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DECOLORIZATION AND BIODEGRADATION STUDIES OF THE TEXTILE EFFLUENT TREATED BY *TRAMETES VERSICOLOR.*

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ABSTRACT: Azo dyes are frequently used in textile industries and accounts for nearly 70% of dye market. Many physical and chemical methods are available to treat textile effluent but they are expensive. But microbiological degradation of textile effluent is an eco-friendly and cost-effective technology. Most of the fungi are robust organisms which are generally more tolerant to high concentration of pollutants. White rot fungi degrade the pollutants by their enzymes which have wide range of substrate specificity. Trametes versicolor was selected for the degradation studies on textile effluent. Spectroscopic analyses were made at every 24 hours interval. Ultraviolet-Visible Spectrophotometric study showed complete disappearance of visible region peak (480nm) indicating the degradation of textile dyes. This was further confirmed by Fourier Transform-Infra Red Spectroscopic studies which indicate the break up of azo bond at 48 hours of fungal treatment.
Key words: Biodegradation, FT-IR study, Textile effluent, T.*versicolor*, UV-VIS studies

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INTRODUCTION

Waste water from textile industries are complex mixtures of many polluting substances such as organo-chloride based pesticides, heavy metals, pigments and dyes [1,2]. Depending on the class of dyes, their release in wastewater effluents can range from 2% for basic dyes to 50% for reactive dyes, which ultimately find their way into the environment [3,4]. The chemical structures of synthetic dye molecules are designed to resist fading upon exposure to light, heat, water, chemicals and microbial attacks and this property render them recalcitrant[5,6]. 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 [7]. Azo colorants make up the largest and most versatile class of dyes with more than 2000 different azo dyes being currently used [8,9]. Azo dyes which comprise of about 70% of dye market are difficult to degrade due to their complex structure and synthetic nature [10]. Even very small quantity of dyes in water is highly visible and affects the aesthetic merit of water, water transparency and gas solubility of water bodies. Therefore, it is necessary to remove dyes before the effluent is discharged into the receiving water bodies.
Number of Physical and Chemical methods have been used to treat the dye effluent but they are relatively expensive, require intense energy and forms hazardous by-products [11]. Bioremediation is an attractive technology that utilizes the metabolic potential of microorganisms in order to clean up the environmental pollutants to less hazardous form. They are cost-effective, environmental friendly and do not produce large quantities of sludge [12]. White rot fungi have attracted a lot of attention due to their ability to attack a wide range of recalcitrant compounds including dyes [13,14]. White rot fungi posses a great range of enzymatic systems which degrade a wide range of organic pollutants including dyes and polyaromatic hydrocarbons (PAHs) [15,16].

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 15^0C.

Microorganism and Culture Condition

White rot fungi *Trametes versicolor* 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.versicolor* was maintained on Yeast Glucose Agar at 30^0C as mentioned by MTCC. Fresh culture was made separately in 100ml of Sabouraud's dextrose broth, which contains 40g of dextrose and 10g of peptone per liter of distilled water. The culture was incubated at 30^0C ± 1^0C 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 liters 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 analyzed by UV-VIS Spectrophotometer over a range of 200-800nm and Fourier Transform Infra Red (FT-IR) Spectrometer over a range of 4000 cm^{-1} to 400cm^{-1}.

For decolorization study, 5 ml of sample was drawn, centrifuged at 4000rpm 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 105^0C 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 Spectrophotometer

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 peak decreases whereas in biodegradation, either the visible peak disappears completely or new peaks will appear [17]. Dye adsorption can also be clearly judged with the fungal cells. Fungus becomes deeply colored because of the adsorbed dyes, whereas those retain their original color when biodegradation occurs [18]. Figure1 shows the overlaid UV-VIS spectra of raw effluent and *T.versicolor* treated effluent at an interval of 24 and 48 hours. The insertion spectrum in figure 1 shows the visible peak at 480nm.

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 [19]. And no peak was obtained at 480nm even on dilution.

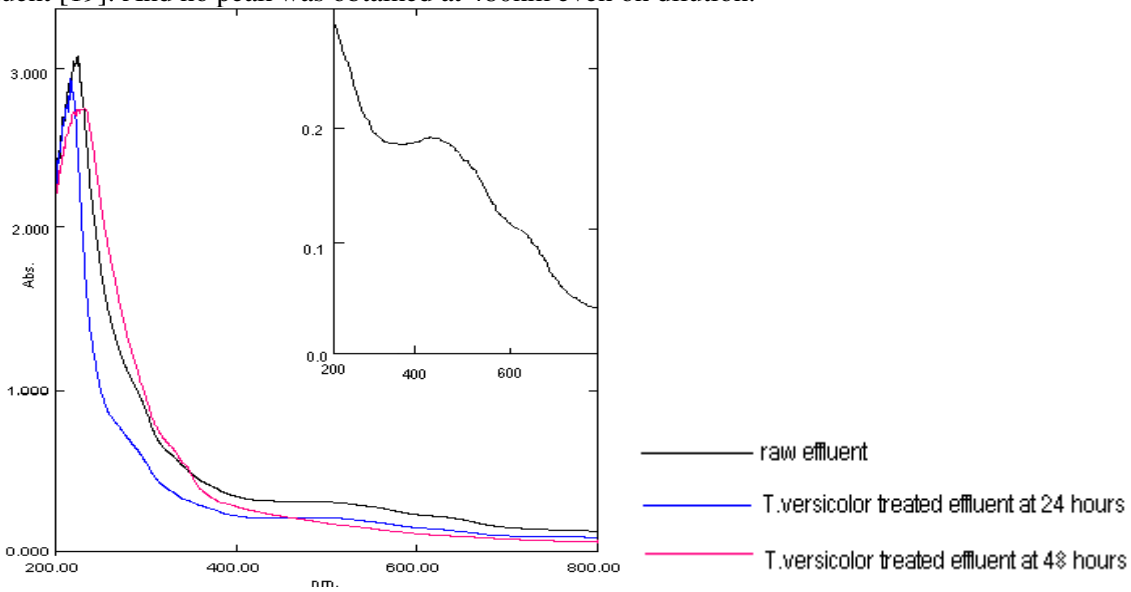

Fig. 1. Overlaid UV-VIS spectra of raw and *T.versicolor* treated effluent.

Degradation Study by FT-IR Spectrometric Analysis

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} represent 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 [18]. 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 [20,21]. The peak at 622 cm^{-1} corresponds to C-Cl stretching. Weak bands at 479 cm^{-1} and 467 cm^{-1} correspond to S-S stretching in disulfides.

As the fungal treatment with *T.versicolor* begins, new peaks appear at 2926 cm^{-1}, 1560 cm^{-1} and 1508 cm^{-1} assigned to C-H stretching, N=O stretching in nitro aromatic compounds and N-H bending in aromatic amines. New peaks at 1498, 1490, 1457 cm^{-1} correspond to C-H bending and C=C stretching in aromatic nuclei. A new peak at 1349 cm^{-1} indicates the C-N stretching vibration in aromatic amines. The peaks correspond to C-N stretching in amides (1419 cm^{-1}) and C-N stretching in nitro aromatic compounds (875 cm^{-1}) decrease in intensity. All other peaks corresponding to free N-H stretching in amides (3449 cm^{-1}), N-H stretching in amines (2360, 2344 cm^{-1}), C≡C stretching (2138 cm^{-1}), C=O stretching and N-H bending in amides and C=C stretching (1653, 1636 cm^{-1}), Azo bond stretching (1570 cm^{-1}), C-H stretching in aromatic compounds (1122 cm^{-1}), C-O stretching (1021 cm^{-1}) and C-Cl stretching (620 cm^{-1}) remain as in the control sample.

After 48 hours of fungal treatment, peak at 3438 cm^{-1} indicates N-H stretching in amides and Sodium salts of amino acids. New peaks at 2852 and 2515 cm^{-1} appear which have been assigned to O-H stretching in acids and C-H stretching. The Azo peak at 1570 cm^{-1} disappears and new peak at 1593 cm^{-1} corresponding to N-H bending in amines/ amides and is the characteristic peak of carboxylate ions of Sodium salts of amino acids[22]. The peak corresponding to N-H bending in aromatic amines is also present at 1506cm^{-1}. The peak at 1417 cm^{-1} represents C-N stretching in amides and symmetric COO^- stretching of Sodium salts of amino acids. All other peaks over the range of 1500-1400 cm^{-1} disappear. A new peak at 928 cm^{-1} corresponding to O-H deformation in carboxylic acid appears. New peak at 780 cm^{-1} and a peak at 646 cm^{-1} correspond to N-O stretching and O=N=O bending in nitrites. Even though the Azo peak disappears at 48 hours of treatment, the aromatic amines (1506 cm^{-1}) are present in the effluent. The intensity of other peaks increases.

After 72 hours, the peak corresponding to C-H stretching shifts to 2739 cm^{-1}. The C-N stretching of aromatic amines (1354 cm^{-1}) present on 3rd day also. The intensity of peaks at 1636 and 1589 cm^{-1} become stronger. A new peak appears at 1042 cm^{-1} has been assigned to S=O stretching [21,23]. The intensity of the peaks increases as the treatment proceeds.

On 6th day of treatment, a peak at 1560 cm^{-1} appears which corresponds to N=O stretching in nitrites. Multiple peaks at 1497, 1489, 1472, 1456 cm^{-1} represent C-H bending and C=C stretching of aromatic nuclei. The peaks at 1585 cm^{-1} and 1418 cm^{-1} indicate asymmetric and symmetric COO^- stretching of Sodium salts of amino acids. The peaks at 854, 781cm^{-1} and 647 cm^{-1} correspond to nitrites. As the treatment progresses, the intensity of peaks increases which confirms the predominance of amides, amines, amino acids, nitrites in the absence of Azo peak in the effluent.

On final day of treatment, the peak at 1585 cm^{-1} disappears and a new peak arises at 1565 cm^{-1} which has been assigned to N=O stretching in nitrites and strong peak at 1416 cm^{-1} has been assigned to N=O stretching in nitrosamine[24]. Figure 2 shows the overlaid FT-IR spectra of raw textile effluent and effluent treated by *T.versicolor* at different intervals.

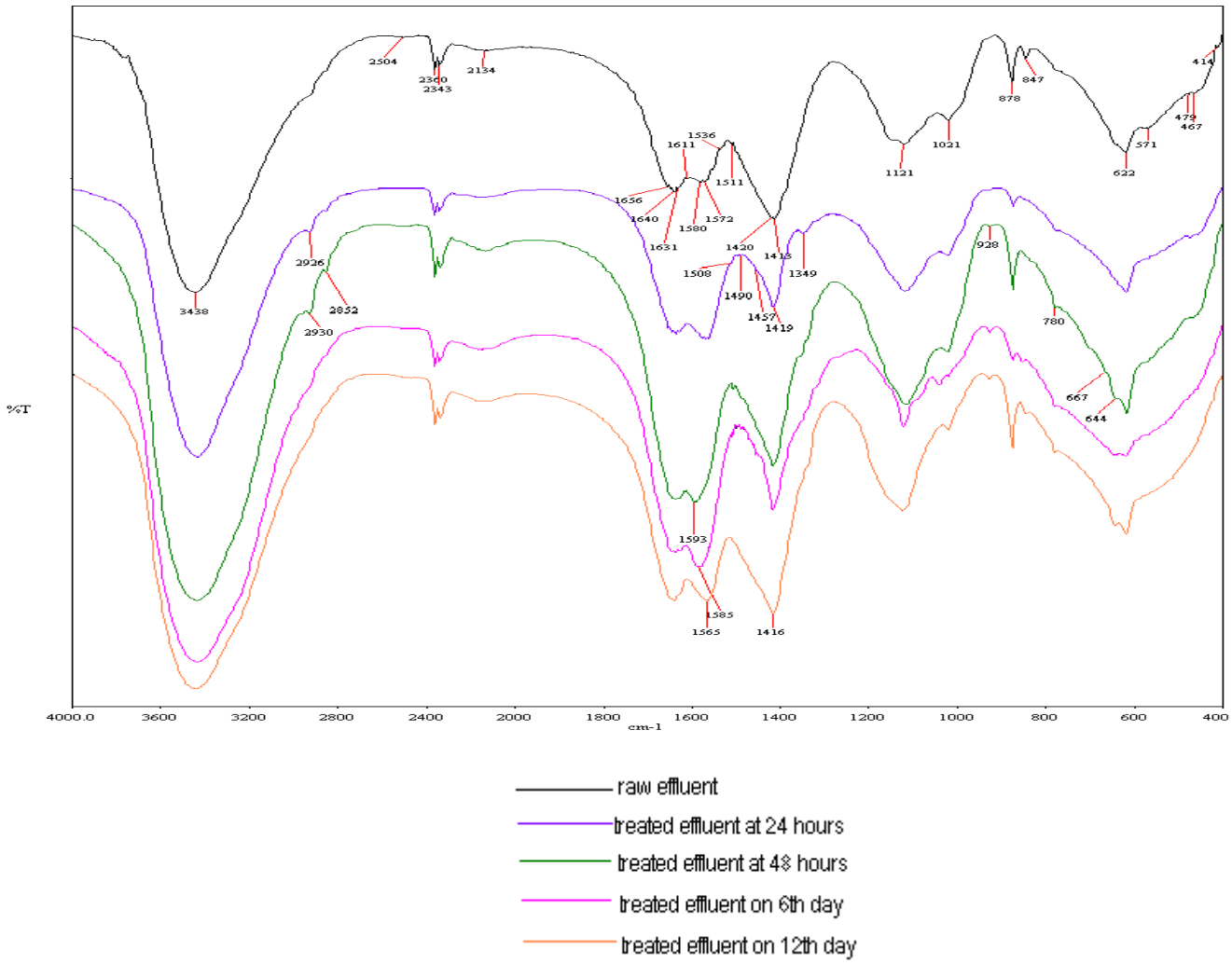

Fig. 2. Overlaid FT-IR spectra of raw and *T.versicolor* treated effluent at various intervals.

The absence of peaks near 1350 and 1500 cm^{-1} corresponding to C-N stretching and N-H bending in aromatic amines and the absence of Azo peak at the end indicates that the extracellular enzymes secreted by the *T.versicolor* breaks the Azo dye and degrades the aromatic amines. The appearance of peaks at 1565, 1416, 781 and 646 cm^{-1} indicate that the metabolites were nitrites and nitrosamines.

CONCLUSION

T.versicolor degrades the textile effluent by secreting extracellular enzymes. UV-VIS study indicated that the decolorization of the textile effluent was due to the biodegradation. 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.

REFERENCES

[1] Mechichi H, Mechichi T, Dhouib A, Sayadi S, Martinez A T & Martinez M J. 2006. Laccase purification and characterization from *Trametes trogii* isolated in Tunisia: decolorization of textile dyes by the purified enzymes, Enzyme and Microbiol Technol, 39, 141-148.

[2] Pratistha Dwivedi Rajesh Singh Tomar, 2017. Microbial degradation and decolorization of azo and anthraquinone textile dyes, Int J Pharm Bio Sci Apr ; 8(2): (B) 989-998.

[3] Khalid A, Arshad M & Crowley D E, 2008. Decolorization of azo dyes by *Shwanella* sp. Under saline conditions, Appl Microbiol Biotechnol., 79, 1053-1059.

[4] Amoozegar M A, Hajighasemi M, Hamedi J, Asad S & Ventosa A, 2011. Azo dye decolorization by halophilic and halotolerant microorganisms, Ann. Microbiol., 61, 217-230.

[5] Silvia Romero, Blanquez P, Gloria Caminal, Font X, Sarra M, Gabarrell X & Terasa Vincent, 2006. Different approaches to improve the textile dye degradation capacity of *Trametes versicolor*, Biochem Eng J, 31, 42-47.

[6] Meenu Chhabra, Saroj Mishra & Sreekrishnan T R, 2008. Mediator assisted decolorization and detoxification of textile dyes/dye mixture by *Cyathus bulleri* laccase, Appl Biochem Biotechnol, 151, 587-598.

[7] Zeroual Y, Kim B S, Yang Y W, Blaghen M & Lee K M, 2007. Decolorization of some azo dyes by Immobilized *Geotrichum* sp. Biomass in fluidized bed bioreactor, Appl Biochem Biotechnol., 142, 307-316.

[8] Mezohegyi G, Bengoa C, Stuber F, Font J, Fabregat A & Fortuny A, 2008. Novel bioreactor design for decolorization of azo dye effluents, Chem Eng J, 143, 293-298.

[9] Olukanni O D, Osuntoki A A & Gbenle G O, 2009. Decolorization of azo dyes by a strain of micrococcus isolated from a refuse dump soil, Biotechnol, 8(4), 442-448.

[10] Jin X C, Liu G Q, Xu Z H & Tao W Y, 2007. Decolorization of a dye industry effluent by *Aspergillus fumigatus XC6*, Appl Microbiol Biotechnol., 74,239-243.

[11] Phurge S S, Kalyani D C, Surwase S N & Jadhav J P, 2011. Eco-friendly degradation, decolorization and detoxification of textile effluent by a developed bacterial consortium, Ecotoxicol Environ Saf, 74, 1288-129.

[12] Saratale R G, Saratale G D, Chang J C & Govindwar S P, 2011. Bacterial decolorization and degradation of azo dyes: A review, Journal of Taiwan Institute of Chemical Engineers, 42, 138-157.

[13] Chhavi Rani, Jana A K & Bansal A, 2011. Studies on the biodegradation of azo dyes by White rot fungi *Daedalea Flavida* in the absence of external carbon source, International Proceeding of Chemical, Biological and Environmental Engineering, 6, 147-150.

[14] Jilani K, Ashger M, Bhatti H N & Mushtaq Z, 2011. Shake flask decolorization of direct dye solar golden yellow R by *Pleurotus ostreatus*, J Chem Soc Pak., 33(2), 209-214

[15] Dominguez A, Couto S R & Sanroman S M, 2005. Dye decolorization by *Trametes hirsuta* immobilized into alginate beads, World J Microbiol Biotechnol, 21, 405-409.

[16] Gao D, Du L, Yang J, Wu W M & Liang H, 2010. A Critial Review of the application of White rot fungus to environmental pollution control, Crit Rev Biotechnol, 30(1):70-77.

[17] Lamia Ayed, Mahdhi A, Cheref A & Bakhrouf A, 2011. Decolorization and degradation of azo dye Methyl Red by an isolatd *Sphingomonas paucimobilis*: Biotoxicity and metabolites characterization, Desalination, 274, 272-277.

[18] Jadhav U U, Dawkar V V, Kagalkar A N, Govindwar S P, 2011. Effect of metals on decolorization of Reactive Blue HERD by *Comamonas sp. UVS*, Water Air Soil Pollut, 216, 1-4, 621-631.

[19] Zille A, Gornacka B, Rehorek A & Cavaco-Paulo A, 2005. Degradation of azo dyes by *Trametes villosa* laccase over long periods of oxidative conditions, Appl Environ Microbiol, 7(11)**,** 6711-6718.

[20] Parshetti G, Kalme S, Saratale G & Govindwar S P, 2006. Biodegradation of Malachite Green by *Kocuria rosea MTCC1532*, Acta Chim. Slov, 53, 492-498.

[21] Patil P S, Shedbalkar U U, Kalyani D C, Jadhav J P, 2008. Biodegradation of Reactive Blue 59 by isolated bacterial consortium PMB11, J Ind Microbiol Biotechnol, 35, 1181-1190.

[22] Silvertein R M & Webster F X, 1997. Infrared Spectroscopy, Spectrometric identification of Organic Compounds, Sixth edition, (John Wiley & Sons Inc, New York), 104.

[23] Rajeshwari K, Subashkumar R & Vijayaraman K, 2011. Biodegradation of mixed textile dyes by bacterial strains isolated from dye waste effluent, Journal of Environmental Toxicology, 5 (2), 97-107.

[24] Telke A A, Kalyani D C, Dawkar V V & Govindwar S P, 2009. Influence of Organic and Inorganic compounds on oxidoreductive decolorization of Sulphonated azo dye C.I. Reactive Orange 16, J Hazard Mater, 172, 298-309.

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