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Full Length Research Paper

Bio-decolourization of textile effluent containing Reactive Black-B by effluent-adapted and non-adapted bacteria

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Release of coloured textile effluents is undesirable in the aquatic environment as they reduce light penetration, thereby affecting aquatic life and limits utilization of the water media. Microbial bioremediation is an alternative treatment option available other than the commonly employed physico-chemical and biological methods to treat these toxic effluents. This study investigated the potentials of certain selected effluent soil-adapted and non-adapted bacteria to decolourize an actual textile effluent that contained the diazo dye compound Reactive Black-B. Five effluent-adapted and four non-adapted bacterial isolates were tested. The results show that effluent-adapted strains were better candidates for decolourizing the effluent than the non-adapted species.

Key words: Textile effluent, water pollution, azo dye, Reactive Black-B, biodecolourization, microbial bioremediation, bacteria.

INTRODUCTION

Environmental pollution caused by the release of a wide range of compounds as a consequence of industrial progress has now assumed serious proportions. Management of water pollution is at present one of the major challenges for environmentalists. More than 10,000 different textile dyes with an estimated annual production of $7x10^5$ metric tonnes are commercially available worldwide (McMullan et al., 2001). 2% of these dyes are directly discharged as aqueous effluents and 10% are subsequently lost during textile colouration process (Pearce et al., 2003). Colour is one of the most obvious indicators of water pollution, and discharge of highly coloured synthetic dye effluents can be damaging to the receiving water bodies (Nigam et al., 1996).

The release of coloured compounds into water bodies is undesirable not only because of their impact on photosynthesis of aquatic plants but also due to the carcinogenic nature of many of these dyes and their breakdown products (Weisburger, 2002). These dyes lin-ger in the environments for longer periods if let out with-out adequate treatment. Hydrolysed Reactive Blue-19 has a half-life of about 46 years at pH 7 and 25°C (Hao et al., 2000). Several combinations of treatment methods have been developed in order to effectively process textile wastewater; decolourization being one them. Treatment of dye wastewater involves physical/chemical methods such as coagulation, precipitation, adsorption by activated charcoal, oxidation by ozone, ionizing radiation and ultra filtration. These methods are costly, less efficient, has limited application but also generate wastes which are difficult to dispose off (Chen et al., 1999).

Microbial decolourization and degradation is an environment friendly and cost-competitive alternative to chemical decomposition processes (Verma and Madamwar, 2003). Moreover decolourization and degradation can also detoxify the effluent effectively without leaving any residues. The aim of the present work was to screen selected species of effluent soil-adapted and non-adapted bacteria for their ability to decolourize an actual textile effluent containing Reactive Black-B and to explore their potential as active azo dye degraders.

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MATERIALS AND METHODS

Selection and screening of micro-organisms

Vertical soil sample was collected under prescribed aseptic conditions from a dye contaminated area in Chinnakarai, Tirupur, Tamil Nadu, India. A sample of non-contaminated soil was taken from Kottar area of Nagercoil, Tamil Nadu. The samples were serially diluted (Waksman, 1922; Warcup, 1955) and spread over minimal media composed of glucose (3%), yeast extract (0.6%), KH_2PO_4 (0.6%), $M_gSO_4.7H_2O$ (0.02%), Na_2Co_3 (1.0%), agar (2.0%) at a neutral pH (Senan and Abraham, 2004) and 30°C for 3 days. Plates showing maximum growth and load were isolated and selected for identification of bacterial strains. The bacterial isolates were purified and characterized based on Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

Collection of textile effluent

Textile dyeing industry effluent containing synthetic diazo compound namely Reactive Black-B was collected from a dyeing unit in Arulpuram, Tirupur. The concentrated solution from the dye bath was collected in a 10 liter plastic can and refrigerated till use.

Effluent treatment with bacteria

Standard inocula of the isolated bacterial species were inoculated into 500 ml conical flasks containing 250 ml of textile effluent medium under orbital sharing condition (150 rpm/min) at 28 ± 2°C under strict sterile conditions. Two separate flasks were set to observe combined action of effluent-adapted and non-adapted bacteria separately on the effluent. Effluent medium without bacterial inoculum was treated as control.

Decolourization studies

3 ml of each of the treated suspension was centrifuged at 3000 rpm for 30 min in a laboratory centrifuge (REMI). The Optical Density (OD) was recorded spectrophotometrically (SPECTRONIC 60). Decolourization was monitored at 24 h interval for 10 days. The decolourization percentage (%D) was calculated using the formula:

$$\%D = \frac{OD_{Zero\ Day} - OD_{Sample}}{OD_{Zero\ Day}} \times 100$$

RESULTS

Identity of bacterial isolates

Five effluent-adapted and 4 effluent-non-adapted bacterial strains were isolated for the decolourization studies. Identity of the bacterial isolates are given in Tables 1 and 2

Microbial decolourization

Among effluent-adapted isolates, maximum decolourization percentage 44.2% was obtained on the 9th day with *Arthrobacter* sp. and on the 10th day with *Pseudomonas aeruginosa.* The non-haemolytic *Alcaligenes* sp. caused 42.63% decolourization on the 10th day. A minimum of 35.68% decolourization was observed in the effluent treat-ed with *Bacillus* sp. (Table 3).

Among effluent non-adapted species, *Pasteurella* sp. caused a maximum decolourization of 41.73% on the 7th day followed by 39.03% with *Pseudomonas* sp. *Kluyvera ascorbata* attained a maximum of 19.76% decolourization only on the 10th day whereas all the other effluent non-adapted bacteria achieved the same within 8 days of inoculation (Table 4).

DISCUSSION

Azo dyes represent a major group of dyes causing environmental concern because of their colour, biorecalcitrance, potential toxicity and carcinogenicity to animals and human (Levine, 1991; Conneely et al., 1999). Azo dyes are widely used in textile industries because of their ease of synthesis, versatility and cost-effectiveness (Griffiths, 1984). Though many of these dyes are banned for commercial sale and use, they are still largely available and used in textile industries because they are cheap and applicable to a large variety of textiles.

Synthetic azo dyes frequently found in textile effluents pose a great environmental threat. Due to inefficiencies of the industrial dyeing process, 10 - 15% of the dyes are lost in the effluents of textile units rendering them highly coloured (Vaidya and Datye, 1982; Boer et al., 2004). These dyes are xenobiotics and their degradation in nature is rather difficult. It is difficult to remove the dyes from such effluents since they are stable to light, heat and oxidizing agents. Most current physical and chemical technologies do not achieve total decolourization of coloured effluents or they have operational difficulties or are too expensive and traditional biological wastewater treatments have low removal efficiencies (Robinson et al., 2001). In recent years, considerable interest has been generated in studying microbial azo dye degradation (Banat et al., 1997). Environmental biotechnology relies upon the pollutant degrading capacities of naturally occurring microbial consortium in which bacteria plays a central role (Liu and Suffita, 1993; Stolz, 2001).

In the present study, decolourization proceeded gradually even up to 10th day in effluent-adapted bacterial treatment whereas in non-adapted bacterial treatment, there was rapid decolourization during initial days of exposure that slowed down abruptly on the 5th to 7th days of treatment. Control showed no decolourization which confirmed decolourization was as a result of metabolic activities of the introduced microbes and not due to abiotic factors (Oranusi and Ogugbue, 2005).

When each pure culture was tested individually, they showed remarkable decolourization potential. There was much lesser percentage decolourization when the adapted and non-adapted isolates were tested separately in combined treatments. Synergistic role of the bacterial

Table 1. Biochemical characteristics of effluent-adapted bacterial isolates.

S.No.	Test	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
1	Gram staining	Gram - ve Bacillus	Gram - ve irregular rod	Gram + ve irregular rod	Gram - ve Bacillus	Gram + ve regular spore formers
2	Indole test	-ve	- ve	- ve	-ve	+ve
3	Methyl Red (MR) test	-ve	- ve	- ve	-ve	+ve
4	Voges-Proskauer (VP) test	-ve	-ve	-ve	-ve	-ve
5	Citrate test	-ve	+ ve	- ve	- ve	+ ve
6	Triple Sugar Iron Agar (TSI) test	Acid slant/ Acid Butt	Acid slant/ Acid Butt	Acid slant/ Acid Butt	Acid slant/ Acid Butt	Acid slant/ Acid Butt
7	Mannitol Motility (MM) test	- / +	d / +	+ / +	- / +	- /+
8	Urea Hydrolysis test	-ve	- ve	- ve	-ve	-ve
9	Nitrate Reduction test	+ve	+ ve	+ve	+ve	+ve
10	Oxidase test	+	-	-	+ve	-
11	Sugar Fermentation test					
	(a) Lactose	-ve	+ve	+ve	-ve	-ve
	(b) Adonitol	-ve	-ve	-ve	-ve	-ve
	(c) Dextrose	-ve	-ve	-ve	-ve	-ve
	(d) Trehalose	-ve	-ve	-ve	-ve	-ve
	(e) Melibiose	-ve	-ve	-ve	-ve	-ve
	(f) Raffinose	-ve	-ve	-ve	-ve	-ve
	(g) Arabinose	-ve	-ve	-ve	-ve	-ve
	(h) Sucrose	-ve	-ve	-ve	-ve	-ve
	(i) Cellobiose	-ve	-ve	-ve	-ve	-ve
Identified organism		Non-Haemolytic *Alcaligenes* sp.	*Eubacterium sp.*	*Arthrobacter* sp.	*Pseudomonas aeruginosa*	*Bacillus* sp.

D = Doubt.

Table 2. Biochemical characteristics of effluent non-adapted bacterial isolates.

S.No.	Test Name	Round, White colony	Rhizoidal colony	Cream colony	White colony
1	Gram staining	Gram – ve short rod	Gram – ve rod	Gram – ve rod	Gram – ve rod
2	Indole test	+ve	- ve	- ve	+ve
3	Methyl Red (MR) test	+ve	- ve	- ve	+ve
4	Voges-Proskauer (VP) test	-ve	-ve	-ve	-ve
5	Citrate test	-ve	+ ve	+ ve	+ ve
6	Triple Sugar Iron Agar (TSI) test	Acid slant/Acid Butt	Acid slant/Acid Butt	Acid slant/Acid Butt	Acid slant/Acid Butt
7	Mannitol Motility (MM) test	+ / +	- / +	- / +	+ / -
8	Urea Hydrolysis test	-ve	+ve	- ve	-ve
9	Nitrate Utilization test	+ve	+ve	+ve	+ve
10	Catalase test	+ve	+ve	-	-
11	Coagulase test	+ve	-ve	-	-
12	H_2S production test	-ve	-ve	-ve	-ve
13	Oxidase test	-ve	-ve	+ve	+ve
14	Lysine Decarboxylate test	+ve	+ve	+ve	+ve
15	Lactose utilization	-ve	-ve	-ve	Weak + ve
16	Blood Agar Plate	αHC	αHC	-	-
Identified Organism		*Kluyvera ascorbata*	*Bacillus* sp.	*Pseudomonas* sp.	*Pasteurella* sp.

αHC = Alpha Haemolytic Colony.

Table 3. Percentage decolourization of textile dyeing industry effluent containing Reactive Black-B treated with 5 effluent-adapted bacteria (A, B, C, D and E) for 10 days.

Days	% Decolourization					
	Non Haemolytic *Alcaligenes* sp. (A)	***Eubacterium* sp. (B)**	***Arthrobacter* sp. (C)**	***Pseudomonas aeruginosa* (D)**	***Bacillus* sp. (E)**	**A+B+ C+D+ E**
0	-	-	-	-	-	-
1	5.3	5.4	5.3	5.3	5.4	5.4
2	5.3	19.71	17.49	15.13	21.78	12.86
3	29.86	21.71	23.97	21.81	23.86	15.16
4	29.86	21.78	29.86	25.93	23.86	25.93
5	33.8	21.78	33.8	31.83	23.86	30.08
6	33.8	30.08	39.1	33.8	28.01	30.08
7	37.33	31.95	40.86	33.8	31.95	31.95
8	37.33	33.82	42.63	35.56	31.95	33.82
9	39.1	35.68	**44.2**	39.1	33.82	35.68
10	**42.63**	37.55	44.2	**44.2**	35.68	35.68

Table 4. Percentage decolourization of textile dyeing industry effluent containing Reactive Black-B treated with 4 effluent non-adapted bacteria (a, b, c and d) for 10 days.

Days	% Decolourization				
	***Kluyvera ascorbata* (a)**	***Bacillus* sp. (b)**	***Pseudomonas* sp. (c)**	***Pasteurella* sp. (d)**	**a+b+c+d**
0	-	-	-	-	-
1	0	5.26	19.7	9.4	7.79
2	0	7.9	26.02	14.29	10.33
3	2.62	10.31	26.02	21.05	20.29
4	5.24	15.13	28.07	27.26	27.9
5	7.86	30.04	28.07	34.77	31.7
6	7.86	30.04	33.64	36.65	31.7
7	10.24	30.04	**39.03**	**41.73**	31.7
8	15	30.04	39.03	41.73	31.7
9	17.38	30.04	39.03	41.73	33.51
10	19.76	30.04	39.03	41.73	33.51

isolates in decolourizing the effluent was ruled out and antagonism among the microbes was observed as the reason for reduced decolourization in combined treatments. The results contradicted the observation of Knapp and Newby (1994) who suggested a synergistic role of bacterial species in decolourization.

Effluent-adapted *Bacillus* sp. gave 35.68% reduction in colour whereas the non-adapted isolate of the same species showed 30.04% colour removal. The results agree with that of Olukanni et al. (2006). Similarly, effluent-adapted *P. aeruginosa* showed 44.2% decolourization which was better than non-adapted *Pseudomonas* sp. (41.73%). An NADH-dependant azoreductase of the strain *Bacillus* sp. SF was found to be responsible for decolourization of azo dyes (Maier et al., 2004). The role of enzymes has been stressed in decolourization of azo dyes. Enzymes involved in the degradation of azo dyes are mainly peroxidases (Goszczynski et al., 1994) and laccases (Abadulla et al., 2000).

Chung and Stevens (1993) suggested microbial decolourization requires an unspecific enzymatic capacity ubiquitously found in a wide variety of micro-organisms. Some researchers had observed that decolourization of azo dyes follow the first-order kinetic model (Carliell et al., 1995; Willetts and Ashbolt, 2000; Van der Zee et al., 2001) whereas some others found zero-order kinetics (Dubin and Wright, 1975; Brown, 1981; Harmer and Bishop, 1992). Meschner and Wuhrmann (1982) pointed out cell permeability as a rate-limiting factor in the microbial reduction of sulfonated azo dyes.

The microbes utilized carbon, nitrogen and sulphate found in effluent medium for their nutrition. Decolourization percentage could be further increased and prolonged by supplementing the effluent medium with other cheaper

effective carbon or energy source such as sucrose, starch and hydrolysed starch. Ability of the microbial isolates to utilize starch as a co-substrate could be encouraging from commercial point of view (Moosvi et al., 2005).

Conclusion

Effluent-adapted bacteria were found more capable in decolourizing the effluent than the effluent-non-adapted ones. Further, it is suggested that genetic improvement and manipulation of the potential adapted and non-adapted bacterial azo dye degraders could remediate the textile effluent efficiently and cost-effectively.

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