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Communal action of microbial cultures for Red HE3B degradation

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ABSTRACT

The consortium PMB11 consisting of three bacterial species, originally isolated from dye contaminated soil was identified as *Bacillus odysseyi* SUK3, *Morganella morganii* SUK5 and *Proteus* sp. SUK7. The consortium possessed the ability to decolorize various textile dyes as well as mixtures of dyes. PMB11 could decolorize Red HE3B (50 mg l^{-1}) with 99% of decolorization within 12 h in nutrient broth, while in mineral medium it could decolorize up to 97% within 24 h. Induction in the activities of various oxidative and reductive enzymes indicates the involvement of these enzymes in decolorization. Biodegradation of the dye was monitored using UV–vis spectroscopy, HPLC and FTIR analysis. The Red HE3B degradation pathway was proposed by GC–MS analysis. Various metabolites formed after the degradation were identified as 2,5-diaminobenzene 6-aminotriazine, aniline 2-sulfate, aniline 3-sulfate, 2-amino 5-chlorotriazine and naphthalene. Phytotoxicity studies revealed that metabolites formed after degradation were significantly less toxic in nature.

1. Introduction

The textile dyeing and finishing industry produces large volumes of high strength aqueous waste continuously, leading to major environmental pollution problems. Annual consumption of dyes is around 7×10^4 tones throughout the world, out of which 50% of dyes are lost in manufacturing and processing units [1]. In India an average mill producing 60×10^4 m of fabric, discharges approximately 1.5 million liters of effluent per day [2].

Azo dyes are characterized by the presence of one or more –N=N–(azo) bonds. Reactive group of azo dyes are mostly used in textile dyeing due to their superior fastness to the applied fabric, high photolytic stability, and resistance to microbial degradation. However, reactive dyes exhibit low levels of fixation with the fiber and about 10–20% of total dyes used in dyeing process remain left in the effluent [3,4]. The discharge of highly colored dye effluents from industries to nearby water bodies can result in to serious water pollution problems because color is the first contaminant to be recognized in water. Hence color has to be removed before discharging the wastewater into a receiving water body or on the land.

Moreover the physical and chemical methods have disadvantages of being highly expensive, coupled with the formation of sludge and the emission of toxic substances [5]. Microbial decolorization has received much attention, because of its ecofriendly nature. Currently an extensive research is focused to find out optimal microbial biomass, which is as cheap as possible for removal of contaminating dyes from large volumes of polluted water [6]. Recent research has exposed the survival of a wide variety of microorganisms including white-rot fungi, bacteria and mixed cultures capable of decolorizing wide range of dyes [7,8]. Microbial consortium from separately isolated strains can be constructed artificially to give optimum performance for the selected ecological function. Microbial consortia are usually used without analyzing the constituent microbial populations for environmental remediation. Complexity of the microbial consortium, enables them to act on a variety of pollutants [9]. Several bacterial consortia capable of decolorizing azo dyes have been reported earlier [10–15]

In the present study, we have focused our attention on the decolorization and degradation of reactive textile dye. This article reports studies on Red HE3B decolorization by individual bacterial strains as well as consortium PMB11. Red HE3B is one of the diazo dyes and has a very large consumption rate in the textile dyeing processes. The dye is soluble in the water and has a λ_{max} of 510 nm. Various experimental conditions have been optimized for decolorization of Red HE3B and effect of media compositions on decolorization was also investigated.

2. Materials and methods

2.1. Chemicals

Red HE3B and other reactive dyes (Navy Blue RX, Navy Blue HE2R, Red HE8B, Red HE18, Red 6BI, Magenta MB, Green HE4BD and Methyl orange) used in decolorization studies were obtained

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from the textile industry Ichalkaranji, India. 2,2′-Azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) was obtained from Sigma Aldrich, USA. Nicotinamide adenine dinucleotide reduced disodium salt (NADH) and catechol from SRL Chemicals, India. Bacteriological peptone, yeast extract, beef extract, glucose and lactose were obtained from Hi-media Pvt. Ltd., Mumbai, India.

2.2. Media composition

Decolorization experiments were carried out in the synthetic medium containing ($g\,l^{-1}$): K_2HPO_4 3, Na_2HPO_4 6, NH_4Cl 4, NaCl 5, $MgSO_4$ 0.1 and supplemented with yeast extract 5. Synthetic medium was used to study the effect of carbon and nitrogen sources on decolorization in the 100 ml culture broth. Additionally (0.5%) organic and inorganic carbon and nitrogen sources were used individually as well as in combination. In addition, the decolorization of Red HE3B was also studied in nutrient broth having composition ($g\,l^{-1}$): peptone 10, NaCl 5 and beef extract 3.

2.3. Experimental procedure

2.3.1. Isolation and identification

Isolation of bacterial species was carried out from soil contaminated with waste water of textile processing and dye manufacturing unit, Ichalkaranji (India), by enrichment culture technique. The morphologically distinct bacterial strains were selected for the dye decolorization study. Pure cultures were maintained on the nutrient agar medium having composition ($g\,l^{-1}$): peptone 1, NaCl 5, beef extract 3 and agar 2.5. Consortium was developed by mixing each bacterial isolates (5% v/v having optical density 0.60 at 620 nm) in 100 ml medium and the flasks were incubated at 30 °C for 24 h [16].

2.3.2. Decolorization studies

Consortium PMB11 was grown in 100 ml synthetic medium with yeast extract for 24 h at 30 °C and routinely used for decolorization purpose. Red HE3B ($50\,mg\,l^{-1}$) was added to the culture broth containing 24 h grown bacterial consortium. Abiotic controls without bacterial consortium were kept to analyze abiotic loss of the dye. The aliquots (3 ml) were withdrawn, centrifuged (6000 rpm, 10 min) to separate bacterial biomass and absorbance of the supernatant was measured at respective wavelength. The percent decolorization was calculated using the formula; % Decolorization = $A - B/A \times 100$; where A is initial absorbance and B is observed absorbance.

2.3.3. Various textile dyes and dye mixture decolorization

The percentage decolorization of the different dyes as, Navy Blue RX, Navy Blue HE2R, Red HE8B, Red HE18, Red 6BI, Magenta MB, Green HE4BD and Methyl orange ($50\,mg\,l^{-1}$) and the mixture of dyes, were determined by using synthetic medium supplemented with yeast extract. Various dye mixtures were prepared by using combination of five different dyes (1 mg of each dye) and added to the 24 h grown 100 ml bacterial consortium PMB11.

2.3.4. Analytical methods

The supernatant samples of the individual bacterial strains and consortium obtained at 0 h and after decolorization were subjected to spectral analysis. UV–vis spectral analysis was carried out using UV–vis spectrophotometer (Hitachi U 2800, Tokyo, Japan) and changes in its absorption spectrum (400–800 nm) were recorded. Metabolites produced after biodegradation of the Red HE3B were extracted with equal volumes of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and solvent was evaporated in rotary evaporator. The dried residues obtained were dissolved in small volume of HPLC grade methanol and the sample was used for FTIR, HPLC and GC–MS analysis. FTIR analysis was carried out using Perkin Elmer 783 Spectrophotometer. HPLC analysis was performed in an isocratic Waters 2690 system equipped with dual absorbance detector, using C_{18} column (4.6×250 mm) and HPLC grade methanol as a mobile phase.

GCMS analysis used a QP2010 gas chromatography coupled with mass spectrometer (Shimadzu). The analysis was performed in the temperature-programming mode at an ionization voltage 70 eV. Temperature of the Restek column (0.25 mm, 30 mm; XTI-5) was initially kept at 40 °C for 4 min, then raised up to 280 °C with rate of $10\,°C\,min^{-1}$, and held for 4 min. The temperature of injection port and the GC–MS interface was maintained at 280 and 290 °C respectively. The flow rate for helium as a carrier gas was $1\,ml\,min^{-1}$. NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC–MS was used for comparison of retention times and mass spectra of degradation metabolites based on their fragmentation pattern.

2.3.5. Enzyme assays

Cells were harvested (10,000 rpm at 4 °C for 20 min) and suspended in 50 mM potassium phosphate buffer (pH 7.4). Cell suspension ($100\,mg\,ml^{-1}$) was gently homogenized and sonicated, keeping the sonifier output at 40 A, giving 5 strokes each of 30 s, with 2 min time intervals (Sonics vibra Cell, Germany) at 4 °C. This cell homogenate was used as crude enzymes source. Activities of enzymes were assayed spectrophotometrically at room temperature where reference blank contained all the components except enzyme (0.2 ml), in triplicates and average rates were calculated. One unit of enzyme activity was defined as a change in absorbance unit/min mg protein.

Laccase, aminopyrine *N*-demethylase and azoreductase activities were assayed by the procedure reported earlier [16,18]. Tyrosinase activity was determined by modified procedure of [19], by monitoring the formation of catechol quinone at 495 nm in a reaction mixture. NADH-DCIP reductase activity was determined using a procedure reported earlier [20]. The reduction of DCIP was calculated using the extinction coefficient of $19\,mM\,cm^{-1}$.

2.3.6. Phytotoxicity study

The degradation products of the dye, Red HE3B, extracted with equal volume of ethyl acetate were dried and dissolved in water to form the final concentration range 700 ppm for phytotoxicity studies. The phytotoxicity study was carried out at room temp (30 ± 2 °C) in relation to *Phaseolus mungo* and *Triticum aestivum* seeds (10 seeds) by watering separately 5 ml samples of Red HE3B and its degradation product (700 ppm) per day. Control set was carried out using plain water at the same time. Length of plumule (shoot), radical (root) and germination (%) was recorded after seven days.

2.3.7. Statistical analysis

Data were analyzed by One-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparisons test. Values are mean of three experiments. Readings were considered significant when P was ≤ 0.05.

3. Results and discussion

3.1. Isolation and identification

Morphologically different bacterial strains isolated from dye contaminated soil possessed the ability to decolorize the textile dye Red HE3B. The strains were identified as, *Bacillus odysseyi* SUK3, *Mrganella morganii* SUK5 and *Proteus* sp. SUK7 by 16S rRNA gene sequence analysis. The nucleotide sequences of strains were

Table 1.1
Decolorization of different reactive textile dyes.

Sr. No.	Name of dyes[a]	% Decolorization	Time (h)
1	Navy Blue RX	92	12
2	Navy Blue HE2R	91	6
3	Red HE8B	88	12
4	Red HE18	90	22
5	Methyl orange	87	12
6	Red 6BI	85	36
7	Magenta MB	89	36
8	Green HE4BD	83	36

[a] 50 mg l^{-1}.

deposited in the GenBank under the accession number EU760699, EU760698 and EF541142 respectively.

3.2. Decolorization of Red HE3B

Red HE3B is one of the diazo dyes and has a very large consumption rate in the textile dyeing processes. When pure cultures of these isolates were tested individually for their decolorization capacity in the liquid medium, these cultures showed decolorization of Red HE3B (50 mg l^{-1}) with variation in percentage decolorization. The individual strain *B. odysseyi* SUK3, *M. morganii* SUK5 and *Proteus* sp. SUK7 showed the ability to decolorize Red HE3B (50 mg l^{-1}) up to 85%, 89% and 88% within 120, 60 and 68 h respectively, further more incubation showed no significant change in decolorization percentage because the produced metabolites may interfere with the enzyme activities responsible for the biotransformation. Whereas, the developed bacterial consortium PMB11, showed 99% decolorization of Red HE3B (50 mg l^{-1}) within 12 h. When bacterial cultures were mixed and inoculated together in liquid medium, increased decolorization of Red HE3B up to 99% was observed with reduction in the time required for decolorization as compared to individual bacterial strains. Previously, consortium PMB11 was reported for 92% decolorization of Navy Blue RX (Reactive blue 59) with in 3 h [16]. The observed results conclude that, the synergistic action of the bacterial strains might be responsible for faster and efficient decolorization of the dye Red HE3B. The use of developed microbial consortia PMB11 was found to be advantageous than the pure cultures in the decolorization of dye Red HE3B. The advantage of mixed cultures is apparent as some strains can collectively carry out biodegradation that cannot be achieved by individual pure strains.

3.3. Various textile dyes and dye mixture decolorization

Consortium PMB11 decolorized eight textile dyes and three mixtures of dyes in synthetic medium, supplemented with yeast extract. Various textile dyes were screened for the decolorization by using consortium PMB11. The percentage decolorization as well as time required for the decolorization was found to be varied for different dyes (Table 1.1). Minimum decolorization was observed for Green HE4BD which was up to 83% within 36 h, whereas Navy Blue RX showed maximum decolorization up to 92% within 12 h. Textile industry effluent is a mixture of various dyes, so it is necessary to prove the ability of consortium PMB11 to decolorize mixture of dyes. Three dye mixtures, viz., mixture no.1, 2 and 3 showed 55%, 70% and 74% decolorization respectively, after 24 h of treatment with consortium PMB11 (Table 1.2). Dyes of different structures and classes are often used in the textile processing industry and therefore, the effluents from the industry are markedly variable in composition. Extent of decolorization of dyes varies with each dye; which is because of the structural differences of dyes [21–25]. Consortium PMB11 had the capacity to decolorize all tested dyes (Navy Blue RX, Navy Blue HE2R, Red HE8B, Red HE18, Red 6BI, Magenta MB, Green HE4BD and Methyl orange) as well as mixtures of dyes. As the textile effluent contains mixture of various dyes with varying concentration, utilization of the consortium PMB11 for the treatment of the textile wastewater will prove its worthiness.

Table 1.2
Decolorization of different dye mixtures .

Mixture of dyes no.	Name of dyes	λ_{max} (nm)	% Decolorization
1	Navy Blue RX + Navy Blue HE2R + Red HE3B + Red HE18 + Green HE4BD	550	55
2	Navy Blue RX + Navy Blue HE2R + Red HE3B + Red HE18 + Methyl orange	540	70
3	Navy Blue RX + Navy Blue HE2R + Red HE3B + Red HE18 + Red HE8B	480	74

3.4. Effect of medium components

Various media components were found to affect the decolorization of Red HE3B. Table 2 shows the effect of various carbon and nitrogen sources on the decolorization within 24 h. The consortium PMB11 was unable to decolorize Red HE3B in the synthetic medium. Decolorization of Red HE3B in the medium supplemented with peptone (71%) and glucose (70%) resulted in better decolorization efficiency compared to that of beef extract (67%), while incorporation of lactose was found to diminish the decolorization (33%). When yeast extract was added as the sole source of carbon and nitrogen in the synthetic medium, 97% decolorization was observed. The results obtained in the present study match the previous observations, where, consortia PDW showed optimum decolorization when carbon–nitrogen source was yeast extract [12]. Dyes are deficient in carbon content and biodegradation without any extra carbon source is found to be very difficult [26]. The bacterial culture was unable to decolorize the dye in absence of co-substrate, which indicates that availability of supplementary carbon source is necessary for growth and decolorization of dyes [11,27]. Addition of supplementary carbon and nitrogen sources for the decolorization of certain textile dyes was reported earlier in case of, *Sphingomonas* sp. strain ICX [28], *Rhodopseudomonas palustris* [24] and *Pseudomonas* sp. SUK1 [25].

On the other hand, the ability of the culture to decolorize Red HE3B in the presence of different nitrogen sources was also studied. Ammonium nitrate (37%), potassium nitrate (36%) and urea (37%) showed poor decolorization ability. Moreover, when nitrogen sources were supplemented along with yeast extract in the medium, decolorization was significantly increased.

Table 2
Decolorization of Red HE3B (50 mg l^{-1}) by consortium PMB11 in different media.

Sr. No.	Media	% Decolorization
1	Synthetic medium (SM)	No decolorization
2	SM + Glucose	70
3	SM + Peptone	71
4	SM + Lactose	33
5	SM + Yeast extract	97
6	SM + Beef extract	67
7	SM + Ammonium nitrate	37
8	SM + Potassium nitrate	36
9	SM + Urea	37
10	SM + Yeast extract + Ammonium nitrate	67
11	SM + Yeast extract + Potassium nitrate	71
12	SM + Yeast extract + Urea	90

Table 3.1
Enzyme activities in control cells.

Enzyme	SM + YE	Nutrient medium	SM + YE + Urea
Lignin peroxidase [a]	0.025 ± 0.001	0.20 ± 0.003	0.065 ± 0.001
Laccase [a]	0.087 ± 0.001	0.047 ± 0.003	0.107 ± 0.001
Tyrosinase[a]	–	0.014 ± 0.002	0.018 ± 0.001
DCIP reductase[b]	4.131 ± 0.011	3.23 ± 0.02	9.61 ± 0.001
Aminopyrine *N*-demethylase[c]	6.591 ± 0.005	29.25 ± 0.03	10.04 ± 0.02
Azo reductase[d]	0.107 ± 0.001	0.282 ± 0.006	0.01 ± 0.001

Table 3.2
Enzyme activities in induced state (after dye decolorization).

Enzyme	SM + YE	Nutrient medium	SM + YE + Urea
Lignin peroxidase [a]	0.081 ± 0.002**	–	0.056 ± 0.001
Laccase [a]	0.029 ± 0.001	0.046 ± 0.007**	0.067 ± 0.001
Tyrosinase [a]	0.03 ± 0.001	0.028 ± 0.002**	–
DCIP reductase[b]	2.78 ± 0.001	4.896 ± 0.001**	12.31 ± 0.011**
Aminopyrine *N*-demethylase [c]	0.04 ± 0.001**	20.72 ± 0.001**	25.74 ± 0.05**
Azo reductase[d]	0.212 ± 0.001**	1.05 ± 0.006**	0.428 ± 0.001**

[a] enzyme unit/min mg protein.
[b] μg DCIP reduced/min mg protein.
[c] *n* moles of formaldehyde librated/mg protein.
[d] μM of Methyl red reduced mg of protein^{-1} min^{-1}. Values are mean of three experiments ±SEM. Significantly different from control cells at $^{**}P < 0.01$ by One-way (ANOVA) with Tukey–Kramer comparison test.

3.5. Enzyme analysis

Decolorization of Red HE3B was observed in different media, with variation in time required for the optimum decolorization. This ensures that, different enzymes get induced in different media, during the decolorization process. The biotransformation enzymes viz. lignin peroxidase, laccase, tyrosinase, aminopyrine *N*-demethylase NADH-DCIP reductase and azo reductase were studied in three different mediums; synthetic medium with yeast extract, nutrient broth and synthetic medium with yeast extract along with urea (Table 3.1). In the synthetic medium with yeast extract, levels of lignin peroxidase, tyrosinase, aminopyrine *N*-demethylase and azo reductase increased significantly, while in nutrient medium all five studied enzymes were induced after decolorization of dye except lignin peroxidase (Table 3.2). Fast decolorization of the dye in nutrient broth was observed as a result of induction of all studied biotransformation enzymes. The enzymatic status of an organism might depend on the constitutive nutrients available for its growth.

The several reports have showed that the lignin modified enzymes are efficiently involved in dye decolorization [29–31]. Similarly, various reductases such as, NADH-DCIP reductase [20], azoreductase [18] have been reported earlier for the biotransformation of dyes. Potential applications of oxidoreductases have also been illustrated for the dye decolorization [32]. Demethylation reactions for the biodegradation of Methyl violet [33] and Crystal violet [34] were reported previously. Induction in the activities of various enzymes studied, concludes the communal action of these enzymes in the process of the dye decolorization

3.6. Analysis of the biodegradation product

3.6.1. UV–vis spectral analysis

Analysis has been used to confirm that decolorization process of Red HE3B was due to biodegradation. Spectrophotometeric analysis of Red HE3B showed maximum absorbance at 510 nm and decrease in the absorbance of samples withdrawn after decolorization using bacterial consortium (Fig. 1). If dye removal is attributed to biodegradation, either the major visible light absorbance peak would completely disappear or a new peak will appear [35], indicating that the color removal by the isolated bacterial strains and its consortium is due to the biodegradation.

3.6.2. Fourier transforms infrared spectroscopy (FTIR) analysis

The FTIR spectral comparison between Red HE3B and test sample which was extracted after dye decolorization experiments confirmed the biodegradation of the dye into different metabolites by consortium PMB11 (Fig. 2A) and individual bacterial strains (Fig. 2B). The FTIR spectrum of Red HE3B showed the presence of different peaks at 3471 cm^{-1} which showed CH stretching of single bridge alcoholic or phenolic compound, at 3099 and 2937 cm^{-1} showed NH stretching of amides and CH stretching of asymmetric alkanes respectively. Peak at 2096 cm^{-1} displayed NH_3 stretching of amino acid. Peak at 1485 cm^{-1} (C=C stretch) for disubstituted aromatic compounds and 1213 cm^{-1} for (S=O stretch) sulfur compounds. Peak at 1051 cm^{-1} showed S=O stretching of sulfuric acid and peak at 765 cm^{-1} (CH stretch) for benzene ring with three adjacent hydrogen atoms. The FTIR spectrum of the test sample i.e. of the products formed after decolorization showed peaks at 3236 cm^{-1} showed –N=O stretching of nitrites, at 2951 and 1445 cm^{-1} for CH stretching of asymmetric alkanes. Trisubstituted alkanes (C=C stretch) indicating peak was displayed at 1666 cm^{-1}. Peak at 1325 cm^{-1} displayed for S=O stretching of sulfur containing

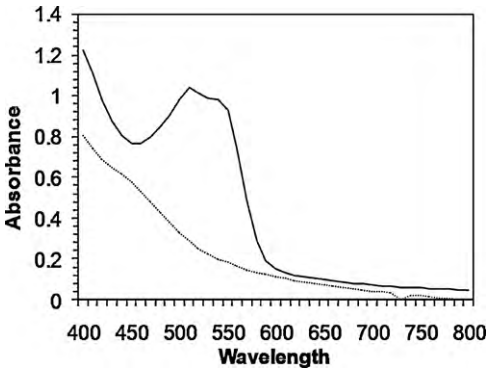


Fig. 1. UV–visible spectra of Red HE3B (–) and after decolorization by consortium PMB11 (····).

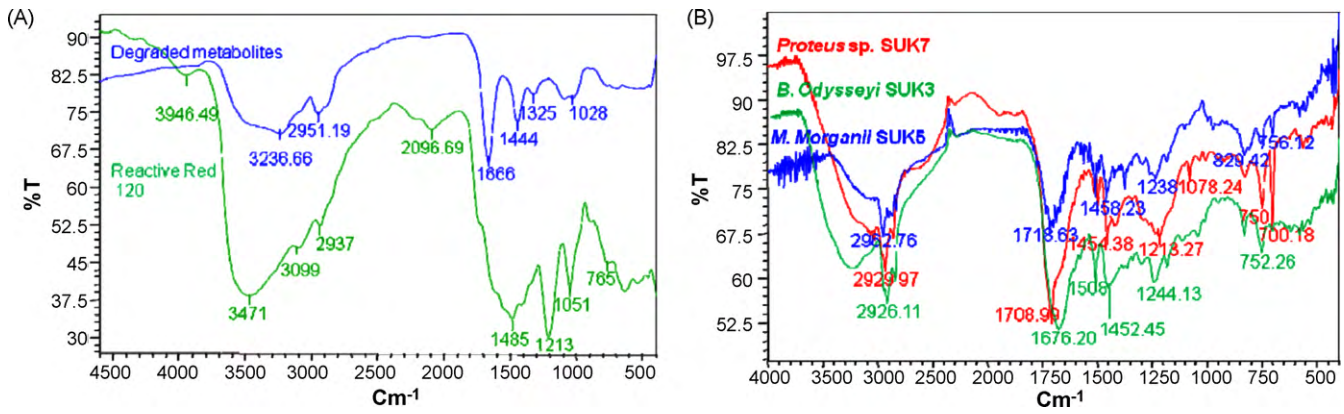


Fig. 2. (A) FTIR analysis of Red HE3B and degradation metabolites by consortium PMB11. (B) FTIR analysis of degradation Red HE3B metabolites byindividual bacterial strains.

compound. Peaks at 1028 and 1093 cm^{-1} showed C–OH stretching of primary alcohol and CH deformation of pyridine respectively. The FTIR spectrum indicated that the peaks of metabolites formed after degradation, were significantly different than those present in the spectrum of the dye during the biotransformation process.

3.6.3. High performance liquid chromatography (HPLC) analysis

HPLC chromatogram of Red HE3B and the metabolites formed after its degradation by consortium as well as individual bacterial stains showed peaks at different retention times. The HPLC analysis of control Red HE3B showed a peak at retention time 1.724 min(Fig. 3A(a)), whereas metabolites obtained after degradation of dye showed additional five peaks at retention times 1651, 2.029, 2.146, 2.787 and 3.189 min(Fig. 3B(b)). Degradation of Red HE3B by *B. odyssey* SUK3, *M. morganii* SUK5 and *Proteus* sp. SUK7 showed peaks at different retention times. HPLC chromatogram of metabolites formed after degradation of Red HE3B by individual bacterial stains showed peaks with significant variation compared to consortium (Fig. 3B). HPLC analysis confirmed the biodegradation of Red HE3B in to different metabolites, the observed peak pattern with individual strain and consortium is significantly different from each other.

3.6.4. GC–MS analysis

The GC–MS analysis showed the probable metabolites produced during the Red HE3B biotransformation process in the nutrient medium (Table 4). Azo dyes can be cleaved symmetrically or asymmetrically, with an active site available for an enzyme to excite the molecule.

The pathway was proposed based on the GC–MS results which indicated the asymmetric cleavage of the sulfonated azo dye Red HE3B (Fig. 4). It was subjected to asymmetric cleavage by peroxidases to give 2,5-diaminobenzene aminotriazene, intermediate I and azo bond containing as intermediate II. Then action of azoreductase was leading to the breaking of the azo bonds to form aniline 2-sulfate, aniline 3-sulfate, 5-aminoanphthal sulfate, and 2-amino

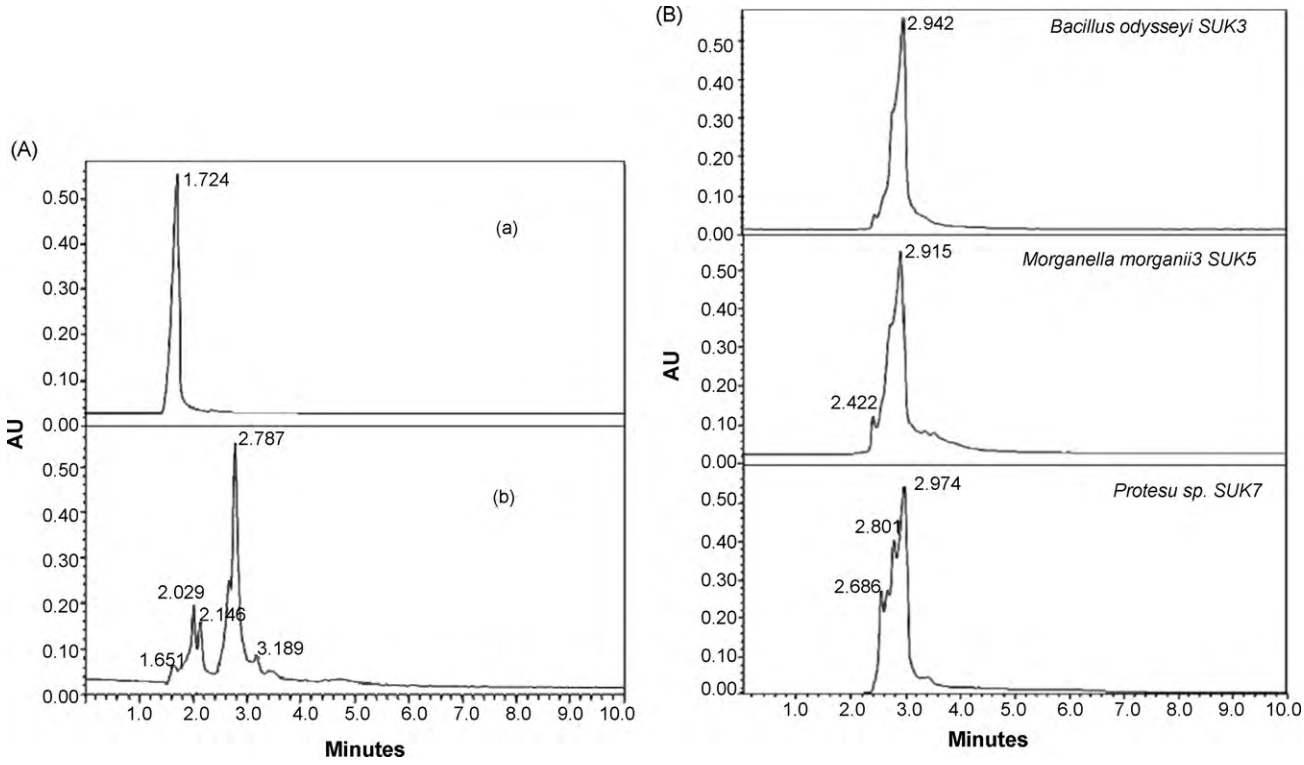


Fig. 3. (A) HPLC elution profile: (a) Red HE3B, (b) its degradation metabolites by consortium PMB11. (B) HPLC elution profile of Red HE3B and its degradation metabolites by individual bacterial strains.

Table 4
GC mass spectral datasheet of metabolites formed after degradation of Red HE3B.

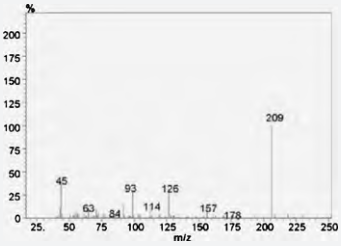
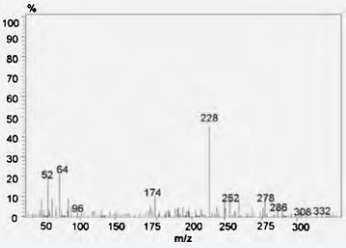
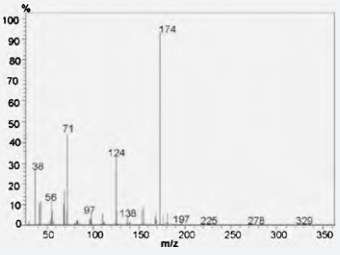
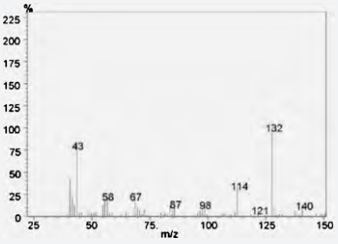
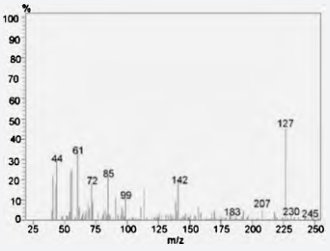
Sr. No.	Molecular weight of metabolite (*m*/*z*)	Retention time (min)	Name of metabolites	Mass peaks
1	209	26.87	2,5-diaminobenzene aminotriazene	
2	228	22.28	2-aminoanphthal 6-sulphonate	
3	174	22.28	5-sulphonateaniline	
4	132	21.18	2-amino 5-chlorotriazine	
5	127	21.80	Naphthalene	

Table 5
Phytotoxicity study of Red HE3B and its degraded metabolites.

Parameters studied	*Phaseolus mungo*			*Triticum aestivum*		
	Water	Red HE3B[a]	Metabolites[a]	Water	Red HE3B[a]	Metabolites[a]
Germination (%)	100	60	100	100	50	100
Plumule (cm)	7.4 ± 0.80	3.4 ± 0.23	5.1 ± 0.23[$]	3.7 ± 0.74	2.8 ± 0.31	3.5 ± 0.23[$$]
Radicle (cm)	3.16 ± 0.24	2.1 ± 0.12	3.02 ± 0.10[$]	11.12 ± 0.58	4.4 ± 0.21	9.5 ± 0.31[$$]

[a] 700 ppm concentration of Red HE3B. Data was analyzed by One-way (ANOVA) Test and mentioned values are the mean of 10 germinated seeds of three sets SEM (±). Seeds germinated in degradation products are significantly different from the seeds germinated in Red HE3B at [$]$P < 0.05$, [$$]$P < 0.01$ when compared by Tukey–Kramer multiple comparison test.

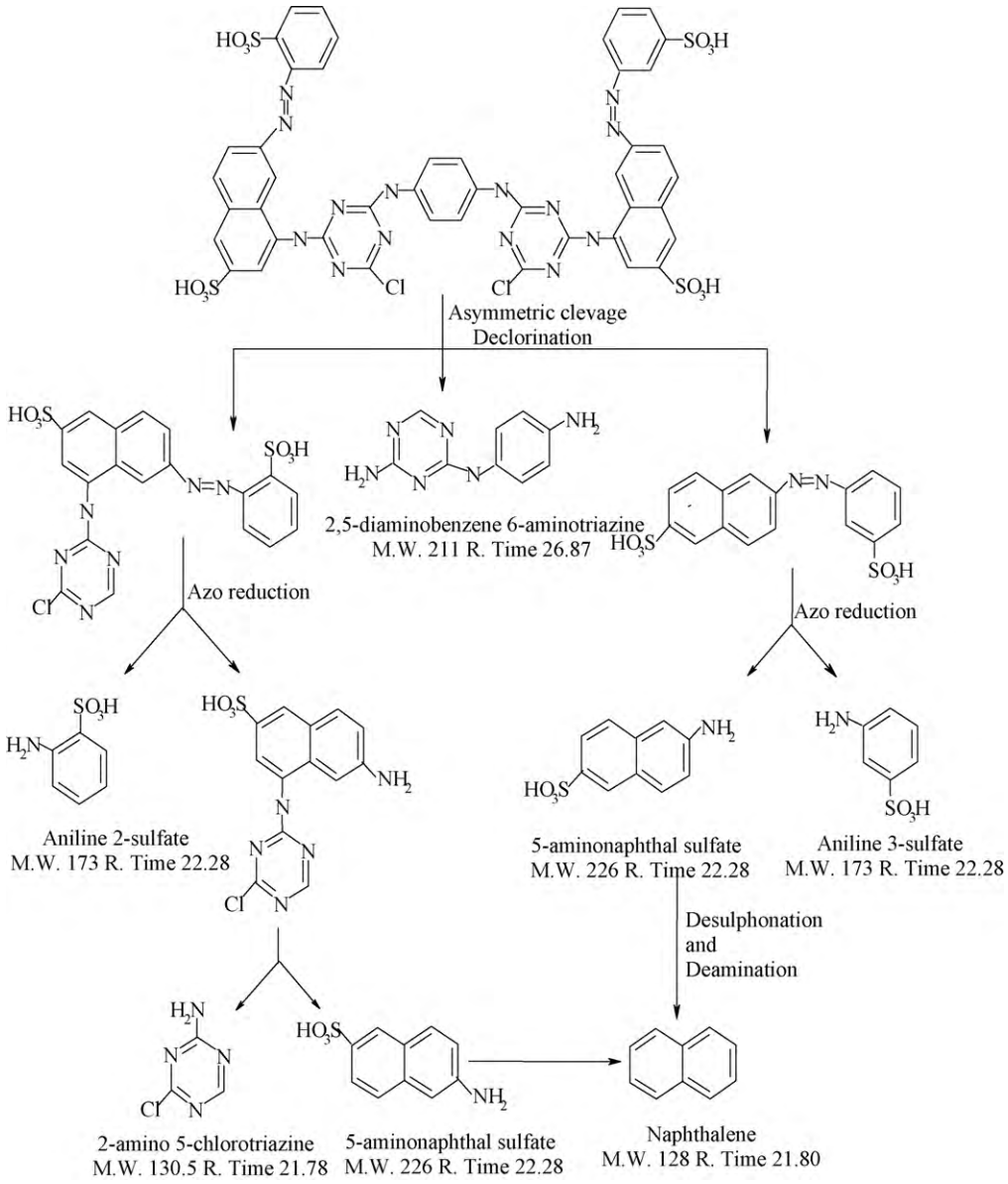


Fig. 4. Proposed pathway for the biodegradation of Red HE3B.

5-chlorotriazine. Further 5-aminonaphthal sulfate was subjected to deamination and desulphonation and gave naphthalene.

3.7. Phytotoxicity study

Germination of *P. mungo* and *T. aestivum* seeds was 60% and 50% respectively with Red HE3B treatment, also its degradation metabolites and plain water showed 100% seed germination. The mean of plumule length and radicle length of *P. mungo* were 7.4 ± 0.8 and 3.16 ± 0.24 cm, respectively, and in case of *T. aestivum*, 3.7 ± 0.74 and 11.12 ± 0.58 cm respectively, in water as a control. The length of plumule and radicle were significantly affected by Red HE3B than its degradation metabolites. In presence of the dye, the seeds showed the mean of plumule length and radicle length of *P. mungo* to be 3.4 ± 0.23 and 2.1 ± 0.12 cm, respectively and in case of *T. aestivum*, 2.8 ± 0.31 and 4.4 ± 0.21 cm, respectively. In contrast, when treated with degradation metabolites, the plumule length and radicle length were found to be in *P. mungo*, 5.1 ± 0.23 and 3.02 ± 0.10 cm; and in *T. aestivum*, 3.5 ± 0.23 and 9.5 ± 0.31 cm, respectively with 100% germination.

The length of plumule and radicle were significantly affected by Red HE3B than its degradation metabolites, indicating less toxic nature of degradation metabolites as compared to dye (Table 5). Hence phytotoxicity studies revealed biodegradation of Red HE3B by bacterial consortium PMB11 resulted in the detoxification of dye.

4. Conclusions

The use of microbial consortia was found to be advantageous than pure cultures in the decolorization of dyes. The treatment systems having mixed microbial populations are more effective due to concerted metabolic activities of microbial community, as well necessity of proper combination of bacterial strains in consortium. As the catabolic activities of microorganisms in mixed consortium complement each other, the utilization of microbial consortium offers considerable advantages in the decolorization and degradation of synthetic dyes. The involvement of enzymes in dye decolorization might depend on the constitutive nutrients available for growth of bacteria. The communal action of various biotrans-

formation enzymes leads to the faster decolorization of the dye Red HE3B. The final product of dye degradation were aniline 2-sulfate, aniline 3-sulfate, 2-amino 5-chlorotriazine, 2,5-diaminobenzene 6-aminotriazine and naphthalene. The phytotoxicity study revealed the less toxic nature of degradation metabolites.

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