Mycofiltration based Optimized Decolorization of Azo Dyes

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Abstract

One of the major environmental problems threatening the aquatic life is the presence of textile dye in the waste water. Reactive navy blue is one of the widely used dyes in the textile industries and also toxic pollutant to the environment. Decolorization of these textile dyes is one of major fields of interest in research. One of the methods which can be used for decolorization of these toxic dyes is mycofiltration. It is a form of mycoremediation where the fungal mycelium is used as a biofilter to remove the toxic pollutants. In this study fungal species Aspergillus niger and Rhizopus stolonifer were evaluated for efficiency in decolorization of the Reactive blue 171.

Decolorization studies were carried out to determine the optimum, pH, temperature, inoculum volume, incubation duration, glucose concentration and dye concentration. Both A. niger, R. stolonifer and mixed culture of A. niger and R. stolonifer showed good dye decolorization ability after 5-7 days. The decolorization was much more effective at pH 7 and in the presence of 3% glucose concentration, A. niger grew well in 100 mg/L dye concentration, resulting in 67% decolorization rate in 7 days. R. stolonifer showed dye decolorization after 5 days and high decolorization rate was observed in 200 mg/L. Experimental studies were conducted with pilot reactor setup consisting of mycofilter inoculated with R. stolonifer and its ability to decolorize the dye was determined. We can conclude that both A. niger and R. stolonifer are efficient enough to decolorize the industrial dyes and can be used in the biological treatment of dyes.

Keywords: Mycofiltration, Decolorization, Mycoremediation, *Aspergillus niger*, *Rhizopus stolonifer*.

Introduction

Textile dyes are one of the major environmental pollutants extensively used in the textile industries. Due to inefficient dyeing process, high amounts of the dyes are lost in effluents and are directly discharged into the water bodies which cause the formation of aromatic amines under anaerobic conditions[13]. They are highly toxic and carcinogenic which pose a major threat to aquatic life and do not degrade easily due to their highly complex structure[3,6]. Most commonly used synthetic dyes are azo dyes, they are mainly responsible for the coloration purpose and of recalcitrant nature. Degradation and decolorization of these dyes are difficult due to their highly complex structure and synthetic nature[15,18].

In order to remove the textile dyes from wastewater, many treatment methods have been used but even after treatment, the effluent passes through the systems without any change. Physical and chemical methods like ozonation, flocculation, irradiation, oxidation, filtration etc. are very expensive, need high energy requirement and may form hazardous by-products[16]. Unlike these methods, biological treatment is used with the help of microorganisms like bacteria, fungi and yeast for efficient removal of the dye from the wastewater[1,9]. Many types of fungi species are highly capable of decolorization of the azo dye as they can produce both intracellular and extracellular enzymes. Fungal species like *Phanerochaete chrysosporium*[5], white rot fungi and *Trametes versicolor*[10] are highly efficient in the decolorizing the textile dye. One of the methods in which textile dyes can be treated is mycofiltration. It is a method in which the fungal mycelium is used as a fungal biofilter to remove the toxic pollutants[7,11].

In this study, the main objective was to evaluate the decolorization potential of the industrial dye by *Aspergillus niger*, *Rhizopus stolonifer* and their mixed culture. In addition, an experimental study was designed and conducted using a pilot scale reactor consisting of mycofilter made up of sugarcane husk inoculated with *R. stolonifer*. The fungal culture inoculated in the sugarcane husk was selected based on its ability to decolorize the dye. The dye solution was filtered through the mycofilter and was evaluated to determine the effects of mycofiltration.

Material and Methods

Microorganisms: *Aspergillus niger*, *Rhizopus stolonifer* fungal species were soil isolates kindly provided by Apex Laboratories, Guindy, Chennai.

Culture conditions: The fungal cultures were inoculated in Potato Dextrose Broth (PDB) and were kept in rotary shaker at 27ºC for 5 days for good fungal mat growth.

Dyes: The reactive navy blue used in this study was one of commonly used textile dyes. The molecular weight is 1418.93 and its chemical formula is $C_{40}H_{23}O_{19}N_9Na_6S_6Cl_2$. Its maximum light absorption wavelength is 596 nm.

Methods

Decolorization studies: Decolorization was carried out and the reaction mixture consisting of 100 mg/L of reactive navy

blue in PDB was inoculated with 100 µL of the fungal cultures and incubated at 27 °C for 5 days. Then the reaction mixture was centrifuged for 20 min at 4000 rpm and the supernatant was taken to measure the dye decolorization rate. The dye decolorization rate was measured by observing the absorbance of dye which decreases in the case of decolorized sample at the maximal wavelength 596 nm[4]. The dye decolorization rate E is expressed in terms of percentage as in eq. 1:

$$E = \frac{A_o - A_t}{A_o} \text{ X } 100 \quad (1)$$

where A_o is the initial absorbance and A_t is the final absorbance.

Optimization of physiochemical parameters: To determine the operating conditions on the dye decolorization rate, the experiments were done at different parameters like pH levels (3, 5, 7, 9 and 11), temperatures (27 °C, 37 °C, 47 °C), dye concentrations (50, 100, 150 and 200 mg/L), inoculum volumes (50, 100, 150 and 200 µL), glucose concentration (1%, 2%, 3% and 4%) and incubation time (4, 6, 8 and 10 days). The fungal cultures were inoculated into the PDB consisting of dye concentration 100 mg/L.

UV-visible spectrophotometric analysis: The dye sample and the decolorized dye sample were taken and centrifuged at 4000 rpm for 20 min. Then the supernatant was collected and the absorption spectrum was recorded from 300 nm to 800 nm. By using UV-visible spectrophotometer, the changes in the UV-visible spectra before and after decolorization of the dye sample were studied.

Experimental studies

Pilot scale reactor: The experimental study was designed based on the dye decolorization potential of the fungal species *Rhizopus stolonifer*. Mycofilter was made by inoculating *R. stolonifer* in the sugarcane husk and the dye solution (100 mg/L) was pumped into the top end of the mycofilter and in the outflow vessel, the filtered solution was collected. The decolorization of the dye was found by observing the absorbance of dye at wavelength 596 nm. The dye decolorization rate in the filtered sample was observed by measuring the absorbance of the dye in which the filtered sample was analysed in 3 consecutive days.

Fourier transform infrared spectroscopy: The dried dyes and its degraded metabolites obtained from solvent extraction methods were subjected to FT-IR analysis using Jasco FT-IR 6300 spectrometer which has a wave number accuracy of 0.07 cm^{-1}. The transmittance percentage was observed from 400-4000 cm^{-1}. The peak differences in the dyes and its degraded metabolites are observed and noted[2].

Results and Discussion

Decolorization studies: The decolorization studies show that the *A. niger* decolorized the dye at the rate of 50% and *R. stolonifer* could decolorize the dye solution (100 mg/L) at rate of 62% in 5 days in the case of Reactive blue 171. For both fungal species, visual observation of decolorization of dye was done from 3rd-5th day of incubation.

Physiochemical parameter optimization using OFAT approach: Parameters like pH, temperature, inoculum volume, dye concentration and glucose concentration were optimized using the OFAT approach. This approach is mainly used to the find the optimal conditions for the decolorization. The optimization parameter was determined only for reactive blue 171 dye as the decolorization rate was higher compared to other dyes in both *A. niger* and *R. stolonifer*.

Effect of inoculum volume on decolorization: The optimum inoculum volume required for higher dye decolorization rate was found to be 100 µL for 5 mL of the reaction volume i.e. 2% inoculum volume [fig. 1a]. The combined culture of *A. niger* and *R. stolonifer* showed high decolorization rate at 150 µL inoculum volume. 100 µL inoculum volume of *A. niger* showed high decolorizing activity and 100 µL *R. stolonifer* showed high decolorizing activity. These results suggest that the optimal inoculum volume required for the decolorization of dye is 100 µL. Vaithanomsat et al[19] reported 2% (w/v) fungal inoculum size for the reduction of 600 mg/L reactive black 5 under pH 5.

Effect of pH on decolorization: The effect of pH on decolorization of dyes by *A. niger* and *R. stolonifer* shows that both the organisms were capable of decolorizing the dye over the pH range of 3.0-9.0. The best decolorization was achieved at pH range 7.0-9.0 [fig.1b]. The rate of color removal of dye was lower at strong alkaline condition (pH 11). The optimal conditions for the dye decolorization were achieved at pH 7. pH optima in the range of pH 3.0-5.0 in the case of *Aspergillus fumigates*, *A. niger* and *P. chrysosporium*[9] has been reported. Results showed that both *A. niger* and *R. stolonifer* could decolorize the dye at pH 7 thereby making them suitable for treatment of textile dyes.

Effect of temperature on decolorization: The optimal incubation temperature was studied [Fig. 1c] and the optimal temperature for the dye decolorization was found to be 37°C. Temperature above and below 37°C showed lower decolorization rate. The combined culture of *A. niger* and *R. stolonifer* showed higher decolorization rate than individual culture and the optimum growth temperature for the fungal growth is about 25-35°C. It has been reported that the temperatures for optimum growth, enzyme activities and dye decolorization for most of the white-rot fungi were found between the range over 25-37°C[9].

Effect of time course on decolorization: Optimal incubation time for the reactive blue 171 and the decolorization rate was studied. It has been reported that decolorization of the dye for *A. niger* was observed between 7th-9th day of incubation and for *R. stolonifera*, the

decolorization was observed between 9th-12th day of incubation and the color of the fungal mat changed to the color of dye. The optimal incubation time for the reactive navy blue M3R was found to be between 7-9 days of incubation. The decolorization rate was lower in 5th day and 11th day of incubation time which must be due to the cell viability, loss of nutrient source etc. [fig. 1d].

Effect of glucose concentration on decolorization: The effect of glucose concentration on decolorization is shown in fig. 1e. Addition of glucose enhanced the decolorization activity and the results showed that high decolorization rate was achieved at 3% glucose concentration. Low decolorization rate was seen in 1% and 4% glucose concentration as low glucose concentration could not meet the growth requirements of the fungi and higher glucose concentration may inhibit the decolorizing activity of the fungi. The optimum glucose concentration for the dye decolorization was found to be 3% glucose concentration.

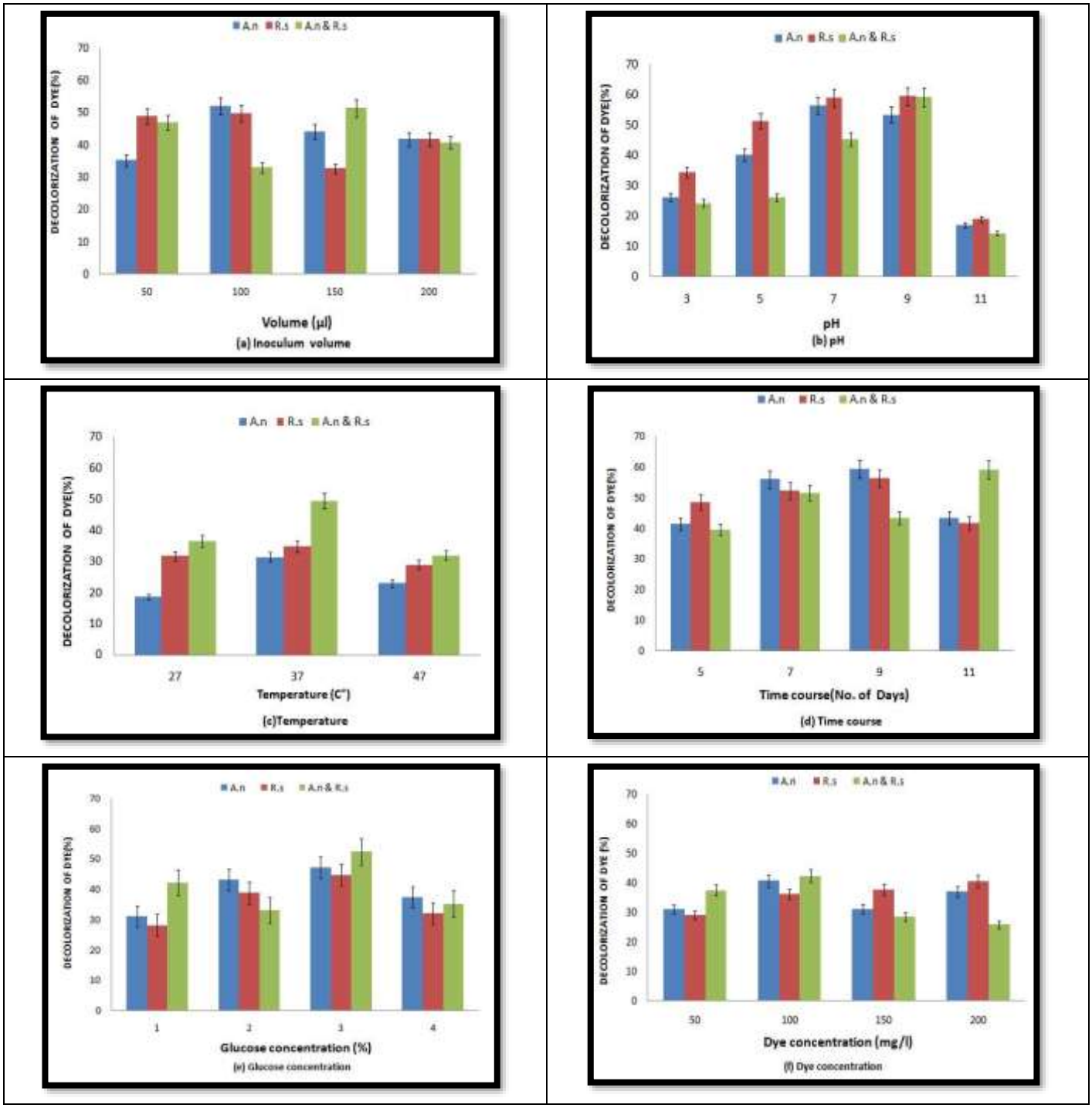

Figure 1: Optimization of operating conditions- (a) Inoculum volume, (b) pH, (c) Temperature, (d) Incubation duration, (e) Glucose concentration, (f) Dye concentration

Effect of dye concentration on decolorization: The effect of the dye concentration on the decolorization of the dye showed that the maximal decolorization rate was achieved at 100 mg/L dye concentration. *A. niger* showed high rate of decolorization at 100 mg/L than other dye concentrations and *R. stolonifer* showed higher decolorization rate at 200 mg/L dye concentration and the combined culture of both fungi showed higher decolorization at 100 mg/L (fig. 1f). Namdhari et al[8] showed that high concentration of the dye is toxic and affects the metabolite activity. These results suggest that *A. niger* and *R. stolonifer* maximal dye treated concentrations were found to be 100 mg/L and 200 mg/L respectively.

UV-visible spectrophotometric analysis: The UV -Visible spectroscopy is one of the primary techniques to determine the decolorization of dye and the disappearance of the sharp peak indicates the decolorization of the dye shown in figure 2[16]. The UV-visible spectrum of navy blue M3R (fig. 2) showed a maximum peak at 600 nm. The decolorized samples showed decreased OD values.

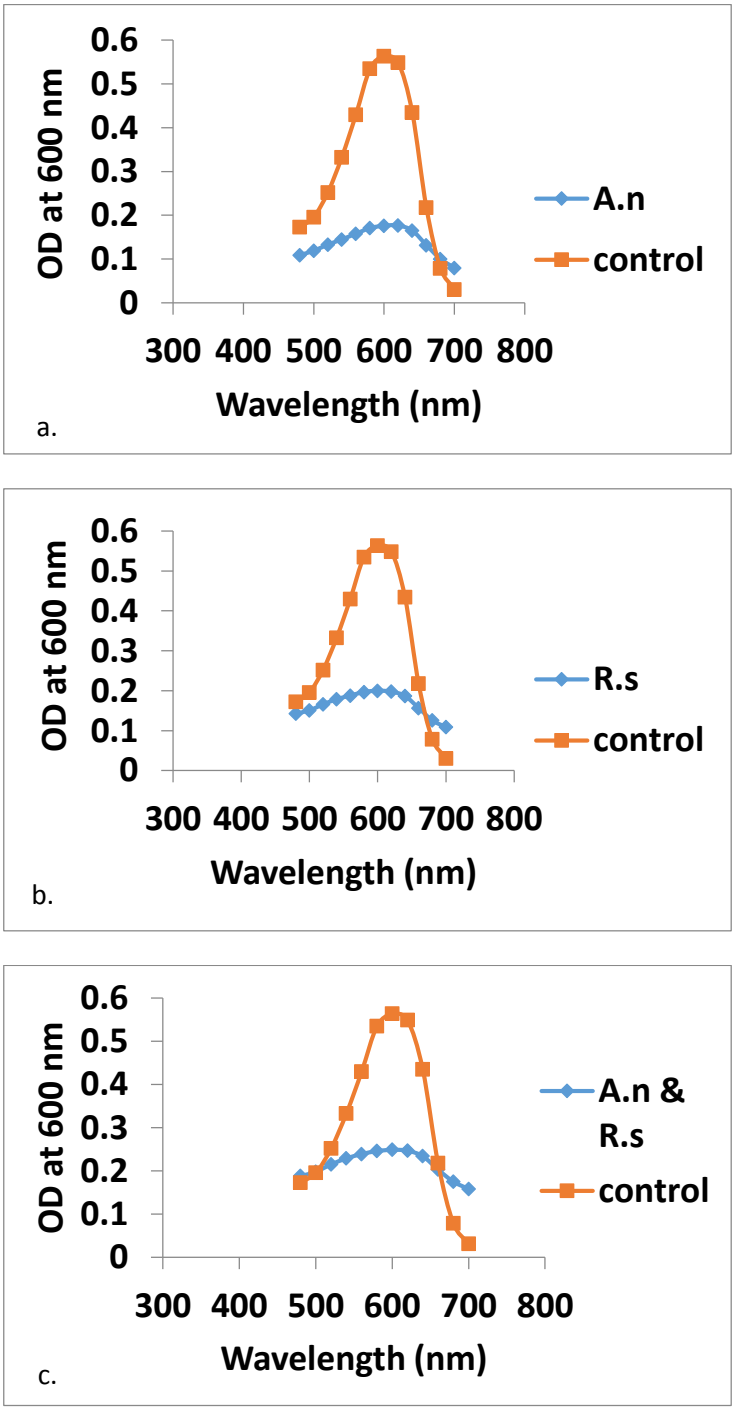

Figure 2: UV-Visible spectrum of Reactive navy blue and its decolorized samples using a. *A. niger*, b. *R.stolonifer* and c. mixture of *A.niger* and *R.stolonifer*

Pilot scale reactor: The filtered solution obtained from the mycofilter showed 79% decolorization rate. Rogers[11] had reported that mycofiltration reduced the *E.coli* population in the wastewater. In this study, reactive blue 171 dye was selected to experimentally determine the ability of the mycofilter to decolorize it and the rate of decolorization was studied (fig. 3).

FTIR analysis: FTIR is an analytical tool which reveals information on both the type and the strength of interaction[12]. The FTIR analysis was determined for reactive blue 171 and the decolorized sample. The FTIR spectra for dye sample and the decolorized sample is shown in figure 4 and the interpretation of the peaks is given in table 1. The peak at 1411.64 in the dye indicates azo bond. This peak is missing in the decolorized metabolites FTIR. This indicates that the azo bonds have been broken in the decolorized metabolites.

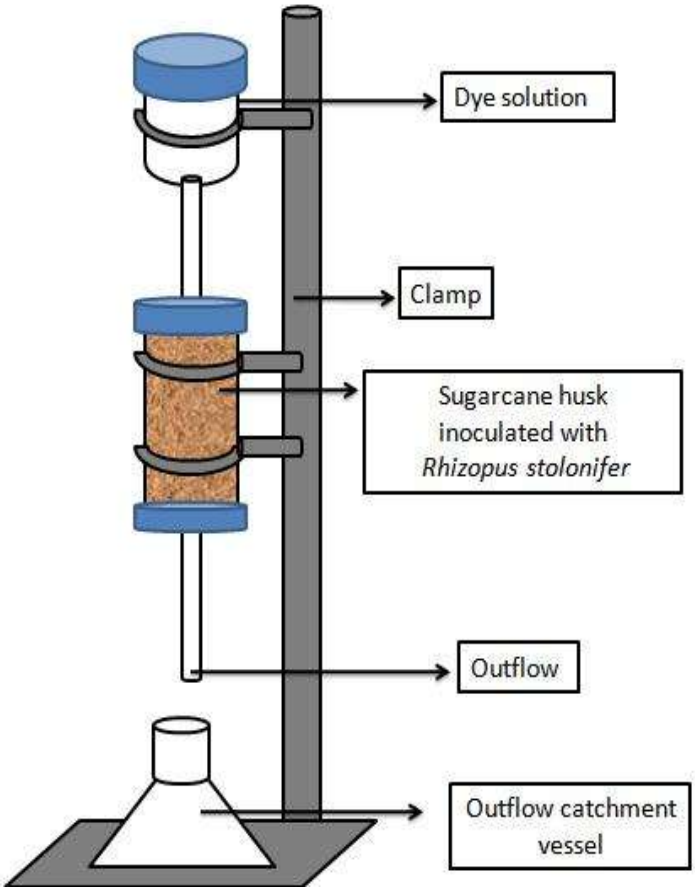

Figure 3: Diagrammatic representation of the experimental setup (Pilot scale reactor)- Mycofilter

Table 1
Interpretation of FTIR peaks in dye and degraded metabolite samples

Sample	Peak (cm^{-1})	FTIR Analysis
Dye	3301.64	N-H
	2932.23, 2288.13, 2072.14, 1986.32	C-H stretching
	1650.77	C=N
	1411.64	N=N
	1263.6	C-N
	1080.91	C-O
	715.461	C-Cl
Degraded metabolites	3460.63	O-H stretching
	2961.16, 2318.02, 1986.36	C-H
	1738.61	C=O
	1366.32	NO_2 stretching
	1216.86	C-O
	948.806	C-H bend

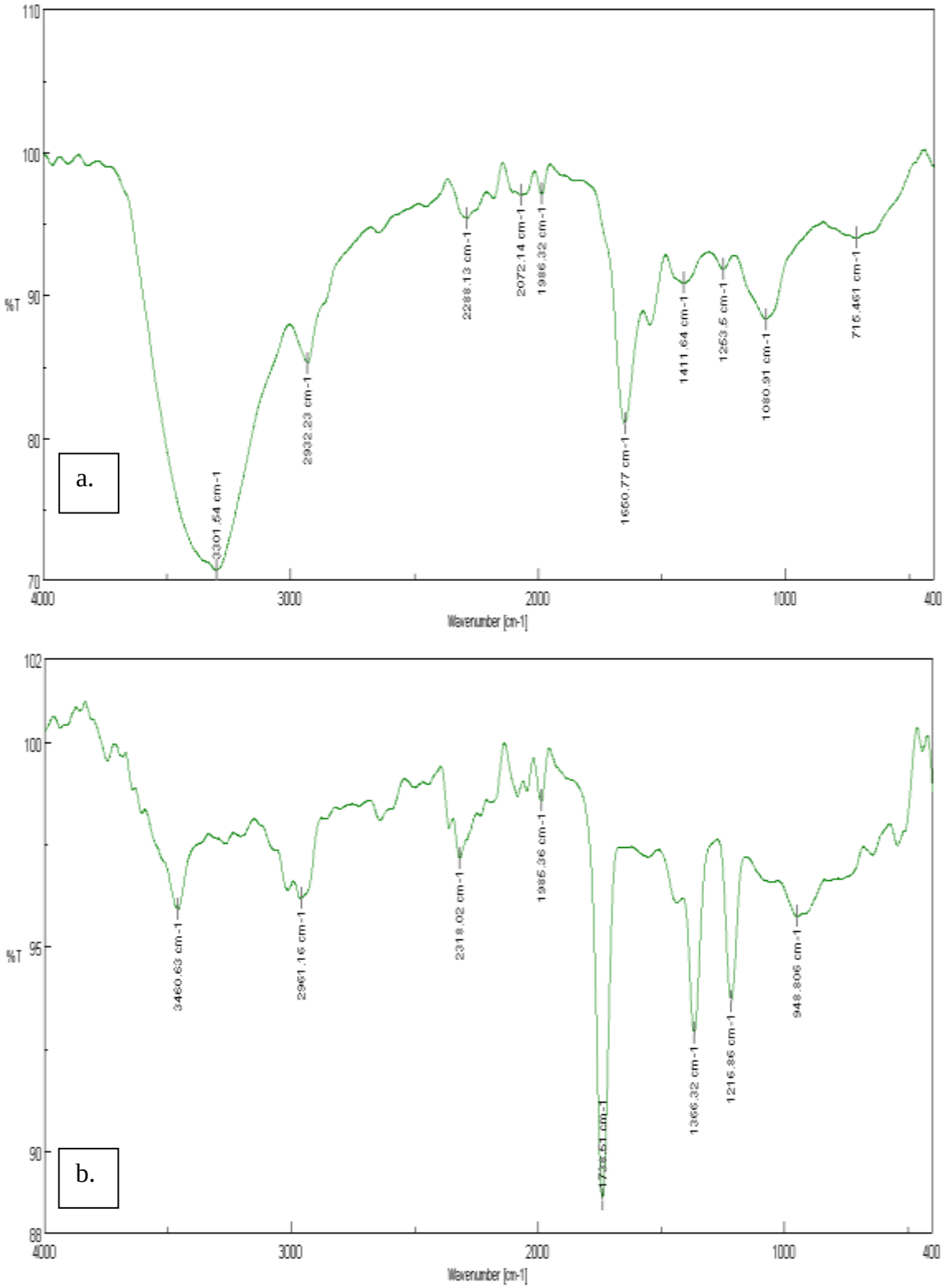

Fig. 4: FTIR spectra of a. Reactive navy blue solution and b. decolorized sample obtained through mycofiltration

Conclusion

In this study both *Aspergillus niger* and *Rhizopus stolonifer* and mixed culture of *A. niger* and *R. stolonifer* showed high decolorizing activity against reactive navy blue dye commonly used in textile industries. Decolorization studies were done and optimal conditions for the dye decolorization were also determined. Disappearance of sharp peak in the UV-visible spectra of the decolorized sample showed the decolorizing activity of the fungi. Experimental studies conducted determined the ability of mycofilter to decolorize dyes in real time. Hence this study concludes that both the fungal species *A. niger* and *R. stolonifer* have practical application potential that can be used to decolorize the industrial dyes.

The mycofilter was effective enough to decolorize the azo dye and hence it can be applied in the biological treatment of industrial dyes to reduce pollution in the environment.

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