International Journal of Biotechnology and Biochemistry
ISSN 0973-2691 Volume 13, Number 2 (2017) pp. 147-153

http://www.ripublication.com

Bioremediation of textile azo dyes by newly isolated *Bacillus* sp. from dye contaminated soil

Sneha S. Jaiswal* and A. V. Gomashe

P.G. Department of Microbiology, Shivaji Science College, Congress Nagar, Nagpur, Pin Code. 440012, Maharashtra, India.

**Correspondence author*

Abstract

During the last years several bacterial strains have been isolated that decolorize azo dyes aerobically. In the present work *Bacillus* sp. which possessed the ability to decolorize textile azo dyes (Acid red 2 and Acid orange 7) aerobically isolated from the dye contaminated soil of the local dying houses in Nagpur (India). Decolorization was monitored spectrophotometrically and physic-chemical parameters such as pH, time and temperature were optimized. *Bacillus* sp. decolorized 90% of Acid red 2 (100mg/L) at pH 6 at 37^0C within 72 hours and 99% Acid orange 7 (100mg/L and pH 6-8) at 30^0C within 48 hours under shaking condition. The decolorization potential of this bacterium can be utilized for the dye removal from wastewater aerobically.

Keywords: Bioremediation, Textile Azo dyes, Acid red 2, Acid orange 7, Decolorization, *Bacillus* sp.

INTRODUCTION:

Water pollution due to dyeing industry is the matter of great concern since large quantity of colored effluent is discharged into the water bodies. The dye effluent is highly toxic as it contains certain chemicals such as dyes that could be toxic, carcinogenic or mutagenic to living organisms. Major contribution to color in the effluent is of azo dyes which are in purified form is seldom directly mutagenic or carcinogenic, except for some azo dyes with free amino groups [1]. However,

reduction of azo dyes, i.e. cleavage of the dye's azo linkage(s), leads to formation of aromatic amines and several aromatic amines are known mutagens and carcinogens.

Also, it is difficult to degrade the mixtures of the wastewater from textile industry by conventional treatment processes. Hence, economical and eco-friendly approaches are needed to remediate dye-contaminated wastewater from various industries. Among the various bioremediation technologies, decolorization using microbial cells has been widely used.

The anaerobic reduction of azo linkages converts the azo dyes to usually colorless but potentially harmful aromatic amines. The produced aromatic amines must be converted to non harmful products which is possible only under aerobic conditions.

Although during the last years, several bacterial strains have been described that aerobically decolorize azo dyes by reductive mechanisms [2], it is evident that bacteria are rarely able to decolorize azo compounds in the presence of oxygen, as a result very few reports exists on the aerobic decolorization of azo dyes [3, 4, 5, 6].

Therefore, present study focused to investigate the feasibility of aerobic treatment of two textile azo dyes (Acid red 2 and Acid orange 7) by newly isolated *Bacillus* sp. isolated from dye contaminated soil of the local dying houses in Nagpur (India).

MATERIALS AND METHOD:

Chemicals:

All the microbiological media and medium ingredients including the textile dyes, Acid red 2 and Acid orange 7 used in the present study were purchased from Himedia (Mumbai, M.S. India).

Isolation, screening and identification of dye decolorizing bacteria from soil:

The dye decolorizing bacteria were isolated from the soil of local dying houses in Nagpur-India. 10 gm of soil sample was suspended in 100 ml of complete medium broth supplemented with Acid red 2 (100mg/L) and Acid orange 7 (100mg/L) individually and acclimatized for 5 days at 30^0C at 150 rpm. Complete Medium Broth contained: Peptone 5 g/L; Yeast extract 3 g/L; Glucose 2 g/L; NaCl 5 g/L; $MgSO_4$.$7H_2O$ 0.1 g/L; K_2HPO_4 5 g/L; KH_2PO_4 1 g/L. The pH of the medium was adjusted to 7.0.

The dye decolorizing bacteria were isolated from acclimatized soil sample by its serial dilution and plating appropriate dilutions on Nutrient agar medium containing, Peptone 5 g/L, Beef extract 3 g/L, Sodium chloride 5 g/L, Agar 15 g/L, Dye (Acid red 2 / Acid orange 7) 100 mg/L, Extra Agar 20 g/L (pH 7.0). All the bacterial isolates

were studied by inoculating them in complete medium broth supplemented with dye. The inoculated medium was incubated at 30^0C (and/or 37^0C) under shaking condition at 150 rpm for 1-5 days. The decolorization was visually observed. The isolates showing considerable decolorization of the dyes were selected for further investigation. The selected bacterial isolates were identified on the basis of morphological and biochemical tests according to Bergey's Manual of Systematic Bacteriology [7].

Dye decolorization assay:

Decolorization activity was determined in 100 ml of nutrient broth supplemented with 10 mg of dye (Acid red 2 and Acid orange 7 individually) and 10 % (v/v) inoculums of selected isolate [8]. Dye containing uninoculated medium served as control. Inoculated medium and control was incubated at 30^0 C for 1-3 days on rotary shaker at 150 rpm. About 5 ml samples were withdrawn aseptically and centrifuged at 10,000 rpm for 15 minutes. Absorption was measured using supernatant of Acid red 2 at 470 nm and Acid orange 7 at 482 nm using UV-Vis spectrophotometer (Spectrascan -UV 2700). The decolorizing activity was expressed in terms of percent decolorization which was determined by using the formula [9]:

% decolorization ={Initial Absorbance - Final absorbance/Initial Absorbance} X100

Dye decolorization optimization:

Decolorization of Acid red 2 and Acid orange 7 by bacterial isolate was optimized with respect to temperature (20^0C, 30^0Cand 37^0C), pH (5-9) and time (24-96 hours). All the flasks were incubated at mentioned conditions on rotary shaker (150 rpm) for 1-4 days.

RESULTS AND DISCUSSION

Isolation, screening and identification of dye degrading bacteria:

All the isolates were screened for the ability of decolorization of Acid red 2 (100mg/L) and Acid orange 7 (100 mg/L) in Complete Medium Broth. On the basis of visual screening a bacterial isolate was found to decolorize the dyes under study significantly. The bacterial isolate was presumably identified as *Bacillus* sp. on the basis of morphological and biochemical characters (Table 1).

Table1. Identification of isolated dye decolorizing bacteria from soil

Gram's nature	Shape	Motility	Glucose fermentation	Sucrose fermentation	Lactose fermentation	Mannitol fermentation	Indole production	Methyl red	Voges-Prausker	Citrate utilization	Catalase	Oxidase	TSI
Positive	Rod	+	Acid only	Acid only	Acid only	Acid only	-	-	-	+	+	-	A/A H_2S+

Numerous microorganisms including bacteria have been isolated and characterized for degradation of various azo dyes [10].

Optimization of decolorization process:

The decolorization ability of *Bacillus* sp. was observed across a range of pH (5-9). The maximum decolorization of Acid red 2 (90%) was recorded at pH 6. Acid orange 7 exhibited nearly 99% decolorization at pH values between 6-8, where at pH 8 maximum decolorization (99.01%) was observed. At pH 7 the strain exhibited percentage decolorization value of 79.64 % for Acid red 2 and 98.51% for Acid orange 7 (Fig. 1).

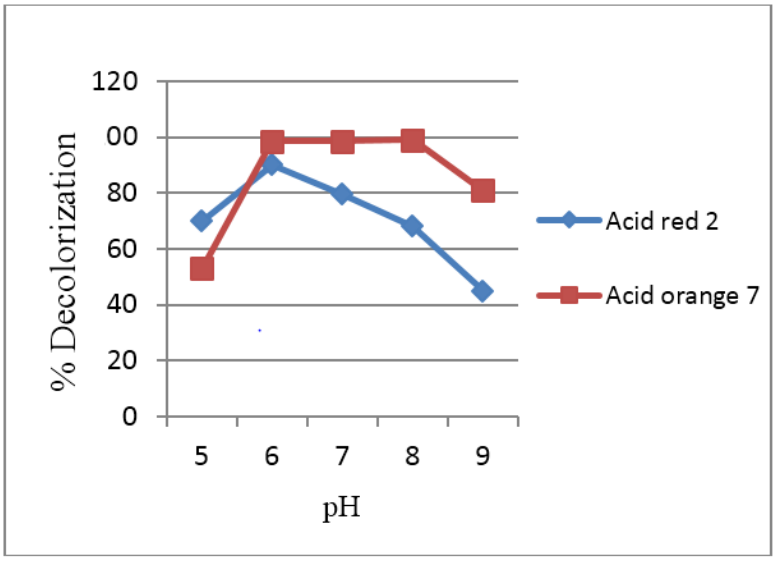

Fig.1 Effect of pH on decolorization potential of by *Bacillus* sp.

These findings are in agreement with the studies in which maximum decolorization of Methyl Red was achieved by Micrococcus strain R3 in pH range of 6-8 [11] and the decolorization of Acid Orange dye by Staphylococcus hominis RMLRT03 strain was found in the pH range of 6-8 [12].

The optimum temperature at which effective decolorization observed was found to be at 37^0C for Acid red 2 and 30^0C Acid orange 7 (Fig. 2).

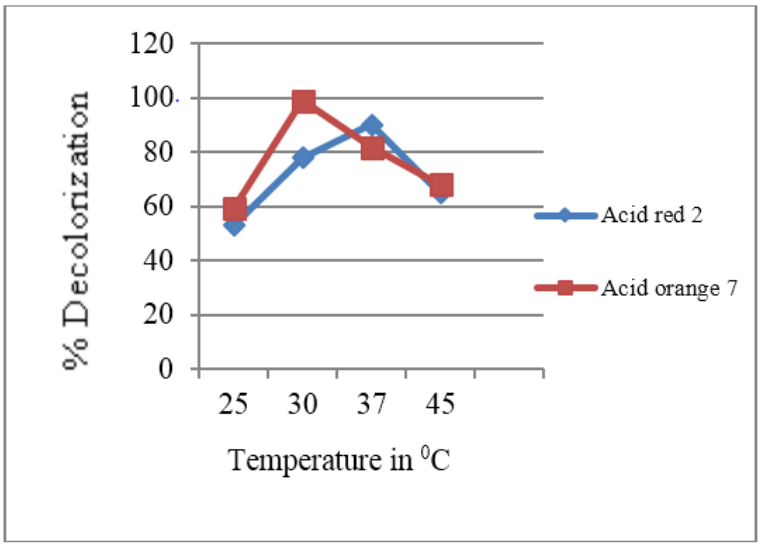

Fig.2 Effect of Temperature on decolorization potential of *Bacillus* sp.

A. hydrophilla decolorized Red RBN dye in the range of 20–35°C [13]. The studies of bacterial consortium JW-2 showed maximum 93% decolorization of Reactive Violet 5R at 37°C [14].

Effective decolorization of Acid red 2 and Acid orange 7 by *Bacillus* sp. was observed after 72 hours and 48 hours of incubation period respectively. The studies on decolorization of acid red by *Acinetobacter radioresistens* showed decolorization percentage of more than 70% at 48 hours of incubation [15].

CONCLUSION:

The textile azo dye Acid red 2 is degradable under aerobic conditions by *Bacillus* sp. isolated from dye contaminated soil from local dye house of Nagpur. Physical parameters (pH, temperature and time) had significant effect on dye decolorization. *Bacillus* sp. showed highest decolorization of Acid red 2 dye effectively during optimization. Therefore, it can be concluded that the *Bacillus* sp. can be of good potential use for the aerobic treatment of azo dye-containing wastewater based on its ability to remove colour.

ACKNOWLEDGEMENT:

The authors are thankful to the Shivaji Science College, Nagpur for its support to carry out present research work.

REFERENCES:

[1] Coughlin, M.F., Kinkle, B.K. and Bishop, P.L., 2002, "Degradation of acid orange 7 in an aerobic biofilm", Chemosphere, 46, pp. 11-19.

[2] Sarayu, K. and Sandhya, S., 2010, "Aerobic biodegradation pathway for Remazol Orange by Pseudomonas aeruginosa", Appl. Biochem. Biotechnol., 160, pp. 1241-1253.

[3] Chang, J.S., Chou, C. and Chen, S.Y., 2001, "Decolorization of azo dyes with immobilized Pseudomonas luteola", Water Sci. Technol., 43, pp. 261-269.

[4] Buitron, G. M., Quezada and Moreno, G., 2004, "Aerobic degradation of the azo dye acid red 151 in a sequencing batch biofilter", Biores. Technol., 92, pp. 143-149.

[5] Banat, I.M., Nigam, P., Singh, D., Marchant, R., 1996, "Microbial decolorization of textile dye containting effluents: A review", Bioresour. Technol., 58, pp. 217–27.

[6] Brown, M.A., Devito, S.C., 1993, " Predicting azo dye toxicity", Crit. Rev. Env. Sci. Tec., 23, pp. 249-324.

[7] Jaiswal S., Gomashe, A.V. and Agrawal, S., 2014, "Decolorization potential of Bacillus sp. for removal of synthetic textile dyes", Int. J. Curr. Microbiol. App. Sci., 3(12), pp. 83-88

[8] Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holf, J.G., 1984, "Bergey s manual of systematic bacteriology", Vol. I., Williams and Wilkins, Baltimore, U.S.A.

[9] Deepak, K.S., Harvinder, S.S., Manjinder, S., Swapandeep, S.C., Bhupinder, S., 2004, "Isolation and Characterization of microorganisms capable of decolorizing various triphenylmethane dyes", J. Basic Microbiol 44 (1), pp. 57-68.

[10] Saratale R.G., Saratale G. D., Chang J. S., Govindwar S. P., 2011, "Bacterial decolorization and degradation of azo dyes: A review", Journal of the Taiwan Institute of Chemical Engineers 42, pp.138–157

[11] Olukanni O.D., Osuntoki A., Gbenle G.O., 2009, "Decolorization of azo dyes by strain of Micrococcus isolated from a reuse dump soil", J. Biotechnol 8, pp. 442–8.

[12] Singh R. P., Singh P. K., Singh R. L., 2014, "Bacterial Decolorization of Textile Azo Dye Acid Orange by Staphylococcus hominis RMLRT030", Toxicol Int. 1(2), pp. 160–166.

[13] Chen K.C., Wu J.Y., Liou D.J., Hwang S.C., 2003, "Decolorization of the textile dyes by newly isolated bacterial strains", J Biotechnol. 1, pp. 57–68.

[14] Moosvi S., Kher X., Madamwar D., 2007, "Isolation, characterization and decolorization of textile dyes by a mixed bacterial consortium JW-2", Dyes

Pigments.74, pp. 723–9.

[15] Ramya M., Iyappan S., Manju A., Jiffe J.S., 2010, "Biodegradation and Decolorization of Acid Red by Acinetobacter radioresistens", J. Bioremed. Biodegrad., 1:105