Bioremediation of Textile Wastewater

Diksha Saxena[1], Vishal Kumar Sandhwar[2], Shivendu Saxena[3]

Department of Chemical Engineering

Parul Institute of Technology, Parul University, Vadodara - 391760, Gujarat, India

Email: *diksha.saxena8882@paruluniversity.ac.in[1], vishal.sandhwar8850@paruluniversity.ac.in[2], shivendu.saxena8938@paruluniversity.ac.in[3]*

DOI:- https://doi.org/10.47531/SC.2022.39

Abstract

Textile wastewater contaminated with dye and soil samples were collected from a small scale industry located at Bhadohi, Uttar Pradesh, India. Study was conducted for screening and isolation of bacteria capable for decolorizing textile dyes. Reactive Black 5 and Reactive Blue 4 dyes were used in the study. Decolorization abilities of isolates were studied and optimizations of various physiochemical parameters (effect of pH, contaminant concentration, inoculum concentration) were examined. Products after dye degradation were analyzed by UV-VIS techniques. Bacterial identification tests (Gram Staining Test, Indole Test, Methyl Red Test, Voges-Proskauer Test, Citrate Utilization Test) were conducted in order to find the family of bacteria. Results show that the optimum pH for dye decolorization was 7.0 for 10, 15 and 20 ppm concentration, Reactive Blue 4 at 10 ppm concentration was showing maximum decolorization after 36 hours, 14 ml inoculum was showing maximum decolorization for Reactive Black 5 and Reactive Blue 4 dyes after 48hours.

Keywords: - *Bioremediation, Textile wastewater, Gram Staining Test, Indole Test, Methyl Red Test, Voges-Proskauer Test, Citrate Utilization Test.*

INTRODUCTION

Industralization and modernization produces large amount of effluent from industries to the environment, which in turn creates more pollution. Mostly synthetic dyes are used in textile dyeingindustries, food industries, cosmetics, paper printing and pharmaceutical industries. 2/3rd of the textile industries were covering gross dye stuff market. During production, approximately10-15%ofthedyestuffislost directly to wastewater that finally harm the environment [3]. Among synthetic dyes,azo dyes causes environmental concern because of their color and toxic nature to animal and human also producing carcinogenic substances [2,3]. Toxic effluents released directly to the water bodies by the industries and causes a great threat to the marine lifeas they undergo chemical and biological changes. In order to treat the effluents from textile and dyeing industries, broadly classified methods are categorized as physical, chemical and biological. Physical and chemical methods which include adsorption, flocculation, coagulation, ion- exchange, chemical oxidation, irradiation, and ultra-filtration are not sufficient

mainly due to high BOD, COD, heat, color pH, presenceofmetalsandthesemethodsrequire high capital costs. Decolorization of dyes by biological means is a cost-effective tool for extracting dye from the nature. The aim of the present study deals with isolation and identification of bacteria (from naturally dye contaminated wastewater and soil samples) which have the ability to decolorizeanddegradesomeazodyesused in textileindustry.

MATERIALS AND METHODS

Collection of samples Textile dye wastewater

The textile dye wastewater and contaminated soil sample was collected from a private small-scale industry Bhadohi, Uttar Pradesh, India. The wastewater was stored in refrigerator at 4± 1 °C in airtight plasticcontainers.

Dyes and media

Two different dyes were used for studying bioremediation process including Reactive Black 5, Reactive Blue 4. Nutrient broth and nutrient agar media were used forculture and isolation of bacterial isolates. Dyes were purchased from Sigma-Aldrich and Alfa Aesar respectively. All chemicals and minimal salt media (MSM) used inthis study were purchased from SRL Pvt. Ltd. Mumbai, India.

Isolation and identification of bacteria from the textile dye effluent

Isolation for bacterial culture was performed by using MSM having glucose and yeast extract containing two azo dyes namely Reactive blue 4 and Reactive black 5 with the initial dye concentration of 10 ppm.The enrichment was carriedoutin100 ml MSM medium by adding 30 ml and 20 ml of textile wastewater sample and soil sample respectively. After every 3rd day of incubation a sample of medium was streake dontosterilenutrientagarplates and incubation was performed at 37oC for 24to 48 h, and 1 ml of the enriched culture was alsotransferredtofreshmedium.Attheend of incubation 1ml sample was diluted from each flask and plated on the agar medium. The pure cultures of bacterial strains were maintained on nutrient agar plate by keeping it at4^{o}C.

Screening of dye decolorizing bacteria Grown culture of 2 isolates were used to inoculate in 500 ml flask containing100 ml Nutrient Broth supplemented withReactive Blue 4 and Reactive Black 5 (10 ppm) dye. Under static conditions inoculated flasks were incubated. 1.5 ml sample was taken out from the flask and centrifuged at 8000 rpm for 15min.

COLONY CHARACTERIZATION

1. Biochemical test

These tests were performed to identify the bacterial species and its family. Reagents required for different biochemical tests were preparedandstoredat4^{o}Cinrefrigerator.

2. Gram staining test

This test helps in differen tiating the bacteria into Gram Positive and Gram Negative Bacteria, which contributes in the classification of microorganisms.

3. IMViC Test

The term "IMViC" states "I" stands for indole test; "M" stands for methyl red test; "V" stands for Voges-Proskauer test, and "C" stands for citrate test.

4. Assay of decolorization

In terms of percentage decolorization, dye decolorization by bacterial activity was calculated. Removal of biomass was carried out by the

centrifugation at 8,000 rpm for 15 min. With the help of UV-visible spectrophotometer, degree of decolorization of the dye was measured at its maximum absorbance wavelength using. Formula used for the decolorization assay was calculated accordingly-

$$\% \, Decolorization$$

$$initialabsorbance - finalabsorbance$$

$$= initialabsorbance$$

RESULTS AND DISCUSSIONS

Screening and identification of bacteria This paper compiled many results like various bacterial identification tests were performed on the basis of which bacterial families have to be decided, kinetic study was performed in order to know ther ate and order of equation, equilibrium study of different bacterial colonies with different dyes were performed so that we can check the ability of bacterial strains, different parameter study like pH, temperature, incubation time, contaminant concentration, and inoculums concentration were performed in order to know the bestfit conditions for a particular bacteria. Results are summarized asbelow:

(A) Bacterial identificationtest

Test Name	*Indicator*	*Result*
Gram staining test	Pink color bacteria appears	Negative
Indole test	Red color ring appears	Negative
Methyl red test	Yellow color media formation	Positive
Voges-Proskauer Test	Red-brown color appears	Negative
Citrate utilization test	Blue color appears	Negative

(A) Equilibrium study by BD1 and BD2

Table 2: Summary of identification tests for black dye

Test Name	*Indicator*	*Results*
Gram staining test	Pink color appears	Negative
Indole test	Red color ring formation	Positive
Methyl red test	Yellow color media appears	Negative
Voges-Proskauer Test	Red brown color appears	Positive
Citrate utilization test	Blue color appears	Positive

Equilibrium study for blue dye BLUE DYE BY BD1 AT 10 PPM

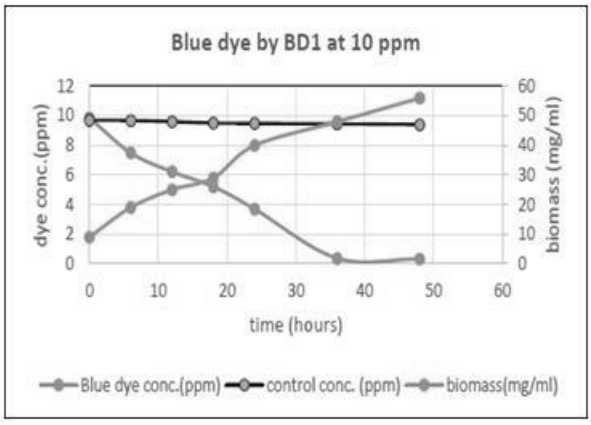

Fig 1: Plot of dye conc. with time, biomass and control conc.

Bluedyebefore Decolourization

Blue dyeafter Decolourization

BLUE DYE BY BD1 AT 20 PPM

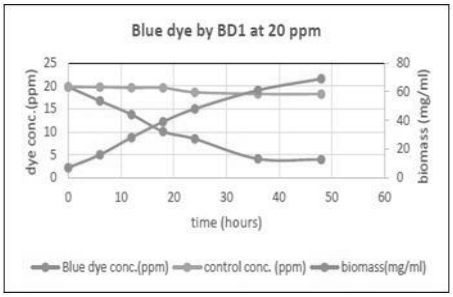

Fig 2: Plot of blue dye with time, biomass and control conc.

BLUE DYE BY BD2 AT 20 PPM

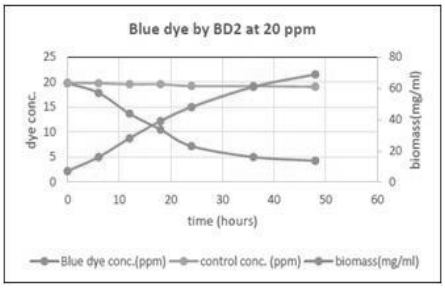

Fig 3: Plot of blue dye with time, biomass and control conc.

Bluedyebefore Decolourization

Blue dyeafter Decolourization

(C) Optimization parametersstudy

Effect of pH on blue dye at 10, 15 and 20 ppm.

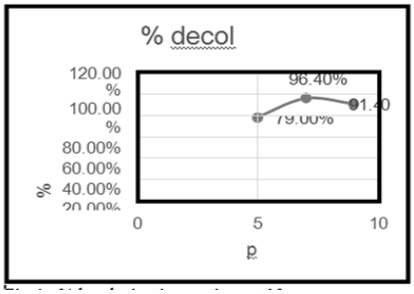

Fig 4: %decolorizationvs time at 10ppm

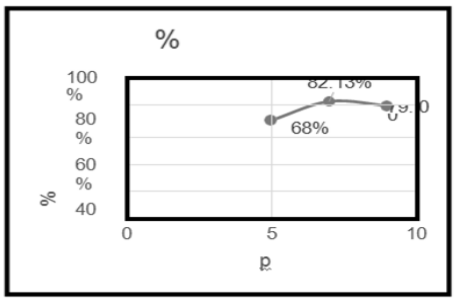

Fig 5: %decolorizationvs time at 15ppm

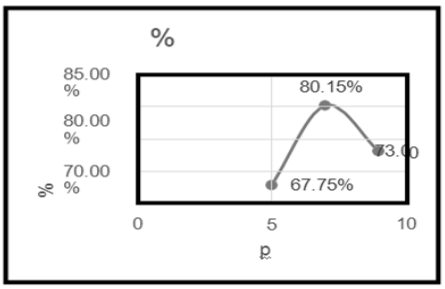

Fig 6: %decolorizationvs time at 20ppm

Equilibrium study for black dye

BLACK DYE BY BD2 AT 10 PPM

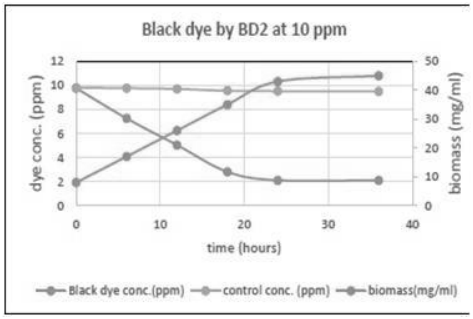

Fig 7: Plot of dye conc. with time, biomass and control conc.

BLACK DYE BY BD2 AT 20 PPM

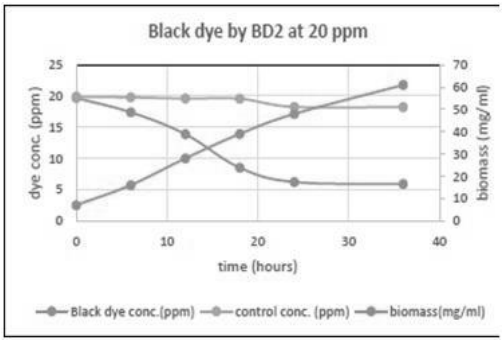

Fig 8: Plot of dye conc. with time, biomass and control conc.

Bluedyebefore Decolourization **Blue dyeafter Decolourization**

BLACK DYE BY BD1 AT 20 PPM

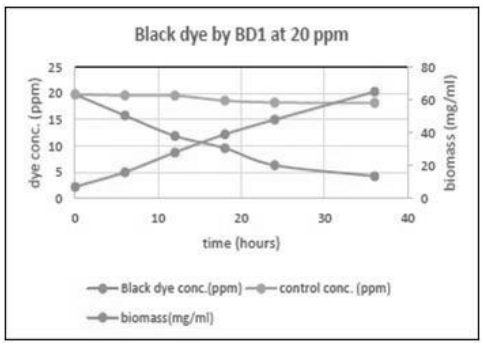

Fig 9: Plot of dye conc. with time, biomass and control conc.

OPTIMIZATION PARAMETERS STUDY

Effect of pH on black dye at 10, 15 and 20 ppm.

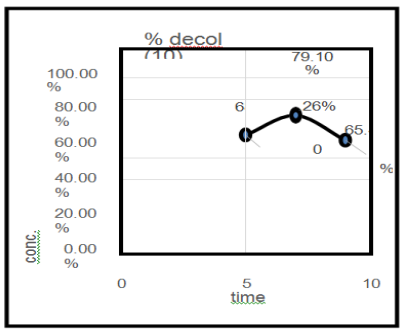

Fig 10: %decolorizationvs time at 10 ppm

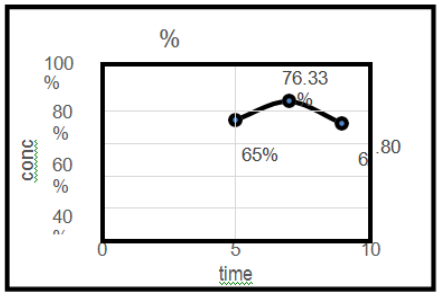

Fig 11: %decolorizationvs time at 15ppm

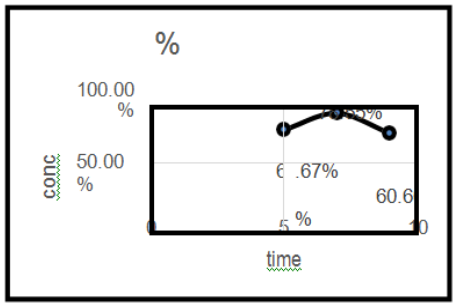

Fig 12: %decolorizationvs time at 20ppm

CONCLUSION AND FUTURE PERSPECTIVES

In this study, isolation of bacteria, and characterization of a bacteria capable of degrading harmful dyes were performed. Factors effecting degradation of dyes was found to bedyeconcentration, pH, incubation time and temperature. The advantages of this biological treatment are low cost, rapid degradation, and simple handling. Future work should be the toxicity test for the wastewater that is under treatment, design of efficient bioreactors for the treatment of dye wastewater. The technique of removing dyes bybioremediation has proved to be very effective method in an eco-friendly way. Future work to isolate new microorganisms having potential to degrade wide range of textile dye stuff and simultaneously creating an environment free from textile dye pollution is needed.

REFERENCES

1. Babu, B.R., Parande, A.K., Raghu, S. Kumar, T.P., 2007. Cotton textile processing: waste generation and effluent treatment. The J. Cotton Sci. 11,141–153.
2. Andre, B.D., Fransisco, J.C., Jules, B.L., 2007. Review paper on current technologies for decolourization of textile effluent. Colourage 40,35–38.
3. Sandhya, S., Padmavathy, S., Swaminathan, K., Subrahmanyam, Y.V., Kaul, S.N., 2005.

Microaerophilic-aerobicsequentialbatchreactorfortreatmentofazo dyes containing simulated wastewater. Process Biochem. 40 (2), 885–890.

4. Deepali, 2011. Bioremediation of Chromium (VI) from textile industry's effluent and contaminated soil using Pseudomonas putida. Iranica J. Energy. Environ. 2 (1), 24–31.
5. ISI, 1991. Methods of sampling and test (Physicaland chemical) for water andwaste water: 3025: IS 10500.
6. WHO, 2011. Guidelines for drinking water quality, First addendum to third edition, Volume I.
7. USEPA, 2012. Environmental Protection Agency. National Primary DrinkingWater Regulations.http://www Regulations. http://www.biovir.com/Images/pdf054.pdf (accessed 27 September 2012).
8. CPCB, 2000-2001. Environmental standards for Ambient air, automobiles, fuels, industries and noise. Pollution ControlLaw Series, PCLS/4/2000-2001, Published by Central Pollution Control Board, New Delhi.
9. Shankar, B., 2009. Chromium pollution in the groundwaters of an industrial area in Bangalore, India. Environ. Eng. Sci. 26(2), 305–310.
10. Dutta, K., Bhattacharjee, S., Chaudhuri, B., Mukopadhyay, S., 2002. Chemical oxidation of C.I. Reactive red 2using Fenton like reactions. J. Environ. Monit. 4, 754–760.
11. Fang, H., Wenrong, H., Yuezhong,L., 2004. Biodegradation mechanisms and kinetics of azo dye 4BS by a microbial consortium. Chemosphere 57 (4),293–301, ISSN0045-6535.
12. Asad, S., Amoozegar, M.A., Pourbabaee, A.A., Sarbolouki, M.N., Dastgheib, S.M., Decolorization of textile dyes by newly isolated halophilicand halotolerant bacteria. Bioresource Technol. 98, 2082–2088.
13. Gunasekaran, P., Puvaneswari, N., Muthukrishnan, J., 2006. Toxicity Assessment and microbial degradation of azo dyes. Indian J. Exp. Biol. 44,618–626.
14. Gogate, P.R., Pandit, A.B., 2004. A review of imperative technologies for wastewater treatment II: Hybrid Methods. Adv. Environ. Res. 8,553–597.
15. Verma, P., Madamwar, D., 2003. Decolorizationofsyntheticdyesbyanewly isolated strain of Serratiamaerascens. World J. Microb. Biot. 19,615–618.
16. Mendez-Paz,D.,Omil,F.,Lema,J.M., 2005. Anaerobic treatment of azo dye Acid Orange 7 under fed-batch and continuous conditions. Water Res. 39 (5),771–778.
17. Pandey, A., Singh, P., Iyengar, L., 2007. Bacterial decolorizationand degradation of azo dyes. Int. Biodeter. Biodegr. 59,73–84.
18. Khehra, M.S., Saini, H.S., Sharma, D.K., Chadha, B.S., Chimni, S.S., 2005. Decolorization of various azo dyes by bacterial consortium. Dyes Pigments. 67 (1),55–61.
19. Kalme, S., Jadhav, S., Jadhav, M., Govindwar,S.,2009.Textiledyedegrading laccase from Pseudomonas desmolyticum NCIM 2112. Enzyme Microb. Tech. 44, 65–71.
20. Gomaa, O.M., 2012. The involvement of acetic acid in programmed cell death for the elimination of Bacillus sp. used in bioremediation. J. Genetic Eng. Biot. 10, 185 192.
21. Watnable, K., Baker, P.W., 2000. Environmentally relevantmicroorganisms. J. Biosci. Bioeng. 89, 1–11.
22. Holt, J.G., Krieg, N., Sneath, P.,Staley, J., Williams, S., 1994: Bergey's Manualof Determinative Bacteriology.Lippincott, Williams and Wilkins, Baltimore.
23. Kamaludeen, S.P., Arunkumar, K.R., Avudainayagam, S., Ramasamy, K., 2003.

Bioremediation of chromium contaminated environments.IndianJ.Exp.Biol.41,972– 985.

24. Ansari, M.I., Malik, A., 2007. Biosorption of nickel and cadmium by metalresistant bacterial isolates from agricultural soil irrigated with industrial; wastewater. Short communication, Bio.Res. Technol. 98, 3149–3153.
25. Barragan, B.E., Costa, C., Marquez, M.C., 2007. Biodegradation of azo dyes by bacteria inoculated on soil media. Dyes. Pigments. 75,73–814.
26. Elkarmi, A., Abu-Elteen, K., Khader, M., 2008. Modeling the biodegradation efficiency and growth of Pseudomonas alcaligenes utilizing 2, 4-dichlorophenol as a carbon source Preand Post-exposure to UV radiation. Jordan J. Biological Sci. 1, 7–11.
27. Olukanni, O.D., Osuntoki, A.A., Gbenle,G.O.,2009.Decolourizationofazodyes by a strain of Micrococcus isolated from a refuse dump soil. Biotechnology 8, 442–448.
28. Gupta, G., Keegan, B., 1998. Bioaccumulation and biosorption of lead by poultry litter microorganisms. Poultry Sci. 77, 400.
29. Saranraj, P., Sumathi, V., Reetha, D., 2010. Decolourization and degradation of Directazo dyes and Biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. J. Ecobiotech. 2 (7),7–11.
30. Sriram, N., Reetha, D., Saranraj, P., 2013. Biological Degradation of Reactive Dyes by Using Bacteria Isolated from Dye Effluent Contaminated Soil. Middle-East J. Sci. Res. 17 (12):1695–1700.
31. McMullan, G., Meehan, C.,Conneely, A., Kirby, N., Robinson, T., Nigam, P., Banat, I.M., Marchant, R., Symth, W.F., 2001. Microbial decolourizationand degradation of textile dyes. Appl. Microbiol. Biot. 56,81.
32. Senan, R.C., Abraham, T.E., 2004. Bioremediation of textile azo dyesby aerobic bacterial consortium. Biodegradation 15,275–280.
33. Togo, C.A., Mutambanengwe, C.C.Z., Whiteley, C.G., 2008. Decolourisation and degradation of textile dyes using asulphate reducing bacteria (SRB)-biodigestermicroflora co-culture. Afr. J. Biotechnol. 7 (2),114–121.
34. Jadhav, J.P., Phugare, S.S., Dhanve, R.S., Jadhav, S.B., 2010. Rapidbiodegradation and decolorization of direct orange 39 (Orange TGLL) by an isolated bacterium Pseudomonas aeruginosastrain BCH. Biodegradation 21,453–463.
35. Murugalatha, N., Mohankumar, A.S.A., Rajesh, C., 2010. Textile effluent treatment by Bacillus species isolated from processed food. Afr. J. Microbiol. Res. 4 (20),2122–2126.
36. Phugare,S.S.,Kalyani,D.C.,Surwase, S.N., Jadhav, J.P., 2011. Ecofriendly degradation, decolorization and detoxification of textile effluent by a developedbacterial consortium. Ecotox. Environ. Safe. 74, 1288–96.
37. Saratale, R.G., Gandhi, S.S., Purankar, M.V., Kurade, M.B., Govindwar, S.P., Oh, S.E., Saratale, G.D., 2013. Decolorization and detoxification of sulfonatedazodye C.I.