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Decolorization of Reactive Blue MR, using *Aspergillus* species Isolated from Textile Waste Water

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Abstract

The present investigation focused on the isolation and characterization of fungal strains, which can efficiently decolorize the textile dye, reactive Blue MR. A total of five indigenous fungal strains were isolated from the effluents collected around the discharge site of textile industry situated in Panipat. Effluent samples were also analyzed for their physiochemical properties. Aspergillus allhabadii, A. niger and A. sulphureus were successfully identified using macroscopic and microscopic study referring relevant literature. Decolorization capabilities of these fungal species were evaluated for reactive blue MR dye (100-300mg/L) in carbon limited Czapek Dox broth (0.5%), carried out under static in vitro condition. It was found that A. allhabadii and A. sulphureus showed higher decolorization capabilities (95.13±0.11%), (93.01±0.25%) with 200mg/L dye, but A. niger showed higher decolorization (83.14±0.19%) with 100 mg/L after ten days of incubation. Decolorization efficiency was also investigated in different carbon sources and found sucrose was the best carbon for all the fungal strains. The fungal isolates were found efficient in decolorization, which proves that these indigenous fungi are potential candidates for bioremediation.

Key words: *Aspergillus*, decolorization, physiochemical, textile dye, reactive blue MR, static condition.

Introduction

Synthetic dyes have been used abundantly in textile and dyeing industries because of their ease and cost effectiveness in synthesis, firmness with variety in colors as compared to that of natural dyes. Beside these dyes are also used in leather, pulp and paper, food processing, cosmetics and pharmaceutical industries. But textile industries are alone considered as the major consumer of the dyes in the market.

Over 10,000 dyes with an annual production of over 7×10^5 metric tonnes are commercially available[1]. Reactive dyes are the class of dye most widely used; industrially it has a world market share of 60–70%. Approximately, 10-15% of the dyes used in the textile industries remain unutilized and along with reactive dyes, remaining 50% unutilized had to be released with effluents[2]. The effluent discharged from textile dyeing mill forms the highly concentrated color waste water, consisting of various types of colors. Along with other industrial wastes, the textile industries and dye industry waste has a significant role in the water pollution. The discharge of the wastewater into receiving streams not only affects the aesthetic sense of nature but also interferes with transmission of sunlight into streams, thus reducing photosynthetic activity[3]. In addition to visual effect, they have an adverse impact in terms of chemical oxygen demand, toxicity, mutagenesis and carcinogenicity[4]. Dyes are designed in such a way that they are resistant to light, water and oxidizing agents. Therefore, they cannot be treated by conventional treatment processes such as an activated sludge[5].

So, these dyes must be removed/degraded for the sustainability of environment. Various methods (adsorption, sedimentation, flocculation, floatation, coagulation, osmosis, neutralization, reduction, oxidation, electrolysis and ion-exchange) are usually employed to remove the colors before discharging into the environment. But these methods have some limitations as high cost and disposal of large quantity of sludge with some toxic waste produced during these processes. Therefore, economical and environment friendly techniques are required for the removal or degradation of dye waste from the effluent. Bioremediation can be an effective tool where indigenous microorganism (bacteria, algae and fungi) are used for the treatment of industrial dye effluent. Among these microorganisms, bacteria are most commonly used for various bioremediation processes. A white rot fungus *Phanerochaete chrysosporium* has been used extensively for decolorization of dyes in wastewaters[6] and is correlated with the ability to synthesize lignin degrading exoenzymes such as lignin and manganese peroxidases (MnP)[7, 8] or Laccases[9, 10]. Development of effective dye degradation technique requires a suitable strain and its use under favorable condition for achieving the maximum degradation potential. In recent years there has been an intense research on fungal decolorization of dye waste water. It is thus turning into a promising alternative for replacement or substitute to present treatment processes. Here, this present work is focused on the isolation and characterization of fungal strains, which would efficiently decolorize the textile dye, reactive blue MR.

Material and Methods

Sampling: The effluent samples used for the present study were collected in sterile air tight bottles with filtering through the ordinary filter paper to remove large suspended particles. Standard procedures (spot and grab) were followed during sampling and samples were transported to the laboratory and stored at 4°C.

Media and chemicals: The textile dye (reactive blue MR) used for the decolorization in the present investigation was a gift from Sheena Export, Panipat (Haryana). All media components and chemicals used in the present study were of analytical grade and purchased from Hi-Media Laboratories (Mumbai, India). The chemical structure of the dye is shown in figure-1.

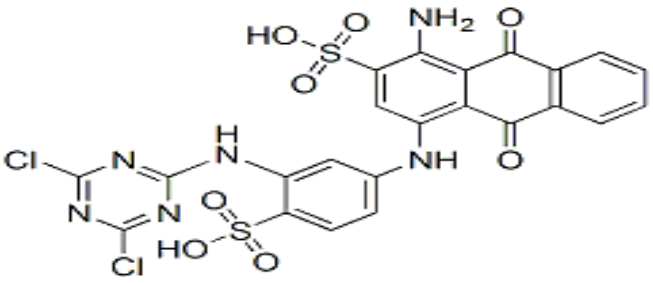


Figure – 1
Chemical structure of Reactive Blue MR

Physiochemical analysis of effluents: Temperature, pH, color and odour of the various wastewater samples were recorded on the spot. Samples collected from the discharge sites were filtered through Whatman no.1 filter paper and their chemical oxygen demand, biological oxygen demand and total dissolved solid was determined using standard procedures[11].

Determination of absorption maxima (λmax) of reactive blue MR: The absorption maximum was determined by using a spectrophotometer. Optical density of dye solution in water was observed at different wavelength between visible regions (340-700nm). The wavelength where, the dye showed maximum absorbance is taken as absorption maximum of the dye; for reactive blue MR it was observed 600 nm.

Isolation and Identification of dye decolorizing fungi: Fungal strains native to the sampled area were isolated on Czapek Dox Agar (CDA) using a dilution plate technique. The following composition of a medium was used (K_2HPO_4, 1.0 g L^{-1}; $NaNO_3$, 30.0 g L^{-1}; KCL, 0.5 g L^{-1}; $MgSO_4.7H_2O$, 0.5 g L^{-1}; $FeSO_4.7H_2O$, 0.01 g L^{-1}; Yeast extract, 5.0 g L^{-1}; Sucrose 30.0 g L^{-1}; Rose Bengal, 0.03 g L^{-1}; Agar, 15.0 g L^{-1}). Fungal strains differing in growth pattern and morphology were isolated and identified using photomicrograph taken with steriobinocular microscope and with the help of taxonomic guides and standard procedure[12, 13, 14, 15]. The identified fungal strains were preserved on CDA slants at 4°C in a refrigerator and were served as stock cultures.

Screening of fungal strains for decolorization on solid-plates: Decolorization abilities of identified fungal strains were tested against reactive blue MR on agar plate. After sterilization of media dye was added aseptically at a concentration of 200mg/L. For each plate, a well was made in the center of the plate and a disc of inoculum placed at the center, and uninoculated medium was maintained as a control. These plates were incubated at 25±2°C and observed for decolorization. The experiments were performed in duplicate for each culture.

Decolorization assay: The ability of fungal strains to decolorize textile dyes was carried out in C-limited Czapek-Dox broth (5 g/L) amended with reactive blue MR (200 mg/L). Erlenmeyer flasks contained 100 ml sterile media with dye and were inoculated with fungal disc (8mm) separately. The flasks were incubated at 25± 2°C for 10 days in static condition. Samples were withdrawn aseptically on alternate days, centrifuged at 5000 rpm for 10 min and the supernatant was scanned in a spectrophotometer at λ_{max} (600 nm) of reactive blue. Two control flasks were also maintained for each fungal strain. One flask contained media (without dye) and inoculated with fungal biomass and second flask contained media with dye and no fungal biomass. Percent decolorization was calculated by applying the formula[16]:

$$Decolorization\ (\%) = Ao - \frac{At}{Ao} \times 100$$

Where, A_0 is initial absorbance of sample and A_t is the absorbance at different time intervals.

To study the effect of different carbon sources (sucrose, glucose and fructose) and dye concentration (100-300 mg/L) in the liquid C-limited Czapek-Dox medium same protocol was applied.

Statistical analysis: Data were analyzed were the mean of triplicates ± standard deviation (SD).

Results and Discussion

Physiochemical characterization of the textile effluent: The effluent discharged by textile industries has leads to a serious problem of groundwater and soils pollution. The physiochemical analysis of sampled textile effluent helped us to measure the pollution level. Thus, the physiochemical parameters test for effluents were conducted and examined. Table-1 showed that effluents have dark black color with pungent smell, relatively high temperature 38°C (measured by a laboratory thermometer), pH 9.7, BOD (492 mg/l), COD (1305 mg/l) and TDS (5867 mg/l). Effluents color is black due to the mixture of various dyes and chemicals used in the dyeing process[17]. The pH of the effluent alters the physiochemical properties of water which in turn adversely affects the biodiversity. High pH is mainly due to the use of carbonate,

bicarbonate, H_2O_2 and NaOH during bleaching process in the textile[18]. Soil permeability gets affected, which results in polluting the underground resources of water[19]. Elevated temperature tends to decrease the solubility of gases in water, which is ultimately expressed as high BOD/COD. TDS values of effluent sample were found higher than the permissible limits as compared to a textile effluent collected from a mill near Hisar (Haryana)[20]. High TDS value reduces the light penetration into the water and ultimately decreases the photosynthesis in aquatic flora. This cause reduction in dissolved oxygen level of water bodies, which results for extremely low purification of wastewater by microorganisms.

Isolation and identification of fungal strains: A total of five morphologically different fungal strains were recovered from the effluents collected around the vicinity of discharged site by employing spread plate technique. Out of the five fungal strains, three were identified after staining with lactophenol cotton blue and microscopic analysis *viz. Aspergillus allhabadii, A. niger* and *A. sulphureus.* These fungal strains may be much adapted to the polluted sites and are utilizing the dyes/xenobiotic compounds as novel growth and energy substrate. The occurrence of fungi in the polluted water depends on the availability of nutrient, oxygen, biological, physical and chemical characteristics of the pollutants. *Aspergillus* species are well adapted to textile waste water and are frequently isolated from effluents and dye contaminated soils[17, 21].

Decolorization assay: Initial screening was done on a solid plate supplemented with sucrose (5 g/L) and reactive blue MR (200 mg/L). A zone of disappearance of dye around the fungal biomass confirms the dye decolorizing activity of the identified fungal strains (figure-2). The results are in accordance with earlier reports of screening of fungi and bacteria showing growth and decolorization on solid culture[22-24]. Further, decolorization potential of all the fungal strains against reactive blue MR was tested in an aqueous medium.

It was found that maximum decolorization efficiency shown by *A. allhabadii* (95.13±0.11%) followed by *A. sulphureus* (93.01±0.25%) and least by *Aspergillus niger* (82.62±0.21%) in static condition after ten days of incubation with the dye (200mg/L) and sucrose as a carbon source (figure-3).

Effect of carbon source on decolorization: Carbon sources such as fructose, glucose and sucrose were used at 5.0g/L to investigate their effect on the decolorization efficiency of the fungal isolates. It was found that highest decolorization shown by *Aspergillus allhabadii* (95.13±0.11%), *Aspergillus sulphureus* (93.01±0.25%) and minimum by *Aspergillus niger* (82.60±.21) in sucrose supplemented medium. The rate of decolorization in fructose and glucose containing medium was found maximum with *Aspergillus niger* (77.89±0.28%) and *Aspergillus sulphureus* (71.87±0.84%). The results of the present investigation depicted that all the tested fungal strains were efficient in decolorization with sucrose supplemented media (figure-4). The primary mechanism of decolorization is due to dye adsorption/degradation by mycelium of fungi with reduction of dye intensity in solution because of changes caused by them[24,25]. Growth media enhances the growth and adsorption/degradation rate by fungi and on addition of carbon or other nutrient sources further increases decolorization process[26, 27]. Furthermore, the rate of dyes removal can be linked with the available co-substrates[28] and with the exponential growth phase[29].

Effect of dye concentration on decolorization: The decolorization efficiency of *Aspergillus allhabadii, A. niger* and *A. sulphureus* were analyzed at 100-300mg/L in liquid media containing sucrose as a carbon source. It was found that highest decolorization shown by *Aspergillus allhabadii* (95.13±0.11%) and *Aspergillus sulphureus* (93.01±0.25%) with 200mg/L but *A. niger* showed higher decolorization (83.14±0.19%) with 100mg/L after ten days of incubation (figure- 5). Generally, the concentration of color compounds found in the effluent or rivers ranged as low as 12 to 16 mg/L. Decolorization of dyes at higher concentration may create an acidic condition, which further facilitate their better removal (enzymatic or by cell wall adsorption) by the fungi[30,31]. It is reported that higher dye concentration strongly inhibits decolorization, which may be due to desorption or toxic effects. The ability of enzyme for recognizing the substrate efficiently at very low concentrations may be present in some waste water[32,33]. The desorption of the dyes from the fungal cells, especially at higher dye concentrations may be due to higher molecular mass, structural complexity and the presence of inhibitory groups, SO_3Na in the dye[34].

Conclusion

The present study results showed that the indigenous fungi haves the ability to remediate the dye from the effluent. It was found that at moderate dye concentration decolorization activity high as compared to higher concentration. It was also found that use of different carbon source also affects the decolorization efficiency level varying with the strains. Further, it can be suggested that dye contaminated sites can potentially be reclaimed by a low cost bioremediation process with native fungal species isolated from the dye disposal sites.

References

1. Zollinger H., Color Chemistry: Syntheses, Properties and Applications of Organic Dyes and Pigments (2nd Edn), VCH Publications, New York, 496 **(1991)**

2. Campos R., Kandelbauer A., Robra K.H., Artur C.P. and Gubitz G.M., Indigo degradation with purified laccases from *Trametes hirsuta* and *Sclerotim rolfsii, J. Biotechnol*., **8,** 131-139 **(2001)**

3. McMullan G., Robinson T., Marchant R. and Nigam P., Remediation of dyes in textile effluent: a critical review on

current treatment technologies with proposed alternatives, *Biosource Technol*., **77,** 247-255 **(2001)**

4. Chung K.T. and Stevens S.E., Decolorization of azo dyes by environmental microorganisms and helminthes, *Environ. Toxicol. Chem*., **12,** 2121-2132 **(1993)**

5. Shaul G.M., Holdaworth T.J., Dempsey C.R. and Dostal K.A., Fate of water soluble azo dyes in the activated sludge process, *Chemosphere*, **22,** 107-119 **(1991)**

6. Kirk T.K., Lamar R.T. and Glaser J.A., The Potential of White rot Fungi in Bioremediation. Biotechnology and Environmental Science Molecular Approaches, Mongkolsuk, S. (Ed.), Plenum Press, UK, 131-138 **(1992)**

7. Koichi H., Yoshio W. and Kazunori N., Decolorization of azo dye by the white-rot basidiomycetes *Phanerochaete sordida* and by its manganese peroxidase, *J. Biosci. Bioengineer*, **95(5),** 455-459 **(2003)**

8. Sharma P., Singh L. and Dilbaghi N., Optimization of process variables for decolorization of Disperse Yellow 211 by *Bacillus subtilis* using Box- Behnken design, *J. Haz. Matter*., **164,** 1024-1029 **(2009)**

9. Rodriguez E., Pickard M.A. and Rafael Vazquez-Duhalt, Industrial dye decolorization by laccases from ligniolytic fungi, *Curr.Microbiol*., **38,** 27-32 **(1999)**

10. Murugesan K., Nam I., Kim Y. and Chang Y., Decolorization of reactive dyes by a thermostable laccase produced by *Ganoderma lucidum* in solid state culture, *Enzyme Microbi. Technol.*, **40**, 1662-1672 **(2007)**

11. Clesceri L.S., Greenburg A.E. and Trussell R.R., Standard methods for the examination of water and wastewater (**17**th Edn), American public Health Association, Washington, DC., USA **(1989)**

12. Gilman J.C., A manual of soil fungi (Revised 2nd Edn), Oxford & IBH publishing Co. **(1944)**

13. Barnett H.L. and Hunter B.B., Illustrated genera of Imperfect Fungi, Burgress Publishing Company, Minneapolis, Minnesota **(1972)**

14. Ellis M.B., Dermatacious Hyphomycetes, Commonwealth Mycological Institute, Kew, Surrey, UK **(1976)**

15. Domsch K.H., Gams W. and Anderson T.H., Compendium of soil fungi, Academic press. A subsidiary of Harcourt Brace Jovanovich, publisher, **(1980)**

16. Olukanni O.D., Osuntoki A.A. and Gbenle G.O., Textile effluent biodegradation potentials of textile adapted and non-adapted bacteria, *African J. Biotechnol*., **5,** 1980-1984 **(2006)**

17. Devi M. and Kaushik B.D., Decolorization of textile dyes and dye effluent by *Aspergillus* Spp., *Indian J. Microbiol*., **45,** 41-44 **(2005)**

18. Wood W.A. and Kellogg S.T., Biomass, cellulose and hemicelluloses, *Methods Enzymol.*, **160,** 632-634 **(1988)**

19. Buckley C.A., Membrane technology for the treatment of dye house effluents, *Water Sci. Techno.*, **25 (10),** 203-209 **(1992)**

20. Vandevivre P.C., Bianchi R. and Verstraete W., Treatment and reuse of wastewater from the textile wet-processing industry: review of emerging technologies, *J. Chem.Technol.Biotechnol.*, **72,** 289- 302 **(1998)**

21. Ponraj M., Gokila K. and Vasudeo Z., Bacterial decolorization of textile dye- Orange 3R, *International J. Adv. Biotechnol. Res.*, **2(1),** 168-177 **(2011)**

22. Selvam K., Swaminathan K. and Chae K.S., Decolourization of azo dyes and a dye industry effluent by a white rot fungus *Thelephora* sp. *Bioresour Technol*., **88,** 115-119 **(2003)**

23. Wesenberg D., Kyriakides I. and Agathos S.N., White-rot fungi and their enzymes for the treatment of industrial dye effluents, *Biotechnol. Adv*., **22,** 281-287 **(2003)**

24. Fu Y. and Viraraghvan T., Dye biosorption sites in *Aspergillus niger, Biores. Technol.*, **82,** 139-145, **(2002)**

25. Knapp J.S., Newby P.S. and Reece L.P., Decolorization of dyes by wood rotting basidiomycete fungi, *Enzyme Microbiol. Technol*., **17,** 664-668 **(1995)**

26. Swamy, J. and Ramsay J.A., The evaluation of white rot fungi in the decoloration of textile dyes, *Enzyme Microbiol. Technol*., **24,** 130-137 **(1999)**

27. Zhang, F.M., Knapp J.S. and Tapley K.N., Decolourization of cotton bleaching effluent with wood rotting fungus, *Water Res*., **33,** 919-928 **(1999)**

28. Ali N., Ikramullah L.G., Hameed A. and Ahmed S., Decolorization of Acid red 151 by *Aspergillus niger* SA1 under different physicochemical conditions, *World J. Microbiol*., **24,** 1099-1105 **(2008)**

29. Sumathi S. and Manju B.S., Uptake of reactive Textile dyes by *Aspergillus foetidus*. *Enzyme Microb*. *Technol*., **27(6),** 347-355 **(2000)**

30. Aksu Z. and Tezer S., Equilibrium and kinetic modelling of biosorption of Remazol Black B by *Rhizopus arrhizus* in a batch system: effect of temperature, *Process Biochem*., **36,** 431-439 **(2000)**

31. Mansur M., Arias M.E. and Copa Patino J.L., The white-rot fungus *Pleurotus ostreatus* secretes laccase isozymes with different substrate specificities, *Mycologia*, **95(6),** 1013-1020 **(2003)**

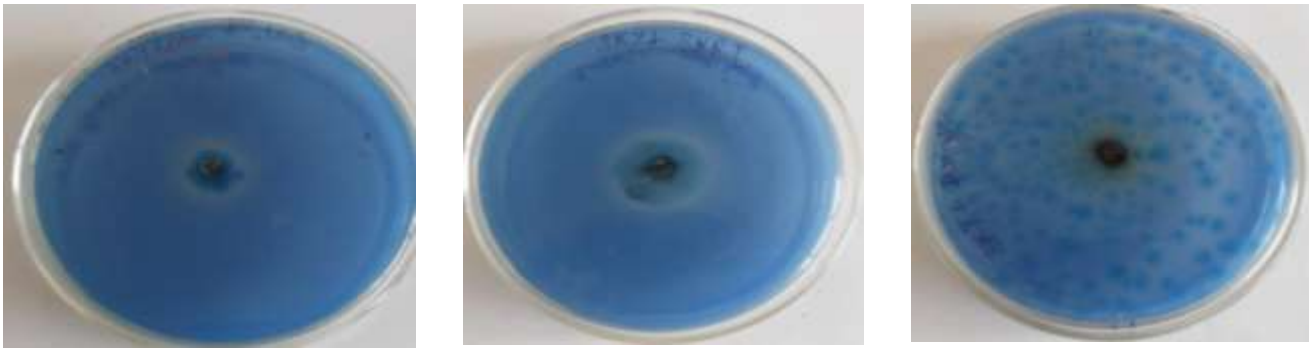
32. Bhatt M., Patel M., Rawal B., Novotny C., Molitoris H.P. and Sasek V., Biological decolorization of synthetic dye RBBR in contaminated soil, *W. J. Microbiol. Biotechnol*., **16,** 195-198 **(2000)**

33. Pearce C.I., Lloyd J.R. and Guthrie J.T., The removal of colour from textile wastewater using whole bacterial cells: a review, *Dyes Pigments*, **58,** 179-196 **(2003)**

34. Hu T.L. and Wu S.C., Assessment of the effect of azo dye Rp2B on the growth of nitrogen fixing cyanobacterium - *Anabena* sp., *Biores. Technol*., **77,** 3-95 **(2001)**

Table – 1
Physiochemical characterization of effluents

S. No.	Parameter	Unit	Effluent
1	Color	-	Dark black
2	Smell	-	Pungent
3	Temperature	^{0}C	38
4	pH	-	9.7
5	BOD	mg/L	492
6	COD	mg/L	1305
7	TDS	mg/L	5867



Aspergillus allhabadii *Aspergillus sulphureus* *Aspergillus niger*

Figure – 2
Fungi showing growth on CDA amended with 200 mg/L dye

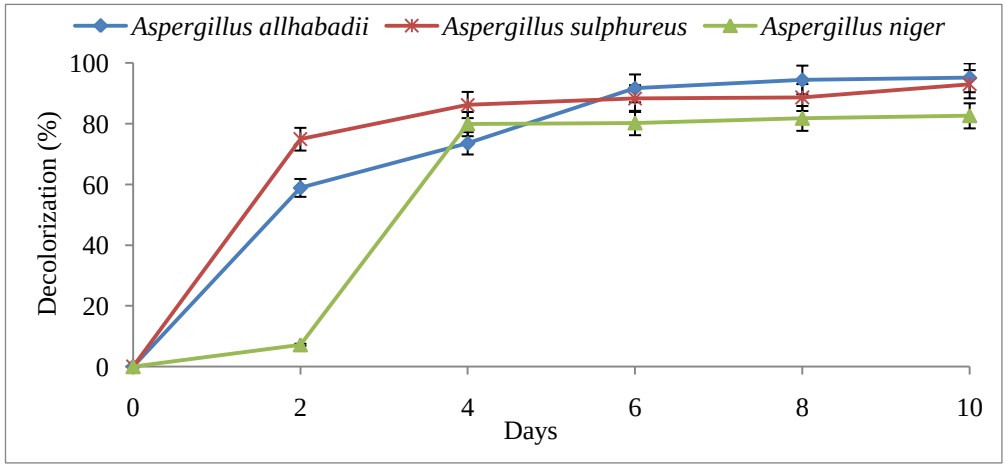


Figure – 3
Decolorization kinetics of *Aspergillus* species in liquid medium

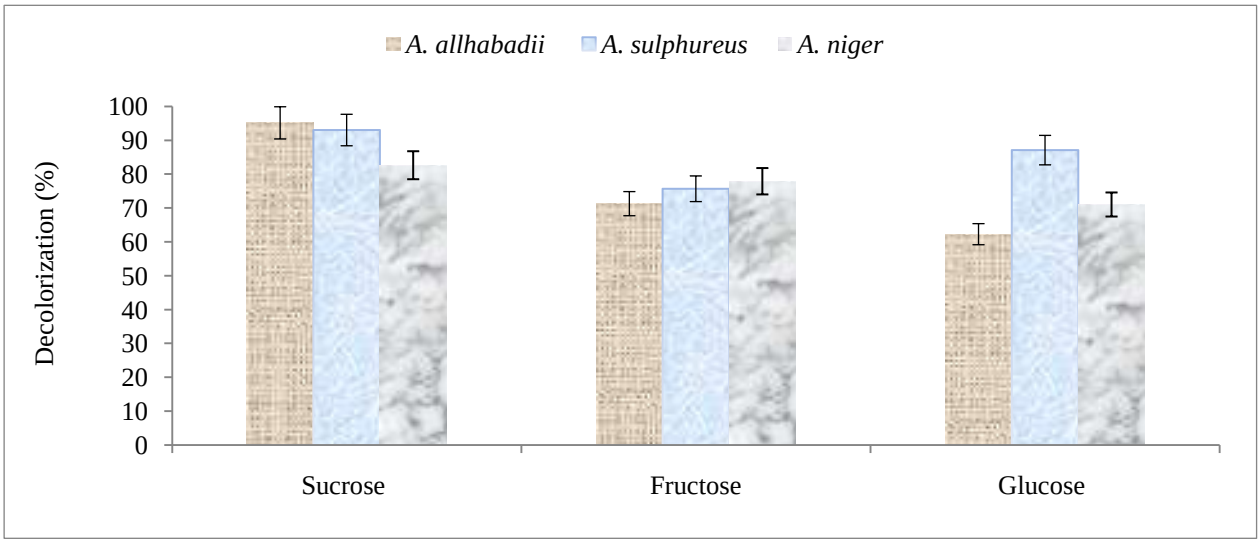


Figure – 4
Effect of carbon source on decolorization of reactive blue MR

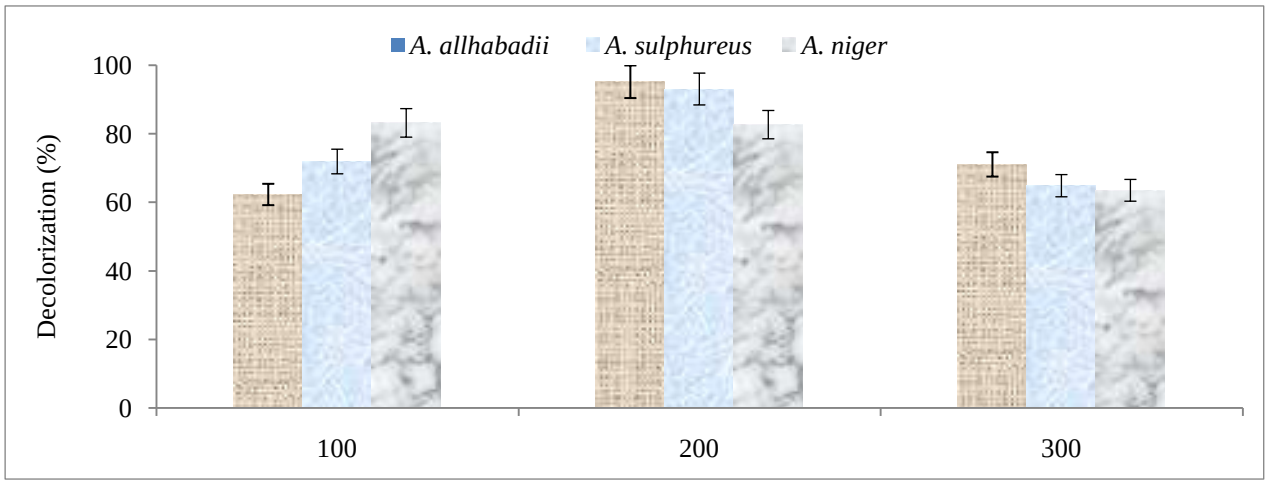


Figure- 5
Effect of dye concentration on decolorization of reactive blue MR