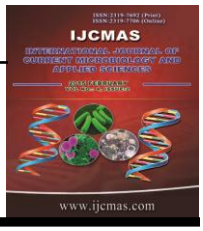
Int.J.Curr.Microbiol.App.Sci (2015) 4(2): 994-1001

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 2 (2015) pp. 994-1001**
http://www.ijcmas.com



Original Research Article

Biodegradation of Different Dye by Bacterial Strains Isolated from Textile Effluents of Western Rajasthan, India

Shellina Khan[1] and Nishi Mathur[2]*

[1]Department of Biotechnology, Faculty of Applied Sciences, JNU, Jodhpur, India
[2]Department of Biotechnology, Mahila PG Mahavidhyalaya, Jodhpur, India
**Corresponding author*

Keywords

Textile Effluents, Biodegradation

A B S T R A C T

The present work was carried out to monitor the efficiency of microbes to decolorize and degrade the dyes present in effluent release in environment from textile industries into non-toxic compound. Five isolates were obtained from the textile effluents using serial dilution. Isolates were evaluated for their capability to decolorize the dyes commonly used in textile industries. The isolates were degrading the dyes up to 50mg/l at 37˚C and pH 7 ±.2. The results revealed that all the bacterial isolates were having good potential to remove the high amount of color. The isolates were utilized for the removal of different concentration (10, 20, 30, 40, 50mg/l) of different dyes such as Malachite Green, Congo Red, Rose Bengal, Methyl Orange.

Introduction

Pollution, due to the textile industry, effluent has increased during recent years. Textile industries are large industrial consumers of waters as well as producers of wastewaters. 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. Water pollution control is at present one of the major areas of scientific activity. There are more than 105 commercially available dyes with over $1*10^6$ ton of dyestuff produced produced annually world wide. (Pandey et al., 2007). Colour present in dye effluent gives a straightforward indication of water being polluted. Major classes of synthetic dyes used include azo, anthraquinone and triarylmethane dyes (Stolz, 2001).

Dyes mainly the Reactive Dyes usually have a synthetic origin and complex aromatic molecular structures, which make them stable and difficult to biodegrade. Reactive Dyes bind to textile fibers, such as cellulose and cotton through covalent bonds (O'Mahony et al., 2002). They are consider as xenobiotic compounds because they possesses azo (N=N) and sulphonic ($-SO_3-$) electron withdrawing groups making the compound less susceptible to oxidation catabolism by bacteria and fungi (Saraswathi and Balakumar, 2009).

Azo dyes have been used increasingly in industries because of their ease and cost effectiveness in synthesis compared with natural dyes (Saratale R.G. et al., 2010). However, most Azo dyes are toxic, carcinogenic and mutagenic. Azo bonds present in these compounds are resistant to breakdown, with the potential for persistence and accumulation in the environment.

Congo Red is an azo dye with a structure 3, 3′-((biphenyl)-4,4′-diylbis(azo))-bis(4-amino-1-naphthalenesulphonicacid) disodium salt. It is intended primarily for the coloration of paper products, used in medicine (as a biological stain) and as an indicator since it turns from red-brown in basic medium to blue in acidic, used to color textile and wood pulp. It is a recalcitrant and act as potent carcinogen and mutagenic because of the presence of aromatic amine group (Cripps C. et al., 1990).

Malachite Green (MG) is a triphenyl methane dye, which is most widely used for coloring purpose, amongst all other dyes of its category (Gupta V.K., 2004). MG has properties that make it difficult to remove from aqueous solutions. If the solution containing MG discharged into receiving streams it will affect the aquatic life and cause detrimental effects in liver, gill, kidney, intestine and gonads. In humans, it may cause irritation to the gastrointestinal tract upon ingestion. Contact of MG with skin causes irritation and redness and pain. Upon contact with eye will lead to permanent injury of human eyes and laboratory animals (Kumar K.V. et al., 2005).

The present study deals with the microbial technology to degrade the different dyes from the dye contaminated areas. The discharge of highly coloured effluents can result in serious environmental damage. To create an ability in bacterium to degrade reactive dyes into non-toxic product. At Different dye concentrations rate of degradation were studied.

Materials and Methods

Sample collection

Waste water samples were collected in screw capped sterilized bottle from textile industries situated in jodhpur Baalotra and Pali. Physiochemical parameters of waste water *viz* .temperature pH, colour and smell were measured.

Dyes and Media

To see the degrading efficiency at different conc. of dyes Methyl Orange, Congo red, Malachite Green and Rose Bengal were used. All chemicals were of highest purity and of analytical grade.

Mineral salt media (E1) containing (g/l): Yeast extract (1.0),NaCl (2.0), MgSO4·7H2O (0.4), MgCl2·6H2O (0.7), CaCl2·2H2O (0.5), KH2PO4 (0.3), K2HPO4 (0.3),(NH4)2SO4 (0.5), and nutrient broth (0.1) with pH adjusted to pH 7 using 5 M sodium hydroxide (NaOH) and hydrochloric acid (HCl) (Khadija, O., et al., 2009).

Isolation of bacteria

For the isolation of bacteria from the effluents collected from various disposal sites of textile dyeing house numerous colonies were obtained through the serial dilution or the streaking plate method on nutrient agar. Each strain was then transfer to nutrient broth and incubated for 24hrs at 37°C. Five bacterial strains were isolated

from water samples and were named as A1, A2, A3, A4 and A5.

Screening of bacteria

Bacterial strains were selected on the basis of decolorising capability using selected dyes. For initial screening 10% aliquot of each isolates were inoculate into micro titre plate each containing 200µL individual dye solution. Decolorizations of dye solution were observed visually after 24 hrs. Strains that showed high decolorizing potential were selected for the further experiments.

Dyes decolorization

Bacterial isolates were transfer to Mineral Salt Medium. Different concentrations of dyes were used. Cultures were incubated at 37C. Effects of dyes on the growth of bacterial isolates were determined.

Analytical methods for dye decolorisation studies:

Decolorization was quantitatively analyzed by measuring the absorbance of the supernatant using a UV-visible spectrophotometer at maximum wavelength. λmax, of 616nm for Malachite Green, 498nm for Congo Red, 525nm for Rose Bengal, 465nm for Methyl Orange. Decolorizing rate was calculated by using the equation (Saratale et al., 2006).

$$\% \text{ Decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Where,

Initial absorbance = initial dye conc. (mg/L), Final absorbance = residual dye conc. (mg/L)

Effect of dye concentration

The various concentrations of dye (10, 20, 30, 40 and 50 mg/l) were added into the culture medium in order to examine the effect of initial dye on the decolourization in static conditions at various time intervals.

Result and Discussion

It is very clear that these industries play a positive role in the Indian economical reformation. Textile dye effluent sample were collected from the disposal site of effluent for screening and isolation of dye degrading bacteria. Since chances of getting microbes having the ability to decolorize the dye effluent is very high.

From initial screening, the isolates showed decolorizing capability were selected for further screening on E1 plate and were chosen for the next step of screening. Screening of dye decolorization for bacterial strains were carried out by series of isolation and identification in Mineral Salt medium using different dyes at different concentrations. At the end of 48 hrs of the incubation period all the isolates showed maximum decolorization at pH 7, under static condition.

All the five bacterial strains viz.A1,A2,A3,A4 and A5 showed their potentiality to degrade al the four dyes used during present investigation. However, efficacy of various bacterial strains was found to be varied at different concentrations in degradation of particular dye. It is clear from Table-1,Fig-1 that bacterial strain A4 resulted in almost 90 per cent degradation of malachite green at 50 ppm conc. Followed by strains A2,A3 and A5 which decolorized 80 per cent malachite green at same conc.Degradation of congo red was done most efficiently by bacterial strains A1,A2 and A3at 10 ppm conc.

Table.1 Decolorization of Malachite Green Dye at different concentration

	10 ppm			20 ppm			30 ppm			40 ppm			50 ppm		
Strain	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**
A1	.2145	.2079	3.07	.2868	.2279	20.53	.3178	.3000	5.60	.6807	.1249	81.65	.9072	.3000	66.93
A2	.2145	.1570	26.80	.2868	.2786	2.85	.3178	.2850	10.32	.6807	.2548	62.59	.9072	.1771	80.47
A3	.2145	.1606	25.12	.2868	.1591	44.5	.3178	.1446	54.49	.6807	.1629	76.06	.9072	.1809	80.05
A4	.2145	.2084	2.84	.2868	.0861	69.97	.3178	.1816	42.28	.6807	.2013	70.42	.9072	.1007	88.89
A5	.2145	.1165	45.65	.2868	.0678	76.34	.3178	.1367	56.98	.6807	.2193	67.78	.9072	.1736	80.86

Table.2 Decolorization of congo red dye at different concentration

	10 ppm			20 ppm			30 ppm			40 ppm			50 ppm		
sample	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**
10-1	.2241	.0131	94.51	.5872	.1785	69.60	.7652	.1476	80.71	.9521	.1893	80.11	1.3692	.2172	48.136
10-2	.2241	.0038	98.30	.5872	.1121	80.90	.7652	.1282	83.24	.9521	.2173	77.17	1.3692	1870	86.342
10-2 pink	.2241	.0092	95.89	.5872	.1214	79.32	.7652	.1131	85.21	.9521	.1743	81.69	1.3692	.1990	85.465
10-4	.2241	.0321	85.67	.5872	.2012	65.73	.7652	.0745	90.21	.9521	.1543	83.79	1.3692	.1420	89.628
10-7	.2241	.0421	81.21	.5872	.1771	69.83	.7652	.1806	76.36	.9521	.1672	82.43	1.3692	.1982	85.524

Table.3 Decolorization of Rose Bengal dye at different concentration

	10 ppm			20 ppm			30 ppm			40 ppm			50 ppm		
sample	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**
10-1	.7582	.5634	25.692	1.287	.9872	23.294	1.864	.9128	51.03	2.376	1.567	34.032	2.953	2.412	16.98
10-2	.7582	.4872	35.743	1.287	.9731	24.39	1.864	.9872	47.03	2.376	1.213	48.923	2.953	2.517	14.76
10-2 pink	.7582	.6825	9.997	1.287	0.303	75.75	1.864	1.158	37.85	2.376	1.987	16.372	2.953	2.104	28.75
10-4	.7582	.3578	52.809	1.287	0.482	62.517	1.864	1.032	44.63	2.376	1.786	24.815	2.953	1.758	40.46
10-7	.7582	.4862	35.874	1.287	.7471	41.95	1.864	1.275	31.57	2.376	1.954	17.761	2.953	2.174	26.37

Table.4 Decolorization of Methyl Orange dye at different concentration

	10 ppm			20 ppm			30 ppm			40 ppm			50 ppm		
sample	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**	**Initial OD**	**Final OD**	**% of removal**
10-1	.1904	.0908	52.311	.5846	.2178	62.744	.9321	.1784	80.86	1.374	.1248	90.922	1.8482	.2812	84.751
10-2	.1904	.0742	61.02	.5846	.1521	73.982	.9321	.1608	82.749	1.374	.0754	94.516	1.8482	.2910	84.254
10-2 pink	.1904	.0610	67.962	.5846	.1174	79.918	.9321	.1991	78.641	1.374	.1832	86.674	1.8482	.1933	89.541
10-4	.1904	.0872	54.202	.5846	.1824	68.799	.9321	.0852	90.859	1.374	.2874	79.095	1.8482	1.804	2.381
10-7	.1904	.0478	74.895	.5846	.2504	57.167	.9321	.4210	57.167	1.374	.2718	80.23	1.8482	.3033	83.589

Fig.1 % color removal of MalaChite Green in static condition

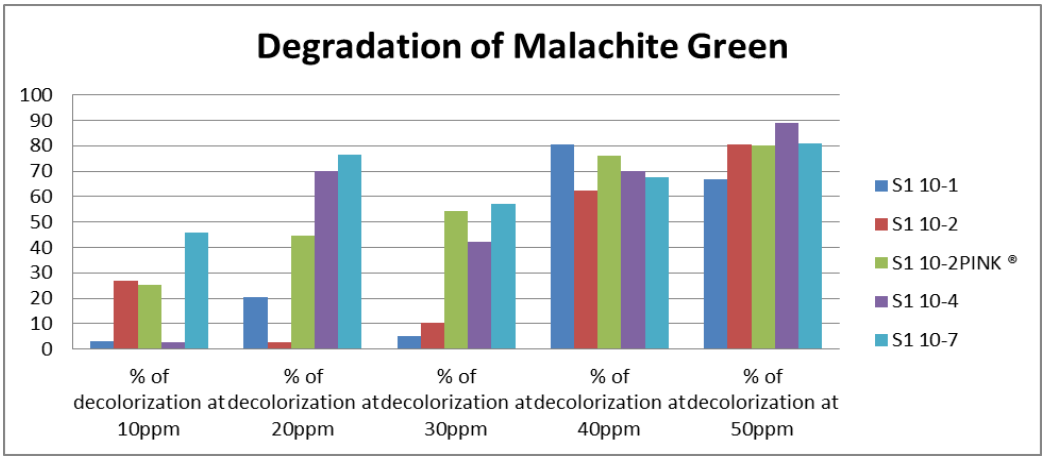


Fig.2 % of removal of Congo Red dye in static condition

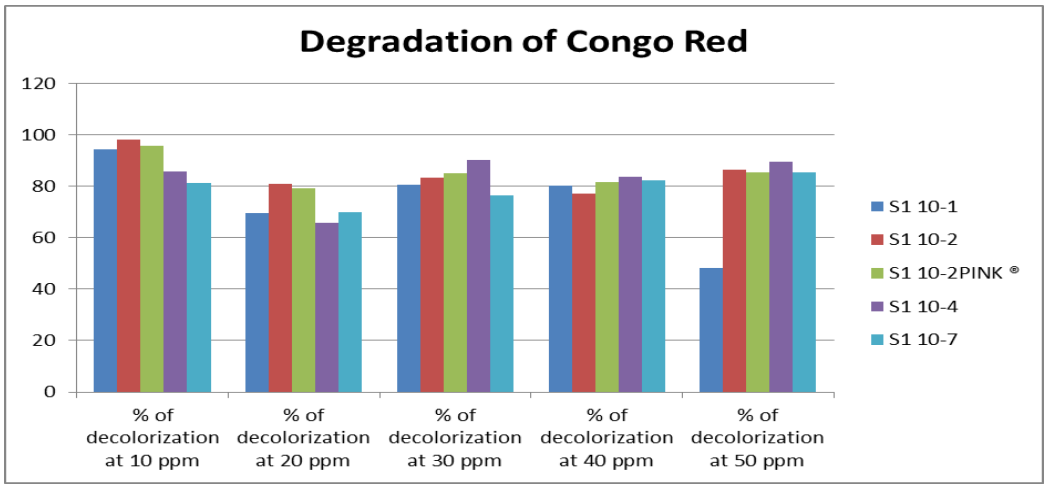


Fig.3 % removal of Rose Bengal in static condition

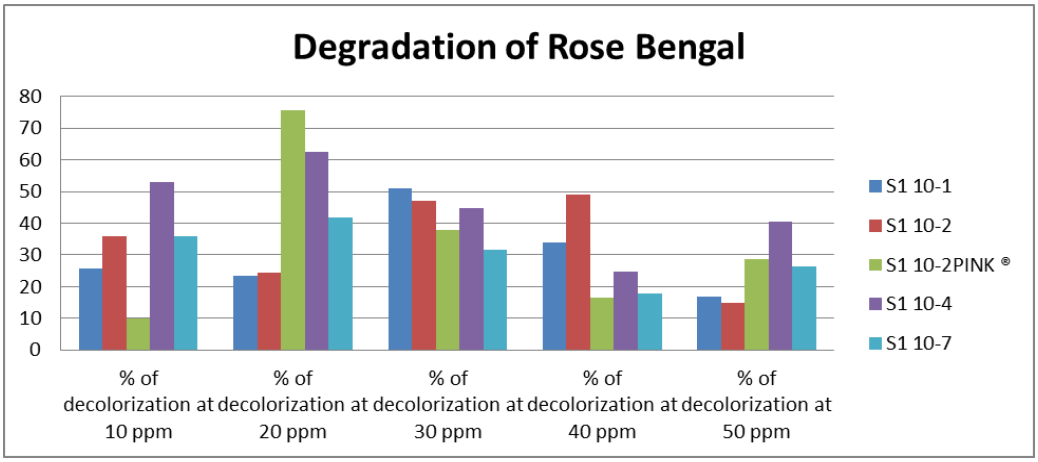
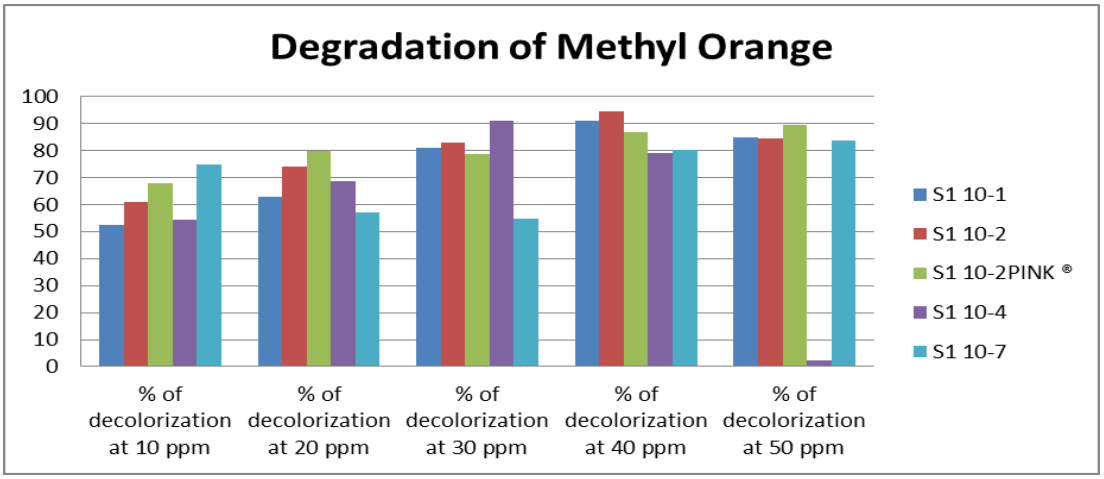


Fig.4 % removal of Methyl orange in static condition



While minimum degradation was observed by strain A1at 50 ppm conc.(Table-2,Fig-2).Similarly maximum decolorization of rose Bengal was observed at 20 ppm conc. by strainA3 followed by strain A4 (Table-3,Fig.3) .Almost ninety per cent degradation of methyl orange was observed by three bacterial strains at various concentrations i.e. by strain A2 at 40 ppm , strain A4 at 30 ppm and strain A3 at 50 ppm (Table-4,Fig.4). Observations clearly suggests degradation potentiality of all the five bacterial strains at various concentrations irrespective iof the dye used, however , efficiency of various bacterial strains was found to varied at different cons. In particular dye.

Few researches successfully obtaining the microbes decolorize dyes but with increasing the concentration of dye bacterial growth decreased. Microorganism capable in utilizing dyes as their carbon source. Azo dyes are deficient of carbon source therefore color removal is feasible only with co metabolite conditions.(Chang et al,. 2004).

Acknowledgment

The author is highly grateful to UGC for the financial support under the scheme of Maulana Azad National Fellowship for Minority students and also thankful to Mahila PG Mahavidhyalaya for providing the infrastructure facility for the research work.

References

Chang, J.S., B.Y. Chen and Y.S. Lin: stimulation of Bacterial decolorization of Azo dyes by extracellular metabolites of *E. coli*. strain No3., *Bioresour. Technol.*, 91, 243-248. (2004).

Cripps C., Bumpus J., Dust S., 1990, *Applied Environmental Microbiology*, 56,1114-1118

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

Jirasripongpun K., Nasanit R., Niruntasook J., Chotikasatian B. 2007, *International Journal Science Technology*, 12, 6–11.

Kumar K.V., Sivanesan S. and Ramamurthi V. (2005) "Adsorption of malachite green onto *Pithophora* sp., afresh

water algae: equilibrium and kinetic modeling" *Process Biochemistry* 40: 2865–2872.

Nirmalarani J., Janardhanan K. 1988, *Agricultural journal*, 75, 41.

O'Moahany, T., Gubal, E., and Tobin, J.M. 2002. Reactive dye biosorption by *Rhizopus arrhizus* biomass. *Enzyme Microb. Technol.*, 31: 456-463.

Pandey, A., Singh, P. and Lyengar, L., 2007. Bacterial decolorization and degradation of azo dyes. *Int .Biodeterior. Biodegrad*, 59: 73-84.

Pinherio HM, Touraud E, Tomas O. Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewater. Dyes Pigments. 2004;61(2):121–39.

Puvaneswari N., Muthukrishnan J., Gunasekaran P. 2006, *Indian Journal of experimental biology* ,44, 618-626.

Saraswathi, K. and Balakumar, S. (2009) Biodecolorization of Azo dye (Pigmented Red 208) Using *Bacillus Fi Rmus* and *Bacillus Laterosporous.* Journal of Bioscience Technology 5, 407-410.

Saratale R.G., Saratale G.D., Chang J.S., Govindwar S.P. 2010, *Biodegradation*, 21, 1999-1015.

Saratale G.D., Kalme S.D. and Govindwar S.P., (2006). Decolorization of textile dyesby *Aspergillus ochraceus*, Ind. J. Biotechnol. 5: 407–410.

Stolz, A. (2001). Basic and applied aspects in the microbial degradation of azo dyes. *Applied Microbiology and Biotechnology* 56, 69-80.