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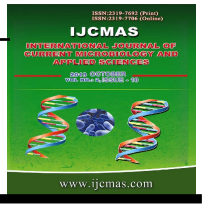

Original Research Article

Rapid decolourization of Disperse Red F3B by *Enterococcus faecalis* and its Phytotoxic Evaluation

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A B S T R A C T

Rapid decolourization potential of a novel bacterial isolate was developed from a mixed culture isolated from a textile industrial ground at Mettuppalayam, Tamil Nadu, India. The isolate decolorized Disperse Red F3B to 94% at 100 mg l^{-1} concentration within 5 hrs under microaerophilic condition at 50°C. The bacterial isolate was determined to be *Enterococcus faecalis* based on the biochemical characteristics and 16S rDNA sequence analysis. Dye decolourization was optimum in Nutrient broth and the biotransformation was confirmed through Ultra Violet-Visble Spectroscopy, Thin Layer Chromatography and Fourier Transformed Infra Red spectroscopy analysis of the initial, intermediate and final decolorized sample. Phytotoxicity testing with the seeds of *Brassica nigra* and *Cyamopsis tetragonolobus* demonstrated more sensitivity towards the native dye, while the products after decolourization did not have any inhibitory effect as seen from germination delay index and morphometric parameters.

Keywords

Biodegradation; azo dye; germination delay index; *Brassica nigra; Cyamopsis tetragonolobus*.

Introduction

Disperse dyes are extensively used in the textile industries since the discovery of synthetic fiber. Disperse dyes are mainly chosen for polyester fibers but they are also used to dye nylon, cellulose triacetate, and acrylic fibers. In some cases, a dyeing temperature of 130°C is required, and a pressurized dye bath is used. Due to such extreme dyeing conditions and low levels of dye fiber fixation, up to 50% of the dye that is present in the original dye bath is lost in to the waste water (Mariana *et al* ., 2004). When such polluted water finds their way into the environment, they retain their colour and structural integrity for a long period of time in the environment proving detrimental to its biotic components (Ajibola *et al* ., 2005; Chinwetkitvanich *et al* ., 2000). Discharge of these dyes into the aquatic environment causes serious damage, since they may significantly affect the photosynthetic

activity of hydrophytes by reducing light penetration (Aksu *et al* ., 2007) and also they may be toxic to aquatic organisms due to their breakdown products (Hao *et al*., 2000). Also, public perception of water quality is greatly influenced by the colour. The presence of unnatural colours is aesthetically unpleasant and tends to be associated with contamination (Waters, 1995).

The three most common groups are azo, anthraquinone and phthalocyanine dyes (Axelsson *et al* ., 2006), most of which are toxic and carcinogenic (Acuner and Dilek., 2004). Azo dyes, which are aromatic compounds with one or more (–N=N–) groups, represent a major group of dyes that are causing environmental concern because of its persistent colour, biorecalcitrance and potential toxic nature to the animals and humans consuming it (Ghasemi *et al* ., 2010; Samarghandi *et al* , 2007; Gharbani *et al* ., 2008; Chen *et al* ., 2004; Hilden Brand *et al* ., 1999; Jadhav *et al*., 2007; Kumar *et al* ., 2006; Martins *et al*., 2002). Several azo dyes induce liver nodules in experimental animals and there is a higher incidence of bladder cancer, spleen sarcomas, hepatocarcinomas and nuclear anomalies in experimental animals and chromosomal aberrations in mammalian cells. Few azo dyes induce liver nodules in experimental animals and there is a higher incidence of bladder cancer in dye workers exposed to large quantities of azo dyes.

Dyes can be removed from wastewater by chemical and physical methods such as including adsorption, coagulation–flocculation, oxidation and electrochemical methods (Lin and Peng., 1994, 1995, 1996). Nevertheless these techniques have been accepted and followed by textile industries; both the physical and chemical methods have many disadvantages in application. These methods involve high energy costs, results in the formation of by-products and accumulation of high loads of sludge (Sarioglu *et al* ., 2007). Accumulation of concentrated sludge leads to practical difficulty in its disposal. Presently, sludge is being deposited in to the lands owned by the textile industries converting them in to waste lands. Conversely, bioremediation can overcome these defects because it is cost saving and environmentally benign. It provides us a feasible solution by making use of the native microorganism. These microorganisms within the soil of the textile industrial areas where the dye effluents containing synthetic compounds are largely found, adapt themselves over the ages for the presence of synthetic dyes due to their persistency in its micro-environment.

Adsorption rather than degradation plays a major role during the decolourization process by fungi and algae, as a result, the dyes remain in the environment. It is well known that bacteria can degrade and even completely mineralize many azo dyes under certain conditions (Asad *et al* ., 2007; Chen *et al* ., 2004; Kapdan and Erten., 2007; Moosvi *et al* ., 2005). Bacterial azo-reductases reduce the azo compounds to their corresponding amines (Bragger *et al*., 1997). Bacterial azoreductases are more active in reducing azo dyes and are capable of converting some azo dyes to mutagenic and carcinogenic amines. Even better, the products of intermediate metabolism during the decolourization process, such as aromatic amines, can be degraded by the hydroxylase and oxygenase produced by bacteria (Pandey *et al*., 2007). There are many number of reports suggesting the use

of native bacterial isolates that have the capacity to decolourize the dyes that are commonly used in the respective textile industries (Asad *et al* ., 2007; Beydilli and Pavlostathis., 2005; Jadhav *et al* ., 2007; Kodam *et al*., 2005; Manu and Chaudhari., 2003; Maximo *et al*., 2003).

Bacterial degradation of dyes is often initiated under anaerobic conditions by an enzymatic biotransformation step (Carvalho *et al* ., 2008; Park *et al* ., 2007). The resulting products such as aromatic amines are further degraded by multiple-step bioconversion occurring aerobically or anaerobically (Barragan *et al* ., 2007; Xu *et al* ., 2006, 2007). Since many aromatic amines are carcinogenic and mutagenic decolourization of the dye containing waste water alone is not sufficient but complete degradation or detoxification is also expected in the process of bioremediation. Recently, a number of investigators report of microorganisms with the ability, not only to decolourize dyes but also detoxify it (Adedayo *et al* ., 2004; Kumar *et al* ., 2006, 2007; Rajaguru *et al* ., 2000).

Azo dye disperse red F3B with a chemical formula, $C_{20}H_{22}N_6O_2S$ (Fig. 1) has a molecular weight of 410.0 (g mol l^{-1}) was selected as the model azo dye for the characterization of the breakdown process during decolourization. Knowledge of biological decolourization of azo dye, disperse red F3B is still inadequate. Much work is still required to isolate new microorganisms capable of degrading a wide range of structurally different dyes and to study their physiological characteristics, in order not only to understand the underlying mechanisms in dye biodegradation, but also for field trials and application.

An assessment of the ecological and genetic impact of the environmental pollutants on the plant populations is of great importance as plants are important commercial products and are consumed by humans. Moreover, plants may be used as biosensors of genetic toxicity of the environmental pollutants.

In this study, we have identified a potential bacterial isolate that is capable of decolourizing and degrading Disperse Red F3B dye to about 94% within 5 h of incubation at 50°C under static conditions. Ultra Violet – Visible (UV-Vis) spectroscopy, Thin Layer Chromatography (TLC) and Fourier Transformed – Infra Red Spectroscopy (FT-IR) results have formed the basis for the degradation results. Percentage of germination and morphometric parameters in *Brassica nigra (L.)* and *Cyamopsis tetragonolobus (L.)* has revealed the eco-friendly nature of the decolourized products that are formed in the process bioremediation.

Materials and Methods

Soil samples were aseptically collected from different sites around United Bleachers (P) Ltd, (UBL), Mettupalayam, Tamil Nadu, India. All the soil samples were mixed and used for the isolation of dye-decolourizing microorganism owing to long-term usage of the location for over decades since the establishment of the industry. The soil samples were serially diluted by following the standard protocol and the dilution series of 10^{-2} to $10^{-7}$was plated in Nutrient Agar medium. Each dilution was maintained in triplicates. All the plates were incubated at 37°C for 24 h. Disperse Red F3B was procured from the same industry. Other chemicals and reagents were of Analytical grade and obtained from Himedia, India.

All decolourization experiments were performed in triplicates. A loopful of each isolated bacterial culture was inoculated into a separate 250 ml Erlenmeyer flask containing the Disperse Red F3B dye (100 mg l^{-1}) in Nutrient broth and incubated for 24 h at 37°C for initial screening of the isolates for the ability to decolourize the dye. The Disperse Red F3B had an intensive red colour and presented the highest absorbance peak (λ max) in the visible spectrum region, at 550 nm. Aliquots of the culture (3 ml) was withdrawn at different time intervals, centrifuged at 5000 rpm for 15 min to separate the bacterial cell mass.

Decolourization was determined by measuring the absorbance of the decolourization medium at 520 nm (λ max) and percentage of decolourization was calculated (Saratale *et al* ., 2006) as follows:

(%) Decolourization = (Initial absorbance – Observed absorbance) / Initial absorbance X 100

The average decolourization rate μgh-1 was calculated (Jadhav *et al* ., 2008) as follows;

Average decolourization rate = C x %D x 1000/ 100 x t

Where C is the initial concentration of dye (mg l^{-1}) and %D is the dye decolourization (%) after time't' in hours (h). A loopful of culture was inoculated in 250 ml Erlenmeyer flask containing 100 ml nutrient broth. The dye was added at the concentrations of 25, 50, 75 and 100 mg l^{-1}. Separate study was carried out for different temperatures (20, 30, 40 and 50) and pH (3-10). Decolourization was also studied under shaking (150 rpm/min) and static conditions. The composition of growth medium for this study was nutrient broth. However, to evaluate the nutrient requirements and to optimize decolourization process of the isolate, the experiments were conducted with nutrient media and minimal media (mg l^{-1}): Glucose 1800; $MgSO_4.7H_2O$ 250; KH_2PO_4 2,310; K_2HPO_4 5,550; $(NH_4)_2SO_4$ 1,980.

The genomic DNA of the strain with best decolourization potential was isolated according to the procedure described by Rainey *et al* (1996). A partial DNA sequence for 16S rRNA gene was amplified by using 5' - ATG GAT CCG GGG GTT TGA TCC TGG CTC AGG-3' (forward primer) and 5'-TAT CTG CAG TGG TGT GAC GGG GGG TGG-3' (reverse primer) (Jing *et al* . 2004). The 16SrDNA has been popular in bacterial gene identification partly because of its relatively small size which hastens sequence analysis and saves time (Madukasi *et al* ., 2011). Amplifications were performed in 50 μl reaction mixtures containing the template DNA, 40ng, 0.2 μM, for each of the primers, dNTPs 200μM, Taq DNA polymerase 2.5U and 10X Taq buffers 5μl. The mixture was subjected to the following amplification conditions; 2 min at 94^{o}C, followed by 30 cycles of 94^{o}C for 1 min, and ended by a final extension step at 72^{o}C for 7 min. The Ploymerase Chain Reaction (PCR) products were electrophoresced on 0.7% agarose gels. The PCR reaction mixture was then sent for sequencing (Chromous Biotech Pvt. Ltd.,). The nucleotide sequence analysis of the sequence was done at Blast-n site at NCBI server http://www.ncbi.nlm.hin.gov/BLAST).

The alignment of the sequence was done by using CLUSTALW program V1.82 at European bioinformatics site

http://www.ebi.ac.uk/clustalw). The sequence was refined manually after crosschecking with the raw data to remove ambiguities and submitted to the NCBI. Physiological characteristics were determined according to the procedures outlined in Bergey's Manual of Determinative Bacteriology.

Samples collected at intermediate and decolourization end point were centrifuged at 10,000 rpm for 15 minutes and the cell-free supernatant was used for the spectrophotometric analysis in Shimadzu UV-1800 (Japan). The dye free medium was used as blank and sterile medium containing 100 mg l^{-1} of Disperse Red F3B was used as the reference for obtaining the spectrum.

The metabolites obtained during the decolourization of the Disperse Red F3B dye was extracted with equal volume of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness in rotary evaporator. The crystals obtained were dissolved in small volume of HPLC grade methanol and the same sample was used for TLC and FTIR analysis. Changes in the absorption spectrum (400-4000) were recorded. TLC analysis was carried out on a silica gel using mobile phase solvent system ammonia, methanol, n- butanol and distilled water (5:2:2:1). The chromatogram was allowed to develop and observed under UV illumination.

The phytotoxicity of the Disperse Red F3B dye was performed to evaluate the toxicity of the dye before and after decolourization in the concentration range of 100 ppm. The study was carried out in *Brassica nigra (L.)* and *Cyamopsis tetragonolobus (L.)*. Emergence of shoot of 0.5cm in length or more was taken as germinated seeds and the germination percentage was determined. Delay index (DI), a normalized parameter, was calculated to compare the performance of the dye and biodegraded dye as given below (Garg and Kaushik., 2007).

$$DI = X/Y$$

Where,
DI = Delay Index
X = delay in germination time over control (no effluent) and
Y = germination time for control

Root length and shoot length of *Brassica nigra (L)* and *Cyamopsis tetragonolobus (L)* were recorded after 15 and 25 day.
The data in this study were analyzed using the SPSS package, and all the values that are represented are the mean ± SE. Students t-tests were used to compare the effect of biodecolourization with that of the native dye. The probability levels used for statistical significance were $P<0.05$ for the tests.

Results and Discussion

From serial dilution of the soil sample taken from the textile industrial area, 46 different isolates were obtained. For the convenience, these isolates were assigned strain numbers from UBL-01 to UBL-46, based on the abundance of the isolates in the serially diluted plates. On screening all the 46 isolates for the ability to decolorize Disperse Red F3B, strain UBL-02 alone exhibited a decolourization rate of 3467µg h^{-1} (Table.1) and was chosen to be used in the latter experiments. Reduction in cell growth and decolourization rate by other bacterial strains might result from the toxicity of disperse red F3B through the inhibition of metabolic activities. Disperse red F3B, like any other azo dyes contains a

Table.1 Average decolorization rates of Disperse Red F3B by individual strains, calculated as μg of dye decolorized per hour**.**

Strain Name	Average decolorization rate (μg h^{-1})
UBL 01	1095
UBL 02	3467
UBL 03	942
UBL 04	1155
UBL 05	1003
UBL 06	1064
UBL 07	790
UBL 08	1064
UBL 09	942
UBL 10	882
UBL 11	1095
UBL 12	821
UBL 13	790
UBL 14	1034
UBL 15	942
UBL 16	1155
UBL 17	1034
UBL 18	730
UBL 19	790
UBL 20	1064
UBL 21	790
UBL 22	882
UBL 23	730
UBL 24	547
UBL 25	1429
UBL 26	273
UBL 27	821
UBL 28	730
UBL 29	1095
UBL 30	1003
UBL 31	942
UBL 32	790
UBL 33	1155
UBL 34	1247
UBL 35	669
UBL 36	1003
UBL 37	790
UBL 38	882
UBL 39	608
UBL 40	1247
UBL 41	730
UBL 42	ND
UBL 43	638
UBL 44	730
UBL 45	2219
UBL 46	1064
ND - No Decolourization	

Table.2 Biochemical profile of *Enterococcus faecalis*

Biochemical Character	Response
N-Acetylglucosamine	+
L-Arabinose	-
Cellobiose	+
Dextrin	+
D-Fructose	+
Galactose	+
D-Glucose	+
Glycerol	+
Glycogen	-
Lactose	+
Maltose	+
Mannitol	+
D-Mannose	+
Melibiose	-
D-Raffinose	-
Ribose	+
Sorbitol	+
Sucrose	+
Growth in 6.5% NaCl	+
Growth in 0.1% Methylene blue milk	+
H2S production	-
Alpha hemolysis	+
Beta hemolysis	+
Lancefield group D	+
Motility	-
Voges-Proskauer	+
Yellow pigment	-
Catalase	-
Esculin hydrolysis	+
+ Positive; *- Negative*	

Table.3 Average decolorization rate of Disperse Red F3B by *Enterococcus faecalis* UBL 02 under optimal conditions, calculated as μg of dye decolorized per hour.

Average decolorization rate (μg h-1)	pH	T^0	Nutrient	Condition	Time taken(hours)
18,800	8	50^0C	Nutrient broth	Static	5 h

Table.4 Phytotoxicity study of disperse Red F3B and its metabolites formed after biodegradation.

Parameters studied	***Brassica nigra***	**D. Red F3B**	**Degraded Product**	***Cyamopsis tetragonolobus****		
	Control			**Control**	**D. Red F3B**	**Degraded Product**
Germination %	100	28.4±1.02	83.6±1.8	100	33.2±1.55	87.25±2.36
Delay index	0	0.33	0	0	0.715	0.28
Shoot length	10.4±0.71[a]	6±0.41[b]	9.2±0.68[a]	15.6±1.1[a]	8.4±0.9[b]	14±1.6[a]
Root length	1.7±0.52[a]	2±0.14[a]	1.6±0.09[a]	3.6±1.1[a]	3.4±1.4[a]	3.3±1.6[a]

*Values followed by same letters in a row are not significantly different ($p \leq 0.05$)

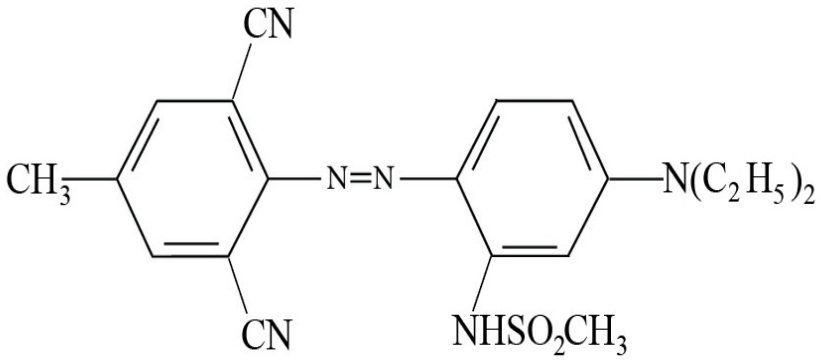

Figure 1. Structure of Disperse Red F3B

sulphonic-acid group on the aromatic ring, which might act as detergent, thereby inhibiting the growth of the microorganisms. Such dyes may affect DNA synthesis since it has also been reported that dyes are inhibitors of the nucleic acid syntheses, or cell growth (Asad., 2007).

The strain UBL-02 was subjected to morphological and physiological characterization (Table 2) and was identified as *Enterococcus fecalis* (Albert and Anciet., 1999). To further confirm the identity of the isolate, 16SrDNA genes was partially sequenced following PCR amplification and it was compared with the sequences deposited in the database (Madukasi *et al* ., 2011*)*. The phylogenetic tree showed that the isolate UBL 02 was an unidentified species and was closely related to *Enterococcus faecalis* species showing similarity of 99%. The sequence has been submitted at the GenBank under the accession no. HM451428.

There was no significant decolourization of Disperse Red F3B in minimal broth by UBL-02 and therefore, further optimization of the conditions was

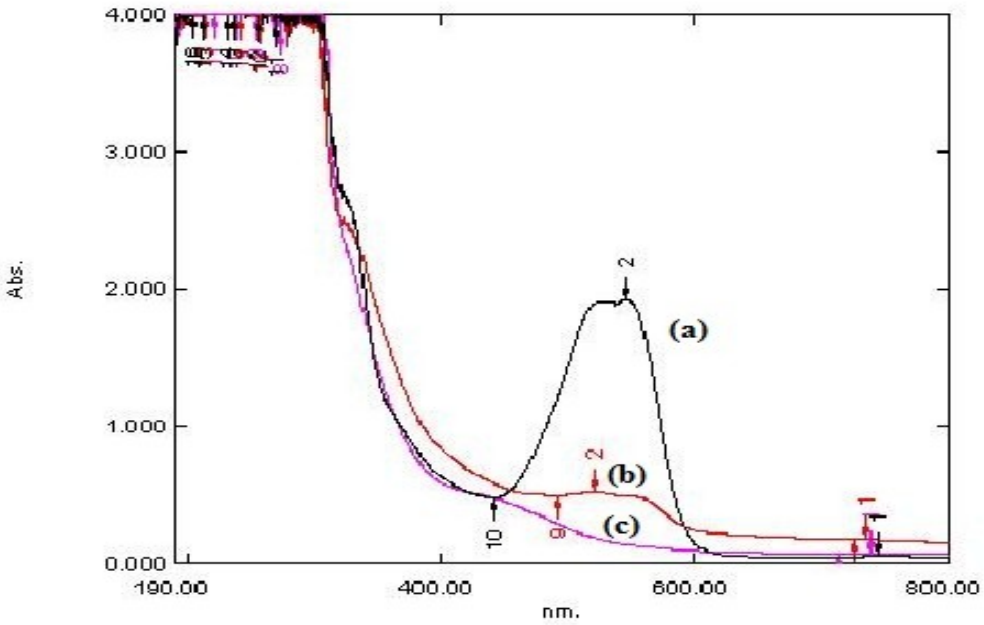

Figure 2. UV-Vis spectra of Disperse red F3B biodegradation at 0 h (a), 2.5 h (b) and 5 h (c).

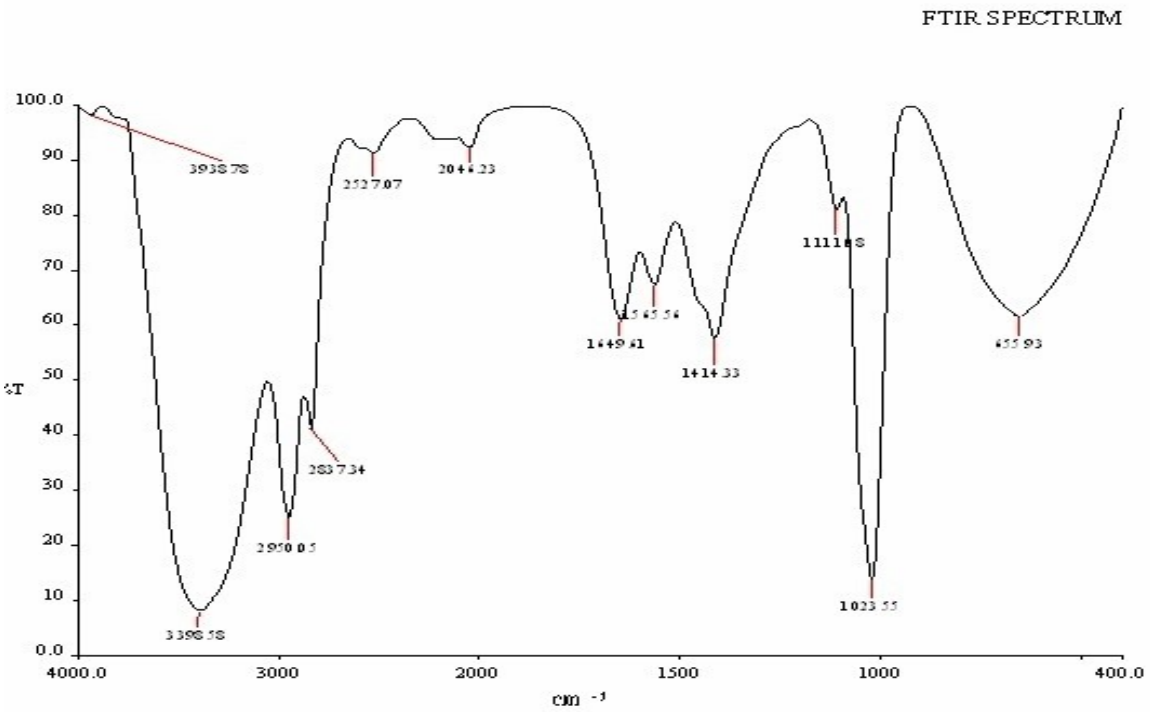

Figure 3. FT-IR spectrum of Disperse Red F3B before decolourazation.

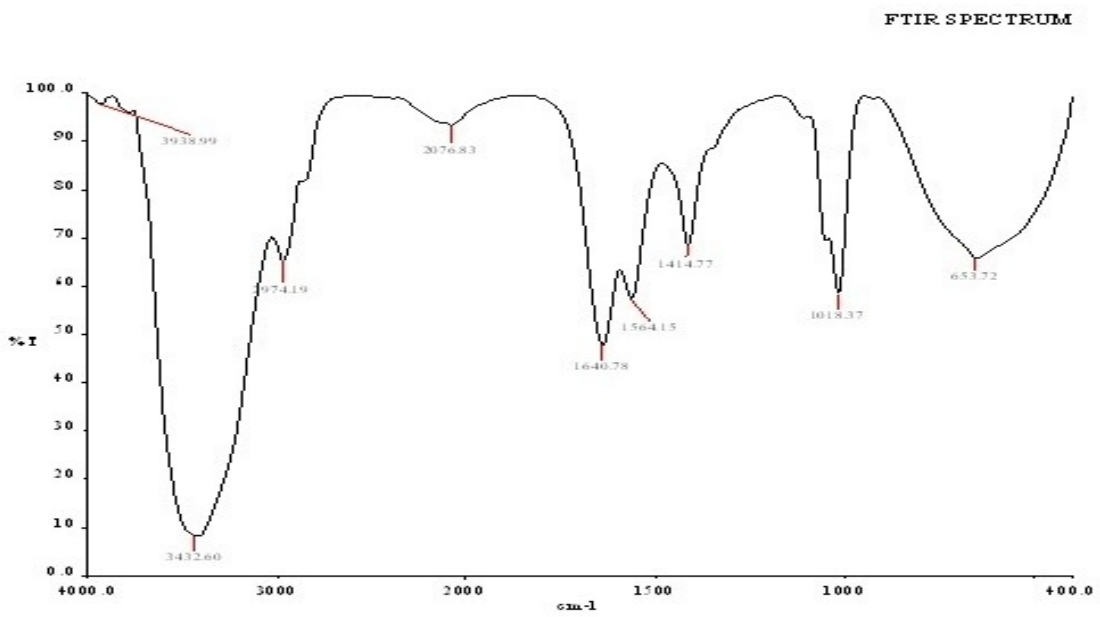

Figure 4. FT-IR spectrum degraded Disperse Red F3B after 5 h.

performed in nutrient broth. Two opinions have been argued for many years: one deems that dyes are not a carbon source since the anaerobic/facultative anaerobic bacteria obtain energy from the glucose instead of the dyes and glucose can enhance the decolourizing performance of biological systems (Sarioglu and Bisgin., 2007); while another deems that glucose can inhibit the decolourizing activity (Chen *et al* ., 2004). The variability may be due to the different microbial characteristics involved. Our results showed that a certain concentration of carbon source was necessary for the *Enterococcus fecalis* UBL 02 decolourizing process. The results clearly indicated that decolourization was greatly affected by addition of yeast extract as nitrogen sources. Metabolism of the yeast extract is considered essential for regeneration of NADH, which is the electron donor for azo bond reduction (Craliell *et al* ., 1995). This is in accordance with other reports of Chen *et al* . 2004; Kodam *et al* .,(2005); Moosvi *et al* ., (2005) stating that, the best decolourization was achieved with the yeast extract as nitrogen source.

The success of bioremediation is dependent on the inherent biodegradability of the pollutant, the accessibility of the pollutant to degrading microorganisms and on the optimization of biological activity (Yousefi Kebria *et al* ., 2009; Sivaraman *et al* ., 2011). When decolourization pattern for the Disperse Red F3B was studied under different conditions, it was found that the dye even at a high concentration of 100 mg l^{-1} was decolourized completely to 94% within 5 h of incubation at pH 8, under 50°C of temperature and static condition with an average decolourization rate of 18,800μgl-1. The average decolourization rate per hour of the *Enterococcus fecalis* UBL 02 has increased 5.4 folds from 3467 μg h^{-1} to 18,800 μg h^{-1} (Table 3) after optimization of the pH, temperature and static conditions.

Our findings show that the capacity of *Enterococcus fecalis* to decolourize

Disperse Red F3B under static and non-aerated condition is similar to that of others (Haiyan *et al* ., 2010; Widhi *et al* ., 2007) on decolourization of reactive red 2 and acid red 27 under static and non-aerated conditions. *Enterococcus fecalis* is also reported for a faster rate of decolourization of reactive red and acid red 2 within 12 h at a conc. of 100 mg l^{-1} (Widhi *et al* ., 2007).

This result suggested that *Enterococcus fecalis* UBL 02 was a type of facultative anaerobe. Oxygen was favorable to the growth of the bacterium but deleterious to the yield process of the degradation related enzyme. The result accorded with the conclusion that none of these decolourizing bacteria are able to efficiently decolourize dyes under aerobic conditions (Khehra *et al* ., 2005; Moosvi *et al* ., 2005). Dissolved oxygen often inhibited the anaerobic decolourization of azo dyes, because oxygen was a preferable terminal electron receptor over the azo groups. During the dye reduction stage, if the extra-cellular environment is aerobic, the high-redox-potential electron acceptor, oxygen, may inhibit the dye reduction mechanism (Pearce *et al* ., 2003).

Decolourization of dyes may take place by adsorption (Aravindhan *et al* ., 2007) or Degradation (Kumar *et al* ., 2007). In the case of adsorption, dyes are only adsorbed onto the surface of bacterial cells, whereas new compounds come into being when dyes are degraded by bacterial enzymes during the degradation process. In adsorption, examination of the absorption spectrum will reveal that all peaks decrease approximately in proportion to each other. If the dye removal is attributed to biodegradation, either the major visible light absorbance peak will completely disappear or a new peak will appear (Yu and Wen, 2005).

The UV-Vis spectra of the media containing the dye before decolourization showed a maximum absorption at 550 nm (0 hour). In the intermediate stage of decolourization, the λ max shifted to 520 nm (2.5 h) and in the final stage after decolourization, the absorption maxima once again disappeared from 520 nm and appeared at 490 nm. Therefore, shifting of peaks from 550 nm to 520 nm and a complete disappearance of peak at 550 nm (5 h) is a clear evidence of molecular rearrangements in the dye structure and biodegradation thereof (Fig. 2). Dye adsorption can be also easily judged by an evidently coloured cell pellet, whereas those retaining their original colours are accompanied by the occurrence of biodegradation (Chen *et al* ., 2004). The observation of *Enterococcus faecalis* cell mass retained their natural colour after decolourization of Disperse red F3B, further confirms that the dye has undergone biotransformation.

The great changes occurring both in UV and visible spectra indicated that the molecular structure of Disperse Red F3B changed evidently after decolourization. The red colour of Disperse Red F3B was caused by the conjugated structure of azo bonds (chromophore) and amino group. It could be presumed that the azo bonds cleaved during the reaction which indicated that the primary chromophore was destroyed (Hui *et al* ., 2009). The absorbance peak at UV spectra did not disappear in the end of decolourization, which indicated that Disperse Red F3B was not completely mineralized while some new metabolites formed in the culture.

The comparison of TLC chromatograms of the media extracted by the organic phase before and after decolourization by the UBL 02 strain under UV light showed that the decolourized sample had two new bands, which might have originated from the dye metabolites. Aromatic amines are the usual decolourization products of azo dyes that appear in the organic phase extract. Comparison of chromatograms of the aqueous phase extracts of the samples before and after treatment also showed the disappearance of the dye band in decolourized media indicating complete decolourization. The parent dye compound disperse ded F3B resolved into a single spot of Rf value 0.156 in TLC.

The solvent extracted sample of the same after decolourization by *Enterococcus fecalis* UBL 02 showed two new spots with Rf values 0.187 and 0.281 respectively.

When the Disperse Red F3B was subjected to FTIR analysis, before and after decolourization, it showed several peaks that represented rearrangements in the molecular structure of the compound. The FTIR spectrum of the control (Fig. 3) displayed a peak at 3432 cm^{-1}, 2950 cm^{-1}, and 1640 cm^{-1} for free $–NH_2$ due to asymmetric stretching vibration, $-CH_3$ for stretching vibration and –OH stretching vibration respectively. A broad peak near to 655 $cm^{-1}$for the monosubstitution phenyl derivative and 1640 cm-1 indicated the aromatic nature of the dye. The peak near 1023 cm^{-1} for S=O indicated sulphoxide nature of the dye. FTIR report of the degradation product (Fig. 4) displayed 3398 cm^{-1} for OH stretching, a peak at 1640 cm^{-1} for C=N and Fermi resonance band at 2950 cm^{-1} for –CH3 indicated the formation of imines. FTIR analysis confirmed the biotransformation of the dye into imines. These results provide obvious evidence of biodegradation of Disperse Red F3B by *Enterococcus fecalis* UBL 02 in the decolourization process, and also supported the earlier conclusion that decolourization by bacteria is due to biodegradation, rather than inactive surface adsorption.

Despite the fact that untreated dyeing effluent may cause serious environmental and health hazards, they are being disposed off in water bodies and this water is being used for agriculture purposes (Jadhav *et al* ., 2010; Kalyani *et al* ., 2008; Saratale *et al* ., 2009). Therefore, evaluation of toxicity of the dye and its degraded product was needed to be tested on the plant species. Disperse Red F3B (100 ppm) showed 28.4±1.02 % and 33.2±1.55 % of germination in *Brassica nigra* and *Cyamopsis tetragonolobus* respectively when compared to the control (Table 4).

Metabolites of Disperse Red F3B did not show inhibition in the germination of *Brassica nigra* and *Cyamopsis tetragonolobu*s. Delay index in this experiment is a measure and an indication of the effect of Disperse Red F3B before and after biodegradation, and demonstrated a complete detoxification of the dye with respect to germination and growth of these two plants. Likewise, shoot length also showed significant reduction in both *Brassica nigra* (6±0.41cm) on day 15 and *Cyamopsis tetragonolobus* (8.4±0.9cm) on day 25 when compared to the control.

However, there was no significant change in the root length. There is not adequate information regarding the precise mechanisms affecting the reduction in germination rate, above ground biomass and shoot height (Sharifi *et al ., 2007)*.

Phytotoxicity studies confirmed the detoxification of the dye with respect to *Brassica nigra* and *Cyamopsis tetragonolobus.* All these experimental demonstration clearly suggests that *Enterococcus fecalis* UBL 02 could be of great importance and use to decolourize complex dyestuff effluent containing various azo dyes.

The strain UBL 02 isolated from the textile effluent polluted soil was identified by 16srDNA gene as *Entercoccus faecalis.* A representative type of azo dye, named disperse red F3B was rapidly decolourized within 5 h under static (microaerophilic) condition, as confirmed by the UV – Vis and TLC analysis. The formation of imines during the microaerophilic stage was confirmed by FTIR analysis, reveling partial mineralization of the dye degradation products. The decolourized products of the dye did not show any significant phytotoxicity when tested on *Brassica nigra* and *cyamopsis tetragonolobus.* Thus the novel isolate used in the present study appears to be a promising alternative to replace or supplement currently available biological treatment process enabling the re-use of biologically treated textile wastewater for irrigation as well.

References

Acuner, E., and Dilek, F. B. 2004. Treatment of tectilon yellow 2G by Chlorella vulgaris. Process Biochemistry . 39: 623–631.

Adedayo, O., S. Javadpour, W. A. Taylor Anderson and Moo-Young M. 2004. Decolorization and detoxification of methyl red by aerobic bacteria from a wastewater treatment plant, World J. Microbiol Biotechnol. 20: 545–550 .

Ajibola, V.O., S. J .Oniye ,C. E. Odeh ,T. Olugbodi and Umeh U. G. 2005. Biodegradation of Indigo Containing Textile Effluent using some strains of Bacteria. Journal of Applied Sciences. 5(5**)**: 853- 855 .

Aksu, Z., N. K. Kilic, S. Ertugrul and Donmez, G. 2007. Inhibitory effects of chromium (VI) and Remazol Black B on chromium (VI) and dyestuff removals by Trametes versicolor. Enzyme and Microbial Technology. 40: 1167–1174 **.**

Albert Manero., and Anicet Blanch, R. 1999. Identification of Enterococcus spp with a biochemical key. Applied and Environmental Microbiology. 65 (10): 4425-4430.

Aravindhan, R., J. R. Rao and Nair, B. U. 2007. Removal of basic yellow dye from aqueous solution by adsorption on green algae Caulerpascal pelliformis. Journal of Hazardous Materials. 142: 68–76 **.**

Asad, S., M.A. Amoozegar, A. A. Pourbabaee, M. N. Sarbolouki and Dastgheib, S. M. M. 2007. Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. Bioresour. Technol. 98: 2082–2088.

Axelsson, J., U. Nilsson, E. Terrazas, T.A. Aliaga and Welander, U. 2006. Decolorization of the textile dyes Reactive Red 2 and Reactive Blue 4 using Bjerkandera sp. Strain BOL 13 in a continuous rotating biological contactor reactor. Enzyme and Microbial Technology. 39: 32–37 .

Barragan, B.E., C. Costa and Marquez, M. C. 2007. Biodegradation of azo dyes by bacteria inoculated on solid media. Dyes and Pigments. 75: 73–81.

Beydilli, M. I., and Pavlostathis, S. G. 2005. Decolorization kinetics of the azo dye Reactive Red 2 under ethanogenic condition: effect of long-term culture acclimation, Biodegradation. 16: 135–146 **.**

Bragger, J. L., A. W. Lloyd, S. H. Soozanehfar , S. C. Bloomfield , Marriootte. C and Martin, G. P. 1997. Investigations into the azo reducing activity of a common colonic microorganism. Int J Pharmaceut. 157: 61-71.

Carvalho, M. C., C. Pereira ,I. C. Goncalves , H. M. Pinheiro , A. R.Santos , A. Lopes

and Ferra M. I. 2008. Assessment of the biodegradability of a monosulfonatedazo dye and aromatic amines. International Biodeterioration & Biodegradation. 62: 96–103.

Chen, H., R. F. Wang , and Cerniglia C. E. 2004. Molecular cloning, over expression, purification and characterization of an aerobic FMN dependent azoreduction from Enterococus faecalis. Protein Exp. Purif. 34: 302–310.

Chinwetkitvanich, S., M. Tuntoolvest and Panswad, T. 2000. Anaerobic decolorization of reactive dye bath effluents by a two-stage uasb system with tapioca as a co-substrate. Water Research. 34 : 2223-2233 **.**

Garg, V. K., and Kaushik, P. 2007. Influence of textile mill wastewater irrigation on the growth of Sorgum cultivars. Applied ecology and environmental research. 6(2): 1-12 **.**

Gharbani, P., S. M. Tabatabaii and Mehrizad, A. 2008. Removal of Congo red from textile wastewater by ozonation . Int. J.Environ. Sci. Tech. 5 (4): 495-500 **.**

Ghasemi, F., F. Tabandeh , B. Bambai and Sambasiva Rao, K. R. S. 2010. Decolorization of different azo dyes by Phanerochaete chrysosporium RP78 under optimal condition. Int. J. Environ. Sci. Tech. 7 (3): 457-464 .

Haiyan Xu., M. Thomas Heinze, D. Donald Paine, E. Carl Cerniglia and Huizhong Chen. 2010. Sudan azo dyes and Para Red degradation by prevalent bacteria of the human gastrointestinal tract. Anaerobe. 16 (2): 114-119 .

Hao O.J., H. Kim and Chiang, P. C. 2000. Decolorization of wastewater. Critical Reviews in Environmental Science and Technology. 30: 449–505 .

Hui Wang., Jian Qiang Su, Xiao Wei Zheng, Yun Tian, Xiao Jing Xiong and Tian Ling Zheng. 2009. Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by Citrobacter sp. CK3. International Biodeterioration & Biodegradation. 63: 395–399 **.**

Jadhav, J. P., D. C. Kalyani, A. A. Telke, S. S. Phugare and Govindwar, S. P. 2010. Evaluation of the efficacy of a bacterial consortium for the removal of color, reduction of heavy metals, and toxicity from textile dye effluent. Bioresource Technology. 101: 165–173 **.**

Jadhav, J. P., G. Parshetti , S. D. Kalme and Govindwar, S. P. 2007. Decolorization of azo dye, methyl red by Saccharomyces cerevisiae MTCC 463. Chemosphere. 68: 394–400 .

Jadhav, U. U., V. V. Dawkar, G. S. Ghodake and Govindwar, S. P. 2008. Biodegradation of direct red 5B, a textile dye by newly isolated Comamonas sp. UVS.J. Hazrd. Mater. 158: 507–516 **.**

Jing X., G. Shaorong, T. Yong, G. Ping, L. Kun and Shigul L. 2004. Antagonism and molecular identification of an antibiotic bacterium BS04 against phytopathogenic fungi and bacteria. High Technol. Lett. 10 (3): 47-51 .

Kalyani, D. C., P. S. Patil, J. P. Jadhav and Govindwar, S. P. 2008. Biodegradation of reactive textile dye red BLI by an isolated bacterium Pseudomonas sp. SUK1. Bioresour Technol. 99: 4635–4641 .

Kapdan, I. K., and Erten, B. 2007. Anaerobic treatment of saline wastewater by Halanaerobium lacusrosei. Process Biochemistry. 42: 449–453 .

Khehra, M. S., H. S. Saini, D. K. Sharma, B. S. Chadha and Chimmi, S. S. 2005. Decolorization of various azo dyes by bacterial consortium. *Dyes and Pigments.* 67: 55–61 **.**

Kodam, K. M., I. Soojhawon, P.D. Lokhande and Gawai, K. R. 2005. Microbial decolorization of reactive azo dyes under aerobic conditions. World J. Microbiol. Biotechnol. 21: 367–370.

Kumar, K., S. S. Devi, K. Krishnamurthi, D. Dutta and Chakrabarti, T. 2007. Decolorization and detoxification of Direct Blue-15 by a bacterial consortium. Bioresour. Technol. 98: 3168–3171 .

Kumar K., S. S. Devi, K. Krishnamurthi, S. Gampawar, N. Mishra, G. H. Pandya and Chakrabarti, T., Decolorization, biodegradation and detoxification of benzidine based azo dye. Bioresour. Technol. 97: 407–413 **.**

Lin, S. H. and Peng, C. F. 1994. Treatment of textile wastewater by electrochemical method. Water Research. 28: 277–282.

Lin, S. H., and Peng, C. F. 1995. Treatment of textile wastewater by Fenton's reagent, Environmental Science. Health Part A. Environ. Sci. Eng. A30 (1): 89–98.

Lin S. H., and Peng, C. F. 1996. Continuous treatment of textile wastewater by combined coagulation, electrochemical oxidation and activated sludge. Water Research. 30: 587–592 .

Madukasi, E. I., Chunhua, H. & Zhang, G. 2011. Isolation and application of a wild strain photosynthetic bacterium to environmental waste management. Int. J. Environ. Sci. Tech. 8 (3):513-522 .

Manu, B. and Chaudhari, S. 2003. Decolorization of indigo and azo dye in semi continuous reactors with long hydraulic retention time. Process Biochem. 38: 1213–122.

Mariana Neamtu., Ayfer Yediler, Ilie Siminiceanu, Matei Macoveanu and Antonius Kettrup. 2004. Decolorization of disperse red 354 azo dye in water by several oxidation processes—a comparative study. Dyes and Pigments. 60 (1): 61-68.

Martins, M. A. M., Queiroz, M. J., Silvestre, A. J. D. and Lima, N. 2002.Relationship of chemical structure of textile dye on the pre adaptation medium and the potentialities of their biodegradation by Phanerochaete chrysosporium. Res. Microbiol. 153: 361–368.

Maximo, C., Amorim, M. T. P., and Costa Ferreira, M. 2003.Biotransformation of industrial reactive azo dyes by Geotrichum sp. CCMI 1019. Enzyme Microb. Technol. 32: 145–151.

Moosvi, S., Keharia, H., and Madamwar, D.2005. Decolourization of textile dye Reactive Violet 5 by a newly isolated bacterial consortium RVM 11.1. World Journal of Microbiology & Biotechnology. 1: 67–672.

Pandey, A., Singh, P., and Iyengar L.2007. Bacterial decolorization and degradation of azo dyes, International Biodeterioration & Biodegradation. 59:73–84.

Park, C., Lee, M., Lee, B., Kim, S. W., Chase, H. A., Lee, J. and Kim, S.2007. Biodegradation and biosorption for decolorization of synthetic dyes by Funaliatrogii. Biochemical Engineering Journal. 36: 59–65 **.**

Pearce, C. I., Lloyd, J. R. and Guthri, J. T.2003. The removal of colour from textile wastewater using whole bacterial cells: a review. Dyes Pigments. **58**, 179–186.

Rainey, F. A., Ward Rainey, N., Kroppenstedt, R. M. and Stackebrandt, E.1996. The genus Nocardiopsis represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of Nocardiopsaceae fam. Nov. International Journal of Systematic Bacteriology. 4: 1088–1092.

Rajaguru, P., Kalaiselvi, K., Palanivel, M., and Subburam, V.2000. Biodegradation of azo dyes in a sequential anaerobic–aerobic system. Appl. Microbiol. Biotechnol. 54: 268–273.

Samarghandi, M. R., Nouri, J., Mesdaghinia, A. R., Mahvi, A. H., Nasseri, S. and Vaezi, F.2007. Efficiency removal of phenol, lead and cadmium by means of UV/TiO2/H2O2 processes, Int. J. Environ. Sci. Tech. 4 (1)**:**19-25**.**

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

Saratale, R. G., Saratale, G. B., Kalyani, D. C., Chang, J. S., and Govindwar S. P. 2009. Enhanced decolorization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium-GR. Bioresource Technology. 100: 2493–2500 **.**

Sarioglu, M., Bali, U. and Bisgin, T. 2007. The removal of C.I. Basic Red 46 in a mixed methanogenic anaerobic culture. Dyes and Pigments. 74: 223–229 .

Sharifi, M., Sadeghi, Y. and Akpapour, M. (2007).Germination and growth of six plant species on Contaminated soil with spent oil. Int. J. Environ. Sci. Tech. 4 (4): 463-470.

Sivaraman, C., Ganguly, A., Nikolausz, M. and Mutnuri, S. 2011. Isolation of hydrocarbonoclastic bacteria from bilge oilcontaminated waters. Int. J. Environ. Sci. Tech. 8 (3): 461-470.

Waters, B. D. 1995. The dye regulator's view. In: Cooper P, editor. Colour in dyehouse effluent. Bradford, UK: Society of Dyers and Colorists. 23–26.

Widhi Handayani., Irene Meitiniarti V. & Krisherawan Timotius.2007. Decolorization of Acid Red 27 and Reactive Red 2 by Enterococcus faecalis under a batch system. World J Microbiol Biotechnol. 23: 1239- 1244 .

Xu, M., Guo, J., and Sun, G.2007. Biodegradation of textile azo dye by Shewanellade colorationis S12 under microaerophilic conditions. Applied Microbiology and Biotechnology. 76: 719–726.

Xu, M. Y., Guo, J., Zeng, G. Q., Zhong, X. Y., and Sun, G. P.2006. Decolorization of anthraquinone dye by Shewanella decolorationis S12. Applied Microbiology and Biotechnology. 71: 246– 251 .

Yousefi Kebria, D. Y., Khodadadi, A, Ganjidoust, H, Badkoubi, I., and Amoozegar, M. A. 2009. Isolation and characterization of a novel native Bacillus strain capable of degrading diesel fuel. Int. J. Environ. Sci. Tech. 6 (3): 435-442.

Yu, Z. S., and Wen, X. H. 2005. Screening and identification of yeasts for decolorizing synthetic dyes in industrial wastewater. International Biodeterioration and Biodegradation. 56: 109–114.