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Original Research Paper

Spectroscopic Studies on the Biodegradation of Textile Effluent By White Rot Fungus *Trametes hirsuta*

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ABSTRACT

Microbiological decolorization and degradation is an environmental friendly and cost-effective alternative to the chemical decomposition methods. Most of the fungi are robust organisms which are generally more tolerant to high concentration of pollutants. White rot fungi have the ability to attack a wide range of recalcitrant compounds including dyes. White rot fungi *Trametes hirsuta* was taken for textile effluent degradation. Spectroscopic studies were carried out at every 24 hours interval. UV-VIS spectrophotometric studies on fungal treated effluent showed that the decolorization was due to biodegradation of the effluent and not because of bioadsorption. From FT-IR study, the break up of azo dye was confirmed with the disappearance of azo peak at 48 hours of the treatment.

INTRODUCTION

Textile industries consume large amount of water and chemicals for finishing and dyeing processes and become the largest contributor to water pollution (Jadhav et al. 2010). Approximately 1, 00,000 different dyes are used in industries and over 7×10^5 metric tonnes of dyes are annually produced worldwide (McMullan et al. 2001, Selvam et al. 2003, Chaube et al. 2010). Azo dyes are frequently used in dyeing and textile industries worldwide (Abd El- Rahim et al. 2009) and also in food, paper making and cosmetic industries (Kim et al. 2007). Azo dyes are synthetic organic compounds characterized by the presence of one or more azo (-N=N-) bonds in association with one or more aromatic systems (Zeroual et al. 2007, Couto et al. 2007). The discharge of these coloured wastewater into the rivers and lakes lead to reduction of sunlight penetration in natural water bodies which in turn decrease both photosynthetic activity and dissolved oxygen concentration and also toxic to living organisms (Sukumar et al. 2009). Therefore, it is necessary to remove dyes before the effluent is discharged into the receiving water bodies.

Many physical and chemical methods including adsorption, coagulation, filtration, oxidation and so on have been used for the treatment of dye containing effluents but they are relatively expensive, require intense energy and form hazardous by-products (Ramya et al. 2007, Phugare et al. 2011). Bioremediation provides an alternative technology to existing technologies because they are cost-effective, environmental friendly and do not produce large quantities of sludge (Nasreen et al. 2007, Saratale et al. 2011). Bioremediation is an attractive technology that utilizes the metabolic potential of microorganisms in order to clean up the environmental pollutants to less hazardous form with less input of chemicals, energy and time (Hartash & Kaushik 2009). The ability of fungi to transport a wide variety of hazardous chemicals has aroused interest in using them in bioremediation (Raju et al. 2007).

White rot fungi posses a great range of different enzymes such as hydrolytic enzymes (cellulase, pectinase, xylanase) and extracellular ligninolytic enzymes (Tisma et al. 2010). This has impelled into alternative methods like bioremediation processes. The so called ligninolytic fungi are particularly suitable for the development of such processes since they produce extracellular lignin degrading enzymes. Although dye molecules display high structural diversity, they are degraded by few enzymes. These biocatalysts are all redox-active molecules and thus exhibit relatively wide substrate specificity (Chacko et al. 2011).

MATERIALS AND METHODS

Sample collection: The textile effluent was collected from the discharge tanks of a textile mill located in Madurai district, Tamil Nadu, India. The effluent was sampled in dry, sterile plastic cans and stored in the incubator at 15°C.

Microorganism and culture condition: White rot fungus *Trametes hirsuta* was procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India and used for decolorization and degradation studies of textile effluent.

Stock culture of *T. hirsuta* was maintained on Yeast Glucose Agar at 30°C as mentioned by MTCC. Fresh culture was made separately in 100mL of Sabouraud's dextrose broth (liquid medium without agar), which contains 40g of dextrose and 10g of peptone per litre of distilled water. The culture was incubated at 30°C ± 1°C for 10 days. Subcultures were made periodically.

Biodegradation studies: After the incubation period, 20mL of the culture broth was drawn and added to a PET bottle containing 1.3 litres of textile effluent. The bottle was aerated for 3 hours a day at room temperature and the samplings were done till complete visual decolorization. 100mL of sample was drawn at an interval of 24 hours and was analysed by UV-VIS spectrophotometer over a range of 200-800nm and by Fourier Transform Infra red (FT-IR) Spectrometer.

For decolorization study, 5 mL of sample was drawn, centrifuged at 4000 rpm for 20 minutes and decolorization was monitored by measuring the absorbance of the supernatant at 480nm using UV-VIS spectrophotometer.

For degradation study, remaining quantity of sample was oven dried at 105°C and mixed with spectroscopically pure KBr at a ratio 1:20. Pellets were fixed in the sample holder and analyses were carried out using Nicolet Avatar 360 FT-IR Spectrometer.

RESULTS AND DISCUSSION

Decolorization study by UV-VIS: After each 24 hours of interval, 5mL of sample was drawn for decolorization studies using UV-VIS spectrophotometer over the range 200-800nm. The spectral scan of raw textile effluent exhibits two peaks, one in the visible region (480nm) and another in the ultraviolet region (219nm). In the case of adsorption, the visible region absorption peak decreases whereas in biodegradation, either the visible peak disappears completely or a new peak will appear (Ayed et al. 2011). Dye adsorption can also be clearly judged with the fungal cells. Fungus becomes deeply coloured because of the adsorbed dyes, whereas those retain their original colour when biodegradation occurs (Jadhav et al. 2011). At 24 hours, the intensity of visible peak (480nm) decreases. As the decolorization process proceeds, the visible region peak at 480nm completely disappears in 48 hours and the peak at 219nm decreases in intensity. After 48 hours, the overall absorbance of the fungal treated effluents increased due to the darkening of enzymatic treatment of the effluent (Zille et al. 2005). And no peak was obtained at 480nm even on dilution. Visually slight colour change of the textile effluent was observed on 8th day of the treatment for *T. hirsuta*. Complete visual colour change was visualized on 9th day in *T. hirsuta* treated effluent. The fungal masses also appeared in their original colour at the end of the treatment. Thus, the decolorization is attributed to biodegradation as the visible region peak completely disappears and the fungal masses are not deeply coloured by the textile effluent.

Degradation study by FTIR: The raw sample of the textile effluent have azo (-N=N-) dyes and aromatic amines which are hazardous and carcinogenic in nature. It also contains amides, amines and aromatic compounds. The FT-IR spectrum of the raw effluent shows the following peaks: peaks at 3438 cm^{-1}, 2360 cm^{-1} and 2134 cm^{-1} corresponding to N-H stretching vibration in amides, N-H stretching in amines and C≡C stretching respectively. The peaks at 1656 cm^{-1}, 1640 cm^{-1} and 1631 cm^{-1} representing C=O stretching and N-H stretching in amides and C=C stretching in alkanes. The peak at 1572 cm^{-1} is the most important peak indicating the presence of azo (-N=N-) bond stretching vibration (Jadhav et al. 2011). Also the peak at 1511 cm^{-1} has been assigned to N-H bending in aromatic amines. The peaks at 1420 cm^{-1}, 1121 cm^{-1} and 1024 cm^{-1} are assigned to C-N stretching in amide/C=C stretching of aromatic nuclei, C-H stretching in aromatic compounds and C-O stretching respectively. The peak at 878 cm^{-1} corresponds to C-N stretching in nitroaromatic compounds. The peak at 847 cm^{-1} which is assigned to C-H deformation of para di-substituted aromatic compounds has confirmed that the substitution is in para position of aromatic compound (Parshetti et al. 2006, Patil et al. 2008). The peak at 622 cm^{-1} corresponds to C-Cl stretching. Weak bands at 479, 467 cm^{-1} correspond to S-S stretching in disulphides.

On the first day of fungal treatment, the intensity of peaks at 3435 cm^{-1},1655 cm^{-1}, 1640 cm^{-1}, 1630 cm^{-1}, 1571 cm^{-1}, 1121 cm^{-1} and 618 cm^{-1} increase. Number of new peaks appear at 1493 cm^{-1}, 1477 cm^{-1} and 1459 cm^{-1} corresponding to C-H bending and a new peak at 928 cm^{-1} indicates the O-H deformation on carboxylic acid. On 2nd day of treatment, the strong peak at 3432 cm^{-1} has been assigned to N-H stretching in amides. The peaks at 1640 $cm^{-1}$and 1414 cm^{-1} increase in intensity, a new peak appears at 1592 cm^{-1} and the azo (-N=N-) peak (1571 cm^{-1}) disappears. The strong peaks at 1592 cm^{-1} and 1414 cm^{-1} are the characteristic peaks of asymmetric and symmetric stretching of carboxylate ions of sodium salts of amino acids (Silvertein & Webster 1997). The intensity of other peaks increase. The disappearance of peak at 1571 cm^{-1} indicates the break up of azo bond.

The intensity of all peaks decreases largely on 72 hours of treatment. Multiple peaks are obtained over 3500-2000 cm^{-1}. The peaks at 3512 and 3423 cm^{-1} correspond to free N-H stretching of primary amides and 3337 cm^{-1} for hydrogen bonded N-H stretching of primary amides. The peaks at

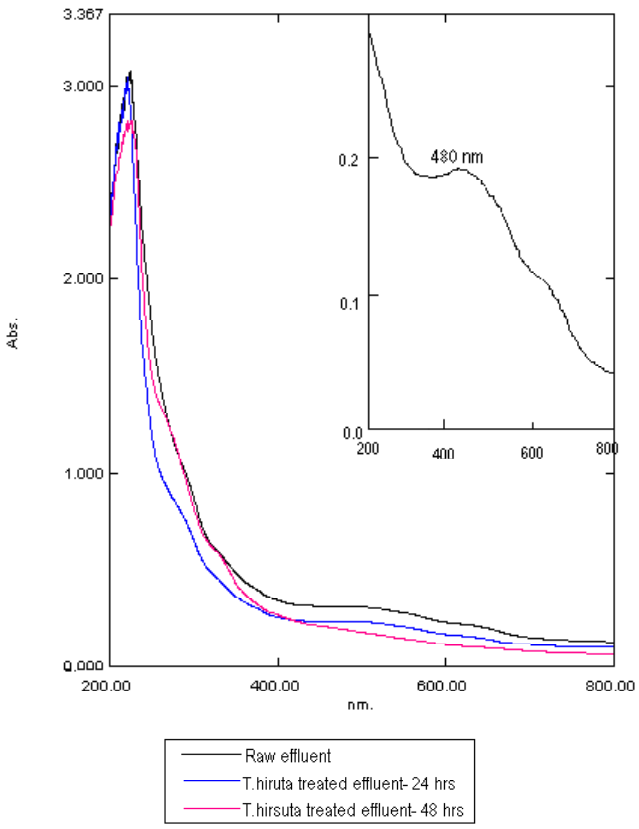

Fig. 1: UV-VIS spectra of raw and fungal treated effluents.

2948, 2864, 2757, 2661, 2562, 2472 cm^{-1} have been assigned to C-H stretching and O-H stretching in acids. A new peak obtained at 1041 cm^{-1} corresponds to S=O stretching (Patil et al. 2008, Rajeshwari et al. 2011). The intensity of peaks at 927 cm^{-1}, 877 cm^{-1} and 651 cm^{-1} becomes much weaker. After three days of treatment, the intensity of peaks increases day by day. On 5th day of the treatment, new peaks appear at 854,780 cm^{-1} and 634, 591 cm^{-1} corresponding to N-O stretching and O=N=O bending in nitrites respectively. Thus, appearance of new peaks at 854, 780, 634, 591 cm^{-1} confirms the presence of alkyl nitrites in the textile effluent treated by *T. hirsuta*.

The complete visual decolorization occurs on day 8; the following peaks observed on 8th day of the treatment are: free N-H stretching in amides (3470cm^{-1}), a new peak at 3390 cm^{-1} assigned to N-H stretching in sodium salt of amino acids, hydrogen bonded N-H stretching in amides (3311 cm^{-1}). Peaks at 2649, 2607, 2501, 2466 cm^{-1} represent O-H stretching in acids and 2354, 2340, 2326 cm^{-1} correspond to N-H stretching in amines. Peak at 2143 cm^{-1} corresponds to C≡C stretching. The peaks at 1640 cm^{-1}, 1632 cm^{-1} and 1583 cm^{-1} appear with much stronger intensity. A new peak appears at 1557 cm^{-1} has been assigned to N=O stretching in nitrites/ nitro aromatic compounds. The peak at 1416 cm^{-1} corresponds to symmetric COO^- stretching of sodium salts of amino acids and N=O stretching in nitrosamine (Telke et al. 2009). The other peaks at 1122, 1042, 928, 877, 847, 781, 644, 618 cm^{-1} remain unaltered. On 9th day of treatment, the peaks at 3390, 3311, 2700-2400 cm^{-1} disappear.

On 10th day of treatment, the peak at 1681cm^{-1} disappears and a new peak appears at 1565cm^{-1} indicating N=O stretching in nitrites and peak at 1416 cm^{-1} corresponds to N=O stretching in nitrosamine. Nitrosamines are produced when the nitrites react with amino group. Under acidic conditions the nitrite forms nitrous acid (HNO_2), which is protonated and split into the nitrosonium cation $N{\equiv}O^+$ and water: $H_2NO_2^+ = H_2O + NO^+$. The nitrosonium cation then reacts with an amine to produce nitrosamine. At the end of the treatment, all the peaks increase in intensity and peaks at 878 and 847 cm^{-1} corresponding to aromatic compounds become sharper and sharper.

Thus, the extracellular enzymes secreted by the White rot fungus *T. hirsuta* breaks the azo (-N=N-) structure of the dye molecules after 48 hours of fungal treatment and on 5th day, nitrites are developed in the effluent and on the course of treatment nitrosamines are produced.

CONCLUSION

The white rot fungus *T. hirsuta* tolerated the alkaline nature of the effluent and is able to degrade the textile effluent. UV-VIS spectrophotometric study indicated that the decolorization of the textile effluent was due to the biodegradation by the enzymes secreted by the white rot fungus. FT-IR results confirmed the results obtained from UV-VIS. In FT-IR spectra, the disappearance of peak corresponding to azo bond stretching and appearance of peaks for nitrites and nitrosamines in the later stages of treatment process confirmed the break up of azo bond and formation of new degradation products in the process of decolorization.

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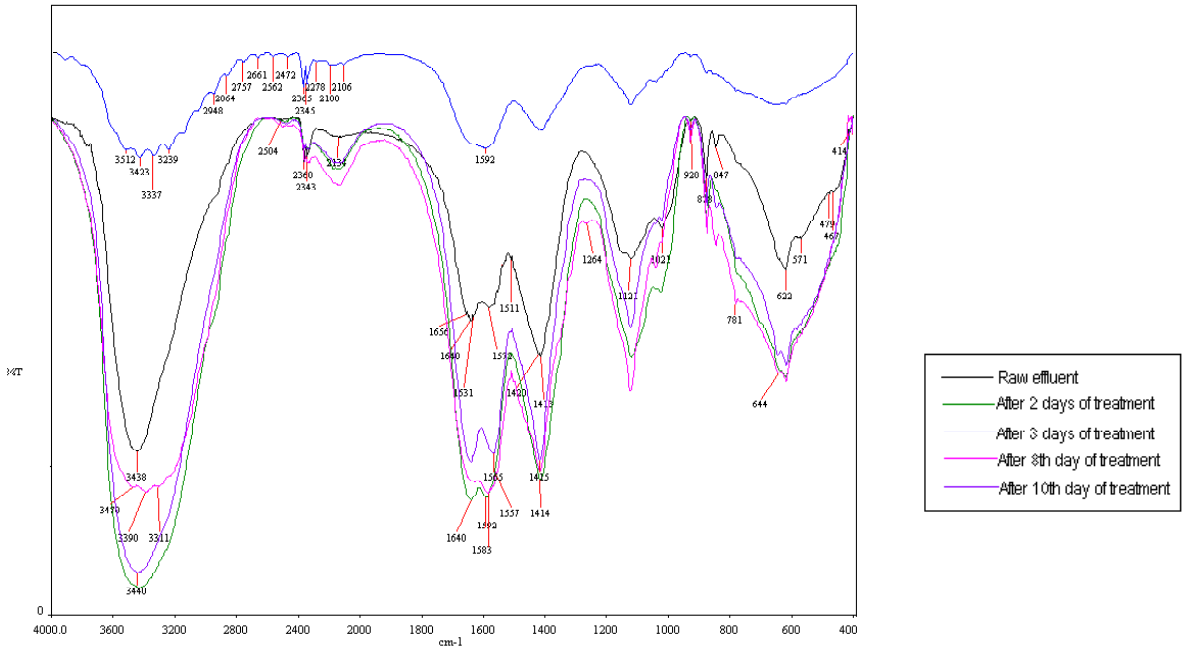

Fig. 2: Overlaid FT-IR spectra of raw and *T. hirsuta* treated effluents at different intervals.

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