International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **1(5),** 46-52, Sept. **(2012)** *I. Res. J. Biological Sci.*

Decolorization of Azo dye Red 3BN by Bacteria

Praveen Kumar G.N. and Bhat Sumangala K.
Department of Biotechnology, Acharya Institute of Technology, Soladevanahalli, Bangalore-560 090, INDIA

Available online at: www.isca.in
Received 7th August 2012, revised 14th August 2012, accepted 17th August 2012

Abstract

Decolorization of azo dye Red 3BN by two bacterial species Bacillus cereus and B. megaterium has been analyzed using mineral effluent, consisting of known concentration of the dye in ZZ medium. Physico chemical parameters like carbon source, nitrogen source, temperature, pH and inoculum volume are optimized for the decolorization process by changing one parameter at a time. Optimal condition for B. cereus was found to be 1% sucrose 0.25% peptone, pH 7, 37°C and 8% inoculum and that for B.megaterium was found to be glucose 1% , 0.25% yeast extract, pH 6, 37°C and 10% inoculum. Extent of decolorization recorded by B. cereus under ideal conditions was 93.64% and that by B.megaterium was 96.88%. The study has confirmed the potential of B. cereus and B. megaterium in the decolorization of Azo dye Red 3BN and opened scope for future analysis of their performance in the treatment of textile effluent.

Keywords: Azo dye, Red 3BN, Bacillus cereus B. megaterium, decolorization.

Introduction

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life[1,2]**.** The textile industry is one of them which extensively use synthetic chemicals as dyes. Wastewaters from textile industries pose a threat to the environment, as large amount of chemically different dyes are used. A significant proportion of these dyes enter the environment via wastewater[1]. Approximately 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced annually, worldwide[3]. Pollution due to textile industry effluent has increased during recent years. Moreover, it is very difficult to treat textile industry effluents because of their high BOD, COD, heat, color, pH and the presence of metal ions[4]. The textile finishing generates a large amount of waste water containing dyes and represents one of the largest causes of water pollution[5], as 10-15% of dyes are lost in the effluent during the dyeing process[6]. The traditional textile finishing industry consumes about 100 liters of water to process about 1 Kg of textile material. The new closed-loop technologies such as the reuse of microbial or enzymatical treatment of dyeing effluents could help reducing this enormous water pollution[7].

Azo dyes have been used increasingly in industries because of their ease and cost effectiveness in synthesis compared to natural dyes. However, most azo dyes are toxic, carcinogenic and mutagenic[8]. Azo bonds present in these compounds are resistant to breakdown, with the potential for the persistence and accumulation in the environment[9]. However, they can be degraded by bacteria under aerobic and anaerobic conditions[10]. Several physico-chemical techniques have been proposed for treatment of colored textile effluents. These include adsorption on different materials, oxidation and precipitation by Fenton's reagent, bleaching with chloride or ozone photo degradation or membrane filtration[11]. All these physical or chemical methods are very expensive and result in the production of large amounts of sludge, which creates the secondary level of land pollution. Therefore, economic and safe removal of the polluting dyes is still an important issue. Bioremediation through microorganisms has been identified as a cost effective and environment friendly alternative for disposal of textile effluent[12,13].

In recent years a number of studies have focused on some microorganisms capable of degrading and absorbing dyes from wastewater. A wide variety of microorganisms are reported to be capable of decolonization of dyes[12,14-26]. The current study has evaluated the potential of two bacterial strains isolated from textile effluent for their decolorization efficiency of the textile dye, Red 3BN under in vitro conditions and optimization of the factors influencing the process.

Material and Methods

Textile dye, Red 3BN and effluent sample were collected from a dying industry located at Peenya, Bangalore (Karnataka). The sample was collected from the effluent disposal site of the industry. All microbiological media and medium ingredients were purchased from HiMedia Laboratories (Mumbai, MH, India).

The dye decolorizing bacteria were isolated from the effluent by serial dilution and plating appropriate dilutions on modified Zhou and Zimmermann (ZZ) agar medium containing (yeast extract-5, glucose-5, $(NH_4)_2SO_4$-0.5, KH_2PO_4-2.66, Na_2HPO_4-4.32, agar-20 [all in gL^{-1}] and Dye (Red 3BN) - 100 mgL^{-1}) and pH 7.0. All the isolated colonies were studied by inoculating them in effluent basal medium containing (yeast extract-5, $(NH_4)_2SO_4$-0.5, KH_2PO_4-2.66, Na_2HPO_4- 4.32, glucose-5 [all in gL^{-1}] and Dye (Red 3BN) - 100 mgL^{-1}) and pH

7.0. The inoculated medium was incubated at 30°C for six days under shaking culture conditions. Dye degrading isolates were identified on the basis of morphological and biochemical tests according to Bergey's Manual of Systematic Bacteriology [27]. The isolates showing more decolorization of the Red 3BN were selected for further studies.

The dye decolorizing activity of bacteria was evaluated using a modified method of Prasad and Rao[28]. Decolorization activity was performed in 100 ml of ZZ medium containing 0.02g of Red 3BN [called as mineral effluent hereafter] and 10% (v/v) inoculum of each isolate separately. Mineral effluent without inoculums served as control. Inoculated medium and control were incubated at 30°C for six days under shake culture conditions. About 2 ml of the samples were withdrawn aseptically from experimental and control media and centrifuged at 8,000 RPM for 15 minutes. The clear supernatant was used for measuring absorption at 600 nm using UV-Vis spectrophotometer (Shimadzu, Japan). The percent decolorization of the mineral effluent was determined by the following formula:

$D= [(A_0-A_1) /A_0] \times 100$

where, D, % of decolorization; A_0, initial absorbance; A_1, final absorbance

Decolorization of Red 3BN by the bacterial isolates was optimized with respect to the effect of carbon source (glucose, sucrose, meso-inositol), nitrogen source (Beef extract, peptone, yeast extract), temperature, pH and inoculum volume. Decolourization under different culture conditions was done by changing the factors one at a time, The basic conditions of culture being 30°C, pH 7.0 under shaking conditions (120 RPM), 10%, (v/v) inoculum in mineral effluent. Duration of the experiment was for six days. In all the experiments mineral effluent without culture inoculum was served as control. After six days the samples were withdrawn and analyzed for percent decolorization of the dye.

The time course of decolorization was carried out under optimum conditions obtained from above studies and the optimum conditions are: for *Bacillus cereus* (1% sucrose, 0.25% peptone, pH 7, 37°C and 8% inoculum), for *Bacillus megaterium* (1% glucose, 0.25% yeast extract, pH 6, 37°C and 10% inoculum). Flasks were incubated up to 144h at their respective temperature and samples were removed after every 24 h and analyzed for decolorization activity as described above.

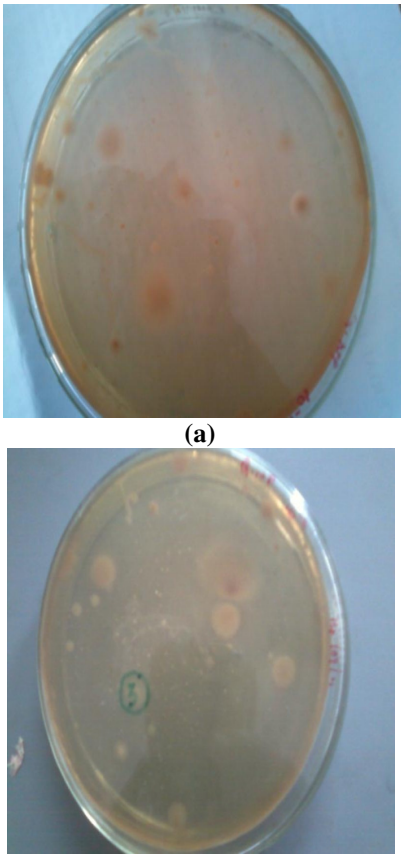
Results and Discussion

The results of the screening and identification of bacteria isolated from the effluent samples are presented in table-1. Morphological and biochemical tests have confirmed the identify of the bacteria as *Bacillus cereus* and *Bacillus megaterium* (figure-1)

Table-1
Identification of dye decolorizing bacteria from effluent

Test	P1	P2
Gram's nature	+	+
Shape	Rod	Rod
Motility	Motile	Motile
Mannitol fermentation	No acid	Acid
Indole production	_	_
Methyl red	_	+
Voges-Prausker	+	_
Citrate utilization	+	+
Catalase	+	+
Oxidase	_	_
Identity of the isolate	*Bacillus cereus*	*Bacillus megaterium*

+ = positive , - = negative



(a)

(b)

Figure-1
Petri plates containing colonies of Bacteria decolorizing Red 3BN (A)- *B.cereus* and (B)- *B. megaterium*

Figure-2 illustrates the effect of different carbon sources on decolorization of Red 3BN by *B.cereus* and *B.megaterium.* Percentage decolorization of the dye recorded for *B.cereus* when glucose was used as carbon source was 16.05% and that for *B.megaterium* was 13.64%. The percentage of decolorization of Red 3BN with sucrose as carbon source was found to be 68%

and 2.36%, respectively for *B. cereus and B. megaterium.* When meso-inositol was used as carbon source 13.64% and 13.83% decolorization of the dye was recorded in cultures of *B. cereus and B. megaterium* respectively. From the above data it is observed that *B. cereus* is more efficient in decolorizing Red 3BN than *B. megaterium* and sucrose is the ideal carbon source for its activity under in vitro condition.

Figure-3 illustrates the effect of different nitrogen sources on decolorization of Red 3BN by *B. cereus and B. megaterium.* Percentage decolorization of the dye recorded for *B. cereus* when beef extract was used as nitrogen source was 7.69% and that for *B. megaterium* was 11,54%. The percentage of decolorization of Red 3BN with peptone as nitrogen source was found to be 66.67% and 57,41%, respectively for *B. cereus* and *B. megaterium.* When the yeast extract was used as nitrogen source 62.5% decolorization of the dye was recorded with both the bacterial species. From the above data it is observed that *B. cereus* is more efficient in decolorizing Red-3BN than *B. megaterium* and peptone is the ideal nitrogen source for its activity under invitro condition.

Figure-4 illustrates the effect of different temperature on decolorization of Red 3BN by *B. cereus and B. megaterium.* Percentage decolorization of the dye recorded for *B. cereus* at 27^{o}C was 50.12% and that for *B. megaterium* was 55.25%. The percentage of decolorization of Red 3BN at 37^{o}C was found to be 60% and 65.12%, respectively for *B. cereus and B. megaterium.* From the above data it is observed that *B. megaterium* is more efficient in decolorizing Red 3BN than *B. cereus* and 37^{o}C is the ideal temperature for its activity under in vitro condition.

Figure-5 illustrates the effect of different pH on decolorization of Red 3BN by *B. cereus and B. megaterium.* Percentage decolorization of the dye recorded for both *B. cereus* and *B. megaterium* at pH 5 was 20.69%. Decolorization of Red 3BN at pH 6 was found to be 31.82% and 68.18%, **respectively** for *B. cereus and B. megaterium.* The percentage of decolorization of Red 3BN at pH 7 was found to be 42.59% and 51.85%, respectively for *B. cereus and B. megaterium.* When the cultures were maintained at pH 8, 12% and 30% decolorization of the dye was recorded in cultures of *B. cereus and B. megaterium* respectively. From the above data it can be inferred that *B. megaterium* is more efficient in decolorizing Red 3BN than *B. cereus* and pH 6 is the ideal for its activity under in vitro condition.

Figure-6 illustrates the effect of different volume of inoculum on decolorization of Red 3BN by *B. cereus* and *B. megaterium.* Percentage decolorization of the dye recorded for *B. cereus* for 2% inoculum was 65.28% and that for *B. megaterium* was 63.45%. The percentage decolorization of Red 3BN with 4% inoculum was found to be 70.12% and 66.65%, respectively for *B. cereus and B. megaterium.* The percentage decolorization of Red 3BN with 6% inoculum was found to be 66.42% and 62.45% respectively for *B. cereus and B. megaterium.* The percentage decolorization of the dye recorded for *B. cereus* with 8% inoculum was 75.32% and that for *B. megaterium* was 68.65%. When 10% inoculums was used 69.43% and 70.85% decolorization of the dye was recorded in cultures of *B. cereus and B. megaterium* respectively. From the above data it is observed that *B. cereus* is more efficient in decolorizing Red 3BN than *B. megaterium* and 8% inoculum is the ideal volume for its activity under in vitro condition.

Figure-7 illustrates the Time course of dye decolorization of Red 3BN under optimum conditions by *B. cereus and B. megaterium.* Percentage decolorization of the dye recorded for *B. cereus* was 93.64% at 1% sucrose 0.25% peptone, pH 7, 37^{o}C and 8% inoculum and that for *B. megaterium* was 96.88% at glucose 1% , 0.25% yeast extract, pH 6, 37^{o}C and 10% inoculum.

The outcomes of this experiment indicated that *B. megaterium* performed the decolorization process to a better extent than *B. cereus* under a combination of the ideal levels of all factors influencing the process. However, both the species of bacteria can be inferred as good agents for the degradation of Red 3BN.

Azo dyes represent one of the recalcitrant chemicals not being degraded by conventional effluent treatment processes[29]. Bacteria have been recognized as an important and efficient agent for the degradation and decolorization of textile dyes[10-12]. *B. cereus* has been reported to decolorize different azo dyes from textile effluent. Modi *et al.*[30] have reported maltose and peptone as the ideal carbon and nitrogen sources respectively for efficient decolorization of Reactive Red 195 by *B. cereus,* recording 97% reduction in color of the dye in the effluent. In another study Ola *et. al.*[31], have reported decolorization of Cibacron red P4B and Cibacron black PSG to the levels 81% and 75% respectively by this species. Further the study [31] has reported requirement of different carbon and nitrogen sources for maximum decolorization of the two dyes. The current study has revealed 93.64% decolorization of Red 3BN with sucrose and peptone as ideal carbon and nitrogen sources respectively. From the above outcomes of different studies, it can be inferred that the metabolic flux of *B. cereus* alters with the type of carbon and nitrogen sources available in the surrounding environment and efficiency of decolorization of the azo dyes is greatly dependent on these factors.

Degradation and decolorization of the wide range of azo dyes has been reported by different researchers [32-35] who confirmed the role of the bacterial enzyme azoreductase in cleavage of the dye leading to its decolorization. In another study Tripathi and Srivastava[29] have confirmed 94.4% decolorization of another azo dye, orange G by *B. megaterium* and predicted decolorization at ideal conditions using statistical tools, central composite design (CCD). The current study has demonstrated the decolorization of Red 3BN by this species and the level of decolorization was found to be 96.88% at optimum conditions of key parameters analyzed. Therefore, it can be concluded that *B. megaterium* is a highly potential bacterial species capable of degrading and decolorizing wide range of azo dyes.

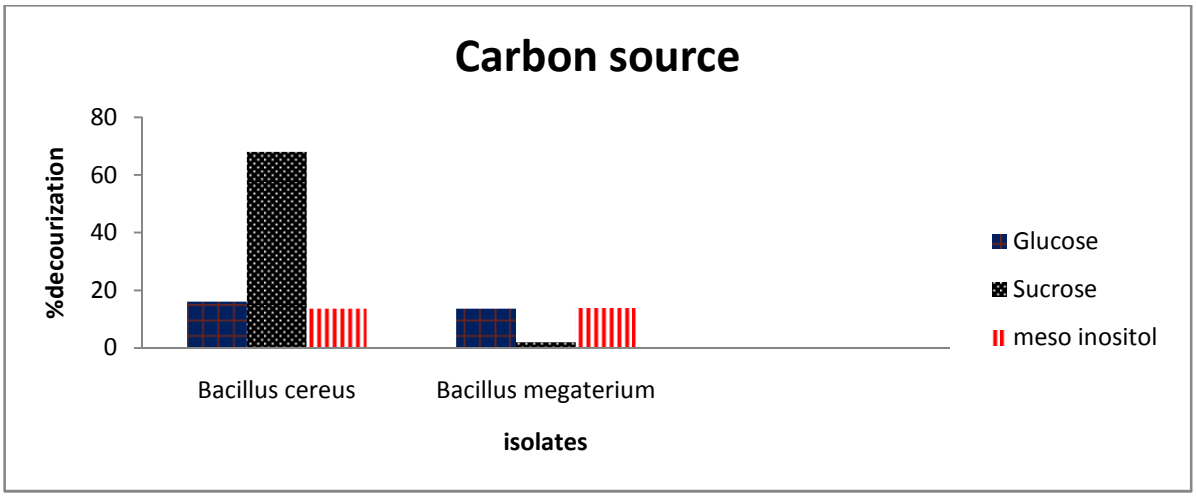


Figure-2
Effect of carbon sources on decolorization of Red 3BN by bacterial isolates (pH 7.0, 30°C , 120RPM, 144h)

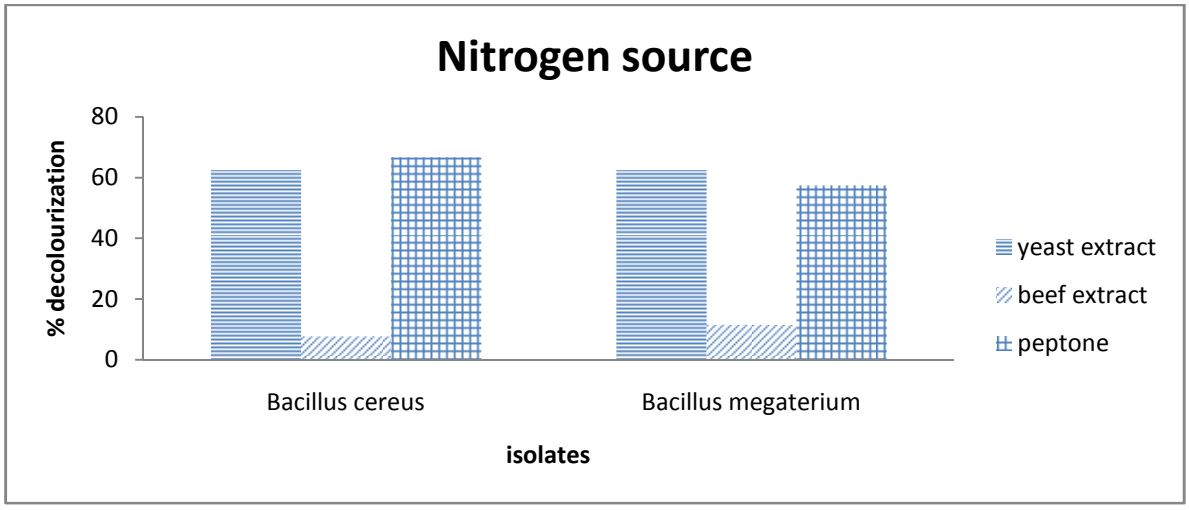


Figure-3
Effect of nitrogen sources on decolorization of Red 3BN by bacterial isolates (pH 7.0, 30°C, 120RPM, 144h)

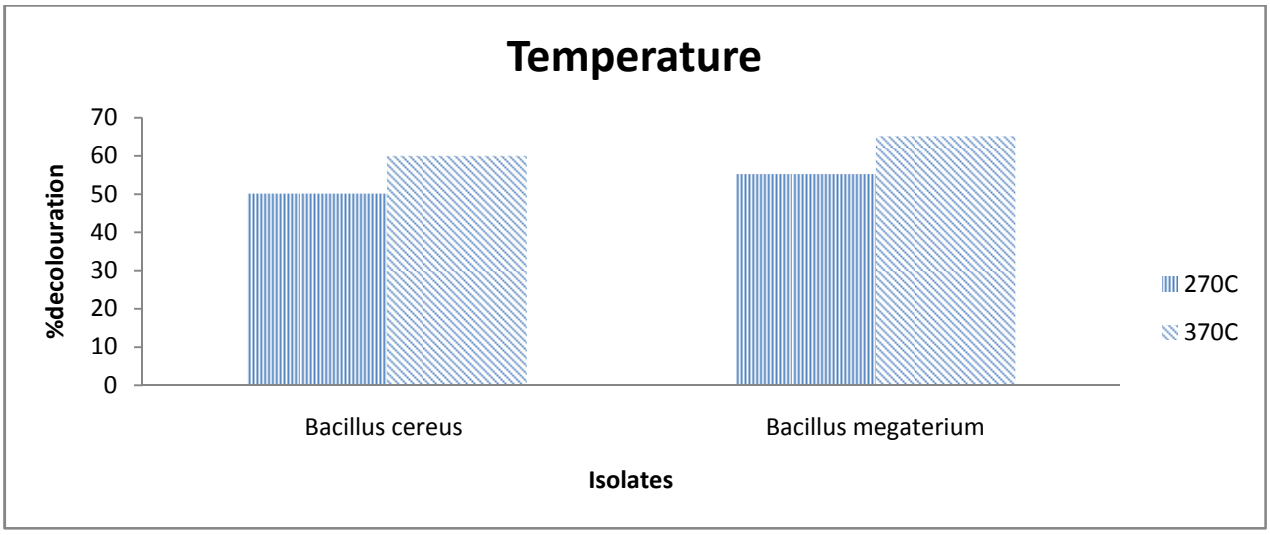


Figure-4
Effect of temperature on decolorization of Red 3BN by bacterial isolates (pH 7.0, 120RPM, 144h)

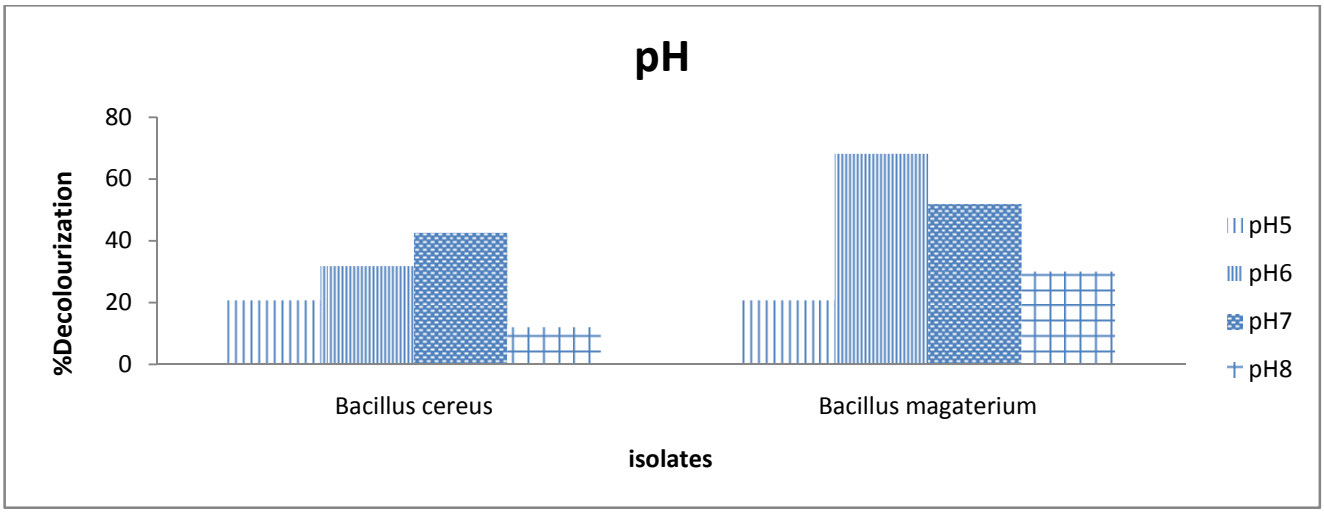


Figure-5
Effect of pH on decolorization of Red 3BN by bacterial isolates (30°C, 120RPM, 144h)

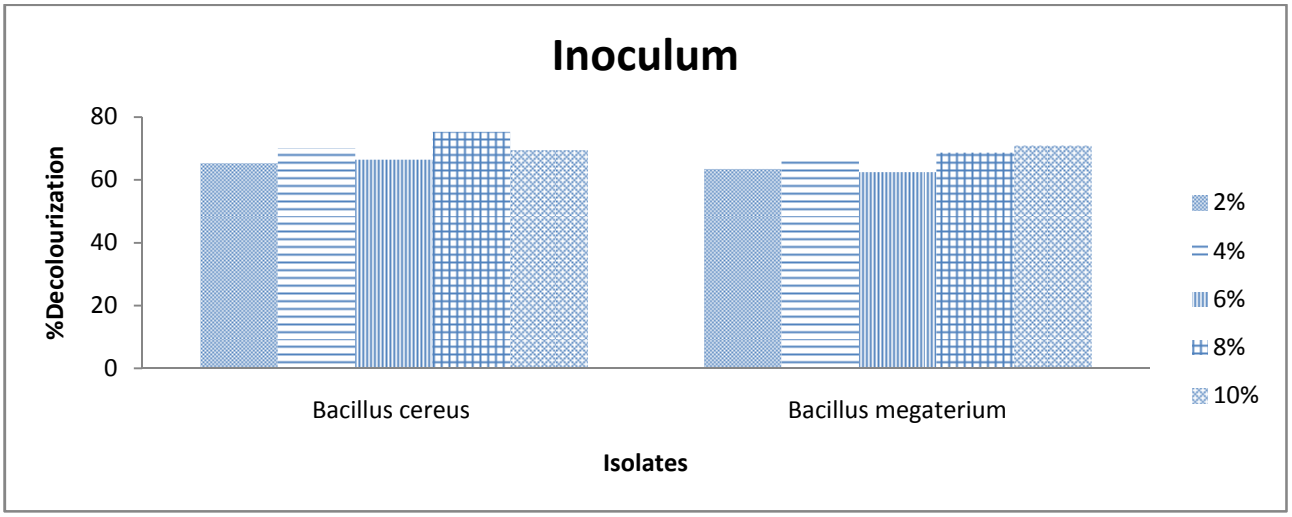


Figure-6
Effect of inoculum volume on decolourization of Red 3BN by bacterial isolates (pH 7.0, 30°C, 120RPM, 144h)

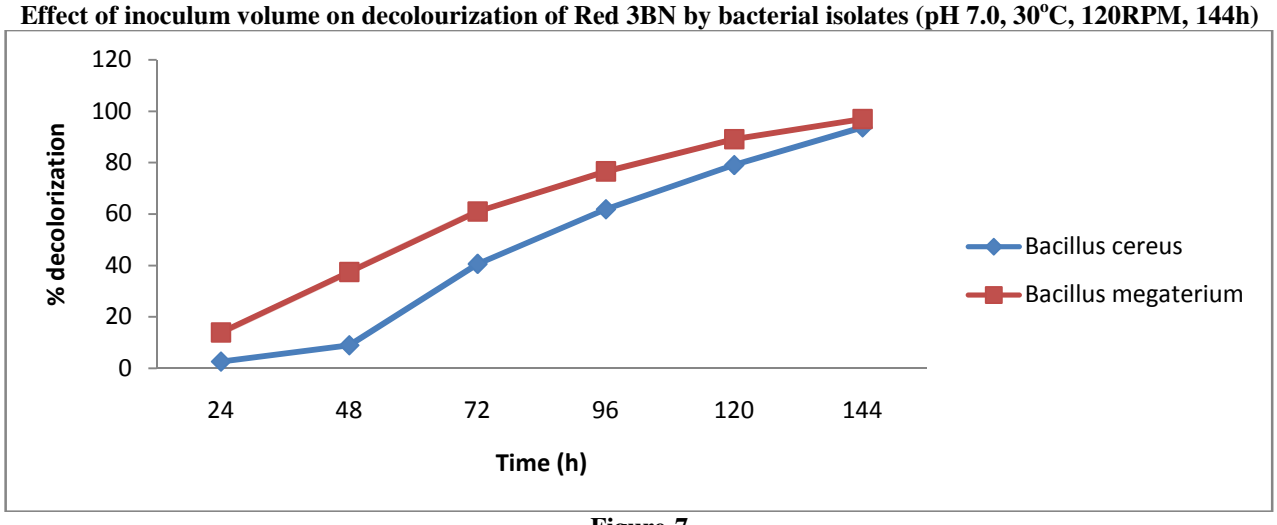


Figure-7
Time course of decolorization of Red 3BN dye by bacterial isolates under optimum condition

Conclusion

Current investigation has confirmed the decolorization of Azo dye Red 3BN by the bacteria *B. cereus* and *B. megaterium* under *in vitro* conditions. Extent of decolorization recorded by *B. cereus* under ideal conditions was 93.64% and that by *B.megaterium* was 96.88%. Thus the study has confirmed the potential of *B. cereus* and *B. megaterium* in the decolorization of the dye indicating their possible application for treatment of textile effluents.

Acknowledgement

The authors gratefully acknowledge the HOD, Biotechnology, Acharya Institute of Technology, Bangalore for providing the facilities for the research work and the Management of AIT for their support and encouragement.

References

1. Moorthi P.S., Selvam S.P., Sasikalaveni A., Murugesan K. and Kalaichelvan P.T., Decolorization of textile dyes and their effluents using white rot fungi, *African J Biotech,* **6(4),** 424-429 **(2007)**

2. Baljeet Singh Saharan and Poonam Ranga, Optimization of cultural conditions for decolourization of textile azo dyes by *Bacillus subtilis* spr42 under submerged fermentation, *Advanced Biotechnology and Research*, **2(1),** 148-153 **(2011)**

3. Rafi F., Fraeankalin W. and Cerniglia C.E., Optimization of cultural condition for decolorization of textile effluent, *Appl Environ Microbiol*, **56**, 2146 **(1990)**

4. Anjali P., Poonam S. and Leela I., Bacterial decolorization and degradation of azo dyes, *Int Biodet Biodegr*, **59**, 73–84 **(2007)**

5. Bhatti H.N., Akram N. and Asgher M., Optimization of culture conditions for enhanced decolorization of Cibacron Red FN-2BL by Schizophyllum commune IBL-6, *Appl Biochem Biotecnol,* **149,** 255-264 **(2008)**

6. Zollinger H., Colour Chemistry Synthesis Properties and Application of Organic Dyes and Pigments, *VCH New York*, 92–102 **(1991)**

7. Abadulla E., Tzanov T., Costa S., Robra K., Cavaco A. and Gubitz G., Decolorization and detoxification of textiles dyes with Laccase from from trametes hirsuta, *Appl Environ Microbial*, **66(80),** 3357 – 62 **(2000)**

8. Pinherio H.M., Touraud E. and Tomas O., Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewater, *Dyes and Pigments*, **61(2),** 121-139 **(2004)**

9. Talarposhti A.M., Donnelly T. and Anderson G., Color removal from a simulated dye wastewater using a two phase anaerobic packed bed reactor, *Water Res,* **35(2),** 425–432 **(2001)**

10. Wong P. and Yuen P., Decolorization and biodegradation of Methyl red by Klebsiella pneumoniae RS-13, *Water Res,* **30(7),** 1736-1744 **(1996)**

11. Robinson T., McMullan G., Marchant R. and Nigam P., Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative (review), *Biores Technol,* **77(3),** 247-255 **(2001)**

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

13. Ponraj M., Gokila K. and Vasudeo Zambare, Bacterial decolorization of textile dye- Orange 3R, *International journal of advanced biotechnology and research ISSN 0976-2612*, **2(1),** 168-177 **(2011)**

14. Chang J.S. and Kuo T.S., Kinetics of bacterial decolorization of azo dye with *Escherichia coli* NO_3, *Bioresource Technology,* **75**, 107–111 **(2000)**

15. Chang J.S., Chou C. and Chen S.Y., Decolorization of azo dyes with immobilized *Pseudomonas luteola, Process Biochemistry,* **36,** 757–763 **(2001)**

16. Fu Y. and Viraraghavan T., Dye biosorption sites in Aspergillus niger*, Bioresource Technology,* **82,**139–145 **(2002)**

17. Saikia N. and Gopal M., Biodegradation of β-cyfluthrin by fungi, *Journal of Agriculture and Food Chemistry,* **52,** 1220–1223 **(2004)**

18. Fournier D., Halasz A., Thiboutot S., Ampleman G., Dominic M. and Hawari J. Biodegradation of octahydro- 1, 3, 5, 7- tetranitro-1, 3, 5, 7-tetrazocine (HMX) by Phnerochaete chrysosporium, New insight into the degradation pathway, *Environmental Science and Technology,* **38,** 4130–4133 **(2004)**

19. Aksu Z.. and Donmez G. , A comparative study on the biosorption characteristics of some yeasts for Remozal Blue reactive dye, *Chemosphere,* **50,** 1075–1083 **(2003)**

20. Gupta V.K., Rastogi A., Saini V.K. and Jain N., Biosorption of copper (II) from aqueous solutions by *Spirogyra* species, *Journal of Colloid and Interface Science,* **296,** 59–63 **(2006)**

21. Acuner E. and Dilek F.B., Treatment of tectilon yellow 2G by *Chlorella vulgaris, Process Biochemistry,* **39**, 623–631 **(2004)**

22. De-Bashan L.E., Moreno M., Hernandez J.P. and Bashan Y., Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae *Chlorella vulgaris* coimmobilized in alginate beads with the microalgae growth- promoting bacterium *Azospirillum brasilense, Water Research,* **36,** 2941–2948 **(2002)**

23. Valderama L.T., Del Campo C.M., Rodriguez C.M., De-Bashan E.L. and Bashan Y., Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga *Chlorella vulgaris* and the macrophyte *Lemna minuscule, Water Research,* **36,** 4185–4192 **(2002)**

24. Yan H. and Pan G., Increase in biodegradation of dimethyl phthalate by *Closterium lunula* using inorganic carbon, *Chemosphere,* **55,**1281–1285 **(2004)**

25. Gupta V.K., Mittal A., Krishnan L. and Gajbe V., Adsorption kinetics and column operations for the removal and recovery of malachite green from wastewater using ash, *Separation and Purification Technology,* **40,** 87– 96 **(2004)**

26. Kumar K.V., Sivanesan S. and Ramamurthi V., Adsorption of malachite green onto *Pithophora* sp., a fresh water algae: equilibrium and kinetic modeling, *Process Biochemistry,* **40,** 2865–2872 **(2005)**

27. Sneath P.H.A., Mair N.S., Sharpe M.E. and Holf J.G., Bergey's Manual of Systematic Bacteriology, *Williams and Wilkins, Baltimore, U. S. A.,* **1**, **(1984)**

28. Arun Prasad A.S. and Bhaskara Rao K.V., Physico chemical characterization of textile effluent and screening for dye decolorizing bacteria, *Global Journal of Biotechnology and Biochemistry* **5**, 80-86 **(2010)**

29. Tripathi A. and Srivastava S.K., Biodegradation of orange G by a novel isolated bacterial strain *Bacillus megaterium* ITBHU01 using response surface methodology, *African Journal of Biotechnology*, **11(7),** 1768-1781 **(2012)**

30. Modi H.A., Rajput G. and Ambasana C., Decolorization of water soluble azo dye by bacterial cultures isolated from the dye house effluent, *Bioresource Technology*, **101(16),** 6580-6583 **(2010)**

31. Ola I.O., Akintokun A.K., Akpan I., Omomowo I.O. and Areo V.O., Aerobic decolourization of two reactive azo dyes under varying carbon and nitrogen source by *Bacillus cereus, African Journal of Biotechnology*, **9(5),** 672-677 **(2010)**

32. Khan J.A., Biodegradation of Azo dye by moderately halotolerant *Bacillus megaterium* and study of enzyme azoreductase involved in degradation, *Advanced Biotechnology*, **10(7),** 21-27 **(2011)**

33. Namdhari B.S., Rohilla S.K., Salar R.K., Gahlawat S.K., Bansal P. and Saran A.K., Decolorization of Reactive Blue MR, using Aspergillus species isolated from Textile Waste Water, *ISCA Journal of Biological Sciences*, **1(1),** 24-29 **(2012)**

34. Kumar Praveen G.N. and Sumangala K. Bhat., Fungal Degradation of Azo dye- Red 3BN and Optimization of Physico-Chemical Parameters, *ISCA Journal of Biological Sciences,* **1(2)**, 17-24 **(2012)**

35. Raj Kumar S., Suresh Kumar R. and Jitender Kumar R., Decolorization of Reactive Black HFGR by Aspergillus sulphureus, *ISCA J. Biological Sci.,* **1(1),** 55-61 (2012)