damage of liver, spleen and lungs, and decreased blood coagulability[8].

Eosin yellow is a pink water soluble acid dye which displays yellow-green fluorescence. It is a heterocyclic dye containing bromine atoms, and is used in the fields of dyeing, printing, tanning, printing ink and fluorescent pigment, etc. Eosin yellow is used in paint and dye industries because of its vivid colour. The toxic nature of the dye is still not quantified much but its high content in living systems is proved to be harmful[9].

Color removal from effluent is one of the most difficult requirements faced by the textile finishing, dye manufacturing, and pulp and paper industries. These industries are major consumers of water and, therefore, cause heavy water pollution. Most of these dyes are harmful when brought in contact with living tissues for a long time. The discharge of such dyes in to the rivers or streams without proper treatment causes irrepairable damage to the crops and living beings, both aquatic and terrestrial[10].

Several methods are employed to treat textile effluents to achieve decolorization including physicochemical methods, such as filtration, coagulation, carbon activated and chemical flocculation[11,12]. These methods are effective but they are expensive and involve the formation of a concentrated sludge that creates a secondary disposal problem[13]. In recent years, new biological processes, including aerobic and anaerobic bacteria and fungi, for dye degradation and wastewater reutilization have been developed. The bacterial reduction of the azo bond is usually nonspecific and bacterial decolorization is normally faster. Microbial decolorization and degradation have appeared as an environment-friendly and cost-competitive alternatives to chemical decomposition processes[14]. Hence, the present study was carried out to isolate bacteria from textile dye effluent contaminated soil samples and to find out their decolorization ability of Eriochrome Black T, Eosin Yellow and Aniline.

Materials and methods

Chemicals

All the chemicals, reagents, biochemical identification test kits and media components used in this study were purchased from Hi-Media, Mumbai, India. All chemicals were of analytical grade, available commercially and used without further purification.

Sampling

Soil and dye effluent samples were collecte in sterilized bottles from Karur, Salem, Erode an Paramakudi, located in Tamil Nadu and kept in col condition till the samples were brought to the laborator

Screening dye decolorizing organisms

One gram of soil sample contaminated wit textile dye wastewater was serially diluted wit sterilized distilled water. Serial dilutions ($_{10}^{-1}$ to $_{10}^{-6}$) the samples collected were inoculated into nutrie agar medium by the spread plate technique. Isolatio of the bacterial strains was carried out with strea plate techniques. The selected bacteria were isolate and tentatively identified using rapid biochemic identification test kit for Gram-negative rods (Hi Media). The isolates were further confirmed b streaking on selective media.

Preparation of dyes

0.1 g of Eriochrome Black.T, 0.1 mL of Eos Yellow and 0.1g of Aniline dye were dissolved in 1(mL of distilled water separately. They were kept stock solutions, and the concentration of those solutio were 1000 ppm.

Absorption maxima for the dyes

The standard solution of the dye was prepare by taking 1 mL of the dye and making upto 100 m with distilled water. The absorption spectra for th three days Eriochrome Black T, Eosin Yellow ar Aniline were determined using colorimetry. Based the absorption maxima the wavelengths chosen we 510 nm for Eosin yellow and 520 nm for the other tw dyes.

Standard graph

Using series of concentrations of the thr dyes separately, the absorbance values were read respective wavelengths. From the absorbance value standard graphs were plotted against the concentrati of dyes.

Inoculation of isolated strains

To the autoclaved media, the pure d Eriochrome Black T, Eosin Yellow and Aniline we added in different concentrations, like 10, 50, 100 a 1000 ppm separately. To each of this medium 0.1 m of pure culture broth was added and kept in the roo temperature for incubation. The absorbance readin

Table 1: Biochemical tests used for the identification of isolated organisms

S.No.	Biochemical test	*Pseudomonas aeruginosa*	*Flavobacterium sp.*
1	Gram's staining	Nil	Nil
2	Catalase test	+	+
3	Motility test	+	Nil
4	Simmons citrate agar test	+	Nil
5	**Acid production from**		
	Glucose	Nil	+
	Maltose	Nil	Nil
	Sucrose	Nil	Nil
	Mannitol	Nil	Nil
6	Gelatin Red test	+	+
7	Methyl Red test	Nil	Nil
8	Voges – Proskauer test	Nil	Nil

(+ indicates positive result)

were taken on 1st, 2nd, 3rd and the 4th day using colorimeter.

Results

Biochemical tests

Table 1 divulges the biochemical tests for the identification of isolated organisms such as *Pseudomonas aeruginosa and Flavobacterium sp.* Gram staining results show that both the organisms are gram negative. Catalase test was found to be positive for both the organisms. Whereas motility and simmons citrate agar test indicated positive results for *Pseudomonas aeruginosa* and negative results for *Flavobacterium sp.* Acid production test from maltose, sucrose and mannitol showed negative result for both *Pseudomonas aeruginosa and Flavobacterium sp.* Whereas acid production from glucose showed negative result for *Pseudomonas aeruginosa* and positive result for *Flavobacterium sp.* Methyl red and Voges-Proskauer tests indicated negative results for both the organisms and the gelatin red test showed positive results for both the isolates.

Viable count

Table 2 depicts the viable count of *Pseudomonas aeruginosa and Flavobacterium sp.* using dyes such as Eriochrome Black T, Eosin Yellow and Aniline. In 1000 ppm sample of each dye, the viable count (CFU/mL) was observed by calculating the colony forming units for each dye. Maximum number of colonies were noticed at 10^4 dilution and minimum number of colonies at 10^6 dilution for both the organisms treated with the 1000 ppm concentration of the three dyes separately.

Decolorization study

In the present study, Fig.1. illustrates the treatment of the dye, Eriochrome Black T using *P. aeruginosa* at 10, 50, 100, 1000 ppm concentrations. Both 10 and 50 ppm concentrations of the dye were completely decolorized in two days while 100 ppm concentration required three days. For *Flavobacterium sp.* treatment 10 and 50 ppm concentrations of the dye were decolorized fully in three days while 100 ppm required four days (Fig. 2).

In Eosin yellow treatment, complete decolorization was noticed after three and four days for 10 and 50 ppm concentrations respectively when *P. aeruginosa* was employed (Fig.3). In the treatment using *Flavobacterium sp.* full decolorization of the dye was achieved in three days for 10 ppm concentration while 50 ppm concentration required four days (Fig. 4).

Fig. 5 shows that *P. aeruginosa* was able to decolorize 10 ppm of Aniline in three days while 50 and 100 ppm of Aniline in four days. *Flavobacterium sp.* needed three days for the complete decolorizsation of 10 ppm concentration and four days for 100 ppm concentration (Fig.6).

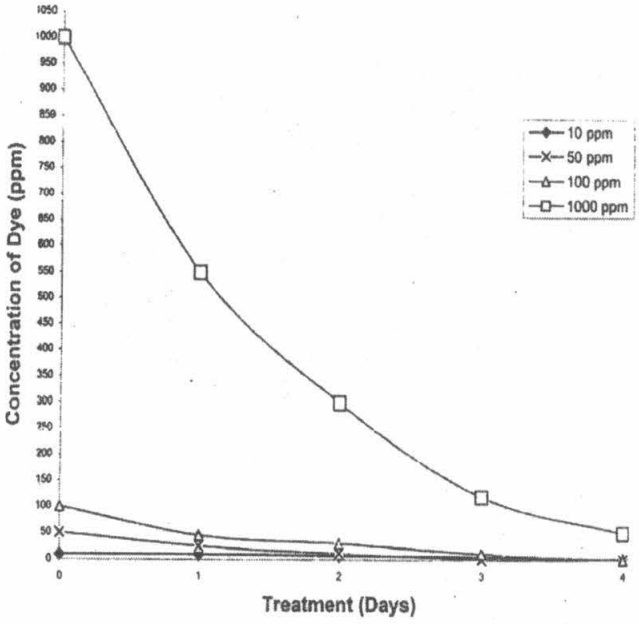

Fig 1

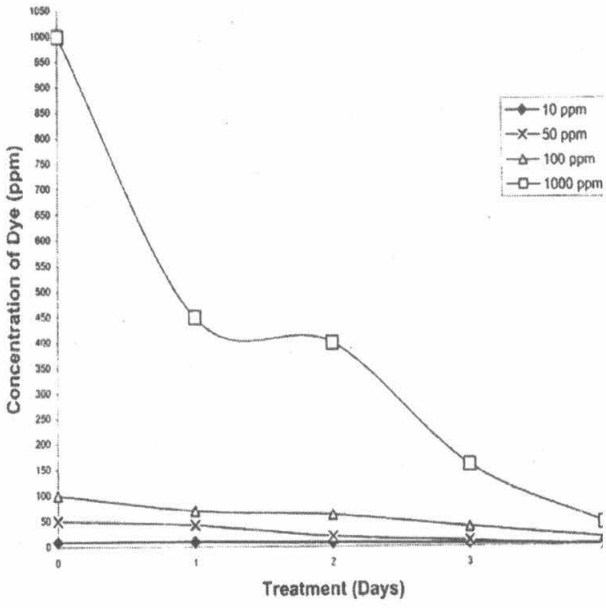

Fig 3

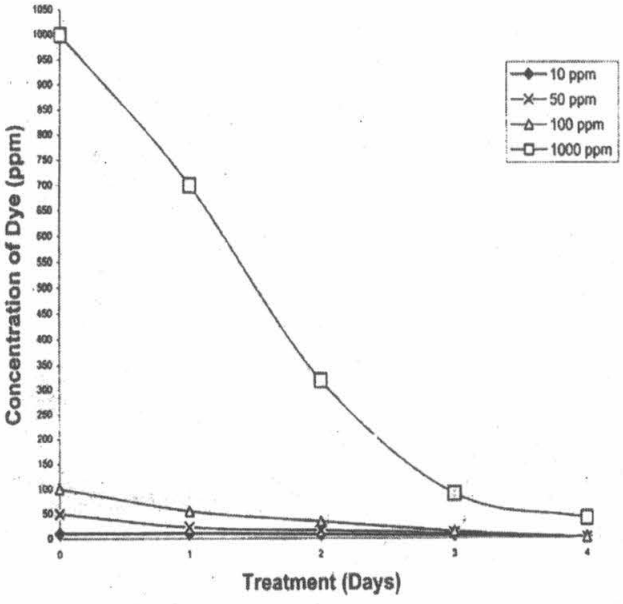

Fig 2

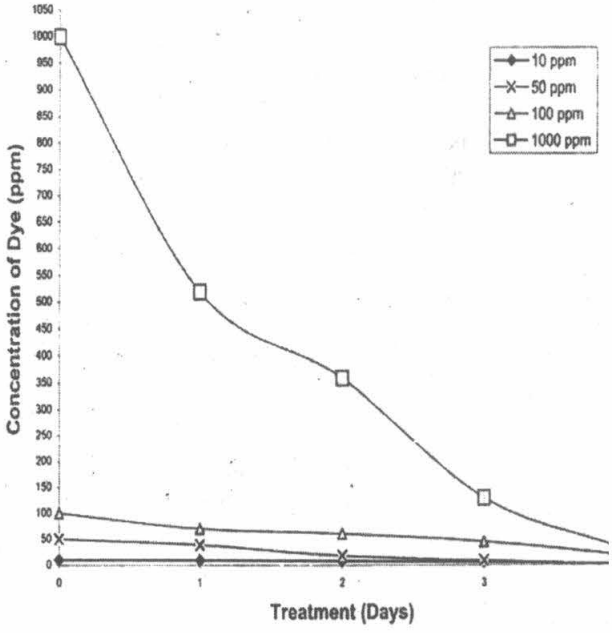

Fig 4

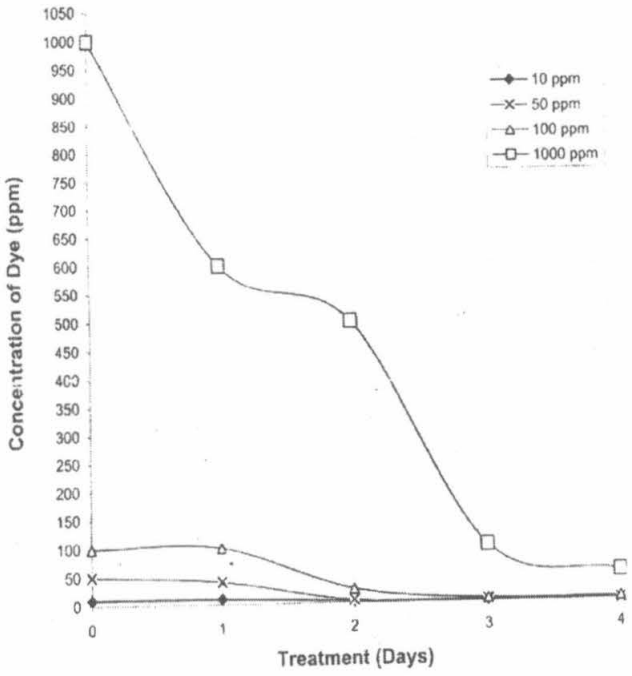

Fig 5

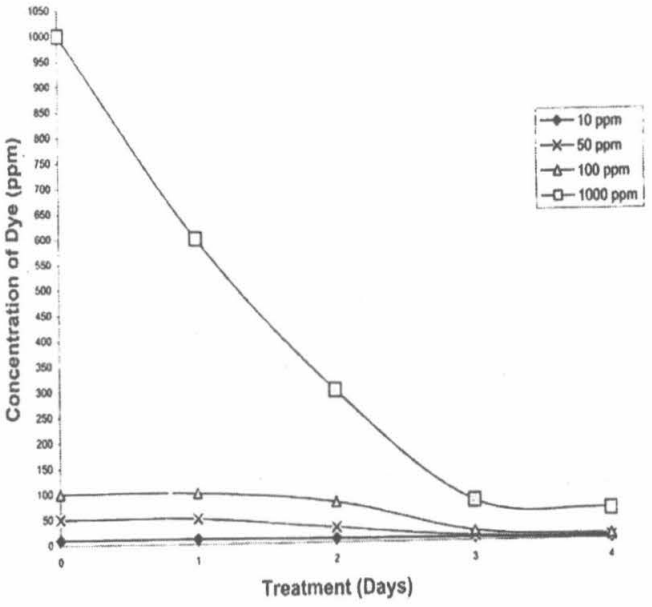

Fig 6

Discussion

Synthetic dyes and pigments released into the environment mainly in the form of effluents by textile, leather and printing industries cause severe ecological problems. These compounds have a great variety of colours and chemical structures and are recalcitrant to microbial attack. Most of the dyes are non-toxic, except for azo dyes which comprise of a large percentage of synthetic dyes, and are degraded into potentially carcinogenic amines[15].

Torang *et al* (2002) and Ahtiainen *et al* (2003) discovered that aniline at very low concentrations can be readily degraded in surface waters (rivers and lakes) [16,17]. However, the presence of the xenobiotic in wastewater at concentration exceeding 100 mg/mL can considerably complicate its biodegradation by traditional activated sludge technology[18]. Because of relatively large scale of aniline release into the environment and costly procedure of its chemical remediation[18], there is an urgent need to find out micro-organisms capable of degrading this xenobiotic. Examples of such organisms were found among various species of bacteria[18,19]

Eosin Yellow is not a single dye but a variety of related dyes and all are derived from fluorescein, which is a useful fluorescent dye widely used to label antibodies but is useless for ordinary light microscopy. By substituting halogens or nitro groups for some hydrogens, a variety of shades of red can be produced from yellowish to bluish e.g. Eosin Yellow (yellowish) changes to Eosin B (bluish) if the bromine groups on positions 2' and 7' are changed to nitro groups. The dyes are also fluorescent but are solely used as red dyes, although the parent dye fluorescein is widely used as a labelling compound in immunofluorescence. The sodium salts of the dyes are all freely soluble in water and fairly soluble in alcohol but will precipitate as eosinic acid if the pH is very low. However, adding dilute acids will improve eosin staining but may over differentiate the nuclear stain.

Degradation of azo dyes by micro-organisms has been extensively documented. Azo dyes can be degraded by lignin – degrading fungi, white rot fungi, bacterial peroxidases, aerobic and anaerobic bacteria possessing azoreductases, and anaerobic bacteria capable of producing reduced flavins and hydroquinones[3,20]. Bacterial biodegradation of azo dyes is often initiated by cleavage of azo bonds by

Table 2: Viable count (CFU/mL) of *Pseudomonas aeruginosa* and *Flavobacterium sp.* after 24 hours from 1000 ppm dye concentration

Name of the dye	*Pseudomonas aeruginosa*		*Flavobacterium sp.*	
	Dilution factor	Number of colonies	Dilution factor	Number of colonies
Eriochrome Black T	10^4	140	10^4	144
	10^5	98	10^5	108
	10^6	89	10^6	97
Eosin Yellow	10^4	130	10^4	134
	10^5	116	10^5	101
	10^6	84	10^6	69
Aniline	10^4	106	10^4	160
	10^5	91	10^5	137
	10^6	62	10^6	98

azoreductases which is followed by the aerobic degradation of the resulting amines[21].

As the azoreductase in some micro-organisms can catalyze the reductive cleavage of azo groups, they have potential advantages in developing biotreatment methods of wastewater containing azo compounds[22]. Azoreductase activity has been identified in several bacteria, such as *Xenophilus azovorans* KF46[23], *Pseudomonas luteola*[24], *Rhodococcus*[25], *Shigella dysenteriae* type I[26], *Klebsiella pnumoniae* RS-13[27], and *Clostridium perfringens*[28]

Russ *et al* (2000) suggested that bacterial membranes are almost impermeable to flavin containing cofactors and hence, restrict the transfer of reduction equivalents by flavins from the cytoplasm to the sulphonated azo dyes. Thus, a mechanism other than reduction by reduced flavins formed by cytoplasmic flavin-dependent azoreductases has to be responsible for sulphonated azo dye reduction in bacterial cells with intact cell membranes[29]. One such mechanism involves the electron transport-linked reduction of azo dyes in the extra-cellular environment. For achieving this, the bacteria can establish a link between their intracellular electron transport systems and the high molecular weight azo dyes. For establishing such a link, the electron transport components have to be localized in the outer membrane of the bacterial cells in gram-negative bacteria, where they can establish direct contact with either the azo dye substrate or a redox mediator at the cell surface[30].

P. aeruginosa can grow under both static as well as aerobic conditions resulting in complete breakdown of aromatic moieties of dye structure. The decolorization efficiency depends upon the presence of peptone and glucose, which are vital to decolorization triggered by enzymatic reduction of azo bonds. Based on the FTIR, NMR, and HPLC data, decolorization of Remazol Orange by *P. aeruginosa* seems to involve a complete breakdown of azo bond to form two aromatic amines, as well as further degradation of the azo bond[31]. Kulla (1981) reported that the simple azo compound (Carboxi orange II) was aerobically degraded by *Flavobacterium sp*[32]. Based on the present study, bacterial isolates, *Flavobactserium sp.* and *P. aeruginosa* can be used as good microbial sources for textile dye waste water treatment.

In the present work, *P. aeruginosa* and *Flavobacterium* sp were employed in the decolorization of Erichrome Black T, Eosin Yellow and Aniline. Among

these two organisms, P. *aeruginosa* was found to be more efficient in decolorizing all the three dyes used. Both the organisms required more than four days for decolorizing the dyes at 1000 ppm concentration. Control showed no decolourization which confirmed that decolourization was the result of metabolic activities of the introduced bacteria and not due to abiotic factors[33].

Conclusion

Wastewater treatment, employing physical and chemical methods, involves enormous cost and continuous input of chemicals which becomes uneconomical and results in further environmental damage. But biotreatment techniques using bacteria are ecofriendly and economic, as much bacterial biomass is available from industries. Color removal of effluents from textile dye industries is possible. Among the two bacterial strains tested in the present work, *P.aeruginosa* was found to be more efficient than *Flavobacterium sp.* For the both the strains, decolorization was achieved within three days for all three dyes at lower concentrations.

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References

1. Andre dos B S, Francisco J C and Jules van B L, Review paper on current technologies for decolourisation of textile wastewaters: Perspectives for anaerobic Biotechnology, *Bioresour. Technol*, 98, 2369–2385 (2007).

2. Zollinger H, Color Chemistry: Syntheses, Properties and Applications of Organic Dyes and Pigments (second ed, VHC Publishers, New York) 1991.

3. Stolz A, Basic and applied aspects in the microbial degradation of azo dyes, *Appl. Microbiol. Biotechnol*, 56, 69–80 (2001).

4. O'Neill C, Lopez A, Esteves S, Hawkes F R, Hawkes D L and Wilcox S, Azo-dye degradation in an anaerobic–aerobic treatment system operating on simulated textile effluent, *Appl. Microbiol. Biotechnol*, 53, 249–254 (2000).

5. Dave P N, Kaur S and Khosla E, Removal of Eriochrome black-T by adsorption on to eucalyptus bark using green technology, *Indian J. Technol*, 18, 53-60 (2011).

6. US Environmental Protection Agency, OPPT Chemical Fact Sheets, Aniline Fact Sheet, Washington, DC, 1994.

7. EC Joint Research Centre. Institute for Health and Consumer Protection, EU Risk Assessment Report: Aniline, European Chemicals Bureau, Ispra, Italy, 2004.

8. Agency for Toxic Substances and Disease Registry. Medical Management Guidelines: Aniline, ATSDR, Atlanta, GA, 2009.

9. Ansari R and Mosayebzadeh Z, Removal of Eosin Y, an Anionic Dye, from Aqueous Solutions Using Conducting Electroactive Polymers, *Iran Polym.* J, 19, 541-551 (2010).

10. Purkait M K, DasGupta S and De S, Adsorption of eosin dye on activated carbon and its surfactant based desorption, *J. Environ. Manage*, 76, 135 (2005).

11. Gogate P R and Pandit A B, A review of imperative technologies for wastewater treatment II: hybrid methods, *Adv Environ Res*, 8, 553–597 (2004a).

12. Gogate P R and Pandit A B, A review of imperative technologies for wastewater treatment I: oxidation technologies at ambient conditions. *Adv Environ Res*, 8, 501–551 (2004b).

13. Maier J, Kandelbauer A, Erlacher A, Cavaco – Paulo A and Gubits G M, A new alkali – thermostable azoreductase from *Bacillus* sp.Strain SF. *Appl. Environ. Microbiol*, 70, 837 – 844 (2004).

14. Ozdemir G, Pazarbasi B, Kocyigit A, Omeroglu E E, Yasa, I and Karaboz I, Decolorization of Acid Black 210 by *Vibrio harveyi* TEMS1, a newly isolated bioluminescent bacterium from Izmir Bay, Turkey. *World JMicrob Biot,* 24, 1375–1381 (2008).

15. Chung K-T and Stevens S E, Degradation of azo dyes by environmental microorganisms and helminthes, *Environ Toxicol Chem*, 12, 2121 – 2132 (1993).

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Biodecolorization of Chosen Dyes Using *Pseudomonas aeruginosa* and *Flavobacterium Sp.*

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Two bacterial strains were isolated from textile dye effluent contaminated sites and tentatively identified as *Pseudomonas aeruginosa* and *Flavobacterium sp.* based on the biochemical tests. These two strains were tested for their efficiency of decolorizing the dyes, Erichrome Black T, Eosin Yellow and Aniline at 10, 50, 100 and 1000 ppm concentrations for four days. Based on the absorption maxima of the respective dyes (510 nm for Eosin Yellow and 520 nm for the other two dyes), absorbance values were determined in a colorimeter during decolorization process. Among the two bacterial strains tested, *Pseudomonas aeruginosa* was found to be more efficient, and complete decolorization was achieved within three days for all the three dyes at lower concentrations.

Key words: *Decolorization, Pseudomonas aeruginosa, Flavobacterium sp, Erichrome Black T, Eosin Yellow, Aniline*

Introduction

Water pollution control is at present one of the major areas of scientific activity. Textile industries are large consumers of industrial water as well as producers of wastewater. With the increased demand for textile products, the textile industry and its wastewater have been increasing proportionally, making it one of the main sources of severe pollution worldwide[1]. Azo dyes, which are aromatic compounds with one or more –N=N– groups, constitute the largest class of synthetic dyes used in commercial applications[2]. According to 1994 estimates, the world production of dyes was around 1 million tons, of which more than 50% were azo dyes[3]. They are considered as xenobiotic compounds that are very recalcitrant to biodegradation processes[2,3] These dyes are widely used in a number of industries, such as textile dyeing, food, cosmetics, paper printing, with the textile industry as the largest consumer. All dyes do not bind to the fabric; depending on the class of the dye, its loss in wastewater could vary from 2% for basic dyes to as high as 50% for reactive dyes, leading to severe contamination of surface and groundwater in the vicinity of dyeing industries[4].

Eriochrome Black T (EBT) is used for dyeing silk, wool, nylon, multifibres after pretreatment with chromium salts, and hence the dye containing effluent also contains heavy metal chromium. Pure EBT is also used as an indicator in complexometric titrations for estimation of Ca^{2+}, Mg^{2+} and Zn^{2+} ions and for biological staining. This dye is hazardous as such and its degradation products, like Naphthaquinone are still more carcinogenic[5].

On the long list of environmentally hazardous chemicals, prominent positions are occupied by organic nitrogen compounds and one among them is phenylamine, otherwise known as aniline. The main sources of this xenobiotic are sewage waters and vapors originating from chemical, tannery, cosmetic or pharmaceutical industry, utilizing aniline as one of the raw materials. Due to its broad range of toxicity and carcinogenicity, aniline has been subjected to stringent legislative control by US Environmental Protection Agency[6] as well as by EC Joint Research Centre[7]. The main toxic effects of aniline start with the formation of methemoglobine in blood, and are expressed by lowering of oxygen transport, anaemia, jaundice,

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16. Torang L, Reuschenbach P, Muller B and Nyholm N, Laboratory shake flask batch test can predict field biodegradation of aniline in the Rhine *Chemosphere,* 49, 1257 – 1265 (2002).

17. Ahtiainen J, Aalto M and Pessala H, Biodegradation of chemicals in a standardized test and in environmental conditions. *Chemosphere,* 51, 529 – 537 (2003).

18. Wang L, Barrington S and Kim J W, Biodegradation of pentyl amine and aniline from petrochemical waste water. *J. Environ. Manage.* 83, 191-197 (2007).

19. Matsumura E, Sakai M, Hayashi K, Murakami S, Takenaka S and Aoki K, Consitutive expression of catABC genes in the aniline-assimilating bacterium *Rhodococcus* sp AN-22: production, purification, characterization and gene analysis of catA, catB , catC, *Biochem.J,* 393, 219 – 226 (2006).

20. Cheng H, Recent advances in azo dye degradation enzyme research, *Curr Prot Pept Sci,* 7, 101–111 (2006).

21. Yeh M S and Chang J S, Bacterial decolorization of an azo dye with a natural isolate of *Pseudomonas luteola* and genetically modified *Escherichia coli, J Chem Technol,* 79, 1354–1360 (2004).

22. Nachiyar C V and Rajakumar G S, Purification and characterization of an oxygen insensitive azoreductase from *Pseudomonas aeruginosa. Enzyme Microb Tech,* 36, 503–509 (2005).

23. Blümel S, Knackmuss H J and Stolz A, Molecular cloning and characterization of the gene coding for the aerobic azoreductase from *Xenophilus azovorans* KF46F. *Appl. Environ. Microbiol,* 68, 3948-3955 (2002).

24. Hu T L, Kinetics of azoreductase and assessment of toxicity of metabolic products from azo dyes by *Pseudomonas luteola, Water Sci Technol,* 43, 261–269 (2001).

25. Chang J S and Lin C Y, Decolorization kinetics of a recombinant *Escherichia coli* strain harboring azo-dye-decolorizing determinants from *Rhodococcus sp, Biotechnol Lett,* 23, 631-636 (2001).

26. Ghosh D K, Mandal A and Chaudhuri J, Purification and partial characterization of two azoreductases from *Shigella dysenteriae* Type I. FEMS, *Microbiol. Lett.* 98, 229-234 (1992).

27. Wong P K and Yuen P Y, Decolorization and degradation of methyl red by *Klebsiella pneumoniae* RS 13. *Water Res,* 30, 1736–1744 (1996).

28. Rafii F and Coleman T, Cloning and expression in *Escherichia coli* of an azoreductase gene from *Clostridium perfringens* and comparison with azoreductase genes from other bacteria. *J Basic Microbiol,* 39, 29–35 (1999).

29. Russ R, Rau J and Stolz A, The function of cytoplasmic flavin reductases in the reduction of azodyes by bacteria, *Appl Environ Microbiol,* 66, 1429–34 (2000).

30. Myers C R and Myers J M, Localization of cytochromes to the outer membrane of anaerobically grown *Shewanella putrefaciens* MR-1, *J Bacteriol,* 174, 3429–38 (1992).

31. Sarayu K and Sandhya S, Aerobic biodegradation pathway for Remazol Orange by *Pseudomonas aeruginosa, Appl Biochem Biotechno,.* 160, 1241-53 (2010).

32. Kulla H G, Aerobic bacterial degradation of azo dyes, in: Microbial degradation of xenobiotics and recalcitrant compounds, (Academic Press, England), 387–399 (1981).

33. Oranusi N A and Ogugbue C J, Effect of pH and nutrient starvation on biodegradation of azo dyes by *Pseudomonas sp. J. Appl. Sci. Environ. Manage.* 9, 39-43 (2005).