



## Removal of a Reactive Dye (Red RB) by the Bacterial Species Isolated from Dyeing Industry Effluents

M. Vasanthy, A. Geetha and G. Seethadevi

PG & Research Department of Environmental Sciences, Govt. Arts College, Ariyalur-621 713, T. N.

### Key Words:

Dyeing industry effluent  
Reactive Dye (Red RB)  
*Enterobacter* sp. (GS5)  
*Escherichia coli* (GS3)  
*Bacillus* sp. (GS10)  
Decolourisation

### ABSTRACT

Removal of reactive dyes from aqueous solution was investigated using bacterial species from dyeing industry effluents. The decolorization was achieved at different dye concentrations, pH and temperature. It was caused by bacterial species such as *Enterobacter* sp. (GS5), *Escherichia coli* (GS3) and *Bacillus* sp. (GS10) and their consortia. More reliable and effective decolorization rates were obtained with 100% *Enterobacter* sp. (GS5) after 96 hours at 10 and 20 ppm. Decolorization has been maximum in the static experimental conditions.

### INTRODUCTION

India's dye industry produces different types of dyes and pigments. Production of dyestuff and pigments in India is close to 80,000 tonnes. India is the second largest exporter of dyestuffs and intermediates among the developing countries after China. The textile industry accounts for the largest consumption of dyestuffs nearing 80%. The textile industry is to satisfy the ever-growing demand in terms of quality, variety, fastness and other technical requirements. However, a recent study conducted under the National Biodiversity Strategy and Action Plan has revealed that chemical colours have all but wiped out India's wonderful vegetables (Mathur et al. 2005).

Colour is a visible pollutant and presence of very minute concentration of colouring substances in water makes it unsuitable for domestic purposes like drinking and other uses. The textile mills require large volumes of water of high purity and generate equally large volumes of wastewater, which is highly coloured and complex in nature (Ho & Mckay 1981).

Main industries that contribute highly coloured effluents are distillery, pulp and paper, tanning, textile, dye manufacturing and paint. Colour inhibits growth of the desirable aquatic biota necessary for self-purification and also reduces dissolved oxygen. When dyeing effluent is discharged into sewer lines, it may cause corrosion. The dyeing effluent treatment is more complex than any other industrial wastewater purification because of the fact that neither two dyeing effluents are alike in character nor can any two effluents be purified or treated by the same treatment. Therefore, effluents must be treated before they are disposed into river bodies or onto land.

The colour imparted by reactive dyes Procion Brilliant Blue MR, Procion Yellow MGR (PYMGR), Procion Orange M2R and their mixture (taupe) was removed using cashew nut hull carbon and commercial activated carbon by performing batch and column experiments. The adsorption has been reported to obey Langmuir and Freundlich isotherm models. The adsorption was found to increase with decrease in initial dye concentration, increased with agitation time and adsorbent concentration (Vasanthy 1996). The removal of colour from wastewater has been reported to be accomplished by flotation, chemical coagulation, chemical oxidation and adsorption (Hu 1996, Zhou & Zimmermann 1993).

The use of microorganisms as adsorbents for dyes also offers a potential alternative to existing methods for purification. The cell wall of microorganisms, which consists essentially of various organic compounds such as chitin, lipids, amino acids and other cellular components, can provide a means for the passive uptake of reactive dyes (Aksu 2001). The authors have earlier used dried activated sludge to remove one important reactive dye, Rhodamine-B (Rh-B) from aqueous solutions. The present work has been carried out to study the feasibility of biosorption of dyes from aqueous dye solution using the bacterial strains isolated from the dyeing industry effluent.

## MATERIALS AND METHODS

**Isolation and identification of microorganisms from the effluent:** Isolation and identification of microorganisms from the effluent was done using the pour plate technique (Buchanan & Gibbons 1974).

**Dilution of the sample:** To 99 mL of the sterile distilled water, 1 mL of the effluent was added aseptically and mixed thoroughly. From this, 1 mL was transferred aseptically to 9 mL of sterile distilled water to obtain  $10^{-3}$  to  $10^{-7}$  dilutions. Then, 1 mL from each dilution was transferred aseptically into sterile petri plates. Nutrient agar medium was used to estimate the total heterotrophic bacteria. The plates were later subjected for incubation at  $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for 24 hours for the isolation of bacterial colonies.

**Bacterial species and growth conditions:** *Enterobacter* sp. (GS5), *Escherichia coli* (GS3) and *Bacillus* sp. (GS10) have been isolated and identified from dyeing industry effluent. These bacterial species were grown at  $25^{\circ}\text{C}$  in nutrient broth medium. The pH of the medium was adjusted to 7.2 before sterilization.

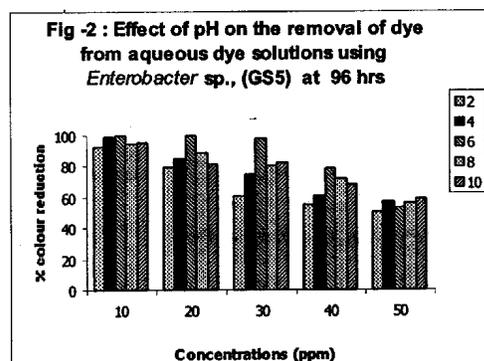
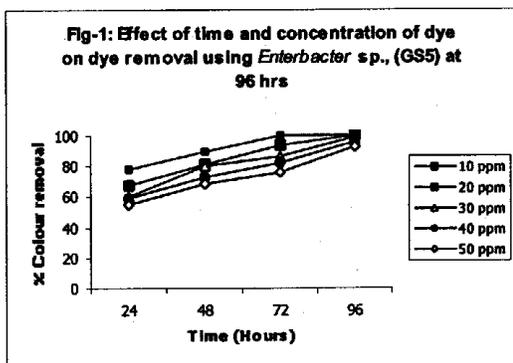
**Batch absorption experiments (Preparation of the bacterial species and dye solutions for biosorption):** Accurately 100 mg of dye was weighed and dissolved in 100 mL sterile distilled water to get 1000 ppm dye solution. A suitable aliquot of the sample solution containing dye was transferred into a 100 mL of volumetric flask and the solution was made up to the mark with double distilled water. The absorbance was measured at 519 nm against a blank. A standard graph was plotted for 10-100 mg/L of dye.

**Effect of contact time and concentration on percent colour removal:** Accurately, 50 mL of aqueous solutions of concentrations ranging from 10, 20, 30 40 and 50 ppm were prepared and dispensed into five sterile flasks. To the flasks, 1 mL of each organism was added as an inoculum under aseptic condition and incubated at  $32^{\circ}\text{C}$ . The pH of the aqueous solutions were adjusted to 2, 4, 6, 8 and 10 with the same initial concentrations.

**Effect of time and bacterial consortia:** The bacterial species such as *Enterobacter* sp. (GS5), *Escherichia coli* (GS3) and *Bacillus* sp. (GS10) were prepared and combinations of GS3 + GS5 + GS10, GS3 + GS5, GS3 + GS10 and GS5 + GS10 were prepared and dispensed into five sterile flasks.

## RESULTS AND DISCUSSION

**Effect of time and initial dye concentration on the removal of dye from aqueous dye solution (Red RB):** The batch experiments have clearly shown (Fig. 1) that the colour imparted by 10 ppm Red RB solution has lost its colour completely after 96 hrs, and the 20 ppm solution has become 100 % colourless after 96 hrs. The maximum of 99% colour removal has been reported after 96 hrs in 30



ppm aqueous dye solution. At the same time, for the other 2 dye concentrations (40, 50 ppm) the colour reduction has reached about 98%.

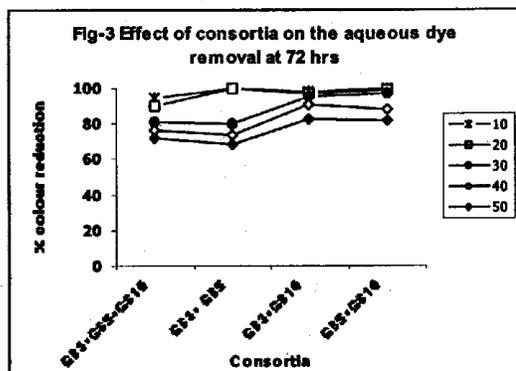
Similarly Nyanhongo et al. (2002) have noticed that the difference in the two culture systems was not significantly different. This indicates that laccase is not involved in the decolourisation of the dye effluent. In contrast, laccase was detected when *Trametes modesta* was used for decolourisation of the textile dyes.

In addition to azo dyes, the ability of bacteria to aerobically metabolize other dye classes has also attracted interest but yielded little success. Sarnaik & Kanekar (1999) described the aerobic mineralisation of the triphenylmethane dye, methyl violet, by a strain of *Pseudomonas mendocina* MCM B-40. Methyl violet, which has some commercial applications in addition to it, recognized the use as a bacteriological and histological stain, was used by the isolate as a sole carbon and energy source.

Under anaerobic conditions many bacteria have been reported to readily decolorise azo dyes. Initially, the bacteria bring about the reductive cleavage of the azo linkage, which results in dye decolourisation and the production of colourless aromatic amines. The potential toxicity, mutagenicity and carcinogenicity of such compounds is well documented (Chung et al. 1992).

**Effect of pH on the dye removal:** The highest decolorization rate was recorded in 10 and 20 ppm after 96 hrs at pH 2, 4 and 6. The moderate reduction of colour at pH 8 and 10 within 96 hrs was 95% and 92% respectively (Fig. 2).

Identification of bacterial strains resistant to mixed dye (Procion Orange M2R and Procion Brilliant Blue MR) was carried out by Geetha (2002). The maximum colour removal was reported to be 100% at 20, 40, 60, 80 and 100 ppm by using *Micrococcus sp.* S5. Five strains showed more than 50% decolorization. Maximum decolouration (72%) was observed with *A. chroococcum* Ala 27. Yatome et al. (1981) observed 96% decolorization of 20  $\mu\text{g/mL}$  of CV (0.852  $\mu\text{g/mL}$ ) in 24 hrs using *Bacillus subtilis* and *Nocardia globulera* respectively (Yatome et al. 1991).



**Effect of consortia on the removal:** The maxi-

mum biosorption capacity was noticed with the consortia containing of *Escherichia coli* and *Enterobacter* sp. after 72 hrs at 10 and 20 ppm. The other consortia such as (GS3 + GS5 + GS10), (GS3 + QS10) and (GS5 + GS10) have reported minimum absorption efficiency with increasing dye concentrations (Fig. 3).

Sorption removal of Procion Orange M2R from aqueous solution using *Pseudomonas* sp. and *Aspergillus flavus* has been found by Shalini Menon (2000). The maximum colour removal was reported to be 82.4%.

## REFERENCES

- Aksu, Z. 2001. Biosorption of reactive dyes by dried activated sludge. Equilibrium and kinetic modelling. *Biochem. Engg. J.*, 7: 79-84.
- Buchanan, R.E and Gibbons, N.E (Eds.) 1974. *Bergey's Manual of Determinative Bacteriology*. English edition, Williams and Wilkins, Baltimore.
- Chung, K.T., Se, S. Jr. and Cernigliz, C.E. 1992. The reduction of azo dyes by the intestinal microflora. *Crit. Rev. Microbiol.*, 18: 175-190.
- Collishaw, P., Cunningham, A.D. and Lindsay, D.P. 2000. The changing world textile market. In: *Book of Papers of International Conference and Exhibition*, Atlanta. George, A. Assn. of Textile Chemists and Colorists, 63.
- Geetha, A. 2002. Identification of bacterial strains resistant to mixed dye (Procion Orange M2R & Procion Brilliant Blue MR). M.Sc. Dissertation, Bharathidasan University, Trichy.
- Ho, Y.S. and McKay, G. 1981. The kinetics of sorption of basic dyes from aqueous solution by *Sphagnum* moss peat. *Can. J. Chem. Engg.*, 76: 822-827.
- Hu, T. L. 1996. Removal of reactive dyes from aqueous solution by different bacterial genera. *Water Sci. Technol.*, 34: 89-95.
- Nyanhongo, G.S., Gomes, J., Gubitz, G.M., Zvauya, R., Read, J. and Steiner, W. 2002. Decolourisation of textile dyes by laccases from a newly isolated strain of *Trametes modesta*. *Water Res.*, 36: 1449-1456.
- Mathur, N., Bhatnagar, P. and Bakre, P. 2005. Assessing mutagenicity of textile dyes from Pali (Rajasthan) using ames bioassay. *Applied Ecology and Env. Res.*, 4(1): 111-118.
- Sarnaik, S. and Kanekar, P. 1999. Biodegradation of methyl violet by *Pseudomonas mendocina* MCMB-402. *Appl. Microbiol. Biotechnol.*, 52: 251-254.
- Shalini Menon 2000. Sorption Removal of Procion Orange M2R from Aqueous Solutions Using *Pseudomonas* sp. S5 and *Aspergillus flavus*. M.Sc. Dissertation, Bharathiar University, Coimbatore.
- Vasanthi, M. 1996. Removal of reactive dyes from aqueous solutions and dyeing industry effluent by cashew nut hull carbon. Ph.D. Thesis, Bharathiar University, Coimbatore.
- Yatome, C., Ogawa, T., Koza, D. and Idaka, E. 1981. Biodegradation of azo and triphenylmethane dyes by *Pseudomonas pseudomallei* 13NA. *Jr. Soc. Dyers Chemists*, 97: 166-169.
- Yatome, C., Ogawa, T. and Matsue, M. 1991. Degradation of crystal violet by *Bacillus subtilis*. *Jr. Environ. Sci. Health*, 826: 75-87.
- Zhou, W. and Zimmermann, W. 1993. Decolourization of industrial effluents containing reactive dyes by actinomycetes, *FEMS Microbiol. Lett.*, 107: 157.