and diluted as necessary for absorbance reading. Untreated dye–liquid media served as a control for each dye.

A standard curve for each dye was plotted at its corresponding maximum absorbance wavelength for concentrations ranging from 0 to 200 mg L^{-1}. Decolorization of each dye was computed at 24-hour intervals over a period of five days via Eq.1.

$$D = ((AC - AT)/AC) \times 100 \quad \text{(Eq.1)}$$

Where D is the decolorization/degradation rate (%), AC is the absorbance reading for the control sample (untreated dyes), and AT is the absorbance reading for the treated sample [29].

5) Growth study of isolates at different dye concentrations

Penicillium oxalicum M6A growth was also studied at various dye concentrations (0.4 g L^{-1}, 0.6 g L^{-1}, 0.8 g L^{-1}, and 1.0 g L^{-1}) in potato dextrose broth supplemented with the two textile dyes. The absorbance was read at 600 nm at 24-hour intervals across a 5-day incubation period [30].

6) Effects of various conditions on dye degradation by the selected isolates

Single-factor optimization was employed to investigate the influence of various parameters—dye concentration, pH, salinity, and temperature—on dye degradation by *P. oxalicum* M6A. The initial dye concentrations were varied (50, 100, 200, 300, and 400 mg L^{-1}) to examine decolorization efficiency. The pH of the dye media was varied from 3 to 11, and 0.1 M NaOH or HCl was used.

In a thermostatic incubator (GEMTOP, ZSH-250 F, China), temperatures were tested in 10 °C increments ranging from 15 °C to 45 °C. Salinity was modified from 0% to 5% NaCl to evaluate the growth of *P. oxalicum* M6A under different salt concentrations.

The decolorizing efficiencies of the dyes (RBBR and IC) were assessed under fixed conditions: 200 mg L^{-1} RBBR, pH 7, 1% salinity, 25 °C, and the same conditions used for Indigo Carmine. Static incubation was conducted for 120 hours, and sampling was performed at 24-hour intervals. All the experiments were conducted in triplicate [30].

7) Identification of dye metabolites of dye degradation

Metabolite extraction and chromatographic separation were conducted to establish the degradation byproducts of the dyes. Multiple analytical techniques, including GC–MS, FTIR, and ultraviolet–visible spectroscopy (UV–VIS), have been utilized.

FTIR analysis was conducted at the National Research Institute for Chemical Technology (NARICT), Zaria,

Kaduna, Nigeria, via an Agilent Technologies Cary 630 FTIR spectrophotometer. This analysis aims to monitor the structural changes and distinctive characteristics of dyes both before and after degradation. *Penicillium oxalicum* M6A was grown at 28 °C in 25 mL of dye solution for 7 days. The samples were filtered, washed three times with distilled water, and dried at 110 °C with the help of a hot air oven for 30 minutes in the presence of potassium bromide. The samples were ground at a ratio of 5:95 w/w for analysis, and FTIR spectral data were obtained across a mid-infrared range of 650–4,000 cm^{-1}.

The active components of the dye metabolites were isolated and characterized via GC–MS analysis on a Shimadzu GC–MS-QP2010 PLUS (Japan) from NARICT. Lyophilized samples were dissolved in GC-grade methanol, filtered through a syringe filter, and injected into the GC–MS instrument following the manufacturer's protocols (SHIMADZU GC–MS) [31]. Helium was used as the carrier gas, and identification of metabolites was achieved through matching retention times and mass spectra obtained via GC–MS software and fragmentation analysis to determine compound structures and mass spectrum values (m/z) [30].

8) Microtoxicity and phytotoxicity studies of dyes and their degradation products

The microtoxicity of the original dyes and degradation products from the GC–MS analysis at 200 mg L^{-1} was assessed. Dichloromethane was used to extract the samples, and the samples were dissolved in sterilized water before being tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus*. The agar well diffusion method was employed on Mueller–Hinton agar plates. Wells 0.9 cm in diameter were created and filled with 200 µL of control (sterilized water), treated, or untreated dyes. The plates were sealed with parafilm and incubated at 30 °C for 24 hours. The zone of inhibition was determined by the difference between the well diameter and the total zone of inhibition [33–34].

The phytotoxicities of the original dyes and degradation products from the GC–MS analysis at 200 mg L^{-1} were determined via the use of *Zea mays* (maize) and *Vigna unguiculata* (cowpea) seeds. The experiments were performed at room temperature, in which 10 seeds per crop were placed on bedded filter papers in Petri dishes and sprayed with 10 mL solutions of the corresponding sample (original or treated dye) daily for 7 days. Distilled water was supplied to the control plants. Toxicity was quantified through percentage germination, plumule length (cm), and radicle length (cm) [34].

9) Statistical analysis

Decolorization of each dye was computed at 24-hour intervals over a period of five days via Eq.1. The results are presented as the mean ± standard deviation of three determinations, which were subjected to one-way analysis of variance (ANOVA) to compare the means of three or more groups to determine if at least one group was significantly different from the other groups, and 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

1) Growth study

The influence of dye concentration on *P. oxalicum* M6A growth was investigated, and the findings are shown in Figure 1. The influence of varying concentrations of dye (0.4 g L^{-1}, 0.6 g L^{-1}, 0.8 g L^{-1}, and 1.0 g L^{-1}) on the growth of *P. oxalicum* M6A was examined in a time-course experiment. The maximum growth of the fungus occurred at 0.8 g L^{-1} PBBR and 1.0 g L^{-1} IC on the fifth day of incubation with the dyes.

The maximum growth of P. oxalicum M6A occurred at 0.8 g L^{-1} in the RBBR and at 1.0 g L^{-1} in the IC after five days of incubation with the dyes. This indicated that the isolates could tolerate the dyes at a minimum concentration of 0.4 g L^{-1}.

The growth of fungi in dye-supplemented media highly depends on the concentration and structure of the dyes employed. At low dye concentrations, some fungi may grow better, as the enzymes involved in degradation are able to degrade dyes faster. At high dye concentrations, the dye becomes toxic to fungi and inhibits their growth in media supplemented with dyes by blocking the active sites of the enzymes involved in the detoxication process [35]. The maximum growth was detected in a growth study of Aspergillus niger under Congo red at 0.25 g L^{-1}. With an increase in the initial concentration of the dye to 1 g L^{-1}, the maximum reduction in growth was observed because of the increase in toxicity at higher concentrations of the dye [36].

Wang et al. [37], in their investigation of the decolorization of Congo Red by Ceriporia lacerate, determined an optimal growth rate at a 0.5 g L^{-1} dye concentration. All these works are consistent with this investigation. As the dye concentration increased, the growth rate decreased. Moreover, with a greater number of dye molecules per unit volume, fungal growth was inhibited as a result of increased stress, and the capacity for degradation by the enzymes decreased due to the reduction in the levels of enzymes [38–39].

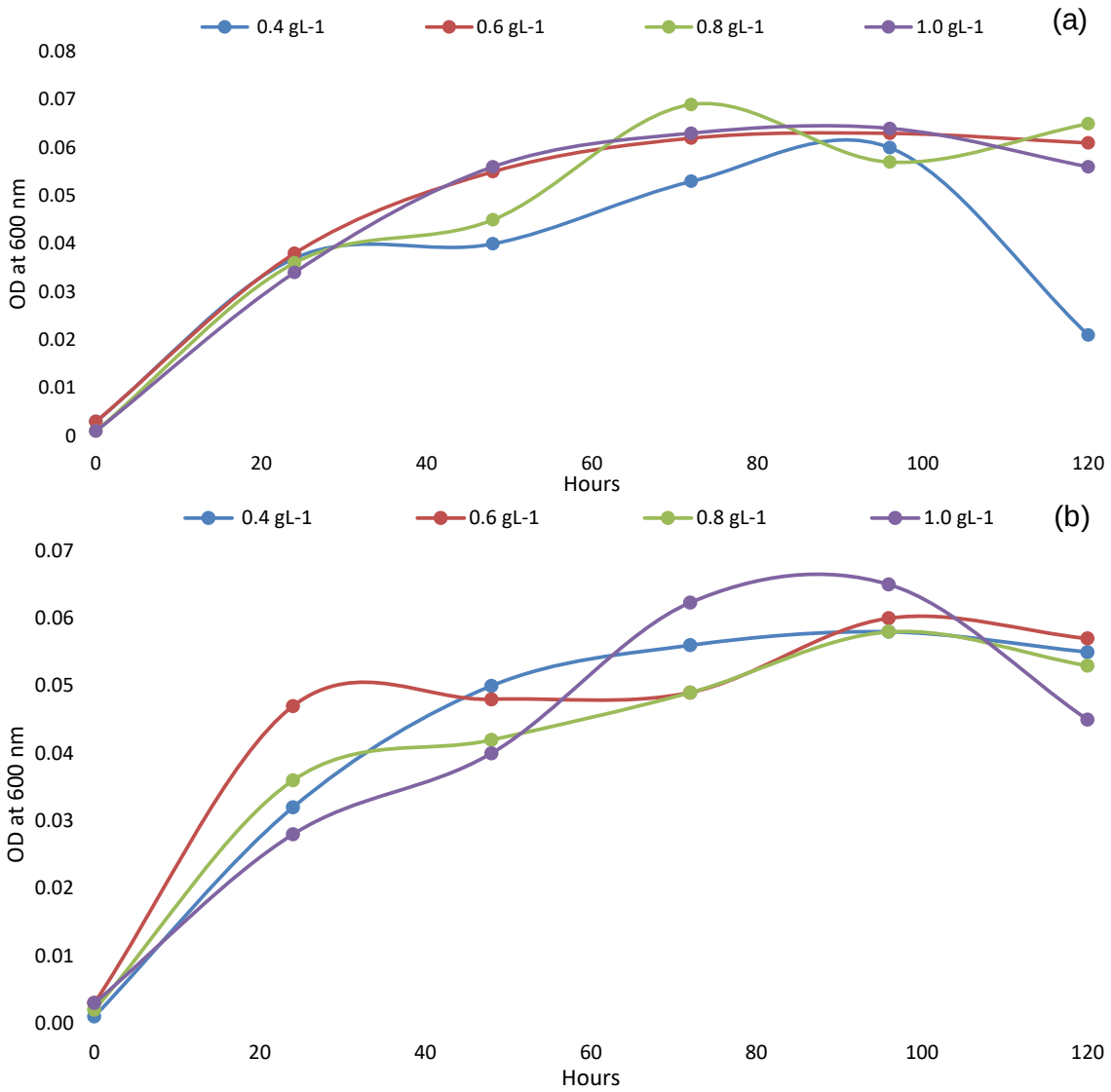

Figure 1 Growth curves of *P. oxalicum* M6A at different concentrations of (a) RBBR and (b) IC at different time intervals.

Table 1 Enzyme activity (U mL^{-1}) of P. oxalicum M6A before and after dye degradation of RBBR and IC

Enzyme	RBBR		IC		Negative control
	Activity before degradation	Activity after degradation	Activity before degradation	Activity after degradation	
Laccase	6.36±0.50	16.14±0.34	8.89±1.0	15.58±0.52	9.43±0.22
Azo reductase	2.14±0.87	ND	3.14±0.37	ND	6.12±0.38
Lignin Peroxidase	3.41±0.27	10.14±0.12	2.34±0.67	12.54±0.33	3.56±0.27
Veratryl alcohol oxidase	1.67±0.06	2.43±0.92	1.67±0.06	2.43±0.92	2.11±0.23
Tyrosinase	1.34±0.44	3.54±0.61	1.34±0.44	3.54±0.61	2.50±0.54
Aldehyde Dehydrogenase	1.45±0.71	8.20±0.46	2.45±0.11	9.31±0.33	2.55±0.17

The activities of different enzymes before and after degradation were relatively high for laccase (16.14± 0.34), lignin peroxidase (10.14±0.12) and alcohol dehydrogenase (8.20±0.46) for RBBR and laccase (15.58± 0.52), and lignin peroxidase (12.54±0.33) and alcohol dehydrogenase (9.31±0.33) for IC, as presented in Table 1. The increases in the activities of oxidative enzymes, particularly laccase, lignin peroxidase, and aldehyde dehydrogenase, reflect their critical functions in the degradation of RBBR and IC dyes. Loss of azo reductase activity upon degradation may be assumed to be a sign of either substrate depletion or enzyme inhibition after reduction. The findings indicate a robust enzymatic system capable of mediating efficient dye degradation through both oxidative and reduction reactions.

2) Effects of various parameters on dye degradation by *Penicillium oxalicum* M6A

The degradation of the dyes was time dependent, as the fungus degraded the dyes better after 72–120 hours (3–5 days) at pH 5, with a degradation percentage above 80%, but degraded poorly at pH values above 5.

Figure 2 shows the time course study of the degradation of dyes at different pH values.

pH is the most significant parameter for dye degradation, as enzymes are maximally active at a specific pH. The degradation of the dyes by *P. oxalicum* M6A was time dependent and decreased at high pH. The dyes were degraded above 80% at an optimum pH of 5 for the RBBR and IC. Parshetti et al. [26] reported that *Aspergillus ochraceus* NCIM-1146 completely decolorized Reactive Blue-25 at pH 5 and that 87%, 81%, and 70% decolorization occurred at pH 3, 7, and 9, respectively. In the present study, the percentage decolorization was lowest at pH 11.

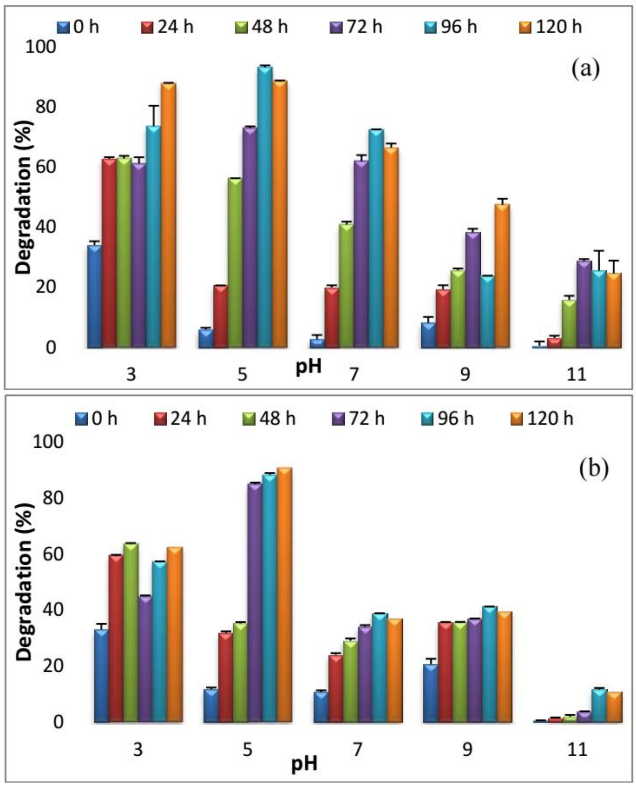

Figure 2 Effects of different pH values on the degradation of (a) RBBR and (b) IC. The error bars represent the standard deviation of the degradation (%) at a given pH and time.

El-Sayeh [39] noted a decrease in the decolorization activity of direct violet dye by *Aspergillus fumigatus* from 94% at pH 5.5 to 47% at pH 3. The optimal pH can increase the rate of color decolorization, and enzymatic degradation of dyes is not supported by highly acidic or alkaline pH values, i.e., 3 and 11. Feng et al. [40] reported that the best dye decolorization rate and cell growth were achieved at pH 6.0 for *M. guilliermondii* A4B and deduced that the isolate could be neutrophilic. At pH 6, a fungal consortium of *Aspergillus tubingensis* and *Penicillium oxalicum* efficiently biodegraded Congo red dye [42-43].

According to Chen et al. [38], the optimum biosorption and biodegradation activities of a *Penicillium* species, *Penicillium simplicissimum*, on triphenylmethane dyes (crystal violet (CV), malachite green (MG), methyl violet (MV), and cotton blue (CB)) were obtained at pH 5.0 and 25 ± 2 °C. *P. oxalicum* at 30 °C and pH 7.0 decolorized Acid Red 183 (AR 183), Direct Blue 15 (DB 15), and DR75 (DR 75) [31].

Figure 3 shows the time course study of the degradation of the dyes at different temperatures. Temperature is an important factor that determines microbial growth, survival, and metabolism. It has an important effect on all microbial survival processes, including water and soil remediation processes.

Penicillium oxalicum M6A degraded RBBR at 35 °C and IC at 45 °C after 120 hours (5 days) of incubation. The difference in the optimal temperature for degradation by the fungus could be due to the different classes of dyes used in this study (anthraquinnone and indole dyes), their chemical structures or the enzymatic machinery involved.

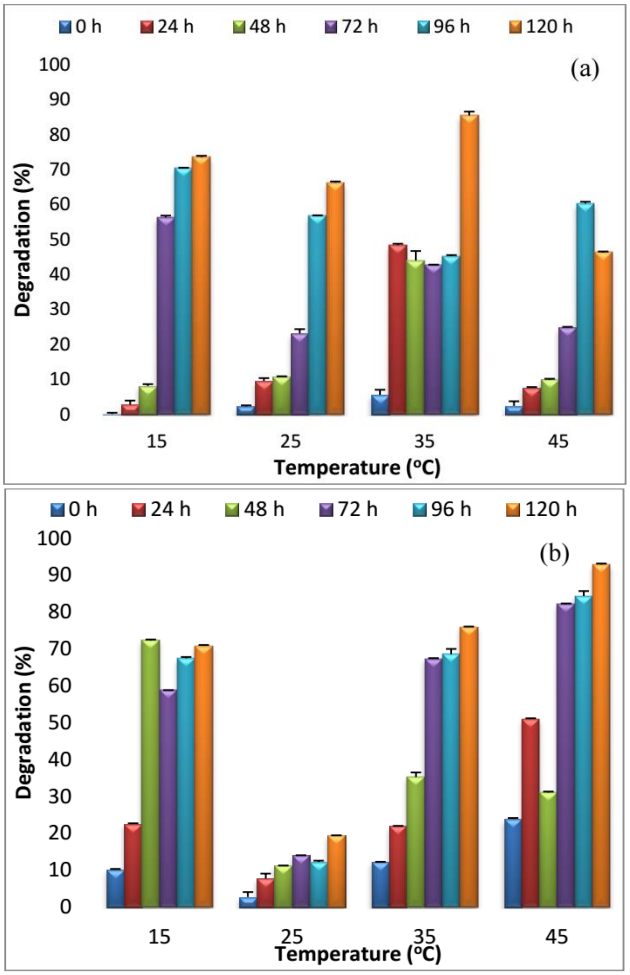

Figure 3 Effects of different temperatures on the degradation of a) RBBR and (b) IC. The error bars represent the standard deviation of the degradation (%) at a given temperature and time.

Al-Tohamy et al. [30] reported that the optimum decolorization efficiency of the halotolerant consortium HYC in relation to temperature was 35 °C (87%), whereas Gharieb et al. [43] reported that the decolorization of DR75 and reactive red dyes was most efficient at 30 °C, with 85.3% and 80.8% color reduction by *M. guilliermondii* and *N. diffluens*, respectively. Both of these studies agree with our work. Ren et al. [44] reported that *P. oxalicum* RJJ-2 degraded 84.88% of erythromycin after 96 h of incubation at 35 °C, even when it was incubated with pharmaceuticals and not dyes.

The decolorization percentages of RBBR and IC at various dye concentrations (ranging from 50 mg L^{-1} to 400 mg L^{-1}) revealed that *P. oxalicum* M6A degraded more than 90% of the four dyes at 50 mg L^{-1} after 5 days of incubation, and the degradation percentage decreased with increasing dye concentration.

Figure 4 shows the effect of dye concentration on dye degradation by *P. oxalicum* M6A.

On the basis of the experimental findings, when the fungus was subjected to 50 mg L^{-1} IC or RBBR, 90% degradation was achieved. Within the same time frame, the degradation capacity of the fungus decreased when the dye concentration was greater than 50 mg L^{-1}. At a dye concentration of 400 mg L^{-1}, the degradation rates were the lowest at 18.20% and 11.78% for the IC and RBBR, respectively. Fetyan et al. [45] reported that Saccharomyces cerevisiae was highly effective in decolorizing direct blue 71 and recorded the highest activity of 100%; this decolorization activity decreased with dye concentrations over 200 mg L^{-1}, which may be because of the toxicity of this higher concentration of dye to the yeast. These studies agree with this work, and this phenomenon may also be explained by the insufficient concentration of biomass for the higher concentration of the dyes to be absorbed by the organisms [46–47].

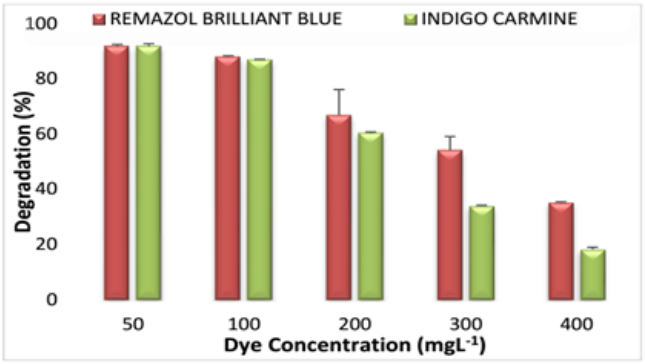

Figure 4 Effects of different dye concentrations on the degradation of dyes. The error bars represent the standard deviation of the degradation (%) at a given dye concentration and time.

Figure 5 shows the decolorization percentages of RBBR and IC at various salt concentrations (1–5%). The maximum decolorization rates of the dyes were at a salt concentration of 1% for the RBBR and 5% for the IC.

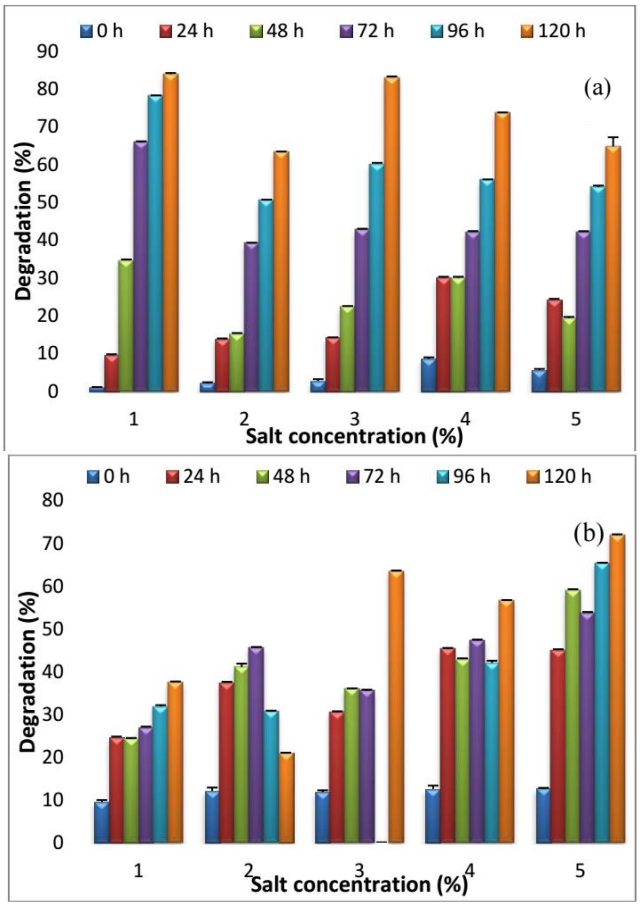

Figure 5 Effects of different salt concentrations on the degradation of (a) RBBR and (b) IC. The error bars represent the standard deviation of the degradation (%) at a given salt concentration and time.

The maximum degradation rate of the dyes by the fungus at different salt concentrations was 1% for RBBR and 5% for IC. The *S. halophilus* and *M. guilliermondii* HYC consortia had RB5 degradation rates between 88.1% and 9.2% at salinity concentrations of 0–5% [30]. Owing to its ability to degrade dyes at high salinity, *P. oxalicum* M6A is suitable for the degradation of textile effluents where their effluents are at high salinity, which makes it difficult for terrestrial fungi to degrade. An increase in salt concentration can cause a significant decrease in enzyme activity and eventually total inactivation [48], as hypersalinity can cause plasmolysis and cell inactivity [49].

3) Identification of the transformation products of RBBR and IC after degradation by *P. oxalicum* M6A via FTIR and GC-MS

The types of vibration and functional groups of the peaks observed in the FTIR spectra for nondegraded RBBR and IC dye and their degraded form by *P. oxalicum* M6A are shown in Figures 6–7. There were

eighteen peaks for the nondegraded RBBR dye and ten peaks for the dye degraded by the fungus. The GC–MS chromatograms of IC degraded by *Penicillium oxalicum* M6A are presented in Figure 8. The GC–MS chromatograms revealed nineteen (19) peaks, some of which were for 4-methylcyclopentadecanone, 9-octadecenamide, and 8-cyclohexadecen-1-one, whereas the GC–MS chromatograms of the metabolites of the degraded RBBR revealed twenty-one (21) peaks, some of which were for 4-amino-2(1H)-pyridinone, 3,7-dimethyl-1-octene, E-2-octadecen-1-ol, and 7-tetradecenal.

The tricyclic anthraquinone structure chromophore cleavage was established by the disappearance of some of the minor absorption bands and shoulders between 1,300–674 cm^{-1} and 2,920–1,990 cm^{-1}, corresponding to aromatic C=C bending, S=O stretching and aromatic C-H bending [42]. The FTIR spectrum of the nondegraded RBBR (Figure 6a) showed intense absorption bands at ~1,610 cm^{-1} and ~1,480 cm^{-1}, related to aromatic C=C stretching vibrations of the anthraquinone chromophore. The peaks at approximately 1,120 cm^{-1} indicate the existence of sulfonic acid (–SO_3H) groups in sulfonated azo and anthraquinone dyes. Certain bands at 1,000–1,300 cm^{-1} were also consistent with the C–N stretching vibrations arising from aromatic amine groups contained clear peaks in the fingerprint region (cm^{-1}). After fungal treatment (Figure 6b), significant transformations were observed. The disappearance of peaks of possibly aromatic C=C and sulfonic acid groups points to the cleavage of the chromophoric nucleus and desulfonation of the dye molecule. The appearance of a wide band near 1,700 cm^{-1} can be attributed to carbonyl (C=O) groups due to the oxidative degradation of the RBBR. The spectra also showed the presence of peaks between 2,850–2,950 cm^{-1}, which can be attributed to C–H stretching vibrations in aliphatic chains and indicate the presence of fewer hydrocarbon fragments. The FTIR results correlate with the compounds identified from the GC–MS analysis of RBBR degradation. Several metabolites corresponded to the observed spectral features: carbonyl compounds such as 3-methylcyclopentanone, 4,5-dimethyl-4-hexen-3-one, and glyceraldehyde were consistent with the new peak at 1,700 cm^{-1} of the FTIR spectrum, confirming the formation of aldehydes and ketones. The detection of 2,4,5-trimethyl-1,3-dioxolane and 4-amino-2(1H)-pyridinone confirmed the cleavage of complex heterocycles and aromatics to oxygen- or nitrogen-substituted, ring-forming fragments, which may account for the weak FTIR bands between 1,200–1,600 cm^{-1}. Long-chain alcohols and fatty acids, including 9,12-octadecadienoic acid, 15-tetracosenoic acid, hexadecanoic acid, E-2-octadecadecen-1-ol, and 13-tetradece-11-yn-1-ol, are responsible for the strong aliphatic C–H stretching bands (~2,850–2,950 cm^{-1}) and establish the fragmentation of the aromatic core into fatty or unsaturated saturated compounds. The FTIR spectra of IC before and after biodegradation (Figure 7) indicate substantial chemical changes. The disappearance of aromatic C=C (726 cm^{-1}) and sulfonate group (1,300 cm^{-1}) peaks and the appearance of carbonyl (1,403 cm^{-1}) and aliphatic traces (3,414 cm^{-1}) establish the effective oxidative degradation of the IC chromophore system. The indole ring structure chromophore cleavage was expressed by the disappearance of several weak absorption shoulders and bands between 1,572–1,073 cm^{-1} and 2,926–1,572 cm^{-1} for aromatic C=C stretching and aromatic C-H stretching. These FTIR findings are also supplemented by GC–MS analysis, which detected a range of aliphatic hydrocarbons, nitrogenous bases, alcohols, esters, and fatty acids in the degraded sample. The presence of pyrimidine-2,4(1H,3H)-dione and 5-amino-6-nitroso-pyrimidine-2,4-dione suggests ring cleavage of indigoid and sulfonated structures, resulting in the formation of nitrogenous heterocycles. 4-Methyl-1,3-dioxane and 6-oxa-bicyclo[3.1.0]hexan-3-ol suggest oxidative ring modifications or rearrangements. 4-Octene and methyl 11-cyclopentylundecanoate suggest alkyl side chain cleavage and methylation reactions, respectively.

Wei et al. [50] reported that the intermediates formed during the degradation of IC in aqueous solution through electrochemical degradation were isatin-5-sulfonic acid (m/z=226), 2-amino-α-oxo-5-sulfobenzeneacetic acid (m/z=242), 2-amino-5-sulfobenzoic acid (m/z=198), and 2-amino-5-hydroxybenzoic acid (m/z=152). The intermediates were different from those found in this study, possibly because of the different degradation methods adopted in both studies. GC–MS analysis revealed that the degradation of the dyes could have occurred via oxidation of the side chains and the aromatic ring and removal of the amines and chromophores in the dyes. We proposed a possible degradation pathway on the basis of the GC–MS metabolites and enzymes identified, as shown in Figure 10.

4) Microtoxicity and phytotoxicity assessment of the dyes and their degradation products

The microtoxicity results of the dyes and their degradation products toward *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus cereus* are presented in Table 2. The results showed that the dyes inhibited the growth of the bacteria used in this study better than their degradation products did. Compared with their degradation products, the zones of inhibition of the dyes were statistically significant. The degradation products of Indigo carmine and RBBR showed no zone of inhibition on *Pseudomonas aeruginosa*. This means that *P. oxalicum* M6A degraded the dyes into less toxic substances. No zone was observed outside the wells with the control, as expected.

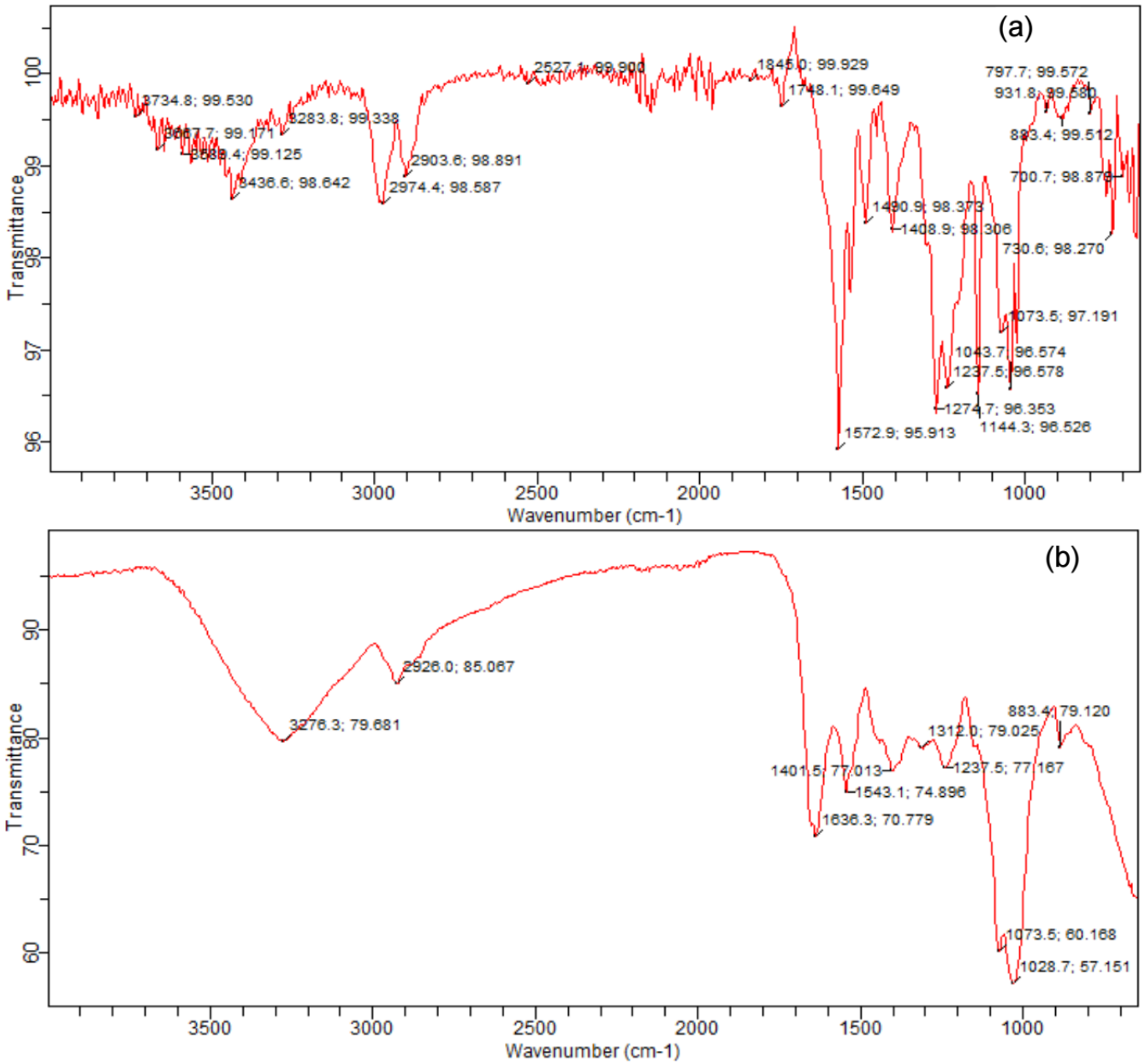

Figure 6 FTIR spectra of (a) nondegraded RBBR dye (control) and (b) RBBR degraded by *Penicillium oxalicum* M6A.

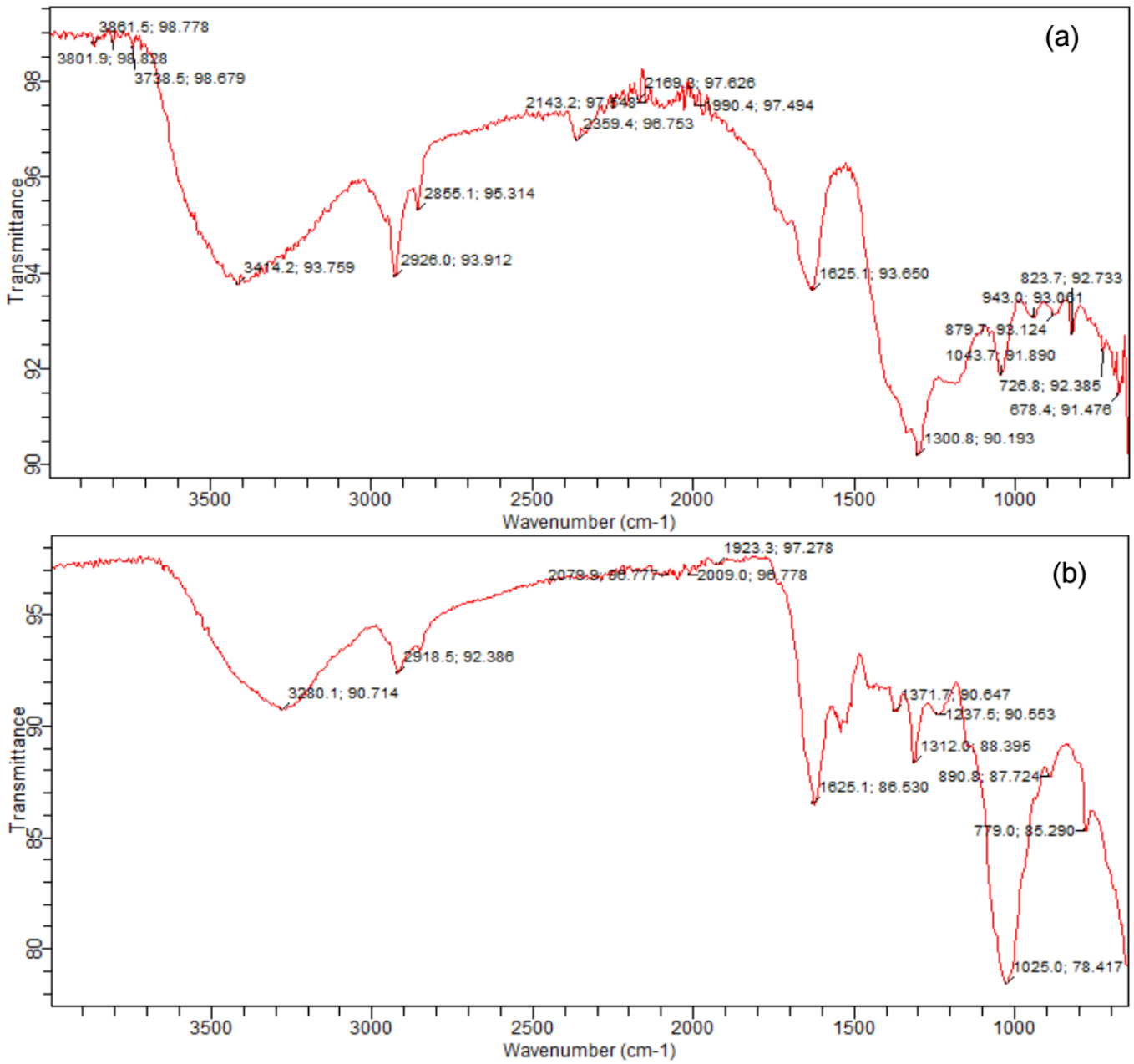

Figure 7 FTIR spectra of (a) nondegraded IC dye (control) and (b) Indigo carmine degraded by *Penicillium oxalicum* M6A.

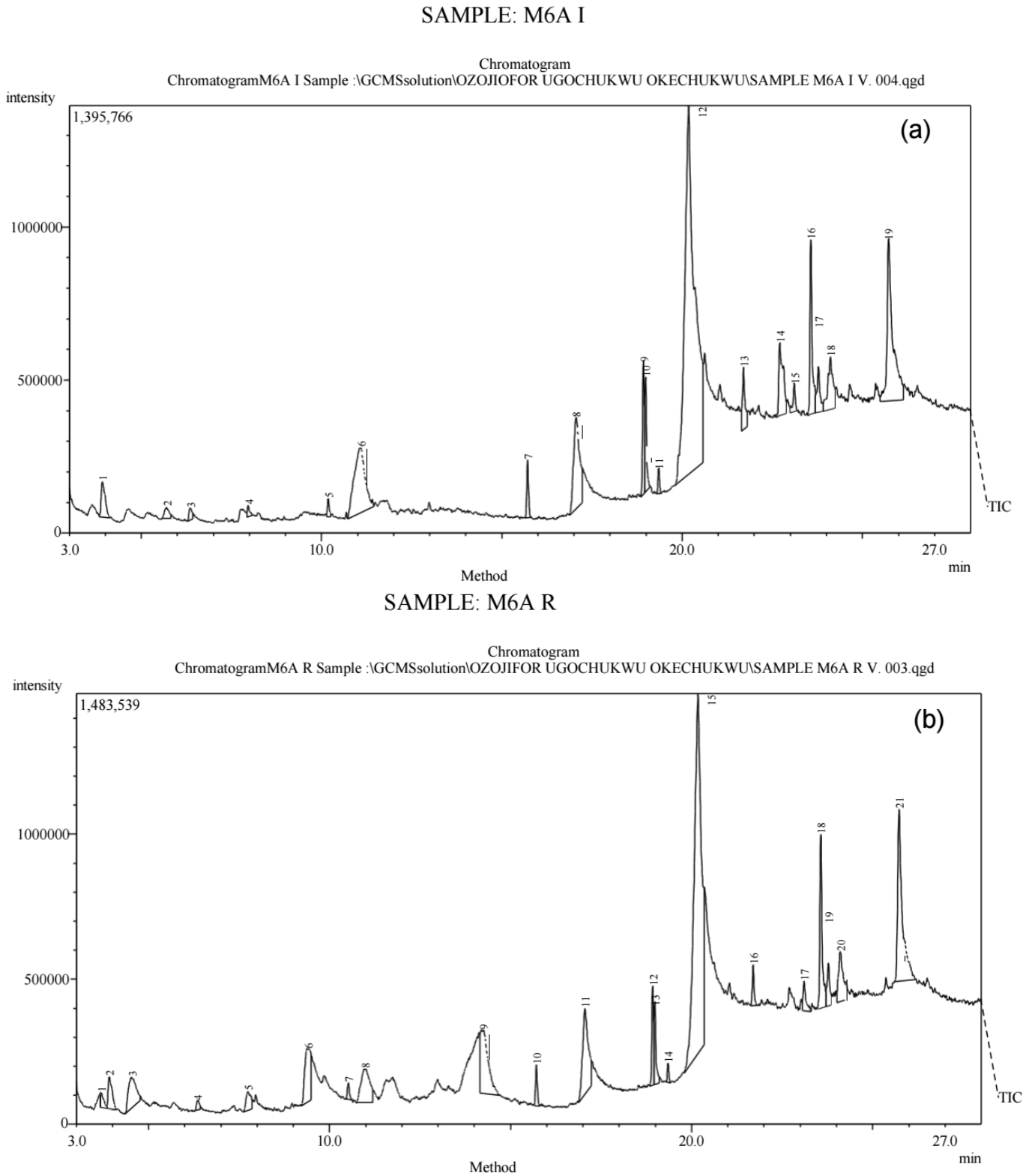

Figure 8 GC–MS chromatograms of (a) IC and (b) RBBR degraded *Penicillium oxalicum* M6A.

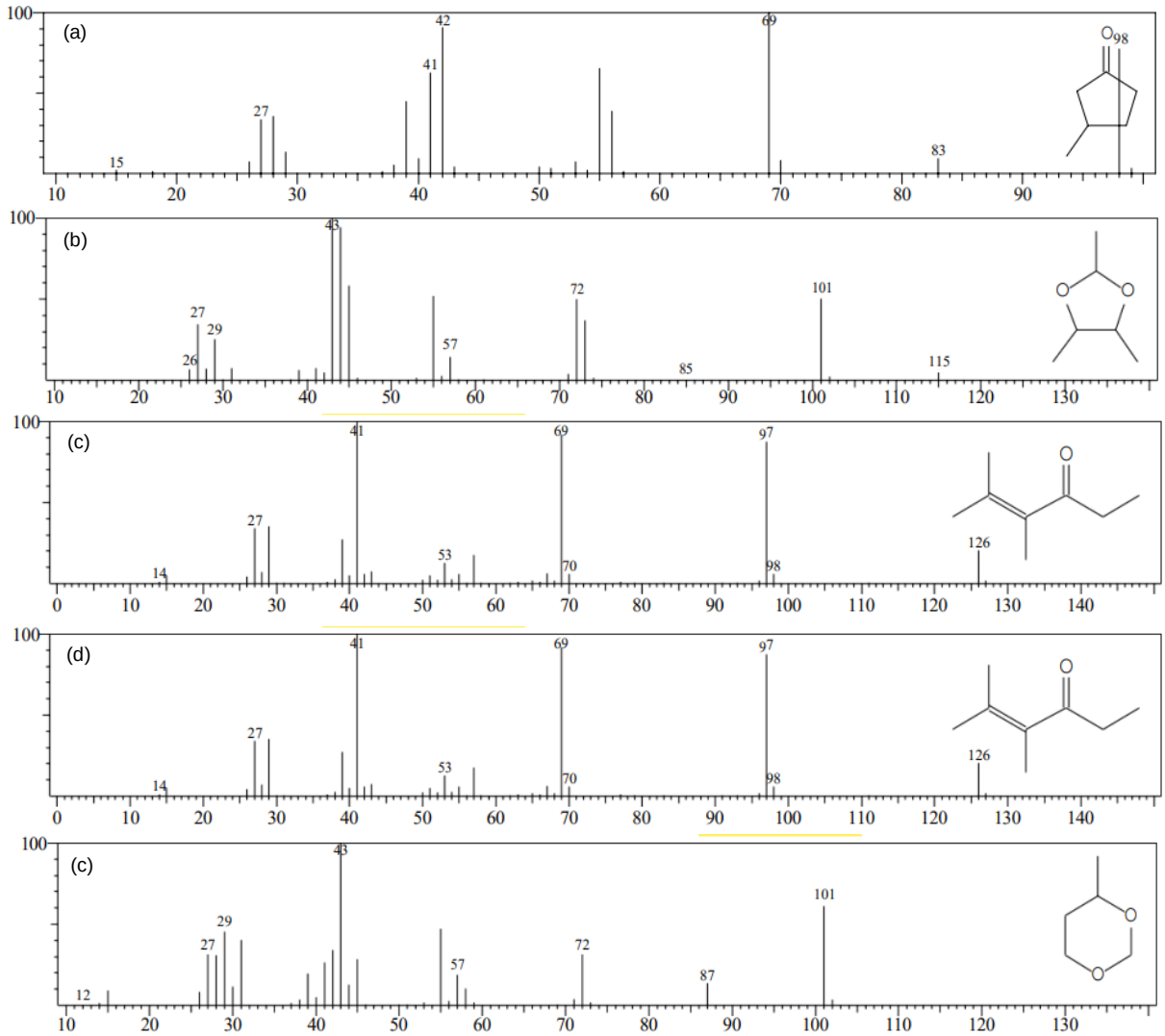

Figure 9 Identification of metabolites of RBBR and IC by GC–MS. (a) 3-Methylcyclopentanone, mass peak (m/z) 98 (b) 2,4,5-Trimethyl-1,3-dioxolane, mass peak (m/z) 116 (c) 4,5-Dimethyl-4-hexen-3-one, mass peak (m/z) 126 (d) Pyrimidine-2,4(1H,3H)-dione, mass peak (m/z) 156 (e) 4-Methyl-1,3-dioxane, mass peak (m/z) 102.

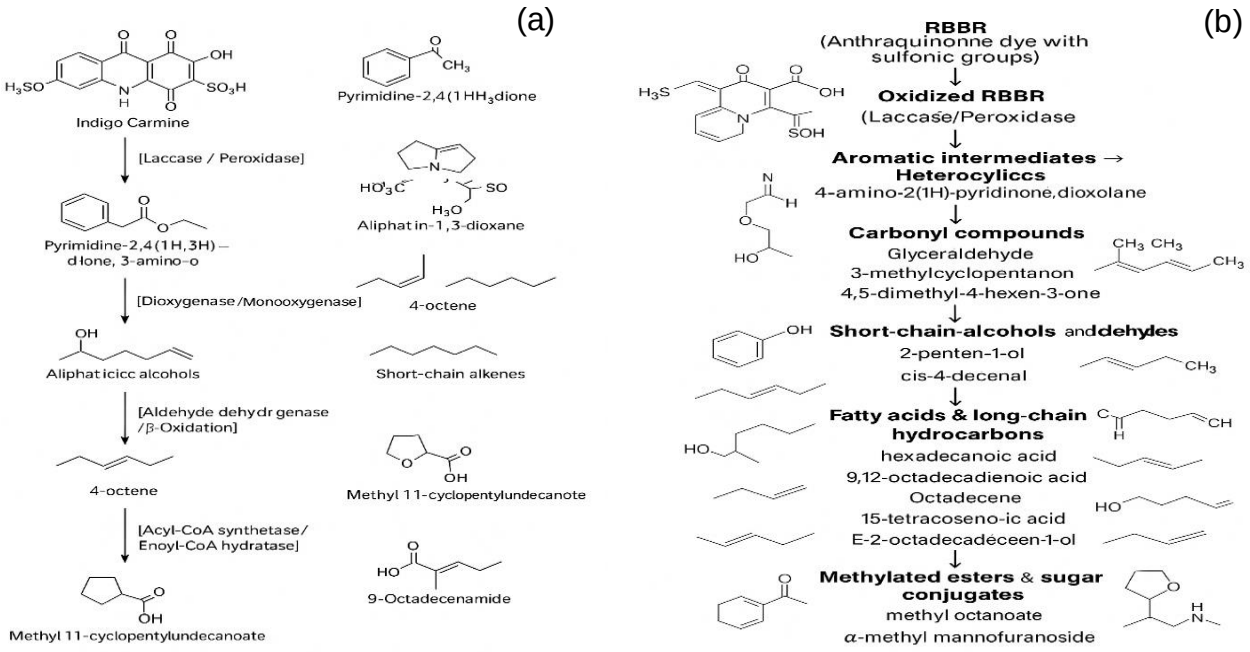

Figure 10 Proposed pathway for the degradation of (a) IC (b) RBBR by the enzymatic machineries in *P. oxalicum* M6A

Table 2 Zone of inhibition (cm) of RBBR, IC and their products of degradation in some bacterial strains

Bacterial strains	Control	RBBR	RBBR-P	IC	IC-P
Staphylococcus aureus	-	0.6±0.3^{c}	0.2±0.1^{ab}	1.83±0.3^{d}	0.3±0.2^{bc}
Pseudomonas aeruginosa	-	0.53±0.2^{b}	-	0.63±0.15^{b}	-
Bacillus cereus	-	1.0±0.2^{b}	0.3±0.2^{a}	0.63±0.2^{b}	0.2±0.1^{a}

Remark: RBBR: Remazol Brilliant Blue R; IC: Indigo Carmine; RBBR-P: Remazol Brilliant Blue Product; IC-P: Indigo Carmine Product; Superscript (a, b, c…) indicates a significant difference across the rows at a 95% confidence interval.

Table 3 Phytotoxicity assessment of RBBR, IC and their degradation products in Zea mays seeds

Parameters	Control	RBBR	Products	IC	Products
Germination (%)	100±0^{c}	66.7±5.8^{a}	66.7±5.8^{a}	60.0±0^{a}	83.3±5.8^{b}
Plumule (cm)	6.4±0.9^{e}	1.2±0.3^{ab}	4.0±0.3^{c}	1.0±0.5^{a}	2.5±0.5^{d}
Radicle (cm)	2.5±0.3^{e}	1.0±0.3^{c}	1.5±0.5^{d}	0.5±0.3^{a}	0.7±0.4^{b}

Remark: RBBR: Remazol Brilliant Blue R; IC: Indigo Carmine; Superscript (a, b, c…) indicates a significant difference across the rows at a 95% confidence interval

Table 4 Phytotoxicity assessment of RBBR, IC, and their degradation products in Vigna unguiculata seeds

Parameters	Control	RBBR	Products	IC	Products
Germination (%)	90±16^{a}	56.7±5.8^{b}	73.3±5.8^{d}	63.3±5.8^{c}	80±0^{d}
Plumule (cm)	4.4±0.9^{c}	1.2±0.3^{a}	4.1±0.5^{c}	1.1±0.1^{a}	2.6±0.8^{b}
Radicle (cm)	2.9±0.7^{b}	1.0±0.3^{a}	2.7±0.8^{b}	2.4±0.2^{b}	2.6±0.9^{b}

Remark: Remazol Brilliant Blue R; IC: Indigo Carmine; Superscript (a, b, c…) indicates a significant difference across the rows at a 95% confidence interval.

Zubbair et al. [51] recorded the microtoxicity of reactive black on *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. From their findings, *P. aeruginosa* and *E. coli* grew in clear zones of 6 mm and 3 mm, respectively, whereas *K. pneumoniae* was resistant to dye toxicity, which corroborates our work.

The phytotoxicity results of the dyes and their degradation products on seeds of *Zea mays* (Maize) and *Vigna unguiculata* (Cowpea) are presented in Tables 3–4. There was a statistically significant difference in the germination percentage and plumule and radicle lengths of the seeds of the two crops irrigated with the untreated dyes and those irrigated with the treated dyes and the control. The percentage germination of the plant seeds of the two crops was highest for the seeds irrigated with the control (sterilized water) (100%), followed by the degradation products of the dyes, whereas the percentage of germinated seeds was the lowest for the seeds treated with the dyes. The plumule and radicle lengths of the seeds of *Zea mays* (Maize) and *Vigna unguiculata* (Cowpea), which are products of dye degradation, were significantly greater than those of the seeds irrigated with nondegraded dyes. The plumule and radical lengths were lowest for *Zea mays* irrigated with IC (1.0 cm and 0.4 cm) and for *Vigna unguiculata* irrigated with RBBR (1.2 cm and 1.0 cm).

Phytotoxicity assays conducted with *Zea mays* (maize) and *Vigna unguiculata* (cowpea) revealed that the untreated dyes IC and RBBR are very toxic to plant systems. Compared with those of the degradation products and the control (sterilized water), the germination percentage, plumule length, and radicle length of the seeds treated with the untreated dyes were greater. Conversely, seeds irrigated with the degradation products of the dyes presented improved germination and growth parameters, which were statistically significant and comparable to those of the control.

These findings clearly indicate that halotolerant, laccase-producing Penicillium oxalicum M6A has the ability to biotransform dyes into less toxic metabolites, minimizing their adverse effects on plant growth. The detoxification achieved underscores the application of fungus-mediated biodegradation as a viable method of mitigating the environmental risks of dye pollution because it is a strategy with particular merit in developing green remediation technologies for industrial dye wastewaters.

These results agree with those of earlier works, such as Bagewadi et al. [33], who also obtained similar results for the degradation of malachite green, Congo red, and methylene blue. The improved germination and growth findings following treatment show that

effluents treated with *P. oxalicum* M6A could be recycled for the irrigation of crops with considerably less environmental risk. These findings demonstrate the potential of *P. oxalicum* M6A as a green bioremediation agent capable of detoxifying harmful textile dyes, thereby facilitating the attainment of a safer wastewater management system and an improved degree of environmental protection.

Conclusions

This study demonstrated the strong biodegradation potential of the isolated marine fungus *Penicillium oxalicum* M6A in the decolorization of the synthetic textile dyes IC and RBBR. Under optimized pH ranges of 5–7, temperatures of 35 °C for the RBBR and 45 °C for the IC, and some salinity ranges, the fungus degraded more than 90% of the dyes in 120 hours. The strain exhibited strong tolerance to saline conditions and various dye concentrations, confirming its applicability for harsh effluent conditions.

Analytical tools such as FTIR and GC–MS detect structural changes and the formation of unique degradation metabolites. In addition, phytotoxicity and microtoxicity testing revealed a significant reduction in toxicity upon fungal treatment, thus determining the environmental safety of the degradation products.

These findings underscore the potential biotechnological value of marine fungi in the context of ecosustainable textile dye treatment. Indeed, *P. oxalicum* M6A is a suitable candidate for the development of ecofriendly and efficient bioremediation procedures in dye-polluted environments. Future research should focus on the elucidation of enzymatic processes and pathways involved in the dye degradation process and explore genetic engineering strategies to increase the efficiency of degradation by the strain and its scalability for industrial implementation.

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