Int. Res. J. of Science & Engineering, 2015; Vol. 3 (3): 107-112 ISSN: 2322-0015

RESEARCH ARTICLE

Decolourization of Congo Red dye by bacteria and consortium isolated from dye contaminated soil

Holey BA

Department of Microbiology, Shri Shivaji Science College, Amravati, MS, India.
Email : bhagyashriholey12@gmail.com

Manuscript Details

Received : 06.04.2015
Revised : 01.05.2015
Re- Revised: 15.05.2015
Accepted: 14.06.2015
Published: 28.06.2015

ISSN: 2322-0015

Editor: Dr. Arvind Chavhan

Cite this article as:

Holey BA. Decolourization of Congo Red dye by bacteria and consortium isolated from dye contaminated soil. *Int. Res. J. of Science & Engineering,* 2015; Vol. 3 (3):107-112.

ABSTRACT

Dyes are widely used in the textile, rubber product, colour, printing, pharmaceutical and paper industries. Azo dyes are regularly used in the textile printing industries. This is the major cause of environmental pollution, because large amount of dye wastewater is discharged from the printing units. Therefore there is need of degradation of dyes. It is observed that several combined anaerobic and aerobic biological treatments utilising bacteria are in progress to enhance the biodegradation of textile dyes. The bacteria are capable of decolourizing Congo Red dye. Various bacterial strains such as *E.coli, Salmonella sp., S.aureus, Proteus sp., Pseudomonas sp*. and *Bacillus subtillis* were isolated and identified. The decolourization of Congo Red dye by the bacterial species was observed in various concentrations (100 to 500µg/ml) of Congo Red (CR) dye. The maximum decolourization was observed up to300µg/ml. Bacterial Consortium shows maximum decolourization that is 98% at 100µg/ml concentration of Congo Red.

Keywords: Consortium, Congo Red, Bacillus, Aerobic, Yeast, fungi

INTRODUCTION

Azo dye is a synthetic dye that has the azo group of two nitrogen atoms (N=N) connecting aromatic ring compounds. Azo dyes are relatively large molecules with high affinity especially for fibers (Aileen *et al*., 2009). Congo red (sodium salt benzidenediazo-bis-1 naphtylamine -4-sulfonic acid) has been reported to be a carcinogenic direct diazo dye used for colourartion of paper products (Ripps *et al*., 1990; Jaladoni-Buan *et al*., 2010).

The dye infested soils are detrimental to the growth of plants also. Extensive work has been carried out on the pollution problems associated with the discharge of dye effluent from industries. It has been documented that the safe method for azo biodegradation is combined aerobic treatment (Mabrouk and Yusuf, 2008; Olukannit et al. 2006). Many organisms such as *Bacillus, Ecoli, Klebsiella, Enterobacter, Pseudomonas* and a group of *fungi, yeast* have been studied for their decolorization of congo red dye (Chen *et al*., 2003; Jaladoni-Buan *et al*., 2010).

MATERIALS AND METHODS

Congo Red

An analytical grade CR obtained from S.D. Fine chemicals Ltd was utilized in this study. A known concentration of CR dye (100µg to 500 µg) added to nutrient broth, sterilized and decolourization study was carried out.

Collection of dye contaminated soil

The dye using effluent soil was collected from the surroundings of dyeing shops, Gol market Wardha. The sample was collected in a plastic container. Then the sample was brought to the laboratory as early as possible and was subjected for various microbiological studies.

Isolation and Identification of bacterial strains

Isolation and identification of bacteria were carried out by plate counting technique. One gram of soil and sludge were weighed individually and suspended in 99ml of sterile distilled water. One ml was pipetted and serially diluted with 10 ml distilled water up to 10-6 dilutions. From this 0.1ml of was taken and spread onto nutrient agar medium containing the following chemicals: Beef extract 3gmL-1, Nacl 5gmL-1, Peptone 5gmL-1 Agar 20gmL-1 and incubated at 37°C for 24hrs. Five different bacterial colonies were observed, further purified by streaking method and were identified by biochemical tests.

Effect of pH on dye decolourization

Eighteen hrs old culture was inoculated in conical flasks containing 100 ml nutrient broth maintained at pH (3-13) and each flasks were prepared with 300µg of Congo Red dye and incubated at 37 ˚C. At different time intervals aliquot (5ml) of the culture medium was withdrawn and supernatant obtained after centrifugation was used for analysis of percent (%) decolourization by measuring Optical Density at 497nm (Perumal *et al*.,2012).

Effect of time on dye decolourization

Eighteen hrs old culture was inoculated in conical flasks containing 100 ml nutrient broth and each flasks were prepared with 300µg of Congo Red dye and incubated at 37ºC. At different time intervals (24,48,72 and 96 hours) respectively, aliquot (5ml) of the culture medium was withdrawn and supernatant obtained after centrifugation was used for analysis of percent(%) decolourization by measuring Optical Density at 497nm (Perumal *et al*.,2012)

Effect of Bacterial monocultures and consortium on dye decolourization

Six pure cultures *IS1, IS2, IS3, IS4, IS5, and IS6* individually and as a consortium were tested for their ability to decolourize Congo Red by colorimetric analysis. A known concentration of CR (100 to 500µg) dye was added to the nutrient broth in 250 ml conical flask, sterilized, inoculated with 5 loops of individual bacterial culture and decolourization study was carried out. At every 12hrs interval 5 ml aliquot of the decolorized culture broth was collected and centrifuged at 10,000 rpm for 5 minutes. The supernatant was recovered and analyzed calorimetrically at 497 nm. The uninoculated medium with Congo red dye was served as blank. The efficiency of the isolates to decolorize Congo red (Hema *et al*.,2014) was calculated by following the formula:

$$\% \text{ Decolourization} = \frac{\text{Initial O.D - Final O.D X100}}{\text{Initial O.D.}}$$

RESULT AND DISCUSSION

Isolation and identification of bacterial strains

Six bacterial strains were isolated from soil samples collected from the surroundings of dyeing shop. The pure bacterial isolates IS1,IS2, IS3,IS4,IS5 and IS6 are identified as *E. coli, Salmonella sp., S.aureus, Proteus sp., Psedomonas sp. and Bacillus subtilis* by respectively biochemical characteristics. The bacterial strains were cultured individually and as a consortium in 250 ml conical flask with 100 ml of nutrient broth. Bacterial strains showed maximum growth at 18 hrs.

Six monocultures and consortium were inoculated in a nutrient broth with pH range from 3-11 at 300µg/ml CR. At pH 7, 70% Congo Red decolourization was observed in consortium followed by 65% and 62% in IS6 and IS5 respectively. At pH 5, consortium showed 50%, IS6 showed 37% decolourization, and maximum decolourization was observed at neutral pH.

Effect of dye concentration on decolourization

The decolourizing activity of the bacterial consortium was studied using Congo Red at different initial concentrations varying from 100 to 500 µg/ml. The maximum decolourization was observed up to 300 µg/ml. Bacterial consortium showed 98 % decolourization at 100 µg/ml concentration of Congo Red dye at pH 7 and temperature 37°C. IS5 monoculture showed 90 % decolourization at 100µg/ml. At 500 µg/ml percent (%) dye decolourization was dropped to 30%.

Bacterial consortium has shown 98 % decolourization at 100 µg/ml concentration of Congo Red dye at pH 7 and temperature 37°C. *Proteus* sp showed 90 % decolourization at 100µg/ml. This value was almost similar to that reported for CR dye decolourization among various concentrations (50 to 250 ppm). *Proteus* sp showed 90 % decolourization at 50 ppm (Perumal *et al*., 2012).

Table 1: Congo red dye decolourization (conc. 100µg/ml)

Sr. No.	Incubation time (hrs)	Decolourization percentage (%)						
		IS1	*IS2*	*IS3*	*IS4*	*IS5*	*IS6*	*Consortium*
1	24	56	58	52	65	61	64	67
2	48	71	74	67	78	72	69	78
3	72	81	83	73	95	87	79	97
4	96	88	88	77	97	90	89	97.5

Table 2: Congo red dye decolourization (conc. 200µg/ml)

Sr. No.	Incubation time (hrs)	Decolourization percentage (%)						
		IS1	*IS2*	*IS3*	*IS4*	*IS5*	*IS6*	*Consortium*
1	24	50	48	51	55	57	53	71
2	48	63	51	62	65	69	64	78
3	72	69	65	67	70	72	71	80
4	96	70	68	69	70.25	73	72	82

Table 3: Congo red dye decolourization (conc. 300µg/ml)

Sr. No.	Incubation time (hrs)	Decolourization percentage (%)						
		IS1	*IS2*	*IS3*	*IS4*	*IS5*	*IS6*	*Consortium*
1	24	48	42	49	52	55	51	65
2	48	56	49	58	60	59.5	57	70
3	72	61	58	63	65	62	62	72
4	96	63	60	63.5	68	64	63	73

Table 4: Congo red dye decolourization (conc. 400µg/ml)

Sr. No.	Incubation time (hrs)	Decolourization percentage (%)						
		IS1	*IS2*	*IS3*	*IS4*	*IS5*	*IS6*	*Consortium*
1	24	40	39	41	42.40	41	40	49
2	48	45	43.44	45	47	45.20	42	51
3	72	49	48	50	50.90	49.20	48	55
4	96	50	48.70	50.10	52	50	48.90	55.57

Table 5: Congo red dye decolourization (conc. 500µg/ml)

Sr. No.	Incubation time (hrs)	Decolourization percentage (%)						
		IS1	*IS2*	*IS3*	*IS4*	*IS5*	*IS6*	*Consortium*
1	24	21	20	22	23	24	22.50	34
2	48	23	21	24	25	26	25	36
3	72	25	23	25	27	28	26	38
4	96	26	23.50	25.60	29	29	26.90	39

Table 6: Effect of pH on dye decolourization(300µg/ml) for 48 hours:

Sr. No.	Incubation time (hrs)	Decolourization percentage (%)						
		IS1	*IS2*	*IS3*	*IS4*	*IS5*	*IS6*	*Consortium*
1	3	21	23	24.50	27	26	23	35
2	5	30	32	35	36.50	36	31	49
3	7	56	49	58	65	62	59	70
4	9	33	29	38	45	41	40	50
5	11	19	18	16.50	20	19	17	40

Table 7: Effect of time on dye decolourization (300µg/ml):

Sr. No.	Incubation time (hrs)	Decolourization percentage (%)						
		IS1	*IS2*	*IS3*	*IS4*	*IS5*	*IS6*	*Consortium*
1	24	48	42	49	52	55	51	65
2	48	56	49	58	60	59.5	57	70
3	72	61	58	63	65	62	62	72
4	96	63	60	63.5	68	64	63	73

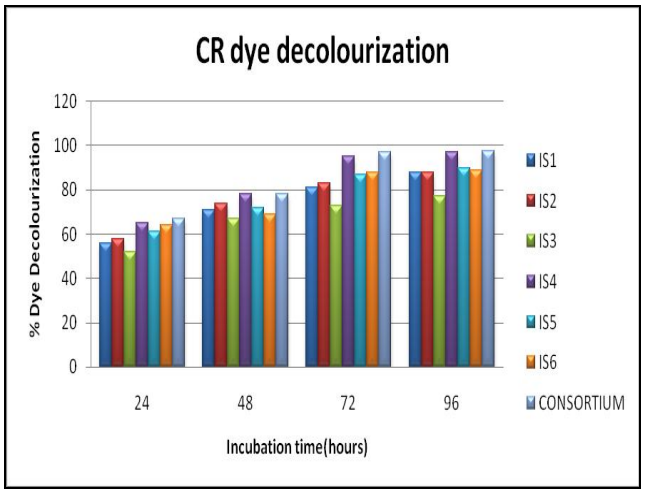

Fig.1: Congo red dye decolourization (conc.100µg/ml)

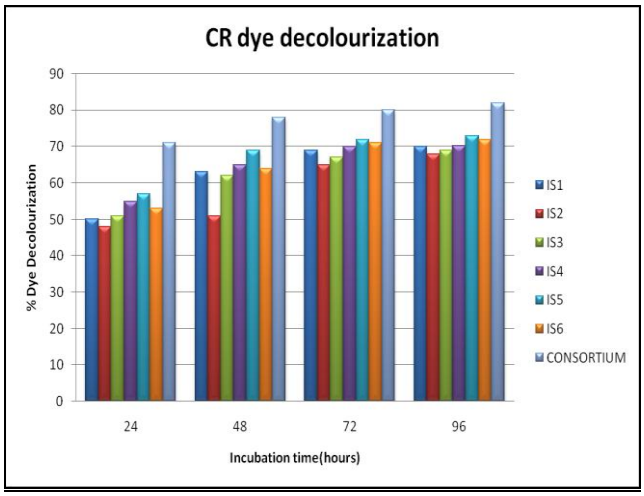

Fig. 2: Congo red dye decolourization (conc.200µg/ml)

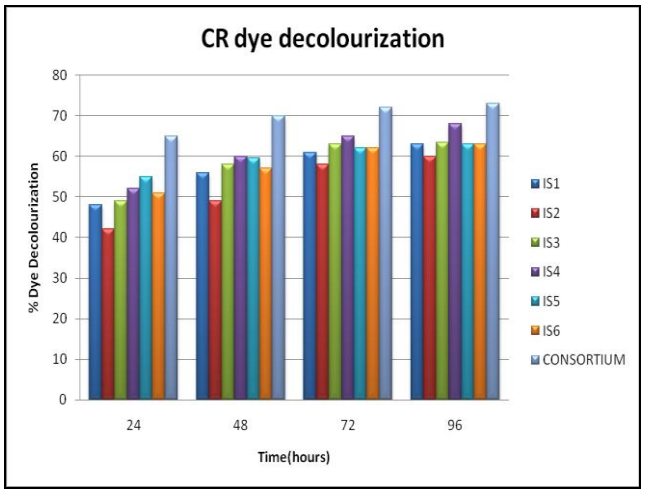

Fig.3: Congo red dye decolourization (conc.300µg/ml)

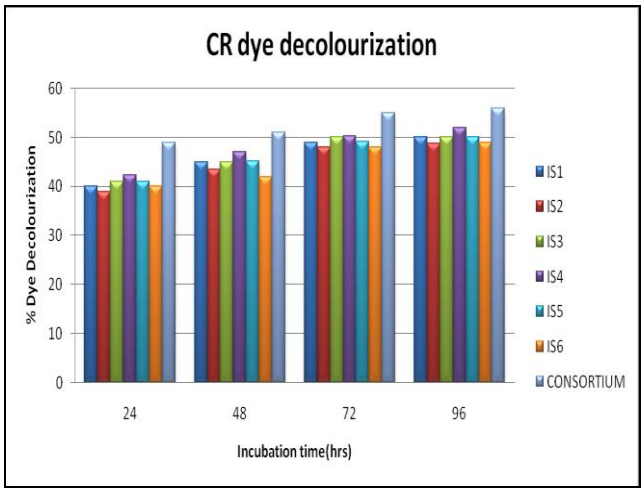

Fig.4: Congo red dye decolourization (conc.400µg/ml)

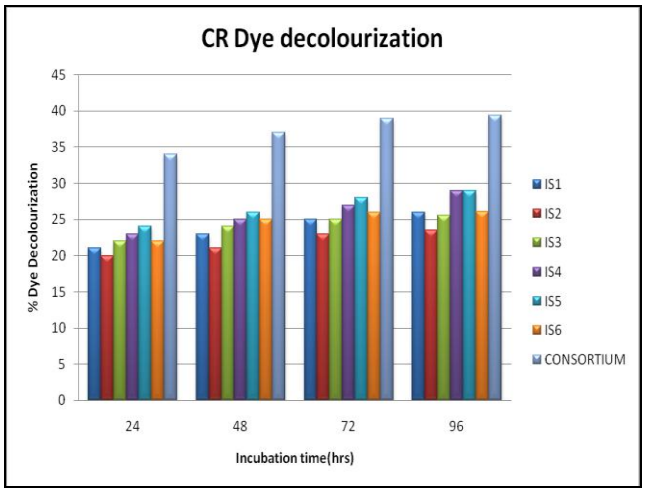

Fig. 5: Congo red dye decolourization (conc. 500µg/ ml)

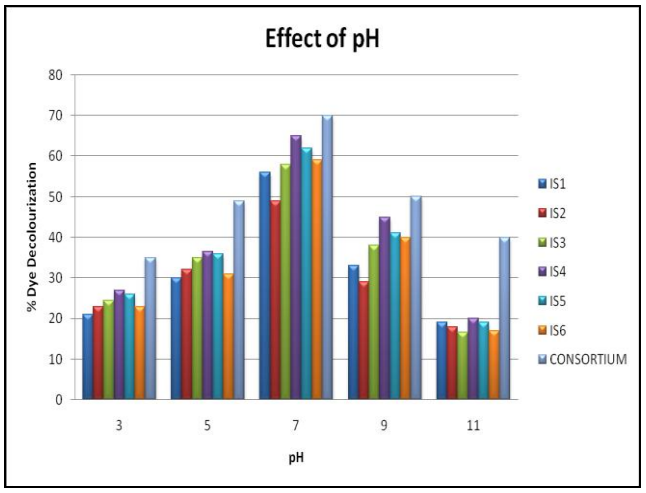

Fig. 6: Effect of pH on dye decolourization (300µg/ml) for 48 hours:

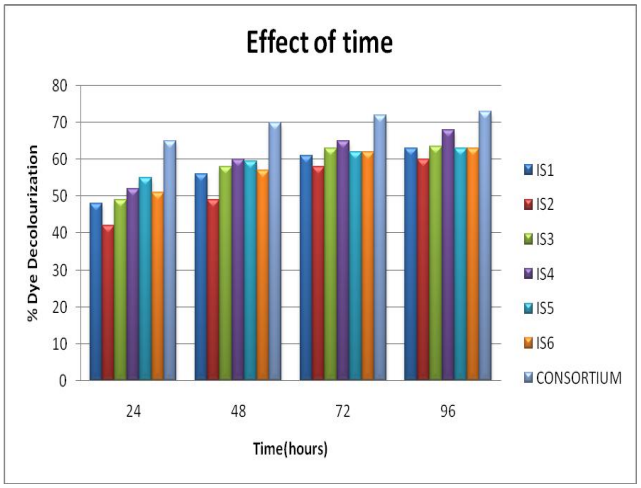

Fig. 7: Effect of time on dye decolourization (300µg/ml):

The effect of pH was also observed. At pH 7, 70% CR decolourization was observed in consortium followed by 65% and 62% in *Proteus and Pseudomonas* respectively. At pH 5, consortium showed 50%, *Pseudomonas* gave 37% decolourization, and maximum decolourization was observed in neutral pH. This value was almost similar to that reported for azo dyes decolourization, showed 70 % decolourization at pH 7 (Aileen *et al*.,2009).

Nosheen *et al*., 2010 documented that behaviour of each strain varied for dye decolourization with variation in pH. Similar studies have been carried

out on other azo dye and bacterial combinations to determine optimal conditions for maximum decolourization efficiency (Maier *et al*., 2004; Olukanni *et al*., 2006).

CONCLUSION:

In this present study, *Proteus* sp (IS4), *Pseudomonas* sp (IS5), B.subtilis(IS6), S.aureus (IS3) *E.coli (IS1)* and *Salmonella* sp (IS2) were decolorized CR dye and consortium showed maximum decolorization. Bacterial consortium showed 98 % decolourization at 100 µg/ml concentration of Congo Red dye at pH 7 and temperature 37°C. IS5 monoculture showed 90 % decolourization at 100µg/ml. At 500 µg/ml % dye decolourization was dropped to 30%. Six monocultures and consortium were inoculated in a nutrient broth with pH range from 3-11 at 300µg/ml CR. At pH 7, 70% CR decolourization was observed in consortium followed by 65% and 62% in IS6 and IS5 respectively. At pH 5, consortium showed 50%, IS6 showed 37% decolourization, and maximum decolourization was observed in neutral pH.

REFERENCES

1. Aileen C, Jalandoni–Buan. Characterization and Identification of Congo Red Decolorizing Bacteria from Monocultures and Consortia. *Philippine Journal of Science.,* 2010; 139 (1): 71-78

2. Chen KC, Wu JY, Liou DJ, Huang SCJ. Decolourization of textil dyes by newly isolated bacterial strains. *Journal of Biotechnology,* 2003; 101: 57-68.

3. Cripps C, Bumpus JA, Aust SD. Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium, Applied Environmental Microbiology,* 1990; 56(4): 1114-1118.

4. Hema N, Suresha S. Bioremedation of textile dye effluent by shewanella putrefaciens. *International Journal of Pharmacy and Biological Science*, 2014 ;109-116

5. Jalandoni-Buan AC, DecenaSoliven ALA, Cao EP, Barraquio VL, Barraquio WL. Characterization and Identification of Congo red decolourizing bacteria from monocultures and consortia. *Philippine Journal of Science,* 2010; 139 (1): 71-78

6. Mabrouk MEM, Yusef HH. Decolourization of fast red by *Bacillus subtilis* HH. *Journal of Applied Science and Research,* 2008; 4: 262-269.

7. Maier JA, Kandelbauer A, Erlacher A, CavacoPaulo, Gubits GM. A new alkalithermostable azoreductase from *Bacillus* sp. Strain SF. *Applied Environmental Microbiology,* 2004; 70 : 837-844.

8. Minussi RC, de Moraes SG, Pastore GM, Duran N. Biodecolourization screening of synthetic dyes by white rot fungi in solid medium. Possible role of siderophores. *Letters of Applied Microbiology*, 2001; 33 (1): 21-25.

9. Nosheen S, Nawaz R, Arshad M, Jamil A. Accelerated biodecolourization of reactive dyes with added nitrogen and carbon sources. *International Journal of Agriculture and Biology,* 2010; 12: 426-430.

10. Olukanni OD, Osuntoki AA, Gbenle GO. Textile effluent biodegradation potentials of textile effluent adapted and non adapted bacteria. *African Journal of Biotechnology*, 2006; 5: 1980-1984.

11. Perumal K, Malleshwari RB. Decolourization of Congo red dye by bacterial consortium isolated from dye contaminated soil. *Journal of Microbiology and Biotechnology Research*, 2012; 2(3): 475-480.