

RESEARCH ARTICLE

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Degradation and Detoxification of Congo Red Azo Dye by Immobilized Laccase of *Streptomyces sviveus*

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Abstract

The discharge of textile effluents enriched with reactive azo dyes is of critical importance owing to inability of the dyes to degrade in waste water and their carcinogenic, mutagenic effects to various organisms. This study initiated based on the need to gaze into molecular mechanism of marine bacterial bioremediation process to develop strategies for the decolorization and detoxification of the synthetic azo dyes. The experimental work carried out to explore decolorization and degradation efficacy of laccase derived from marine actinobacteria, *Streptomyces sviveus* by choosing Congo red-21 as model azo dye. The extracellular production of laccase was confirmed with plate assay in medium supplemented with ABTS as substrate. Laccase was purified to homogeneity from 72hrs culture of *Streptomyces sviveus* by Fast performance liquid chromatography and the molecular size of laccase was noticed as 60 kDa. The purified laccase was immobilized with an efficiency of 82% by Calcium alginate method. The crude, purified and immobilized forms of the laccase enzyme was used to decolorize the Congo red-21. Crude laccase enzyme showed 69% of decolorization of Congo red-21 after 48h where as purified and immobilized laccase represented 78% and 92% of colour removal after 24 h respectively. Fourier-transform infrared spectroscopy, High Performance Liquid Chromatography and Gas chromatography–mass spectrometry were used to unravel the molecular mechanism of dye detoxification and also identify nontoxic products released from Congo Red-21 upon administration with immobilized laccase. Based on GC-MS data, it may deduce that immobilized laccase of *Streptomyces sviveus* cleaves the Congo red-21 dye followed by oxidative cleavage, desulfonation, deamination, demethylation process.

Keywords: Marine bacteria, Reactive Azo dyes, Decolorization, *Streptomyces sviveus*, Immobilized Laccase

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INTRODUCTION

The Synthetic azo dyes are the major constituents of fabric colours and consequently accountable for substantial environmental pollution during dyeing process¹. The azo dyes are one of the most popularly used textile dyes owing to their versatility and based on the chromophore structure they are categorized as acidic, reactive, basic, disperse dyes^{2,3}. In the global textile market, the reactive dyes are chiefly used in the dyeing process owing to varied color spectrum and adherence features⁴. Dumping of azo dyes into the water streams and ponds is a great concern in textile effluent treatment due to their effect on the biological oxygen demand (10-30%), photosynthetic potential of under water plants by blocking the light penetration and toxicity to aquatic organisms due to the aromatic amines released during the cleavage of azo bonds^{5,6}.

During the past fifteen years, bioremediation has been in practice as a novel strategy for the decolourization and the biotransformation of the azo dyes into non toxic products. Bioremediation at the present is being considered as a more eco-friendly alternative to the physical and chemical degradation methods of textile effluents while at the same time being cost effective. The efficacy of the bio degradation is dependent on the nature of the organisms available in the dwelling sites.

Literature replete with bioremediation of textile azo dye effluents using bacteria⁷, fungi⁸ yeast⁹, Actinomycetes¹⁰ and Algae¹¹. Research reports on the degradation of azo dyes by bacteria and fungi have revealed the contribution of either intracellular enzymes like azo reductases or extracellular oxidative enzymes like laccase, manganese peroxidases and tyrosinase^{12,13}. Among the oxidative enzymes, laccase attracted the scientific community due to its physico-chemical properties and catalytic function in the bioremediation of textile effluents¹⁴. Laccase is a glycoprotein involved in the direct oxidation of phenolic compounds, indirect oxidation of non-phenolic compounds and coupling reactions¹⁵, Laccases are extensively available in eukaryotes like fungi, plants, insects and mammals¹⁶ and also in bacteria¹⁷. By keeping in view the importance of laccase mediated degradation of azodyes, the research study is formulated to analyze the effect

of immobilized laccases on degradation of reactive red azo dye.

MATERIALS AND METHODS

The reactive azo dye Congo red – 21 was purchased from the local retail vendors situated in Tirupati with coordinates of 13.65°N 79.42°E, Andhra Pradesh, India. Nutrient glucose broth medium inoculated with marine actinomycetes was taken for the analysis of dye degradation potential. Fine grade solvents and chemicals used for the experimental analysis. These were purchased from Sigma and SD - fine. *Streptomyces sviveus* strain KN3 isolated from the marine sediments, Bay of Bengal, Nellore, A.P with coordinates of 14.5131°N and 80.1791°E¹⁸ was exploited for the production of extracellular laccase.

Screening for extracellular laccase

Qualitative screening approach was utilized to detect the extracellular production of laccase by using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), and Guaiacol as substrates. The ABTS plate was prepared by mixing 100ml nutrient glucose agar with 10ml of 0.2mM ABTS and 1ml of CuSO₄. The appearance of green color halos around the bacterial colony after 48h incubation at 30°C, demonstrates the extracellular production of laccase from *Streptomyces sviveus* strain KN3. The production of laccase was further confirmed with guaiacol plate assay. The assay was performed by supplementing 15ml nutrient glucose agar with 0.1% guaiacol and the plates were inoculated with the isolate KN₃ and incubated at 30°C for 48h. The laccase induced oxidation of guaiacol was visualized with formation of reddish brown colour on the guaiacol plates inoculated with *S. sviveus* KN3¹⁹.

Assay for Laccase enzyme

The quantitative analysis of the Laccase was carried out at 420 nm by calculating the oxidation of ABTS. *S. sviveus* KN₃ indicated maximum production of laccase in nutrient glucose broth (pH 7.0) incubated at 30°C. The oxidation of ABTS was performed by adding the culture supernatant of *S. sviveus* (100µl) to the reaction mixture containing 0.1M Acetate buffer pH 4.5 and 10mM ABTS. The reaction mixture was incubated at a temperature of 25°C for 10 minutes and the oxidation was stopped with the 50% (w/v) Tri chloro Acetic acid.

One unit of enzyme is defined as the amount of enzyme required to oxidize 10 μ l ABTS per minute. Triplicates were maintained for the experimental analysis. Lowry method was adopted to determine the protein concentration²⁰.

Purification of laccase enzyme

The selected *S.sviceus* KN3 was grown under the optimum pH, temperature, Carbon and Nitrogen sources for 4 days. After incubation the culture filtrate was subjected to centrifugation for 30 mins at 11308xg. The supernatant acquired from the centrifugation was subjected to ammonium sulphate precipitation of 20 - 60 % saturation by addition of ammonium sulphate and centrifuged at 11308xg. for 30mins at 4°C. The pellet with protein activity was selected for further analysis while the supernatant was discarded. The pellet was dialyzed by dissolving in 10mM potassium phosphate buffer (pH 7.0) and dialyzed for 24hrs at 4°C. After dialysis the sample was centrifuged and the supernatant was selected for further purification by Fast performance liquid chromatography (FPLC) and protein content was determined by the Lowry method .²⁰

Purification of Laccase by FPLC and molecular weight determination

The chromatographic purification steps were performed in a fast protein liquid chromatography (GE Healthcare, Uppsala, Sweden) with a flow rate of 1 ml/min, temperature of 20°C and volume fraction of 50 μ l and the elution was monitored by UV-detector at 280 nm. The dialyzed enzyme was filtered through a 0.45 μ m membrane filter (Millipore, Billerica, MA, USA) and loaded onto a Sephadex G-75 column (GE Healthcare, Uppsala, Sweden) pre-equilibrated with 10 mol/L Tris-HCl (pH 7.0). The bound enzyme was eluted with the 0.3M KCl gradient and fractions were pooled and used for determining the specific activity if laccase with standard substrates (ABTS and guaiacol). Sodium dodecyl sulphate Polyacrylamide gel electrophoresis (SDS – PAGE) with protein molecular weight markers was used in the determination of the molecular mass of the purified laccase²¹.

Immobilization of laccase Enzyme

Calcium alginate method was adopted for the immobilization of laccase enzyme. Sodium alginate solution (4%) was mixed with FPLC purified laccase enzyme solution (1 mg/mL) at a ratio of 1:2 (v/v) and stirred to facilitate complete mixing. The mixture was slowly dropped into CaCl₂ solution

(2% w/v) using a sterile syringe needle and stirred gently for the formation of immobilized enzyme beads. The Calcium alginate beads were washed with distilled water and refrigerated at a temperature of 4°C for the conduction of experimental work. The efficiency of immobilization was measured by with the help of the formula.

$$\text{Percentage of immobilization} = \frac{\text{Initial activity of laccase} - \text{Activity of laccase in Washings}}{\text{Initial activity of laccase}} \times 100$$

Degradation of Congo red dye by Immobilized laccase

Decolorization experiment was carried out in 25ml conical flasks with 10ml of potassium phosphate buffer (PH6.5) amended with 400 ppm reactive Congo red-21 and laccase enzyme (Crude/purified /immobilized enzyme) at different concentrations such as 25 mg/l and 50 mg/l and the samples were incubated at 30°C and 100 rpm for different time points. Control was maintained without the laccase enzyme. The speed of colour elimination of the Congo red-21 was measured at λ max 540 nm using UV-spectrophotometer (Schimadzu UV 1601) at a regular interval of 12h. The percentage decolorization of textile reactive Congo red -21dye was determined¹⁸

Analysis of degradation Products of Congo red dye

The congo red-29 dye sample degraded by immobilized laccase was centrifuged at 11308xg for 15 min and the supernatant containing the degraded products was subjected to liquid - liquid extraction using ethyl acetate as a solvent (1:1 ratio). The organic phase was collected and evaporated by using a rotary evaporator and the products were dried with anhydrous Na₂SO₄. Further the extracted residue was analyzed by FTIR, HPLC, and GC - MS analysis.

Fourier transform infrared spectroscopy analysis (FTIR)

The profile of functional groups in the parent Congo red-21 and laccase degraded dye was assessed through Fourier transform infrared spectroscopy. The molecular signatures of functional groups were recorded with 16 scan speed in the mid-IR region of 400–4000 cm⁻¹. The parent Congo red-21 dye sample and the sample extracted after treatment with laccase was mixed independently with 0.02 g of sample with KBr at a ratio of 1:20

and the spectra were measured using FT-IR Spectrophotometer (BRUKER Model ALPH).

High performance liquid column chromatography (HPLC)

The parent and degraded samples were extracted and dissolved methanol, filtered and purification was carried out on silica C18 (250 X 4.6mm) column in an isocratic mode with methanol and water (1:1) as mobile phase and detected at 316nm using Shimadzu LC solution. The purified products were eluted with a flow rate of 0.75 ml/min.

Gas chromatography and Mass spectrometry (GC-MS)

GC-MS analysis of parent Congo red-21 dye and the enzyme mediated products was carried out using GCMS analyzer equipped with integrated

gas chromatogram. The injector temperature was maintained at 300°C with an oven conditions at 100°C kept constant for 2 min then increased up to 250°C with 10°C/min and raised up to 300°C with 30°C/min rate. NIST library was accessed to detect and identify the chemical compounds based on the mass spectra.

RESULTS AND DISCUSSION

Qualitative screening approach was utilized to detect the extracellular production of laccase by using ABTS and Guaiacol as standard substrates. The appearance of green color halos around the bacterial colony after 48h incubation, demonstrates, the extracellular production of laccase from *S.sviceus* KN3 (Fig. 1a). The production of laccase was further confirmed with guaiacol plate assay. The enzymatic



Fig. 1. Extra cellular production of Laccase by *Streptomyces syiceus* (a) ABTS as substrate (b) Guaiacol as substrate

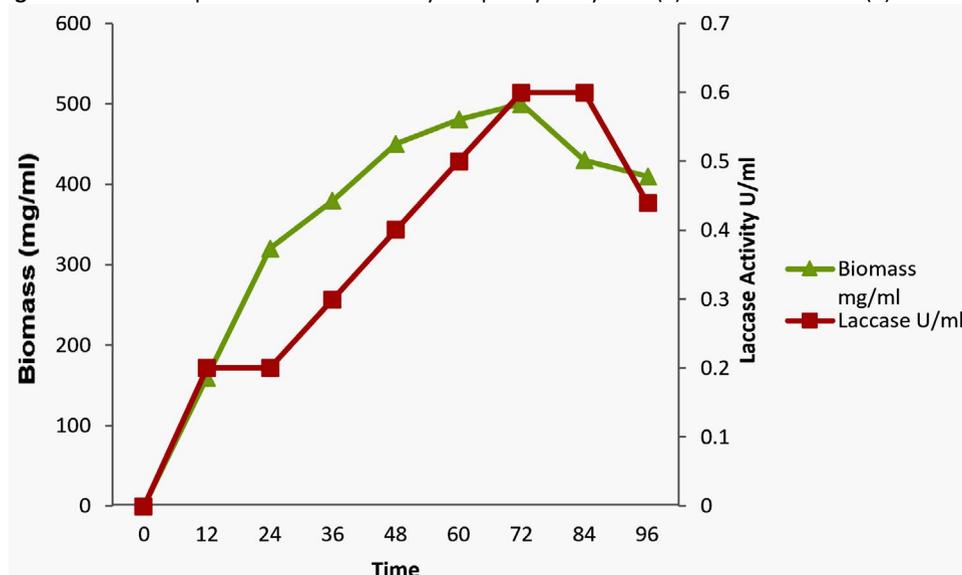


Fig. 2. Effect of time on production of laccase by *Streptomyces syiceus* strain KN3 purification and characterization of Laccase enzyme

reaction of laccase with guaiacol was visualized with the formation of reddish brown colour on the guaiacol plates inoculated with *S.sviceus* KN3(Fig. 1b). Seven different species of *Pseudomonas* were tested for the production of laccase by using standard substrates like syringaldazine or

guaiacol^{22,23}. The laccase activity was reported in *Streptomyces lavendulae*, *Streptomyces psammoticus*, *Streptomyces ipomoeae*, and *Streptomyces sviceus*²⁴. The supernatant of *B. subtilis* demonstrated the oxidation ABTS and guaiacol due to the extracellular production of

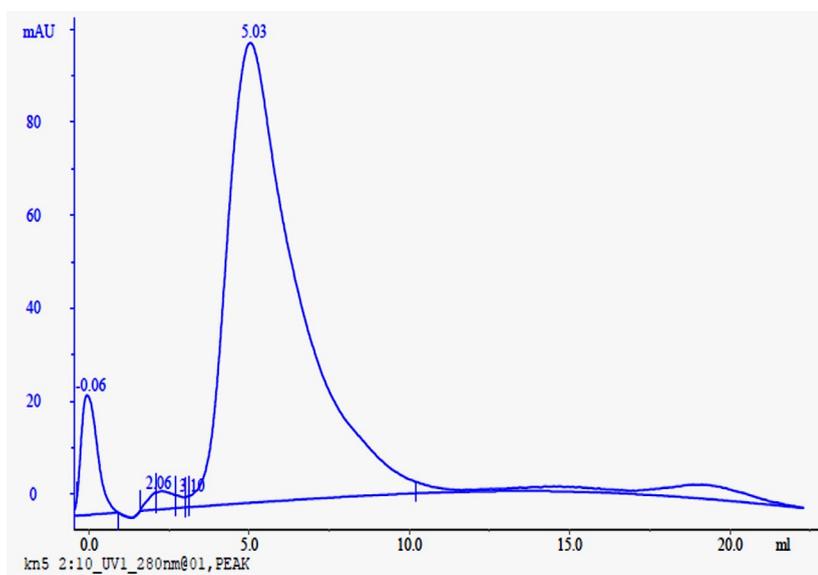


Fig. 3. Purification of laccase from *Streptomyces sviceus* strain KN3 by FPLC

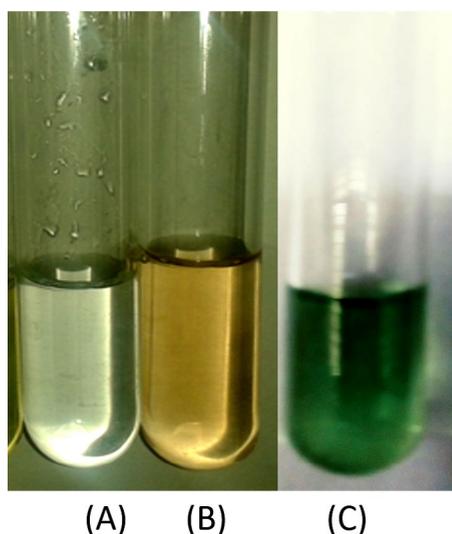


Fig. 4. Reaction of Purified laccase with substrates A) FPLC purified laccase B) Fraction with guaiacol C) Fraction with ABTS

laccase^{25,26} reported the decolourisation activity of laccase against azo dyes and the plate assay has been used to confirm the secretion of laccase in to the extracellular fluid when grown in the nutrient glucose medium.

Production of Laccase from *Streptomyces sviceus* strain KN₃

The extracellular production of laccase by *Streptomyces sviceus* strain KN3 was assessed at different time points. As shown in the Figure. 2 the maximum enzyme yield and biomass was noticed at 72h and considerable production of laccase and biomass was retained up to 84h of incubation. The declined trend was observed at later period and the data showed a linear correlation between laccase production and biomass. Among the textile degrading enzymes, Laccase is considered to be potential bio-remediating enzyme in the treatment of textile dyestuff and textile industry effluents²⁷. The decolorization of azo dyes with laccase enzymes

depends on the source of laccase and number azo bonds in the dye²⁸⁻³¹.

Purification and characterization of Laccase

All the three steps of purification were carried out at 4°C. At the initial step, the culture supernatant of *S.viceus* containing laccase with an initial activity (860U) was concentrated by ammonium sulphate precipitation. The optimum ammonium sulphate fractionation (80% W/V saturation) showed 2.02 U/mg specific activity compared to crude enzyme. The protein pellet obtained after 80% saturation with ammonium sulphate was dialyzed with 10mM potassium phosphate buffer pH 7.0 (Table 1). Further, the dialyzed sample was subjected to purification by Fast protein liquid chromatography (FPLC). The fractions were collected at a flow rate of 1ml/min on gradient

mode using 0.3M KCl as elution buffer. As shown in FPLC chromatogram (Fig. 3) a single peak was resolved at 5.63 min. The fraction collected at 5.63 demonstrated positive reaction with the formation of green and reddish brown complexes with ABTS and guaiacol respectively (Fig. 4).

The purity as well as molecular weight of laccase enzyme was detected by SDS-PAGE. Laccase showed the monomeric structure as visualized by single band with a molecular weight of about 60kDa (Fig. 5). The molecular weight of the laccase enzyme varies depends on the source of the organism³² and the molecular mass of laccase was found to be between 50-130 kDa³³. Few bacterial species with laccase activity have been reported in the degradation of azo dyes^{34,4} However, the laccases isolated from *Streptomyces* are found to be effective for the degradation of reactive azo dyes. And the Laccase enzyme purified from *Streptomyces cyaneus* CECT demonstrated the degradation of azo dyes³⁶.

Decolorization of Congo Red-29 by Laccases

Laccase has been widely applied in the decolourisation of textile dyes compared with azo reductases due to the generation of nontoxic phenolic compounds by free radical mechanism^{37,38}. To evaluate the decolorization efficiency of laccase, the Congo red -21 dye was treated with different concentrations (25mg/l and 50 mg/l) of Crude/purified /immobilized enzyme for different time periods. As shown in the Table 2 the decolorization of congored-21 was commenced within 24 h and the rate of decolorization accelerated with increase in time. Crude enzyme showed 69% of decolorization after 48h where as purified and immobilized laccase represented 78% and 92% after 24 h respectively. The data demonstrates the efficacy of immobilized laccase in the decolourisation and degradation of synthetic azo dyes. *Podoscypha elegans* demonstrated maximum degradation of 70% of Congo red and Rose Bengal after 72 days of incubation³⁹ where as bacterial consortium of *P. rettgeri* strain HSL1 and *Pseudomonas sp.* SUK1 represented the degradation of 22% of 100 mg/L of azo dyes within 48 hours⁴⁰. *Aspergillus niger* exhibited 70% decolourisation of Congo red after 6 days of incubation due to the presence of Lignin peroxidase and Manganese peroxidase and the laccase is totally absent in the culture filtrate of *A.niger*⁴¹.

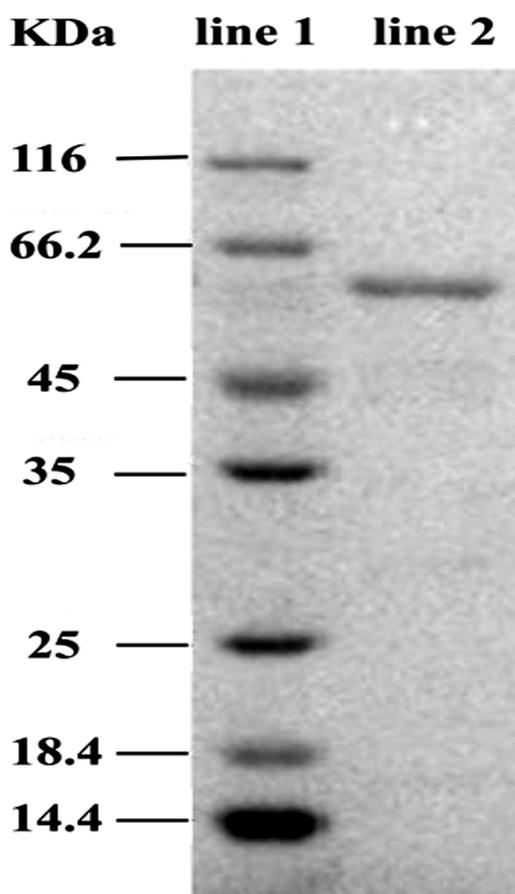


Fig. 5. Molecular weight of laccase derived from *Streptomyces syiceus* strain KN3 line 1: Molecular weight marker Line 2: FPLC purified enzyme

Immobilized Laccases were considered as potential enzymes to be potential candidates in the bioremediation of synthetic dyes as well as textile effluents⁴². Cross-linked aggregates of Laccases enzyme derived from *Cerrena* sp. demonstrated 90% decolorization of Remazol Brilliant Blue Reactive dye⁴³. Similarly, immobilized laccase showed better elimination of colour from Procion Green H4G, Brilliant Blue G, and Crystal Violet on comparison with free enzyme^{17,45}.

Congo red dye degradation by immobilized laccase

Dyes are decolorized either through adsorption or degradation. The dyes are adsorbed

only on the surface of the bacterial cells where as degradation of dyes by bacterial enzymes, results in the formation new products. Different analytical methods were adopted to detect feasible degradation pathway and the metabolites generated from Congo red-21 after treatment with immobilised laccase derived from *Streptomyces sviveus* KN3.

The molecular signatures indicating the functional groups of Congo red-21 parent dye were contrasted with the functional of detoxified Congo red-21 dye. The FTIR spectrum of Congo red-21 control demonstrates molecular signatures at 3250,3100,2370,2080, 1620, 1598,970 and 765

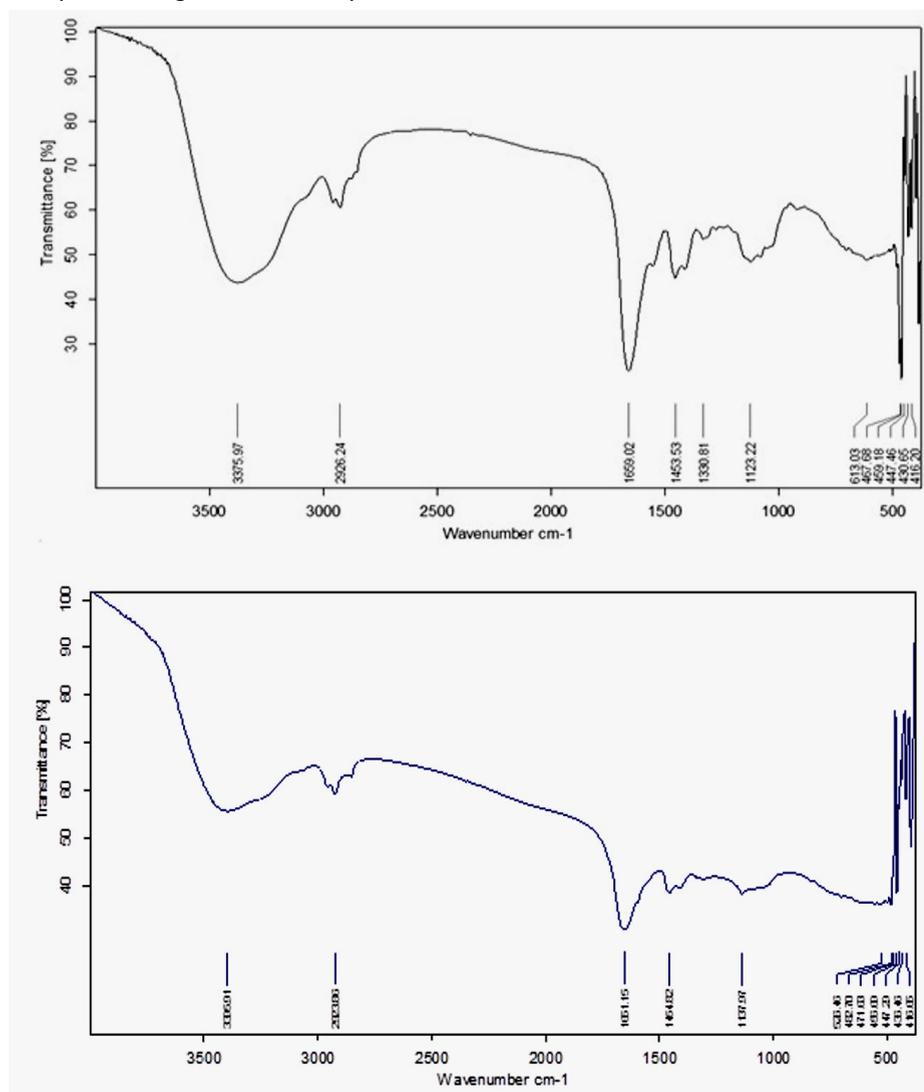


Fig. 6. FTIR spectrum of Congo red-21 (a) control dye (b) Congo red-21 dye after decolorization by immobilized laccase

Table 1. Purification profile of laccase from *Streptomyces viceusstrain* KN3

Purification Steps	Volume (ml)	Total Laccase activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification Fold	Yield %
Crude	1000	860	450	1.60	1	100
Ammonium sulphate	25	700	320	2.02	1.10	82
Dialysed	15	610	192	3.00	1.70	70
FPLC	5	300	30	10.20	5.82	36

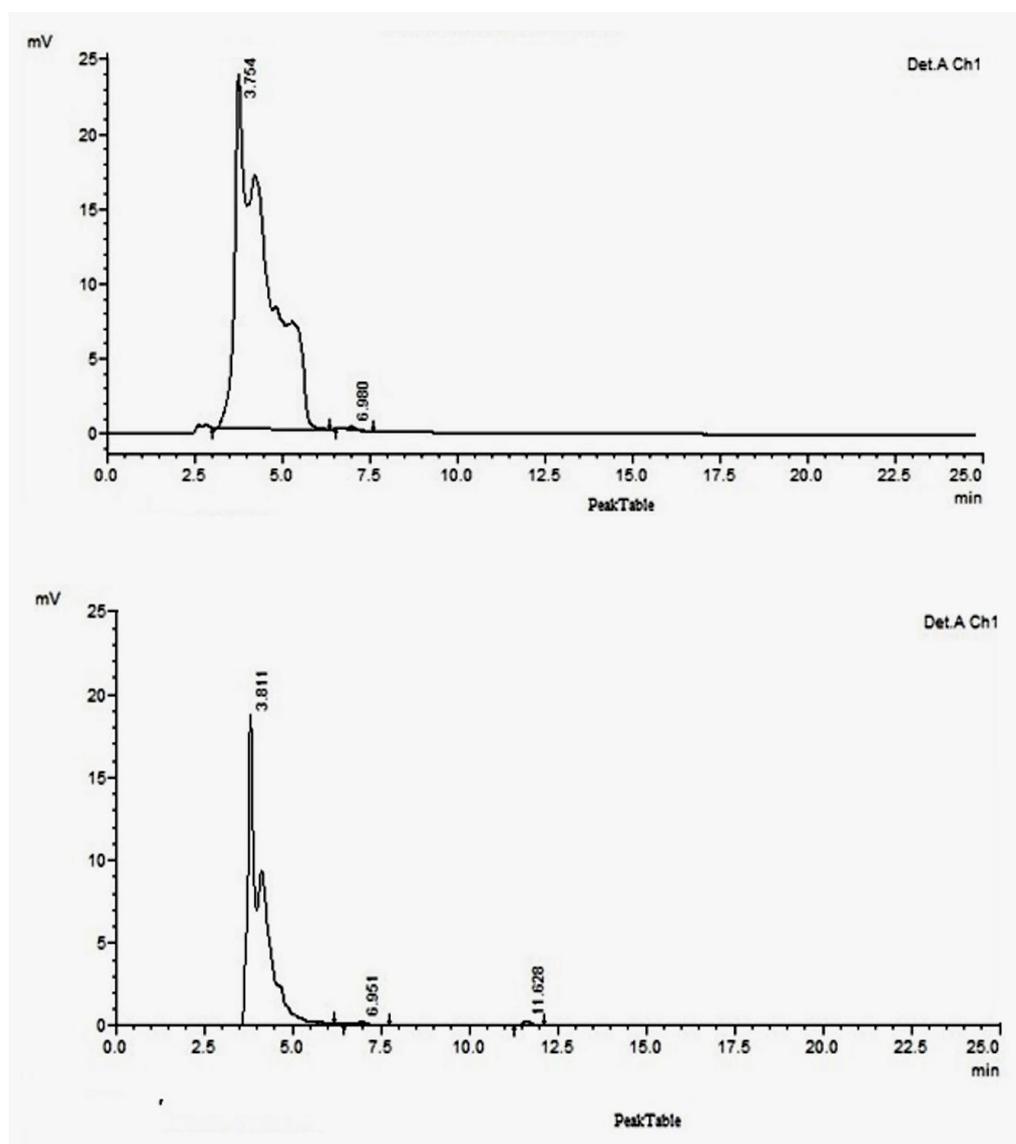


Fig. 7. HPLC chromatograms (A) Congo red-21 parent dye after its degradation by immobilized laccase

cm⁻¹ (Fig. 6a). The molecular signatures from 3250 to 2980 indicates the functional groups such as OH stretch, C-H stretching of aromatic compounds, C≡N stretching, C≡C stretching of and 765 cm⁻¹ demonstrated the aromatic nature of the congo red dye. Specifically, the control dye showed signatures at 1620cm⁻¹ and 1598cm⁻¹ due to the presence of azo linkage and N=N stretching respectively. The FTIR spectrum of extracted metabolites after decolorization by Immobilized laccase demonstrated the absence of IR signatures at 1620cm⁻¹ and 1598cm⁻¹ indicates the cleavage of azo linkage (Fig. 6b). The signature at 1096 cm⁻¹, 1371 cm⁻¹ indicates S-O stretching and S=O

stretching indicated the formation of sulfonates after the degradation by laccases.

These changes in the IR signatures are clear evidence for the degradation of Congo red-21 dye by immobilized laccase. Our spectral data is in agreement with decolorization of textile effluent by *Enterobacter asburiae*⁴⁶. FTIR spectrum provides the information about the type and strength of interactions that occurs with in azo-dyes containing different functional groups after degradation by bacteria. The FTIR technique has been used previously by some workers for analyzing azo-dye degraded products⁴⁷.

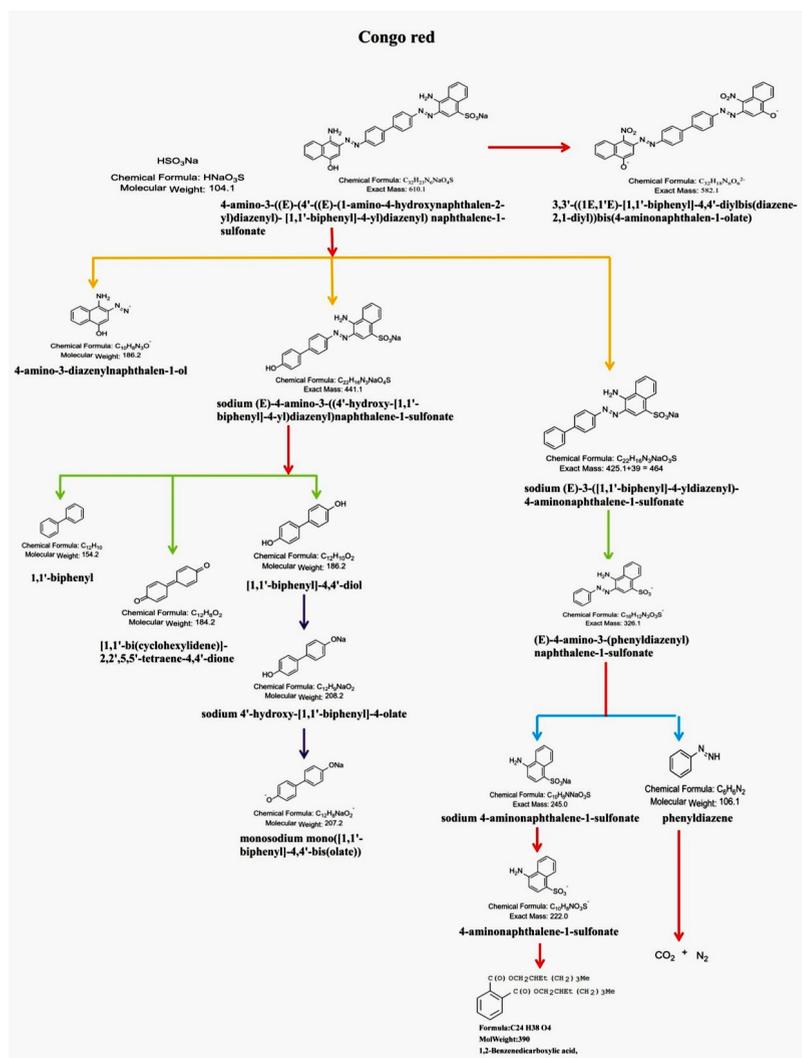


Fig. 8. Proposed pathway of Congo red-21 degradation by Laccase mediated system

Table 2. Congo Red-21 Decolorization by Laccase

Dye	Enzyme	Conc. (mg/l)	decolorization (%)			
			12 h	24 h	36 h	48 h
Congo red-21	Crude	25	12±0.20	25±0.10	35±0.30	52±0.12
		50	22±0.20	44±0.20	53.3±0.10	69±0.20
	Purified	25	43±0.21	62±0.30	71±0.12	80±0.04
		50	58±0.02	78±0.05	84±0.03	84±0.12
	Immobilized	25	68±0.10	82±0.11	92±0.24	96±0.02
		50	78±0.03	92±0.12	96±0.11	96±0.20

HPLC study of Congo red-21 dye and laccase degraded dye was carried out and retention profiles were recorded. Both the samples demonstrated elution peaks at varied retention times. The Congo red -21 dye showed a major peak at 3.754 (Fig.7a) where as dye metabolites of degradation by *Streptomyces sviveus* KN3 exhibited two major peaks at 3.81 and 4.6 and two minor peaks at 6.95 and 11.6 (Fig.7b). Two peaks indicated in degraded sample compared with single peak in parent Congo red-21 dye clearly indicates the degradation of Congo red-21 dye. Our results were supported with the demonstration of new peaks in the decolourised sample of CR by DTS26 due to the production of different intermediate metabolites.

Mass spectrometry is an advanced analytical tool used for the detection of intermediates generated during laccase mediated degradation of reactive synthetic dyes. The proposed enzymatic cleavage pathway involved in the degradation of dyes can be deduced based on the GC-MS data which provides the information about the structure and molecular weight of the degraded products on comparison with NIST data library. GC-MS analysis was performed to characterize the products formed during Laccase mediated degradation of Congo red-21. The Gas chromatogram of Congo red-21 control dye exhibited minor peaks at 1.1, 15.8, 16.8 and 19.5 min. The gas chromatogram of Congo red-21 dye degraded by *Streptomyces sviveus* strain KN3 showed two major peaks at 25.33 and 26.00 min and minor peaks at 16.78, 18.75 and 19.15 min. The structure and molecular weight of identified degraded compounds were validated based on fragmentation pattern and m/z values.

Based on GC-MS data, it may be deduced that laccase cleaves the Congo red-21 dye molecule through asymmetric cleavage, followed by oxidative cleavage, desulfonation, deamination, demethylation process.

The degradation of Congo red-21 dye was initiated with asymmetric cleavage which results in the formation of sodium (E)-4-amino-3-((4'-hydroxy-[1,1'-biphenyl]-4-yl) diazenyl)naphthalene-1-sulfonate (molecular weight 441.1), 4-amino-3-diazenyl naphthalene-1-01 (molecular weight 186.2), Sodium (E)-3-(1,1'-biphenyl)-4-yl diazenyl)-4-aminonaphthalene-1-sulfonate (molecular weight 464). Further, sodium (E)-4-amino-3-((4'-hydroxy-[1,1'-biphenyl]-4-yl) diazenyl)naphthalene-1-sulfonate was transformed to 1,1'-biphenyl (molecular weight 154.2), 1,1-bi(cyclohexylidene)-1,2,2',5,5'-tetraene-4,4'-dione (molecular weight 184.2), -[1,1'-biphenyl]-4,4'-diol (molecular weight 186.2) by desulfonation and deamination and finally results in the formation of monosodium mono (1,1'-biphenyl)-4,4'-bis(olate) (molecular weight 207.2) (Table.3). As shown in the pathway (Fig.8), Sodium (E)-3-(1,1'-biphenyl)-4-yl diazenyl)-4-aminonaphthalene-1-sulfonate was converted into 4-amino-3-phenyldiazenyl naphthalene-1-sulfonate (molecular weight 326.1) which further results in the formation of sodium 4-aminonaphthalene-1-sulfonate (molecular weight 245), and phenyldiazene. The data indicated the accumulation of 1,2 benzendicarboxylic acid (molecular weight 390) as the end product as evidenced from the major peaks of GC-MS chromatograph.

Degradation of azo dye is a complex process and the degradation of azo dyes by laccases

Table 3. GC-MS profile of Congo red-21 degradation products after laccase action

S.No	R.T	Molecular weight	Compound Name
1	18.750	186.2	1,1'-biphenyl]-4,4'-diol
2	19.158	184.2	[1,1'-bi(cyclohexylidene)]-2,2',5,5'-tetraene-4,4'-dione
3	31.050	441.1	sodium (E)-4-amino-3-((4'-hydroxy-[1,1'-biphenyl]-4-yl)diazanyl) naphthalene-1-sulfonate
4	16.783	154.2	1,1'-biphenyl
5	29.117	207.2	monosodium mono([1,1'-biphenyl]-4,4'-bis(olate))
6	31.717	326.1	(E)-4-amino-3-(phenyldiazanyl) naphthalene-1-sulfonate
7	24.042	245.0	sodium 4-aminonaphthalene-1-sulfonate
8	25.33	390	1,2-Benzenedicarboxylic acid
9	26.00	390	1,2-Benzenedicarboxylic acid

has been initiated with asymmetric cleavage of the azo bond⁴⁸. After cleavage, the products are subjected to oxidative cleavage desulfonation, deamination, demethylation and dehydroxylation, depending on the azo dye structure^{48,14}. Research reports demonstrated the degradation through non-specific free-radical mechanism without the cleavage of azo bonds^{50,34}. The Mass spectral data of Congo red -21 degraded by laccase is in agreement with literature reported the asymmetric cleavage of the azo bond⁵². Our results revealed the possible mechanism for the cleavage of congo red-21 dye which involves asymmetric cleavage followed by oxidative cleavage, desulfonation, deamination and demethylation which results in the production of benzene dicarboxylic acid and CO₂. The degradation of Congo red -21 by *Trametes pubescens* exhibited the formation of naphthalene amine⁵³. As per the literature, the degradation of azo dyes is a complicated process and involves asymmetry. In earlier study, we reported the nontoxic nature of degraded products based on their insignificant effect on chromosomal pattern and the germination potential higher plants⁵⁴. The current data confirms the bioremediation potential of immobilized laccase of *Streptomyces sviveus* strain KN3.

CONCLUSION

From the present study, it can be concluded that Congo red dye was effectively degraded by laccase of *S. sviveus*. High decolorisation efficiency was noticed with purified laccase (78%) and immobilised laccase (92%) after 24 h. FT-IR and GC-MS analysis proved the significance of immobilised

laccases in the mineralization and detoxification of the reactive azo dyes, particularly Congo red-21 dye. Thus, degradation by immobilised laccase derived from marine actinomycetes promotes a promising approach in the treatment of textile effluents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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