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ORIGINAL ARTICLE

BIODECOLORIZATION OF DIAZO DIRECT DYE CONGO RED BY FUSARIUM SP. TSF-01

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Abstract:

Azo dyes represent the largest class of synthetic organic colorants listed in the colour index. Many synthetic dyes are toxic, mutagenic or carcinogenic. Congo red is a carcinogenic, recalcitrant and complex diazo direct dye used commonly for the coloration in textile and paper industries. It was selected as model pollutant. The fungal stain Fusarium sp. TSF-01 isolated from textile wastewater sludge was screened for decolorization of Congo red. This strain has given 94.97 and 98.09 % decolorization of a recalcitrant dye, Congo red (100 mgL-1) at agitation and static condition within 3 days respectively. FTIR analysis revealed the degradation of Congo red by this isolate. This study revealed the enormous biodegradation abilities of indigenous microbial flora. Fusarium sp. TSF-01 is an efficient strain for the decolorization of azo textile dyes effluents.

KEY WORDS:

Azo dyes, Congo Red, Fusarium sp. TSF-01, biodecolorization, FTIR .

.INTRODUCTION

Manufacture and use of dyes and pigments is a multibillion - dollar industry. The use of these substances is an integral part of almost all manufacturing processes. Dyes are widely used in industries such as textile, rubber, paper, plastic, cosmetic etc. Among these various substrates textile ranks first in usage of dyes for colouration of fiber. Textile industry is one of the most important industries and in the last few decades have generated a high volume of waste water and inturn released into the environment. Strong colour of textile wastewater is most serious problem of textile waste effluent. It is broadcasted that more than 60% of the dyes world production is consumed by textile industries. The environmental issues surrounding the presence of colour in effluent is continuing problem for dyestuff manufactures, dyers, finishers and water companies because increasingly stringent colour consent standards are being enforced by regulatory bodies to reduce quality of colour in effluent and water courses.

Congo red (C.I. Congo red, M.W. 696.67 g mol^{-1} $C_{32}H_{24}N_6O_6S_2.2Na$) is one of the important sulfonated direct diazo dyes. It is a coloured substance having complex chemical structures and has high molecular weight. Chemically, it is sodium salt of benzidinediazo-bis-1-napthylamine-4-sulfonic acid. It is highly soluble in water and persists in the environment, once discharged into a natural aquatic media. Based on data from experimental animal studies revealed that the substance is presumed to be toxic (Giger, M., 1974). Congo red is benzidine based dye. As such it is expected to metabolize to benzidine which is known to be human carcinogen (Mathur, M., 2005).

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Although many physicochemical techniques of decolourization have been developed over the last 20 years, few have been implemented by the textile industries due to their high cost, low efficiency and inapplicability to a wide variety of dyes. A definitive solution of the colour problem of textile effluents would provide a marked competitive advantage for the industrial sector. Since no single process is able to decolorize all textile effluents, a solution for each situation should be considered, possibly involving a combination of different methods. In recent years number of studies have been focused on some microorganisms capable of decolourizing a wide range of dyes include some bacteria, fungi and algae. The considerable advantages in use of microorganisms for removal of synthetic dyes from industrial effluent includes that the process is relatively inexpensive. It is a simple method. The running cost is low and the end products of complete mineralization are not toxic and ecofriendly.

Over the past decade, many fungal strains have been studied for their abilities to degrade a wide variety of structurally diverse pollutants. Fungal biomasses are capable of treating metal-contaminated effluents with efficiencies several orders of magnitude superior to activated carbon (F-400) or the industrial resin Dowex-50. Additionally, fungal biomasses are susceptible to engineering improvements and regeneration of their capabilities. With regard to organic pollutants, excessive nutrients and dyes, fungi can remove them from wastewaters, leading to a decrease in their toxicities. However, the detoxification rates seem to be dependent on media and culture conditions. The post treatment by anaerobic bioprocesses of effluents that have been pretreated with fungi can lead to higher biogas than the original effluents. In addition to the degradation of organic pollutants, fungi produce added-value products such as enzymes (LiP, MnP, Laccase, amylase, etc.) and single-cell protein (SCP). Most research on fungal capacities to purify polluted effluents has been performed on a laboratory scale, hence there is a need to extend such research to pilot scale and to apply it to industrial processes (Coulibaly et al, 2003).Recently, many studies have also demonstrated that fungi are able to degrade and mineralize a broad spectrum of different dye structures.

Keeping in view, above mentioned facts we have investigated the decolorization and degradation abilities of newly screened strain of Fusarium sp.TSF-01 for Congo red. The IR spectrum of Congo red after biological treatment with the tested organism was also studied.

MATERIALS AND METHODS

Dyes and chemicals:-Congo red ($C_{32}H_{22}N_6Na_2O_6S_2$, M.W. 696.66g/mol) textile synthetic dye was purchased from Hi- Media. Dye solution was prepared by dissolving the dye in distilled water before each experiment. Nutrient media and all other chemicals were obtained from Hi media.

Soil samples were collected from the nearby effluent disposal area of textile units located at Sunil Nagar and Neelam Nagar, Akkalkot MIDC, Solapur, MS, India.

1) Isolation of Fungal strains for decolourisation of Congo red:

The collected soil samples were used for isolation of fungal strains capable of degrading dye by subjecting it to serial dilutions using sterile D/W. 0.1 ml aliquot from each dilution of 10-3 & 10-4 was spread inoculated on sterile modified Sabouraud's Dextrose agar plates containing dye with different concentrations (70 mg/Lit. to 110 mg/ Lit.). All plates were incubated at room temperature for 48-72hours. Dye degradation ability of microorganisms was confirmed by presence of clear zone around the colonies.
Well isolated representative colonies were subjected to purification on Sabouraud's Dextrose agar medium and studied for their morphological, cultural characteristics and then they were transferred on slants of appropriate media. These slants were incubated for 24-48 hours at room temperature until sufficient growth took place & then were stored at refrigeration temperature and maintained as primary & working stock for further studies

2) Decolourisation studies of Congo red:

Selected Fungal isolates from the primary screening were further screened at room temperature by using dyes containing modified Sabouraud's broth by shake flask culture method. A concentration 100 mg/lit of the reactive dyes used as final concentration in the study.

The selected fungal cultures were inoculated &studied at static & shaking condition for 3 days and decolourising activity was measured on each day. Following 24 hrs.of incubation, 5 ml. aliquots of decolourized samples were subjected to filtration followed by centrifugation at 7,000 rpm for 15 minutes. Clear supernatant was withdrawal and subjected for its absorbance measurements.

Measurement of decolourisation (Decolourisation assay):

Congo red decolourising activity was determined by measuring the decrease in the colour intensity as absorbency at 498 nm on each day. Percent decolourisation was calculated as:

$$\% \text{ Decolourisation} = \frac{\text{Initial absorbance} - \text{observed absorbance}}{\text{Initial absorbance}} \times 100$$

3) Biodegradation Analysis:

Biodegradation of Congo Red into different metabolites was confirmed by FT-IR analysis (Parshetti et al., 2006).

FTIR analysis:

Biodegradation of Congo red was monitored by FTIR spectroscopy. For this 100 ml sample was taken after decolourization. Centrifugation was carried out at 10,000 rpm and the metabolites were extracted from supernatant using equal volume of Dichloromethane. The extract was dried over anhydrous Na2SO4 and evaporated to dryness in a rotary vacuum flash evaporator. The treated Congo red dye was characterized by Fourier Transform Infrared Spectrometer (Perkin Elmer Spectrum 65) and compared with control (before treatment) dye. The samples were mixed with spectroscopically pure KBr in the ratio of 1:100 and pressed to obtain IR- transparent pellet. The pellet was placed in sample holder and the analysis was carried out in the mid IR region of 400-4500 cm -1 with 16 scan speed.

4) Identification of promising isolates:

Molecular identification was carried out of this isolate based on 18s rRNA sequencing. The phylogenetic tree was constructed by using Neighbour joining method by Kimura – 2 parameter with 1000 replicates in MEGA 4.0.

RESULTS

1) Isolation of fungal strains for the decolourisation of Congo red from soil samples:
A total number of 12 fungal isolates were obtained from the collected soil samples for dye decolourisation study.

2) Primary screening of the fungal isolates for decolourisation of Congo red:
The results of the primary screening of the isolates for various concentrations of Congo red are presented in Table 5.1. It is seen from Table 5.1 that one of the isolates, TSF-1 showed decolourisation activity and good amount of growth at different concentration of Congo red (70mg/L to 100mg/L). It is further seen that 110mg/L concentration has not supported the growth of TSF-1 and decolourisation. It is also observed that none of the other isolates showed any decolourisation during the incubation period employed, although many of them showed good amount of growth at 70mg/L, 80mg/L, 90mg/L.
Demonstration of the growth and decolourisation of Congo red at all concentrations used qualified TSF-01 isolate as a right candidate for the secondary screening and 100mg/L as the final concentration of dye for further study.

3) Decolourisation of Congo red by TSF-01 isolate:
Results of the TSF-01 isolate for decolourisation of Congo red is shown in Figure 1and Table 1. It is seen that at agitation condition, TSF-01 isolate has brought about 94.97% decolourisation of Congo red within 72 hours of incubation period (Photograph No.1).

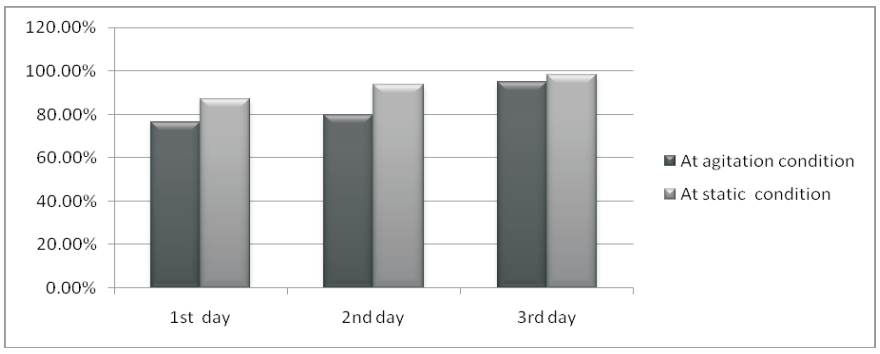
It is further seen that TSF-01 isolate showed maximum growth and decolourisation upto 100mg/L concentration of dye. It is also observed that TSF-01 isolate has brought about 98.09 % decolourisation within 72 hrs. of incubation period at static condition. There is no further significant decolourisation was obtained on further incubation.

This indicates that for maximum decolourisation, aeration of the medium is not mandatory. The isolate may be microaerophilc.

Table 1: Results of decolourisation of Direct Red 28 by TSF- 01 isolate at 100mg/L concentration under Static & Agitation conditions at room temperature.

	% decolorization on	At agitation condition	At static condition
Isolate TSF-01	1st day	76.43%	87.06%
	2nd day	79.77%	93.75%
	3rd day	94.97%	98.09%

Figure 1:- Percent decolourisation of Congo red byTSF-01 isolate at static & agitation condition.

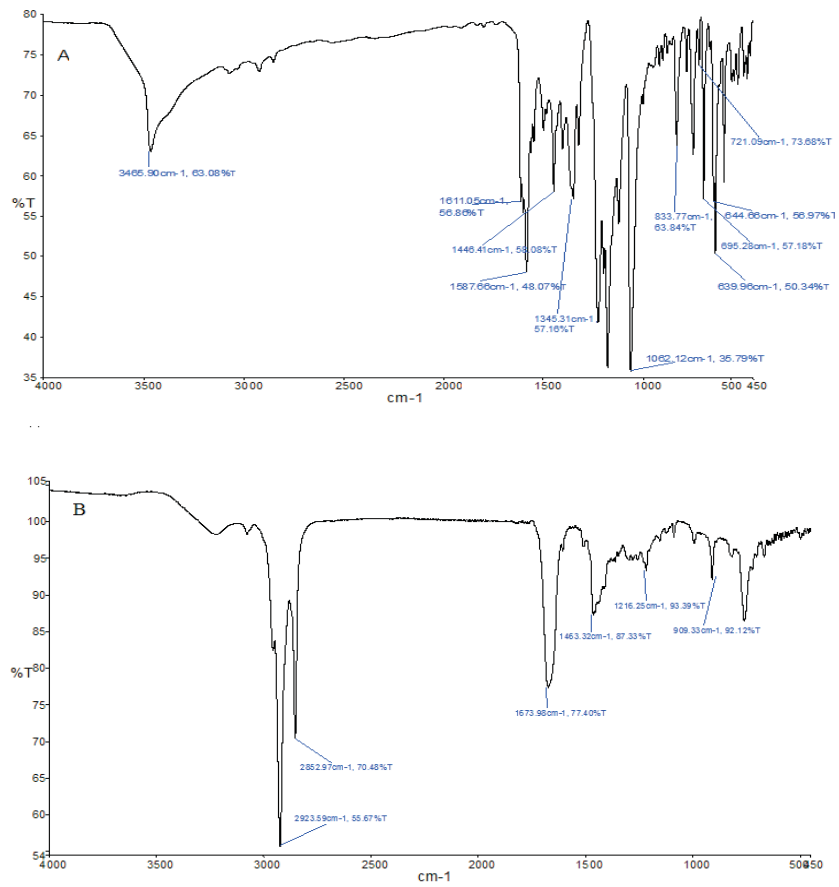


5) Biodegradation analysis:

The difference in FTIR spectrum of Congo red and metabolites obtained after its decolourisation resulted in biodegradation. The FTIR spectrum of Congo red showed specific peaks in fingerprint region for unsubstituted and multisubstituted naphthalene or benzene rings. This was supported by the peak at 639.96 cm-1 for C – H bending vibration, 644.66 cm-1 that corresponds to the C-C bending vibration, 695.28 cm-1 for C – H stretching vibrations for disubstituted aromatic compound, 721.09 cm-1 for CH2 bending vibrations, 833.77 cm-1 corresponds to P- disubstituted ring vibrations, 1062.12 cm-1, 1345.31 cm-1 for S = O stretching vibrations of sulfonic acid, 1446.41 cm-1 for aromatic C=C stretching vibrations, 1587.66 cm-1 for N=N stretching vibrations, 1611.05 cm-1 stretching vibrations of C=C while 3465.09 cm-1 for NH stretching of NH2 group(Figure 2 A).

The FTIR spectrum of metabolites obtained after decolourisation of Congo red showed peaks at 909.33cm-1 for C-C stretching vibrations, 1216.25 cm-1 for C-N stretching vibrations, 1463.32 cm-1 for CH2 bending, 1673.98 cm-1 for C=O stretching vibrations, 2852.97 cm-1 for CH2 symmetrical stretching vibrations and 2923.59 cm-1 for CH2-O asymmetric stretching vibrations as in (Figure 2 B).The absence of peak at 1.587.66 cm-1 for N=N stretching vibrations in the FTIR spectrum of metabolites obtained after decolourisation of Congo red because of the cleavage of azo bond.

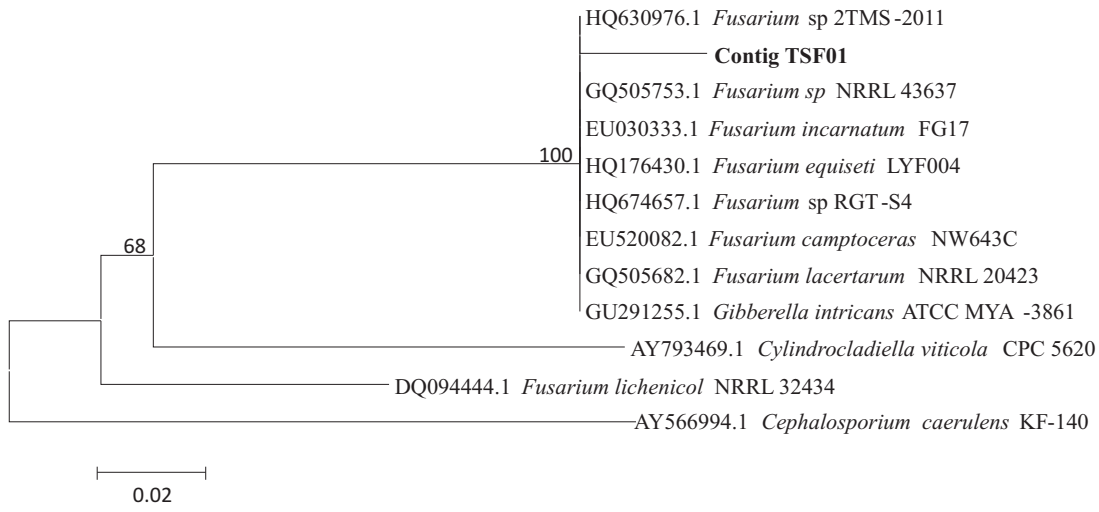
Figure 2: FTIR analyses of Congo red for biodegradation of Congo red By TSF -01 isolate – A) FTIR spectrum of control dye Congo red B) metabolites obtained after decolourisation of Congo red.



4) Identification of TSF- 01 studied for decolourisation of Congo red:

Molecular identification was carried out of this isolate based on 18s rRNA sequencing. The phylogenetic tree was constructed by using Neighbour joining method by Kimura – 2 parameter with 1000 replicates in MEGA 4.0 (Figure 3). According to sequencing similarities and multiple alignments the isolate TSF-01 was named as Fusarium sp.TSF 01. The partial sequence of 18S rRNA of this isolate TSF-01 has been deposited into GeneBank under accession number HE663239.1

Figure 3: Phylogenic tree based on ITS region gene sequences showing relationships among strain TSF-01 and the most close type strain species of Fusarium. Numbers at nodes indicate percentages of bootstrap support based on a Neighbor-joining analysis of 1,000 resampled datasets. Bar 0.02 substitutions per nucleotide position.



DISCUSSION

Based upon these findings, it can be predicted that fungi present in the vicinity of discharged effluent possess a great potential for the use in bioremediation of textile dyes. Biodecolorization of Congo red by fungi has been investigated by a few authors. Dey et al., (1994) reported the Lignin Peroxidase-producing Brown Rot Fungus, Polyporus ostreiformis, and its comparative abilities for lignin degradation and dye decolorization and reported only 18.6% lignin from rice straw in 3 weeks but effected 99% decolorization of Congo red dye in 9 days. Shinde et al. (2013) studied 100% and 96.26% decolourisation of Direct red 28 under shaking and static condition, respectively within 72 hrs. by Aspergillus aculeatus TSF- 05. Shin Kim et al., (1998) reported 77% decolorization of Congo red by fungal strain Pleurotus ostreatus. Novotny et al., (2001) studied biodegradative ability of 103 strains of wood rot fungi. It is worth mentioning, however, that the diazo dye, Congo red appeared to be one of the dyes that was comparatively more resistant to degradation. Irapex lactus caused only 58% decolourisation of this dye during 14 days of investigation. The present study has revealed that isolated Fusarium sp. TSF-01 has brought about 94.97 % decolorization of 100 mg L-1 of Congo red at agitation condition while up to 98.09 % at static condition within 4 days.

CONCLUSIONS

A total no. of 12 fungal strains were isolated from the collected soil samples for the decolourisation of Congo red by using Sabouraud's Dextrose Agar from the nearby effluent discharge areas of textile units. All the isolates were screened primarily for their decolourisation ability by agar plate method and according to the results obtained TSF-01 isolate was selected for further decolourisation of Congo red study. A final concentration 100 mg / L of the dye was used for further study. It was seen that at agitation condition TSF -01 isolate has brought about 94.97% decolourisation of Congo red while 98.09% at static growth condition within 72 hours of incubation period. FTIR analysis has shown the biodegradation of Congo red by TSF- 01.Molecular identification of TSF-01was carried out based on 18s rRNA sequencing as Fusarium sp.TSF 01. The present study indicates that this isolate is an excellent strain for the decolorization of azo textile dyes effluents and it might be a practical alternative in dyeing wastewater treatment.

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