Asian Journal of Water, Environment and Pollution, Vol. 5, No. 4, pp. 115-118.

Short Note

Fungal Detoxification of Azo Dye (Reactive Red 2) by Aerobic Process

A. Mary Saral*, M. Sachidhanandam and M.P. Saravana Kumar

School of Science and Humanities, Chemistry Division, Vellore Institute of Technology, Vellore–632 014, India

✉ sachinvit@gmail.com

Received August 17, 2006; revised and accepted May 17, 2008

Abstract: The decolourization of commercially available azo dye, Reactive Red 2 was studied since, to a certain extent, azo and reactive dyes are carcinogenic. In this research study, a novel fungus, Geotrichum candidum has been tried to degrade the dye colour. Batch studies were conducted in a defined medium with pH 7.0. As decolourization progressed, there was a substantial reduction in pH to 5.23. Maximum biomass growth was observed in the test organism on the 8th day. Maximum decolourization of Reactive Red 2 by Geotrichum candidum of up to 85.2% was seen on the 8th day only. In this case, the mycelium of the test organism also absorbed some amount of colour. Thus, the fungi can be used for decolourizing the colour present in the dye which may have carcinogenic effect on humans and that they have the potential to minimize environment risk.

Key words: Azo dye, decolourization, geotrichum candidum.

Introduction

Rapid industrialization and growth of population has led to the problems of environmental pollution, especially of the aquatic environment with a multitude of contaminants. Among all the pollutants (contaminants), colour appears to have a wide impact on various segments of the environment and has its origin due mostly, to the partially/untreated effluents generated from industries like dye manufacturing, textile, pulp and paper production, tanneries, chemical production, paints, varnishes and a host of others. Of these, textile dye effluents are the major contributors of the colour to the receiving water bodies. Discharge of untreated dye effluents not only impact colour to the receiving water but also interferes with its intended beneficial use.

Development of effective treatment technology for colour removal from dye wastes has been rather baffling. This is primarily due to their diverse and continuously changing character, complex chemical nature, persistent colour, inhibitory and non-biodegradable nature and toxicity.

*Corresponding Author

Literature reveals conflicting findings concerning the capability of textile waste treatment processes such as physical, physicochemical, chemical and biological, especially in terms of colour removal. Colour removal by physical processes is found to be negligible; various physicochemical processes, viz, chemical coagulation, chemical oxidation, adsorption and ion exchange etc., have been found to be of high cost for treating textile dye wastes. Biological treatment methods are cheap and offer the best alternative with proper analysis and environmental control.

The mineralization or complete biodegradation of an organic molecule in water is always a consequence of microbial activity (Alexander, 1980). Eighteen fungal strains able to degrade lignocellulosic material or lignin derivatives were tested with the azo dyes Reactive Black 5. Only the strains of Bjerkandera adusta, Trametes Versicolor and Phanerochaete Chrysosporium were able to decolourise all azo dyes (Heinfling et al., 1997). Laccases are copper-containing enzymes that have very broad substrate specificity with respect to electron donors, e.g. dyes (Abadulla et al., 2000). Chivukula and Renganathan (1995) cited that the azo dye must be electron-rich to be susceptible to oxidation by laccase of

pyricularia ory zae. Therefore, a need exists to develop a novel treatment technology for the textile dye treatment to ensure environmental protection from these harmful pollutants.

The principal objective of this study is: Biodegradation of Reactive Red 2 using a novel fungus, Geotrichum candidum, under aerobic condition.

Materials and Methods

Geotrichum candidum strain was maintained in potato-Dextrose-Agar medium consisting potatoes 200 g and Agar 20 g in 1 L of distilled water (pH 7.0). To ascertain the maximum potential for decolourization, the fungus was grown in Czapek-dox medium consisting of 2 g sodium nitrate, 1 g dipotassium hydrogen phosphate, 0.5 g magnesium sulphate, 0.5 g potassium chloride, 0.01 g ferrous sulphate, 30 g sucrose in 1 L of distilled water (pH 7.0). Basal medium was prepared, sterilized and raised in 250 ml conical flask containing 50 ml, which included 1 ml of spore suspension and 1 ml of dye solution. Sterile control, without inoculums was also maintained under similar conditions.

The commercialized azo dye C.I. Reactive Red 2, $C_{19}H_{10}N_6O_7S_2Cl_2$, was provided by a local manufacturer (Maruti, Kanchipuram). The manufacturer protects the exact composition formulation. The molecular weight (MW) and maximum absorption (A_{max}) for Acid Blue 45 are 568.9 g/mole and 493 nm. Figure 1 shows the structure of the dye.

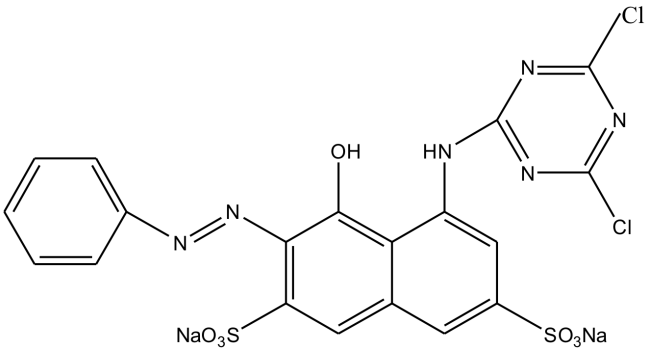

Figure 1: Structure of Reactive Red 2.

Experimental Procedures

A 50 ppm concentration of Reactive Red 2 solution was prepared and filter sterilized. The dye solution was added to conical flasks containing 50 ml basal medium and 1 ml of 5-day old spore suspensions of the test organism, and incubated in a rotary shaker at 150 rpm at 30° C. The experiment was conducted in duplicate. The decolourization exhibited by the test organism was estimated. During the incubation period; samples were withdrawn at 48 hours interval for eight days. They were then analyzed for decolourization, after centrifuging the samples at 12,000 rpm for 15 minutes. The degradation of the dye solution by the organism was monitored in UV-Visible spectrometry (Shimaduz 50) by scanning the culture supernatant. Sterile control was maintained throughout the incubation period and was taken as the blank value in the absorption peaks.

During incubation, the intensity of the dye in solution was reduced due to the fungal adsorption as well as by fungal transformation. Hence, it is necessary to find the dye bound to the mycelium. For this reason, the absorbed dye was solubilized with 10 ml of water and the mycelium was centrifuged. Again the pellet was suspended with 5 ml of distilled water and recentrifuged. The supernatant collected by these two operations were combined and the absorbance values were measured. From this degree of absorption of dye bound to the mycelium was determined. Wet mycelia pellet, separated by centrifugation, was dried at 80°C for five hours in hot air oven and the dry weight was taken. The pH of the supernatant was recorded using pH meter.

Results and Discussion

The decolourization was monitored by scanning the absorbance at wavelength ranging 200-800 nm. The scan profile recorded the highest peak for Reactive Red 2 at 493 nm. The absorbance was assayed at every 48 hours throughout the incubation period. As the decolourization preceded the concentration of dye decreased in terms of their absorbance value. The colour removal of Reactive Red 2 by Geotrichum Candidum was clearly shown in Figure 2 and Table 1. The fungi removed 85.2% of the colour on the 8th day of incubation period by Geotrichum Candidum when the concentration of the dye solution was 50 ppm.

Biomass

Biomass plays an important role in the decolourization of colourants, since it enhances the degradation of the dye Geotrichum candidum on C.I. Reactive Dye 2 showed a maximum value of 0.2213 g/50 ml on the 8th day of incubation with a decolourization efficiency of 85.2% (Table 1).

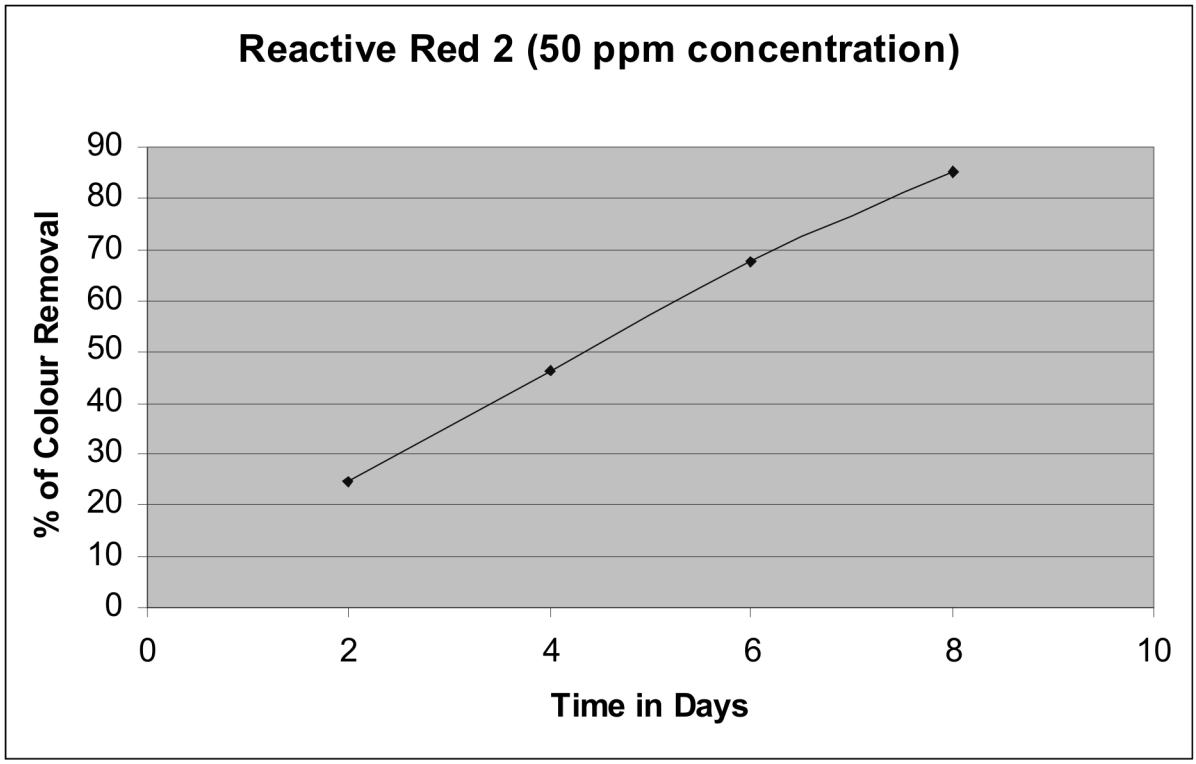

Figure 2: Decolourization of C.I. Reactive Red 2 by Geotrichum candidum

Table 1: Decolourization of Reactive Red 2 by Geotrichum candidum

Incubation Time (Days)	*Biomass g/50 ml*	*Absorbance of Supernatant*	*Absorbance of dye bound*	*Decolourization %*	*Adsorption to mycelium %*	*pH variation during incubation*
Control	–	1.165	–	–	–	7.00
2	0.1327	0.8784	0.6326	24.6%	54.3%	5.72
4	0.1678	0.6267	0.4509	46.2%	38.7%	5.13
6	0.1898	0.3774	0.2284	67.6%	19.6%	4.84
8	0.2213	0.1724	0.0723	85.2%	6.2%	4.23

pH

The study was conducted in a defined medium with pH 7.0, for the test organism, namely, Geotrichum candidum. As the decolourization progressed there was a substantial reduction of pH to 5.2 exhibited by the test organism.

Conclusions

Effective degradation of C.I. Reactive Red 2 was achieved by fungi Geotrichum candidum in an aerobic environment. During the incubation period in the decolourization process by the microorganism, Geotrichum candidum, the pH of the medium always showed a decrease from the initial conditions indicating the release of the degradation products. This process is very simple and economical. In this process, degradation as well as transformation of the dye colour bound to the mycelium is involved in the decolourization of the dye by the test organism.

Acknowledgement

We express our deep and profound sense of gratitude to Vellore Institute of Technology, for providing the necessary facilities throughout this work.

References

Abadulla, E., Tzanov, T., Costa, S., Robra, K.H., Cavaco, P.A. and G.M. Guebitz (2000). Decolorization and detoxification of textile dyes with a laccase from Trametes hirsute. *Applied and Environmental Microbiology*, **66:** 3357-3362.

Alexander, M. and B.K. Lustigman (1966). Effect of chemical structures on microbial Degradation of substituted benzenes. *J.Agr.Food Chem*., **14:** 410-413.

Chivukula, M and V. Ranganathan (1995). Phenolic azo dye oxidation by laccase from Pyricularia aryzae. *Applied and Environmental Microbiology*, **61:** 4374-4377.

Heinfling, A., Bergbauer, M. and U. Szewzyk (1997). Biodegradation of azo and phthalocyanine dyes by Trametes versicolor and Bjerkandera adjusta. *Applied and Environmental Microbiology*, **48:** 261-266.