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Effect of *Termitomyces* sp. on Decolorization and Degradation of Congo Red (CR)

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ABSTRACT: Decoloration and degradation of Congo red by basidiomycete fungi *Termitomyces* sp. was evaluated in this study using fungal biomass as adsorbent. Potential isolate TMS 7 *Termitomyces* sp. isolated from Western Ghats of Tamil Nadu and their biomass production was optimized using four different media. Decoloration study was conducted using different quantity of fungal biomass (0.5-3.5 g) to decolorize different concentration of Congo red (25-250 mg/L) and degradation of congo red was evaluated by using FT-IR. Fungal biomass production was optimized using four different media i.e. Potato Dextrose Agar, Malt Extract agar, Modified Melin Nokrans and Pridham-Gottlieb Modified by Kueti (PGK) and malt extract media recorded maximum growth than other three. Results of decoloration study reveal that least dye concentration (25 mg/L) was degraded up to 99.9 per cent by 0.5-3.5 g fungal biomass and highest dye concentration (250 (mg/L) was decolored to the extent of 55.2% by high fungal biomass of 3.5 g/0.1 L and 3.2 % by 0.5 g biomass during 3 days of incubation at ambient temperature. The degraded products of dye were characterized by FTIR analysis and the results depicted that degraded products of Congo red dye were adsorbed with fungal cell wall material and decolorize the aqueous dye solution. Results inferred that dyedegradation potential of *Terrmitomyces* sp. increased the applicability of this microorganism for detoxification of azo dye from wastewater.

Keywords: Azo dye-Congo red- *Termitomyces* sp.- degradation-FTIR.

INTRODUCTION

Azo dyes are highly colored compounds cause potential threat to environment by reducing the transparency of water bodies. In textile industries, during dyeing process nearly 10-15% of used dyes are released in waste water which is toxic for environment and aquatic life (Machado and Matheus, 2006). Therefore various environmental protection acts were enforced worldwide, which obliges the textile industries to treat the effluents before disposal to environment.

Various physical and chemical methods (coagulation–flocculation, oxidation, and electrochemical methods) have been used to eliminate the colored effluents in wastewater (Daneshvar *et al*., 2004) in the last few decades. However they are generally expensive, of limited applicability and produce large amounts of sludge. Goel and Abhilasha (2016) studied the Degradation of Acid Orange – IV using HCF(III) ions in Aqueous Alkaline Medium, which proved its ability as potential oxidant for the removal of dyes. Ram *et al.*, (2012) studied the Photocatalytic Degradation of Textile Dye reactive Procion Yellow (PY) by Using Titanium Dioxide Nanocatalyst. The experimental results indicated that 83.6% degradation occurred at optimized conditions (Dye concentration 100 ppm, pH 7.8, TiO_2 dose 0.5g/L, UV intensity 25 W/m^2 and time 3.5h). Kumar *et al.*, (2020) studied the photocatalytic degradation of Rhodamine B dye using ZnO Nanoparticles. Photodegradation of Rhodamine B dye in wastewater occurs at a faster rate under UV light. The presence of ZnO acts as a catalyst for the degradation of dye in wastewater.

Biotechnological tools also have been applied for the degradation of various textile dye and it was found that upto 70% color removal was noticed with different microflora (Khadijah *et al.*, 2009). Several microorganisms including fungi, bacteria, yeast and algae can decolorize and even completely mineralized many azo dyes under certain environmental conditions (Pandy *et al*., 2007). Khadijah (2009) reported 1540 bacterial isolates and screened for their ability to degrade selected azo dyes. Kochher and Kumar (2011) study the decolourization of Crystal Violet by *Bacillus subtilis* and decolorization was effective at pH 8, 35°C

with starch and peptone as carbon and nitrogen sources and in static conditions.

The role of fungi in the treatment of waste water has been extensively researched. Fungus has proved to be a suitable organism for the treatment of textile effluent and dye removal. Due to an increased cell-to-surface ratio, fungi have a greater physical and enzymatic contact with the environment. The extra-cellular nature of the fungal enzyme is also advantageous in tolerating high concentration of the toxicants. Many genera of fungi have been employed for the dye decolourization either in living or dead form (Prachi and Anushree, 2009).

Dyes are removed by fungi through biosorption, biodegradation, bioaccumulation and enzymatic mineralization (Lignin peroxidase, Manganese peroxidase, Manganese independent peroxidase and Laccase) (Wesenberg *et al*., 2002). Biosorption process is commonly used for the removal of dyes as well as heavy metal and organic pollution. It is a process in which solids of natural origin are employed for sequestration and isolation of heavy metals from an aqueous environment (Muraleedharan *et al*., 1991). Most biosorption studies have been carried out on microbial systems, chiefly bacteria, microalgae and fungi (Gadd, 2009).

Congo red is a widely used azo dye in the textile dyeing, due its high solubility in water. They contain sulfonic groups and diazo (N=N) groups, hence considered as highly recalcitrant. Various fungal sp. such as *A. niger* (Nedra Asses *et al*., 2018), *Phanerochaete chrysosporium* (Nagarajan and Annadurai, 1999), *Caldariomyces fumago* (Xuelian *et al*., 2013), *Curvularia* sp. (Senthilkumar *et al*., 2014) and *Trametes Versicolor (*Krastanov *et al*., 2013) were reported earlier as Congo red degrading fungi. Dye degradation and decolorization of textile effluent using biological adsorbents like *Termitomyces* sp. is an ecofriendly, sustainable alternative to chemical sorption and other effluent treatment method. Hence present study was conducted to investigate the efficient decolorization of Congo red by *Termitomyces* sp. fungal biomass.

MATERIALS AND METHODS

Study site

Laboratory studies were carried out at department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore (Lattitude: 11°07' 3.36" N, Longitude: 76° 59' 39.31" E), Tamil Nadu during September 2019 to January 2020.

Organisms and culture conditions

Fungal strain *Termitomyces* sp. TMS 7 (Gen Bank accession No. MW694830) was isolated from fruiting body (basidiocarp) of white fungus collected from Western Ghats of Tamil Nadu, India during North east monsoon (October, 2019). Standard culture *Termitomyces albuminosus* (MTCC 1366) was obtained from Indian Institute of Microbial Technology, Chandigarh. The cultures were maintained in Potato dextrose Agar slants at 4°C and sub-cultured periodically.

Biomass production

Fungal biomass production was optimized usingfour different isolation media i.e. Potato Dextrose Agar (PDA), Malt Extract agar, Modified Melin Nokrans (MMN) and Pridham-Gottlieb Modified by Kueti (PGK) to attain high biomass. One disc (5mm) of fungal isolate was transferred to 250 ml Erlenmeyer flasks containing 100 ml of autoclaved culture media. The flasks were incubated at ambient temperature for 15 days. The biomass was determined by calculated the dry mass of mycelia. Mycelia were harvested from the cultivation liquid medium by filtration using whattman No. 1 filter paper and dried of 65°C at 30 min and weighted (g/100 ml).

Preparation of fungal mat

Fungal mat were extracted from *Termitomyces* sp. grown in Malt extract broth after 15 days of incubation at 32°C under static condition and used as an biosorption material for dye decolorization and degradation.

Preparation of dye solution

Azo dye Congo red was taken for this experiment which is chemically sodium salt of benzidinediazo-bis-1-naphtylamine-4 sulfonic acid. Stock solutions were prepared by dissolving one gram Congo red in 1 L of distilled water. The working solutions (25, 50, 100, 150, 200, 250, 500 mg/L) were prepared by successive dilutions of the corresponding stock solutions.

Assessing Congo red decoloration by spectrophotometer

The assay was carried out in 100 ml flask containing 50 ml distilled water along with varying concentrations (25, 50, 100, 150, 200, 250 mg/L) of the azo dye (Congo red). The flasks were sterilized in an autoclave at 121°C at 15 lbs pressure for 15-20 minutes. The flasks were then added with fungal mat (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 & 3.5 g/0.1L) and incubated. Samples were withdrawn at periodic intervals viz., 0hr, 12hr, 24hr, 48hr and 72hr and centrifuged at 8000 rpm for 20 minutes. Decolourization was evaluated by measuring the absorbance of the supernatant at 566 nm using a spectrophotometer. The efficiency of decolorization was expressed as the percentage ratio of the decolorized dye concentration to the initial dye concentration based on the following equation.

% Decolourization = {(Initial absorbance × Final absorbance)/ Initial absorbance)} 100

Detection of vibration and conformational changes by ATR-FTIR

Congo red solution before and after fungal mat treatment (decolourized solution) were centrifuged at

8000 rpm for 20 minutes and the supernatant obtained were subjected to FT-IR analysis. Similarly pure fungal mat and dye adsorbed fungal mat also subjected to FT-IR and spectra were obtained using an ATR-FTIR (Jasco) with a scanning range of 400 cm^{-1} to 4000 cm-1 and resolution of 4 cm^{-1}.

RESULT AND DISCUSSION

Isolation of *Termitomyces* sp. from Western Ghats of Tamil Nadu

Termitomyces is a slow growing fungus mostly isolated from the basidiocarb (fruiting body) of the fungus and spores present in the termite mound soil. It has symbiotic association with termite and hence locally called as termite fungus. Various sp. of termitomyces such as *Termitomyces clypeatus, T. microcarpus, T. albuminosus* are occurred in Western Ghats and southern part of Tamil Nadu. They generally flourish in the termitorium during September –November months. Termite and fungus were in symbiotic relationship and fulfill one another needs. Due to the complex nutrient need, growing Termitomyces in laboratory condition is not easy. Different growing media were used earlier to grow this fungus.

In the present investigation a total of 12 fungal isolates were isolated from fruiting body of termitomyces collected from Western Ghats of Tamil Nadu, India during September 2019 and screened based on ligninolytic enzyme production and biomass production. One best isolate TMS 7 was chosen to study the Congo red decolorization efficiency. TMS 7 further identified as *Termitomyces* sp. by ITS 1 and ITS 4 primers and deposited in NCBI (accession No. MW694830). Karun and Sridhar (2013) reported occurrence of *Termitomyces* sp. from Kodagu District and Dakshina Kannada District, Karnataka during southwest monsoon and post-monsoon seasons (June–November).

Optimization of media for biomass production of *Termitomyces* sp.

Termitomyces is an obligate symbiont associated with Macrotermitinae termites (Nobre *et al*., 2011) and cultivation of this macro fungus is difficult due to its complex ecosystem and nutritional requirement. Many researchers use modified malt extract medium and different substrates (wheat straw, bean straw, saw dust etc.) for cultivating *Termitomyces* sp. (Zeleke *et al*., 2013). Wiriya *et al*., (2014) evaluated different culture media on growth of Termitomyces mushroom. Of ten culture media, malt extract agar was the best for mycelia growth for all isolates.

In the current investigation growth of the fungal isolate TMS 7 was evaluated using four different media. Out of the four different media used malt extract media recorded maximum growth than other three. It exhibited highly significant growth compared with other media. Rate of fungal growth is as follows MEA >PDA> MMN >PGK. The growth of the fungus was slower in PDA medium (2.76(g/100 ml) compared to Malt extract medium (4.85 (g/100 ml). Little/negligible amount of growth was observed in Modified Melin Nokrans (MMN) and least growth was recorded in Pridham-Gottlieb Modified by Kueti (PGK) medium compared to standard culture (*Termitomyces albuminosus*-MTCC 1366) (Table 1).

Table 1: Biomass production of *Termitomyces* sp. in different media.

Media	Biomass (g/100 ml)	
	TMS 7	Std culture
Potato dextrose agar	2.76	1.57
Malt extractagar	4.85	3.61
MMN	1.01	1.27
PGK	0.75	0.81
Mean	**1.874**	1.452
SEd	0.142	0.109
CD	0.302	0.231

Decolorization efficiency

Basidiomycetes fungi is an area of interest because they are lignin degrading fungi, synthesize lignin peroxidase, manganese peroxidase and laccase and thus are able to degrade broad range of recalcitrant, carcinogenic and toxic organic compounds including several dyes (Dixit *et al*., 2012). In the current research effect of *Termitomyces* fungus on Congo red dye decoloration was studied in aqueous solution. Dye concentration ranges from 25-250 mg/L and biomass quantity ranges from 0.5 -3.5 g/0.1 L were taken for this study. Fungal biomass required to degrade Congo red was increased when dye concentration increases otherwise per cent reduction achieved by the least quantity biomass (0.5 g/0.1 L) was reduced when dye concentration increases (Fig. 1).

Least dye concentration (25 mg/L) was degraded up to 99.9 per cent by 0.5-3.5 g fungal biomass. 25, 18 and 3.2 per cent reduction of 150, 200, 250 mg of dye concentration was achieved by 0.5 g of fungal biomass (Fig. 2). Whereas highest dye concentration (250 (mg/L) was decolored to the extent of 55.2% by high fungal biomass of 3.5 g/0.1 L and 3.2 % by 0.5 g biomass (Fig. 3).

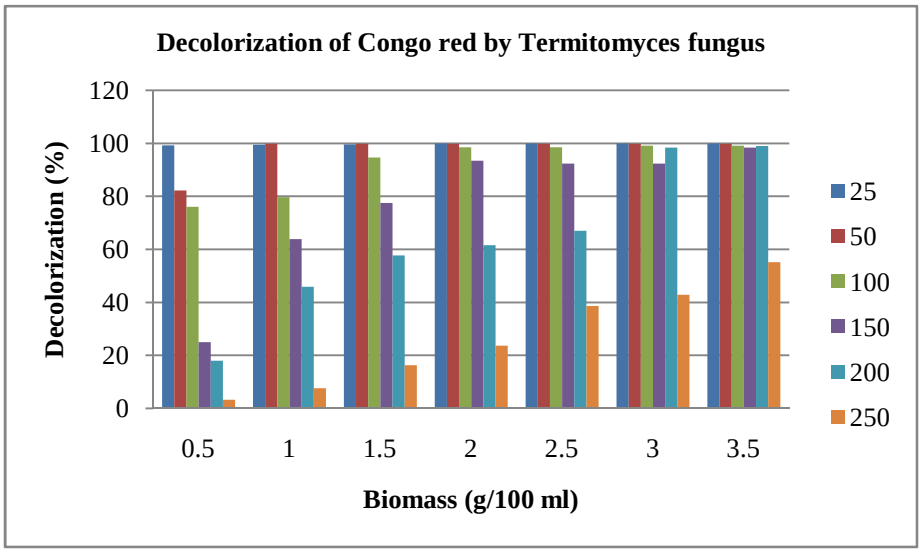

Fig. 1. Effect of different quantity of fungal biomass on varying Congo red concentration.

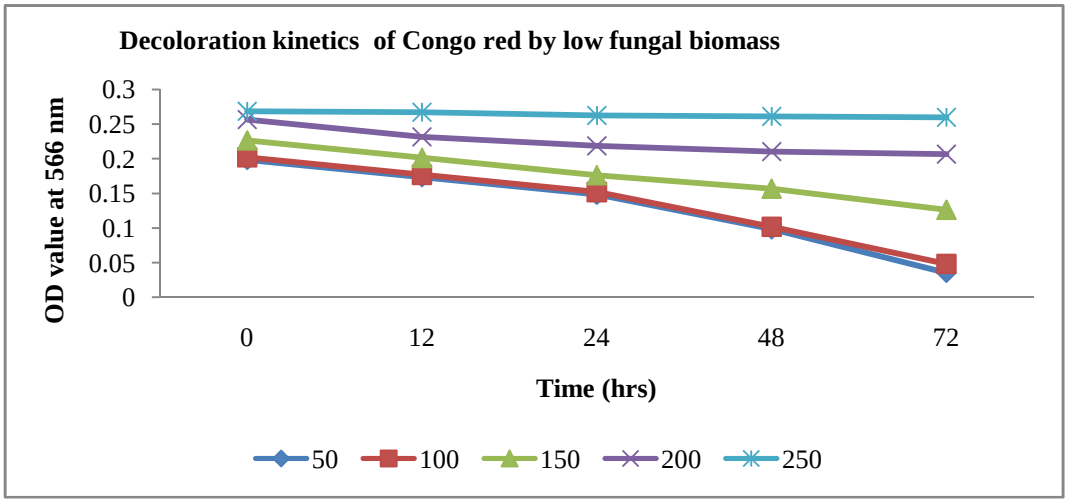

Fig. 2. Decoloration kinetics of Congo red by low fungal biomass.

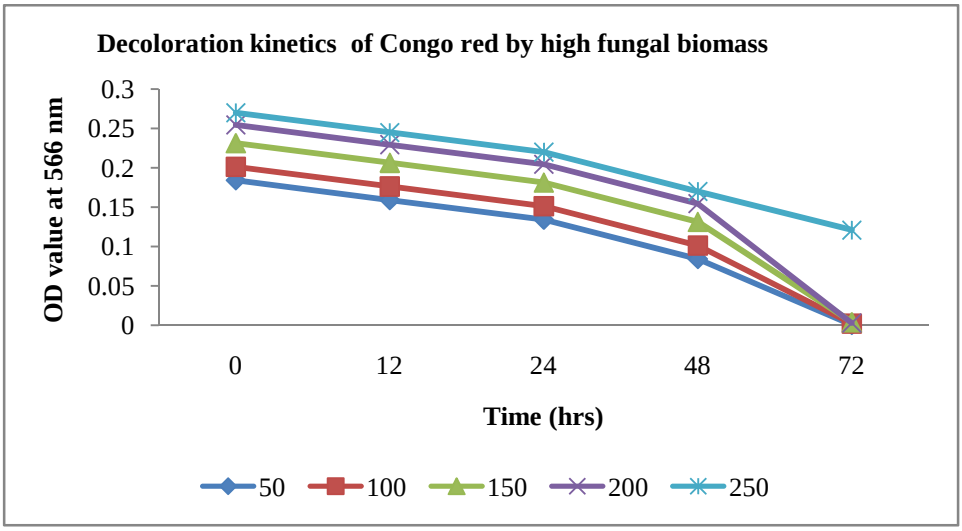

Fig. 3. Decoloration kinetics of Congo red by high fungal biomass.

Graphical representation (Fig. 2) explaining the decolorization maxima attained by 0.5 g fungal biomass in varying dye concentration. It degraded 99.2 per cent of 25 mg dye concentration, 82.2 % of 50 mg concentration, 76.1 % of 100 mg dye, 25 % of 150 mg dye, 18 % of 200 mg dye and 3% of 250 mg dye concentration per litre.

Graphical representation (Fig. 3) explaining the decolorization maxima attained by 3.5 g fungal biomass in varying dye concentration. It degraded 99.94 per cent of 25 mg dye concentration, 99.89 % of 50 mg concentration, 99.06 % of 100 mg dye, 98.4 % of 150 mg dye, 98.94% of 200 mg dye and 55.23% of 250 mg dye concentration per litre.

Decolorization of congo red by *Aspergillus niger* was assessed by Nedra Asses *et al*., (2018) reveals that of 200 mg/L of dye concentration was decolorized above 97% with 2 g mycelia during 6 days at 28°C. Another study recorded decolorization of congo red dye by *Alternaria alternata* CMERI F6 and the decolorization rate was maximal at 25°C, pH 5, 150 rpm and 800 mg/L dye, giving 78% final decolorization efficiency (Chakraborty *et al*., 2013).

Wang *et al*., (2017) reported decolorization of Congo red by *Ceriporia lacerata* ZJSY and decolorization rate was above 90% at 48 h with 3 g mycelia into 20 mL of Congo red solution with the concentration 0.1 mg mL^{-1}. Decolorization study by Kumari (2018) showed that the methyl orange dye was removed by more than 50% in 3 days, Eriochrome Balck T (removed by more than 33%) and HPLC analysis determined several degradation products. These results suggest that this fungi body has potential in color removal from textile waste water containing various azo dyes.

Congo red biosorption on *Termitomyces* fungal mat

Biological treatment of the effluent may become an economically and environmentally attractive alternative to the present physico-chemical methods of treatment. Fungus has proved to be a suitable organism for the treatment of textile effluent and dye removal. The active role of fungi in the treatment of wastewater has been extensively researched and decolorization of various dyes has also been reported to have occurred through both adsorption and degradation by filamentous fungi (Jadhav *et al*., 2010).

In the present investigation effect of fungal biomass on the degradation of Congo red were analyzed through FT-IR. Fig. 4 shows composite representation of FTIR spectra for *Termitomyces* fungal mat and Congo red absorbed fungal mat. Detailed FTIR bands assignments of characteristic infrared bands are shown in Table 2.

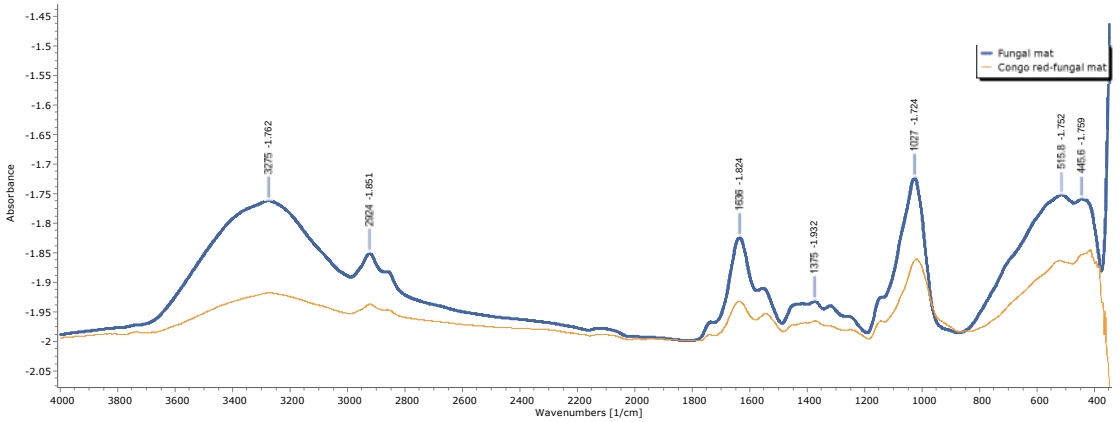

Fig. 4. FTIR spectra of *Termitomyces* hyphal mat, in comparison with Congo red adsorbed fungal mat.

Results of Congo red degradation study using *Ceriporia lacerata* biomass showed the characteristic band at 3275 cm^{-1}, which is attributed to O–H bending vibrations (Wang *et al.*, 2017). *Aspergillus niger* degraded congo red and produces peak at 2937 cm^{-1} corresponds to asymmetric and symmetric stretching of the C- H bond of -CH_2 group. The band at 1641 cm^{-1} is due to the bending of N–H groups of chitin on the cell wall structure of fungal pellets (Ayed *et al*., 2013).

Our present study also recorded similar results. Analysis of *Termitomyces* fungal mat recorded absorbance bands originated at 445.71 and 515.84 cm^{-1} are assigned to C-I stretch and C-Br stretching of alkyl halides with strong intensity. One strong absorbance was observed at 1027.4 cm^{-1} is assigned to C-O stretching. There was a medium intensity CH deformation, indicating alkanes at 1375.4 cm^{-1}. Medium absorbance was found in the double bond region as C=C stretching, Alkenyl at 1636.2 cm^{-1}. Two strong absorbance peaks were observed in the single bond region (2500-4000 cm^{-1}), assigned to Methylene C-H, asym./sym. Stretching at 2923.9 cm^{-1} and sharp, C-H stretch at 3275.3 cm^{-1}.

Table 2: FTIR band assignment of fungal mat before and after Congo red absorption.

FTIR -Position of Bands (cm^{-1})		Type and Origin of Vibrations
Fungal mat	Congo red adsorbed fungal mat	
445.71	415.34	Strong, C-I stretch
515.84	520.02	Strong, C-Br stretch
1027.4	1020.1	Strong, C-O stretching
1375.4	1372.4	Medium, CH deformation alkanes
-	1547.3	Aromatic nitro compounds
1636.2	1638.7	Medium, C=C stretch, Alkenyl.
-	1995.9	Medium, C=C=C
-	2115.5	2100-2270- diamides & azides medium -N=C=N-, -N ,
2923.9	2923.2	Strong, Methylene C-H, asym./sym. Stretch
3275.3	3272.1	Strong, sharp, –C-H stretch 3265-3335
-	3740	Primary amines
-	3828.6	Primary amines

There was shift in peaks of congo red absorbed fungal mat from 445.71 to 415.34 cm^{-1}, 515.84 to 520.02 cm^{-1}, 1027.4 to 1020.1 cm^{-1} , 1375.4 to 1372.4 cm^{-1} , 1636.2 to 1638.7 cm^{-1}, 3275.3 to 3272.1 cm^{-1} and a negligible shift in case of peak at 2923.9 cm^{-1} (2923.2 cm^{-1}) when compared with the bands observed in case of fungal mat.

Also new peaks were observed at 1547.3 cm^{-1} (Aromatic nitro compounds); 1995.9 cm^{-1} (Medium, C=C=C stretching); 2115.5 cm^{-1} (medium intensity diamides & azides, -N=C=N-, -N). Peak observed at 3740 and 3828 cm^{-1} corresponding to primary amines which are widely found in proteins and amino acids. These might be from the fungal cell wall proteins conjugated with disintegrated Congo red dye, because these peaks are not observed in pure fungal mat.

As compared to the control dye, the disappearance of peak at 2120 cm^{-1} indicates the possible breakage of -N=C=N- bond and degradation of CR by the fungus in decolored solution after 5 days. Degraded azo compound was adsorbed with fungal mat, proved by peak appearance at 2115.5 cm^{-1} in Congo red adsorbed fungal mat, which was not present in fungal mat (Table 2.)

Congo red degradation by *Aspergillus niger* was reported earlier (Nedra Asses *et al*., 2018) reveals the adsorption of CR on the fungal biomass induced an increase in some peaks intensity, in particular, those around 3287, 2933, 1649, 1154, and 1034cm^{-1} and appearance of new peaks at 2859, 1379, 1262, and 1154 cm^{-1} also recorded.

CONCLUSION

In the present investigation an attempt was made to degrade and decolorize the mostly used textile dye Congo red by white rot fungus *Termitomyces* sp. complete and partial decolorization was observed in 50 to 250 mg/ 100 ml dye concentration and also degraded products were recorded by FT-IR analysis. Reason behind degradation might be due to the enzyme such as cellobiose dehydrogenase, xylanase and other lignocellulosic enzymes exudated by the fungus. Degraded products were adsorbed with the cell wall material of fungus and there is a strong degradation in the azo bond (azide) also observed. The results indicate that bio sorption and biodegradation of azo dye by *Termitomyces* sp. could be a potential application in the bioremediation of textile effluents containing azo dyes and other toxic compounds with some parameter (pH, temperature, quantity of biomass, organic adsorbents etc.) optimization according to the dye concentration. This investigation gives insight about the azo dye degradation ability of white rot fungus by fungal sorption method however the nature of degraded intermediates of azo dyes and their biodegradability are not yet clear and need further investigation.

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Conflict of Interest: The authors do not have any conflict of interest.

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