ISCA Journal of Biological Sciences ______________________________ *ISCA J. Biological Sci.*
Vol. **1(1),** 55-61, May **(2012)**

Decolorization of Reactive Black HFGR by *Aspergillus sulphureus*

Salar Raj Kumar[1], Rohilla Suresh Kumar[1*] and Rohilla Jitender Kumar[2]
[1]Department of Biotechnology, Chaudhary Devi Lal University, Sirsa, INDIA
[2]Department of Molecular Biology and Biotechnology, CCS HAU, Hisar, INDIA

Available online at: www.isca.in
(Received 25th APril 2012, revised 27th April 2012, accepted, 27th April 2012)

Abstract

The strain Aspergillus sulphureus was evaluated for its ability to decolorize a textile dye, Reactive Black HFGR. The effect of physiochemical parameters (shaking vs static, pH, dye concentration and different carbon source) on the degradation of textile dye was studied in C-limited Czapek Dox broth. It was found that shaking favored to dye decolorization efficiency of fungal strain showed highest decolorization (93.04±1.86%). Effect of different concentration of Reactive Black HFGR dyes ranging from 50 to 300 mg/L had a significant effect on decolorization (69.94±1.09 - 93.04±1.86%) their maximal limits at 200 mg/L and increase in concentration of dye showed a negative effect on the decolorization percentage of the tested fungal strain. There was significant decolorization by the fungal strain with different pH ranging from 4 to 8. However, optimum pH was found to be pH 5. There was an influence of carbon source on decolorization as the fungus showed considerable variation in decolorization activity in the carbon supplemented medium. The fungal strain showed maximum activity with sucrose (93.04±1.86%) followed by glucose (85.31±0.59%) and fructose (78.71±1.44%). Therefore, Aspergillus sulphureus is an efficient strain for the decolorization of reactive textile dyes, and it might be a practical alternative in the dyeing wastewater treatment.

Keywords: *Aspergillus sulphureus*, decolorization, physiochemical, reactive black HFGR, textile dye.

Introduction

Synthetic dyes are manufactured and consumed annually in large quantities in textile, food processing, paper and pulp, cosmetics and pharmaceutical industries. The textile industries account for two-third of the total dye stuff market. Dyes have been used increasingly in textile and dyeing industries because of their ease and cost effectiveness in synthesis, firmness and variety in color compared to that of natural dyes. The most commonly used dye in the textile industry is reactive black HFGR.

Approximately, 100,000 commercial dyes are manufactured[1] and about 10,000 dyes with an annual production of over $7x10^5$ metric tons are commercially available[2]. About 10-15% of dyes used in textile industries remain unutilized[3, 4]. Dyes are designed in such a way that they are resistant to light, water and oxidizing agents and, therefore, cannot be treated by conventional treatment processes such as an activated sludge[5]. Dye colors are visible in water concentration as low as 1 mg/l, whereas textile processing wastewater, normally contains more than 10-200 mg/l of dye concentration[6] resulting in aesthetic problem, affecting photosynthesis in aquatic plants[7] and have toxic and carcinogenic effect in mammals[8].

A major environmental problem facing the textile dyeing and finishing industry is that the industry produces large volumes of high strength aqueous waste continuously. The discharge of wastewaters was containing recalcitrant residues into rivers and lakes lead to higher biological oxygen demand (BOD) causing a serious threat to native aquatic life[7]. The problem of environmental pollution is increasing day by day due to the release of xenobiotic substances into water, soil and air. These substances include organic compounds (pesticides, dyes, polymers etc.) and heavy metal ions. The damage caused by these pollutants to plants, animals and humans cannot be neglected and hence strategies must be developed to solve the problem of environmental pollution on the priority basis. Removal of dyes from the effluents or their degradation before discharge is a great environmental challenge for the industries[9]. Various physical, chemical and biological processes are usually employed to remove these dyes before discharge into the environment. Physical and chemical methods include adsorption, sedimentation, flocculation, flotation, coagulation, osmosis, neutralization, reduction, oxidation, electrolysis and ion-exchange. However, these methods have some limitations like high cost and disposal of the large amount of sludge or some toxic byproduct produced during these processes[10].

Microbial communities are of primary importance in degradation of dye contaminated soils and water as microorganisms alter to dye chemistry and mobility through reduction, accumulation, mobilization and immobilization. Among microorganisms, bacteria, fungi and algae are most commonly used for various bioremediation processes. Bioremediation is a cost effective and environment friendly promising alternative to replace or supplement present treatment processes. A white rot fungus, *Phanerochaete chrysosporium* has been used extensively for decolorization of dyes in wastewaters and correlates to the ability for the synthesis of lignin degrading exoenzymes such as lignin and manganese peroxidases (MnP)[11] or Laccases[12]. The use of species of the genera *Pleurotus*, *Bjerkanera*, *Tremetes*, *Poyporus*, *Phelinus*, *Iprex Lacteus*, *Fungalia trogii*, *Ganoderma* sp., and *Thelephora* sp., have been also investigated[3, 4, 13, 14].

The aim of the present study is to investigate the potential of an indigenous ascomycete, *Aspergillus sulphureus* for the removal of a dye, Reactive Black HFGR from aqueous solution. Various conditions required for decolorization have been optimized and dye decolorization/degradation has been analyzed and confirmed by UV-VIS-spectrophotometer.

Material and Methods

Dye and Chemicals: All media components and chemicals used in the present study were of analytical grade and purchased from Hi-Media Laboratories (Mumbai, India). The textile dyes, Reactive Black HFGR used for decolorization in the present investigation was a gift from M/s Mayur Paliwal Textiles, Panipat (Haryana). The property wise data of the dye are as follows:
CAS No. 17095-24-8, Chemical Name: Reactive Black 5, Synonyms: Dimira Black B; Diamira Black B; Sumifix Black B; Celmazol Black B; Cavalite Black B; Remazol Black GF; Adizol Black B; Remazol Black B; Primazin Black BN; Begazol Black B; Reactive Black HFGR, Molecular formula: $C_{26}H_{21}N_5Na_4O_{19}S_6$, Formula weight: 991.82, Melting point: >300 °C (lit.), Product categories: Organics, Hazard codes: Xn, λ_{max} = 594nm, Structural formula for Reactive Black HFGR is shown in figure 1.

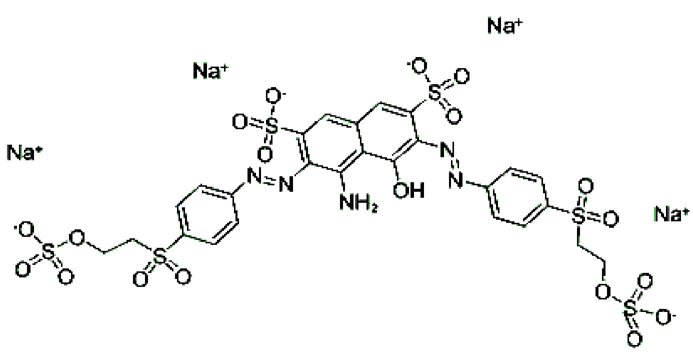

Figure – 1
Chemical structure of textile dye, Reactive Black HFGR

Growth and Decolorization Medium: The Czapek Dox Agar (CDA) medium was used as a growth medium. The following composition of medium was used [K_2HPO_4, 1.0 g L^{-1}; $NaNO_3$, 3.0 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}] for growing the fungus. The decolorization medium is slightly modified C-limited Czapek Dox Broth (5 g/L) amended with 200 mg/L reactive black dyes.

Decolorization study on Agar Plates: Decolorization of dye was studied in petri dishes containing C–limited CDA medium with dye 200 mg/L. All the plates were inoculated at the center with fungal disc (8 mm in diameter) cut from the periphery of the actively growing 5 days old culture grown on plates. The plates were incubated at 25±2°C in BOD incubator. The plates were monitored over a period of 5 days. Abiotic control was also maintained. The radial growth of fungal mycelia, the zone of color change and zone of decolorization on the agar plates were measured after 24 h.

Decolorization Assay: The ability of *A. sulphureus* to decolorize Reactive Black HFGR was carried out in C-limited Czapek Dox broth. Textile dye Reactive Black HFGR (λ_{max} 594nm) was used at 200 mg/L concentration. Agitated cultures of fungal species were grown for 10 days in an incubator shaker at 25±2ºC and 120 rpm. 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} of the dye. Control flask without fungus was also maintained. Percent decolorization was calculated using the formula:

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

Ao and A_t is the absorbance at different time intervals[15]. Above mentioned protocol was followed during the study of the effect of physiochemical parameters on decolorization.

Effect of Physico-Chemical Parameters on Decolorization: Physiochemical parameters such as carbon sources (Glucose, Fructose, Sucrose), static vs shaking conditions, pH (4.0-8.0) and dye concentration (50, 100, 150, 200, 250 mg/L) in the aqueous medium was studied. The Decolorization ability of *Aspergillus sulphureus* at various pH studied and pH 5 was found to be the optimal pH for the decolorization activity. Based on that acidic pH 5 was selected to study the effects of various physico-chemical factors in a decolorization process. The ability of this isolated fungus to decolorize Reactive Black dye (200 mg/l) based on changes in their optical density.

Statistical Analysis: All analysis was conducted in duplicate and results presented here are the mean of duplicate ± standard deviations (SD).

Results and Discussion

Screening of Fungus for Decolorization on solid and liquid medium: *Aspergillus sulphureus* used in this study was isolated from the dye disposal site and identified using microscopic analysis and cultural characteristics. Since the chances of getting microbes having the ability to decolorize the dye effluent is very high. The textile and dyeing industry are one of the industries, which contribute to the soil and water pollution. In the current research work the potential of ascomycete, *Aspergillus sulphureus* for the removal of a dye, Reactive Black

HFGR was studied. Fungal strain was initially screened for their growth and decolorization with Reactive Black HFGR dyes (200 mg/L) on Czapek Dox agar media (CDA). Fungus applied (8mm disc) at the center of the plates and were incubated at 25±2°C for 5 days. The apparent dye decolorization efficiency of native fungal strain was critically visualized and measured after 24 h based on the zone of decolorization around the fungal biomass excluding the size of the inoculum confirm the dye decolorizing activity (Figure- 2). The results are inconsistent with an earlier report of the screening of fungi and bacteria on solid media showing growth and decolorization on solid culture[16]. Further, *Aspergillus sulphureus* were screened for their ability to decolorize reactive black was carried out in liquid media.

Effect of Physiochemical Parameters on Dye Degradation: Fungal treatment of textile dyes and effluents have been found to be influenced by temperature, pH, salts, inhibitory molecules (sulfur compounds, surfactants, heavy metals and bleaching chemicals), carbon and nitrogen sources and other nutrients[17, 18]. The effects of various parameters such as pH, carbon source, dye concentration and agitation were tested in this study.

Effect of Static/Shaking on Decolorization: The Decolorization process was carried out using shaking (120 rpm) and static culture inoculating with fungal disc (8 mm) from fungal colony growing on a CDA plate in Erlenmeyer flasks (250 ml) containing sterilized liquid dye (100 ml) containing medium and incubated in an incubator at 25±2°C for ten days. It was found that the fungus efficient in decolorization in shaking condition (93.04±1.86%) than static (77.33±0.76%). Figure 3 clearly indicating that the shaking condition had a significant impact on decolorization. The extent of per cent decolorization may be increased by treatment under shaking conditions because shaking facilitates the transfer and distribution of nutrients and oxygen between the medium and the microbial cells.

Effect of pH on Decolorization: Effect of pH (4.0-8.0) was investigated, keeping other parameters constant (temp. 25±2°C, rpm 120 and dye conc. 200 mg/L). Figure- 4 showed that the highest color removal was determined at acidic pH 5 (93.04±1.86%) when compared to neutral and basic pH 8. The optimum pH was found 5.0 for maximum removal of dye. The pH has a major effect on the efficiency of dye decolorization, and the optimal pH for color removal is often between 4.5 to 11.5 for most of the dyes and reported sharp changes in decolorization towards both ends of the optimum pH values[16, 19]. The pH significantly influenced the dye biosorption properties of fungi[20, 21, 22]. Higher uptake obtains at lower pH value may be due to the electrostatic attraction between negatively charged dye anions and positively charged cell surface. These results provide an information that acidic pH is required for growth and decolorization.

Effect of Dye Concentration on Decolorization: Generally, the concentration of color compounds found in the effluent or rivers ranged as low as 12 to 16 mg/L. In the present study effect of different concentration of dye (50-300 mg/L) on decolorization was investigated. The Decolorization efficiency of the fungus was found to be highest (93.04±1.86%) with 200 mg/L under shaking condition (Figure- 5). In addition, the growth of fungi was strongly inhibited at dye concentration above 200 mg/L as a higher concentration of dye may be toxic to metabolic activities. Similar finding has been observed that an elevated concentration of dyes is found to be growth limiting[23].

Besides, decolorization of dyes at higher concentration may create an acidic situation, which further facilitate their better removal (enzymatic or by cell wall adsorption) by the fungi[24, 25, 26]. 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 dyes[27].

Effect of carbon source on Decolorization: Three carbon sources such as glucose, fructose and sucrose were used at 5 g/L. The range of activity on decolorization of Reactive Black HFGR with sucrose was the highest (93.04±1.86%) followed by glucose (85.31±0.59%) and fructose (78.71±1.44%) after ten days of incubation in shaking condition (Figure- 6). The primary mechanism of decolorization may be due to dye adsorption/degradation by mycelium of fungi as well as the reduction of dye intensity in solution because of changes caused by them[28, 29].

Growth media enhance the adsorption/degradation capacities of fungi and on the addition of carbon and other nutrient increased decolorization reached up to 92.9% in a short time[30]. Decolorization of dye involved adsorption of the dye compound at the initial stage followed by the decolorization through microbial metabolism[13]. Microorganisms are used for decolorization of dyes and effluents and the fungal biomass proved to be more efficient[24]. Rapid growth of the fungus in C-limited medium with dye indicated that the fungus utilized the dye as the sole source of carbon and produced enzymes to degrade the dyes. No dye decolorization was observed in the control flask without inoculum.

Conclusion

Although decolorization is a challenging process to both the textile industry and the wastewater treatment, the results of this finding suggest a great potential for fungi to be used to remove color from dye wastewaters. The textile dye (Reactive Black HFGR) is degradable under aerobic conditions with a concerted effort of fungus isolated from an effluent disposal site. Physiochemical parameters (pH, carbon source, dye concentration and agitation) had a significant effect on dye decolorization. Further, it can be suggested that the potential of the fungus need to be demonstrated in its application for treatment of dye bearing waste water using appropriate practice, through biotechnological approaches to color removal.

Acknowledgements

The authors are thankful to the Chairperson for providing necessary laboratory facilities to carry out the present research work and M/s Mayur Paliwal Textiles, Panipat (Haryana) for providing the color.

References

1. Zollinger H., Color chemistry syntheses, properties and application of organic dyes and pigments, (1st Edn), VCH Publishers, New York **(1987)**

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. 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)**

4. 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)**

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

6. Hawkes F.R., Hawkes D.L., O'Neill C., Lourenco N.D., Pinheiro H.M. and Delée W., Colour in textile effluents-sources, measurement, discharge consents and simulation: A review, *Journal of Chemical Technology and Biotechnology*, **74,** 1009-1018 **(1999)**

7. McMullan G., Meehan C., Conneely A., Nirby N., Nigam P.R., Banat I.M. and Marchant S.W.F., Mini review: Microbial decolourization and degradation of textile dyes, *Appl. Microbiol. Biotechnol*., **56,** 81-87 **(2001)**

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

9. Baldrian P. and Gabriel J., Lignocellulose degradation by *Pleurotus ostreatus* in the presence of cadmium. *FEMS Microbiol. Lett*., **220,** 235–240 **(2003)**

10. Robinson T., McMullan G. and Marchant R., Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative, *Bioresour. Technol*., **77,** 247-255 **(2001)**

11. Sharma P., Singh L. and Dilbaghi N., Optimization of process variables for decolorization of Disperse Yellow 211 by *Bacillus subtilis* using Box-Behn-ken design, *J. Hazard. Mater*., **164,** 1024-1029 **(2009)**

12. 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 Microbial Technol*., **40,** 1662-1672 **(2007)**

13. Yesilada O., Cing S. and Asma D., Decolorization of the textile dye Astrazon Red FBL by *Funalia trogii* pellets, *Bioresour. Technol*., **81(2),** 155-157 **(2002)**

14. Fu Y.Z. and Viraraghavan T., Removal of a dye from aqueous solution by the fungus *Aspergillus niger*., *Water Qual Res J Can*., **35,** 95-111 **(2000)**

15. 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)**

16. Chen K.C., Wu J.Y., Liou D.J. and Hwang S.C.J., Decolorization of the textile dyes by newly isolated bacterial strains, *J. Biotechnol*., **101,** 57–68 **(2003)**

17. Jacob C.T., Azariah J., Hilda A. and Gopinath S., Decolorization of prociored MX-5B and colored textile effluent using *Phanerochaete chrysosporium*, *J. Environ Biol*., **19,** 259-264 **(1998)**

18. Swammy J. and Ramsay J.A., Effect of glucose and $NH4^{+}$concentrations on sequential dye decoloration by *Tremetes versicolor, Enzyme Microbial Technol*., **25,** 278-284 **(1999)**

19. Chen K.C., Huang W.T., Wu J.Y. and Houng J.Y., Microbial decolorization of azo dyes by *Proteus mirabilis*, *Journal of Industrial Microbiology and Biotechnology*, **23,** 686–690 **(1999)**

20. Hayase N., Kouno K. and Ushio K., Isolation and characterization of *Aeromonas* sp. B-5 capable of decolorizing various dyes, *J. Biosci. Bioeng*., **90,** 570-573 **(2000)**

21. Ramalho P.A., Cardoso M.H., Cavaco-Paulo A. and Ramalho M.T., Characterization of azo reduction activity in a novel ascomycete yeast strain. *Appl. Environ. Microbiol*., **70,** 2279-2288 **(2004)**

22. Ali N., Ikramullah Lutfullah 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)**

23. 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)**

24. 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)**

25. Baldrian P., Purification and characterization of laccase from the white-rot fungus *Daedalea quercina* and decolorization of synthetic dyes by the enzyme, *Appl. Microbiol. Biotechnol*., **63(5),** 560-563 **(2004)**

26. 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)**

27. 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)**

28. Balan D.S.C. and Monterio R.T.R., Decolorization of textile dye indigo dye by lignolytic fungi, *J. Biotechnol*., **89,** 141-145 **(2001)**

29. Chen B., Understanding decolorization characteristic of reactive azo dyes by *Pseudomonas luteola*: toxicity and kinetics, *Process Biochemistry*, **38,** 437-446 **(2002)**

30. Wataru S., Miyashita T., Yokoyama J. and Arail M., Isolation of Azo dye degrading micro organisms and their application to white discharge printing on fabric, *J. Biosci. Bioeng*., **5(88),** 577-581 **(1999)**

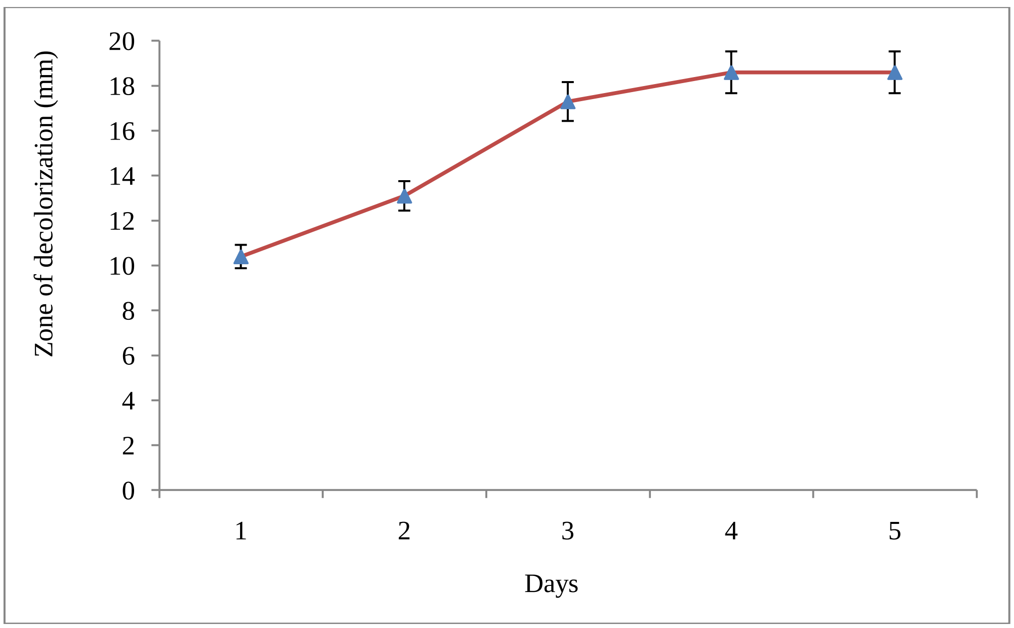

Figure - 2
Solid plate decolorization of Reactive Black HFGR
(5days, 25±2°C, pH 5.0, Dye 200 mg/L, Sucrose 5 g/L,
error bars represents the standard deviation (±) mean of duplicate analysis)

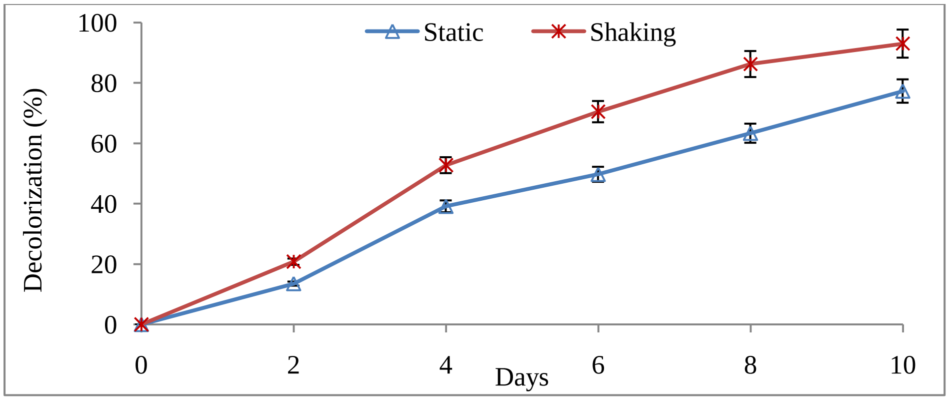

Figure - 3
Effect of shaking/static condition on decolorization of Reactive Black HFGR
(10 days, 25±2°C, pH 5.0, Dye 200 mg/L, Sucrose 5 g/L,
error bars represents the standard deviation (±) mean of duplicate analysis)

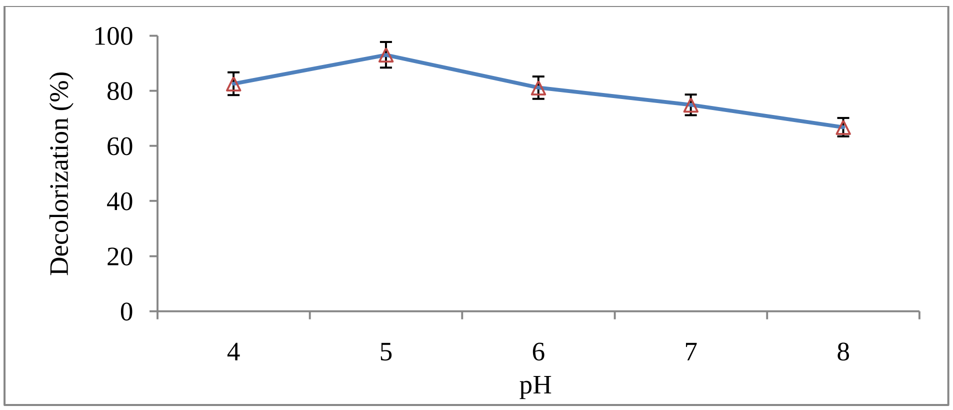

Figure - 4
Effect of pH on decolorization of Reactive Black HFGR
(10 days, 25±2°C, 120 rpm, error bars represents the standard deviation (±) mean of duplicate analysis)

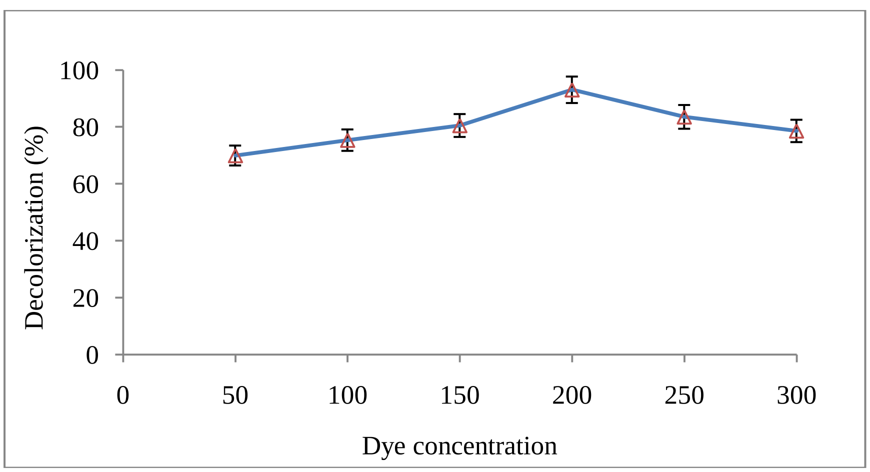

Figure – 5
Effect of dye concentration on decolorization of Reactive Black HFGR
(10 days, 25±2°C, pH 5.0, 120 rpm, Sucrose 5 g/L, error bars represents the standard deviation (±) mean of duplicate analysis)

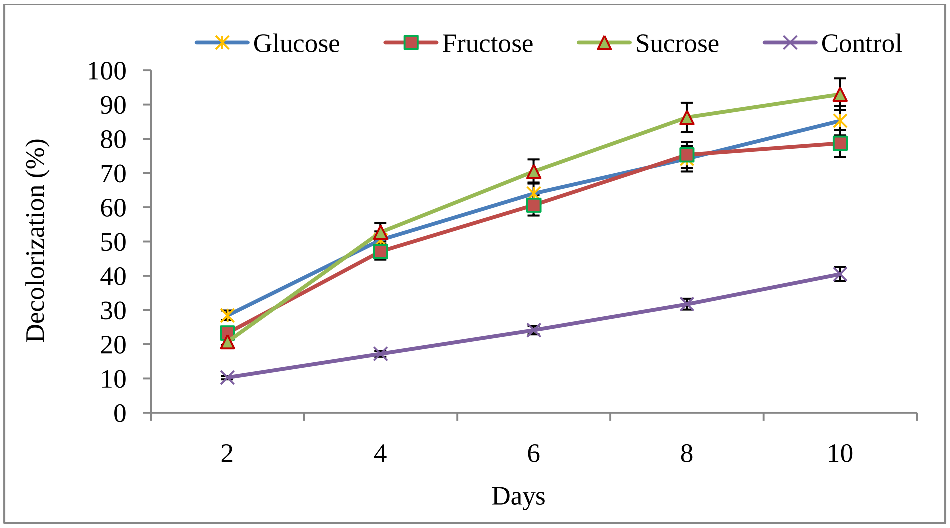

Figure - 6
Effect of carbon source on decolorization of Reactive Black HFGR
(10 days, 25±2°C, pH 5.0, 120 rpm, Dye 200 mg/L, error bars represents the standard deviation (±) mean of duplicate analysis)