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DECOLOURIZATION, DEGRADATION AND DETOXIFICATION OF DYE HOUSE EFFLUENTS BY A DEVELOPED BACTERIAL CONSORTIUM

Darshna K. Patel[1], Devayani R. Tipre[1], Shailesh R. Dave[2*]

[1]Department of Microbiology and Biotechnology, School of Sciences, Gujarat University, Ahmedabad 380009, Gujarat, India.
[2]Xavier's Research Foundation, Loyola Centre for Research and Development, St. Xavier College Campus, Navrangpura, Ahmedabad 380009, Gujarat, India.

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KEYWORDS

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Consortium

Decolourisation

Dye house effluent

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ABSTRACT

Decolourization and degradation of two dye house effluents (DHEs) have been studied using a developed bacterial consortium. Batch experiments were optimized at shake flask level in terms of pH, temperature, culture condition, carbon and nitrogen source. Bacterial consortium was found to be active between pH 6-10 and temperature 25-40 °C under static condition. Among different carbon and nitrogen sources studied, addition of sucrose, fructose, glucose and beef extract were found to support the degradation of both Effluent-2 and 3 (E-2, E-3). Decolourization and degradation profiles of E-2 and E-3 DHEs were studied to optimize the treatment time. Reduction in BOD, COD and ADMI (American Dye Manufacturers' Institute) values were more than 95%, which proved the treatment efficiency of the developed consortium against both the DHEs Moreover, FTIR and HPLC spectral data analysis and enzyme induction pattern confirmed biodegradation of all the DHEs. Intracellular azoreductase, NADH-DCIP reductase and laccase played significant role in degradation. Phytotoxicity and microbial toxicity were reduced in the range of 70-80% and 40-46%, respectively. This results indicated production of non-toxic metabolites. The study gave significant information for the bio-treatment of DHEs containing various heavy metals using a developed bacterial consortium.

* Corresponding author
E-mail: shaileshrdave@yahoo.co.in, srdave@gujaratuniversity.ac.in (S.R. Dave)

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1 Introduction

Water pollution is one of the major areas of concern since last few decade as it has a major impact on all life forms along with aquatic life. Among the major sources of pollutes, coloured waste water generated from the dye houses and textile industries are the most common one (Vikrant et al., 2018). Both these kind of industries consume a huge amount of water and generate coloured effluents. Tehrani-Bagha et al. (2010) reported that among all industrial sectors, coloured wastewater generating industries are found to be the most polluting. Wastewaters containing colours are easily visible and hence pose aesthetic pollution problems. Dye house effluents (DHEs) generally contain dark coloured water along with heavy metals (chromium, copper, molybdenum, zinc, etc.), COD, BOD, TOC, TDS and TSS (Patel et al., 2015). Such metal containing pollutants are known to be hazarus for living beings (Li et al., 2015). Moreover, some portion of dyes are not recovered during the downstream processing and hence dye house wastes are more concentrated as compared to the textile wastewater because textile waste waters get diluted during the washing process of clothes (Patel et al., 2015). Physical and chemical treatment methods are not always possible as they are more costly and practically ineffective. Generation of huge amount of sludge is another problem in such physicochemical treatment methods including advance oxidation process and other conventional treatment methods (Vikrant et al., 2018). Biodegradation is an attractive tool for the treatment of such wastes as it is cost effective and ecofriendly in nature (Stolz, 2001; Shah et al., 2013; Cerron et al., 2015; Raper et al., 2018). Use of various biological organisms including bacteria, fungi, yeast, algae and plants have been studied for their remediation potential (Dave & Dave, 2009; Zabłocka-Godlewska et al., 2015; de Almeida & Corso, 2016; Alizadeh et al., 2017; Ghosh et al., 2017; Swati et al., 2017). Use of consortial system offer a great number of benefits including degradation ability of more than one compound at a time along with co-metabolic activities leading to mineralization of the pollutant compound (Forgacs et al., 2004; Khehra et al., 2005; Sheth & Dave 2010; Bilal et al., 2018). Benefits of using consortium for bioremediation are well reported in the literature (He et al., 2004; Balapure et al., 2016; Patel et al., 2017a; Bilal et al. 2019).

Most of the research work has been focussing only on pure dye or only one industrial effluent and that too mainly the textile wastewater or simulated wastewater, but not the actual DHEs. Different isolates and consortia are reported for degradation of individual dyes or waste. DHEs contain high organic load, high ADMI value, dark colour and presence of toxic metals (Patel et al. 2015). No data are available for treatment of more than one dye containing wastes with developed single bacterial consortium. Keeping these points in consideration bacterial consortium was developed and investigated for decolourization and degradation of two different diverse DHEs. Further, analysis of effluent detoxification was carried out to ensure the safty of the treatment procedure. Present study would be the base for the development of waste water treatment protocol for diverse group of dye containing industrial effluents.

2 Materials and Methods

2.1 *DHEs, media and chemicals*

Two DHEs designated as E-2 and E-3 were procured from Apex dye stuff industries located in Vatva GIDC industrial estate, Ahmedabad, Gujarat, India. Both the effluents were containing mixture of various azo dyes and metals as during that period, only metal complex azo dyes were manufactured by the industry. Treatment of DHEs was carried out in triplicates using 1:10 diluted DHE in Bushnell and Haas (BH) medium containing (g/L): K_2HPO_4, 1.0; KH_2PO_4, 1.0; NH_4NO_3, 1.0; $FeCl_3$, 0.05; $CaCl_2$, 0.02; $MgSO_4$, 0.2 and 0.5% (w/v) yeast extract; pH 7.4±0.2 in a 100 mL Erlenmeyer flasks containing 50 mL of DHE system comprising of 10% (v/v) activated inoculum ($3x10^8$ cells/mL). The preparaed system was kept at 35±2 °C temperature and static incubation condition. ABTS (2,2- azinobis (3-ethylbenzothiazolin-6-sulphonic acid)), NADH (nicotinamide adenine dinucleotide hydrogen), tartaric acid, n-propane, L-tyrosine and other chemicals used in the study were of analytical grade from HiMedia Laboratories, India. Microbial cultures of *Azotobacter* sp. and *Pseudomonas aeruginosa* were obtained from the departmental culture collections.

2.2 Bacterial consortium

An indegeneous bacterial consortium was enriched from a dye polluted site as described in the earlier report (Patel et al., 2017a) and maintained in dye containing nutrient broth at 8±2°C used for further use. Bacteria isolated on nutrient agar plate from the developed consortium were identified on the basis of 16S rRNA gene sequence analysis at Chromous Biotech, Bangaluru, India. All the five isolates were added in equal proportion.

2.3 Physico-chemical analysis of DHEs

Analysis of DHEs for pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solids (TDS) and total suspended solids (TSS) before and after treatment were carried out according to standard methods for water and wastewater analysis (Eaton et al., 1998).

2.4 Optimization of DHEs decolourization

Decolourization of DHEs was optimized using various parameters such as pH (4-12), temperature (25-55°C), culture condition (static and shaking), carbon and nitrogen supplement in BH medium with 0.5% (w/v) glucose, sucrose, lactose, maltose, mannitol, fructose, starch, yeast extract, peptone and beef extract. Decolourization profile of DHEs was studied at an interval of

every 6 h until decolourization remained constant. Decolourization rate was calculated in terms of percent decolourization per hour (Patel et al. 2017b).

2.5 Analysis of colour removal

Decolourization of DHEs was measured by ADMI value calculation using Tristimulus filter method (Eaton et al., 1998) and comparison of UV-Vis spectra of DHEs before and after treatment. ADMI removal was calculated using Equation 1.

$$\%\ \text{ADMI removal} = \frac{(\text{Initial ADMI} - \text{Final ADMI})}{\text{Initial ADMI}} \times 100 \quad (1)$$

Where initial ADMI value at 0 h and the final ADMI value after a particular reaction time.

2.6 Cell free extract preparation

Cells from activated consortium were harvested by centrifugation at 8000 g for 10 min. The supernatant obtained after centrifugation was directly used as a source of extracellular enzymes. The harvested cells were homogenized after suspending in 50 mM potassium phosphate buffer at pH 7.4, and sonicated (Sartrious, Germany) at an amplitude of 70% with ten strokes each of 30 s with a 2 min interval at 4 °C followed by centrifugation at 10000 g for 20 min at 4 °C to remove the cell debris (Patel et al., 2017a). The extra- and intracellular fractions were used as a crude enzyme for respective extracellular and intracellular enzyme assays. Reading of triplicate experiments were used for statistical analysis.

2.7 Enzyme assay

Oxidoreductive enzymes including lignin peroxidase (LiP), laccase, tyrosinase, azoreductase (Azo) and NADH-DCIP reductase (NADH-DCIP red) from intracellular as well as extracellular fractions were studied for their induction pattern in decolourization of DHEs. Azoreductase (Khan et al., 2014), NADH-DCIP reductase (Lade et al., 2012), Laccase (Shah et al., 2012; Agrawal et al., 2014) and Tyrosinase (Kadam et al., 2011) enzymes activities were determined by standard protocole. One unit of reductive enzyme activity was defined as the amount of enzyme required to reduce 1µM substrate per minute. One unit of oxidative enzyme was defined as the amount of enzyme to increase 1.0 absorbance unit under standard assay conditions.

2.8 Analysis of biodegradation

The untreated and treated DHEs were centrifuged at 10,000 g for 10 min and supernatants were extracted using double volumes of ethyl acetate, and dried on a rotary vacuum evaporator at 45 °C. The extracted metabolites were subjected to FTIR and HPLC analysis. FTIR analysis was carried out in the range of 600-4000 cm^{-1} with 16 scan speed (Bruker, Germany). For HPLC analysis, extracted metabolites were dissolved in spectroscopy grade methanol and injected in a C18 column (250 mm × 4.6 mm, 5 mm) equipped with dual wavelength detector by the isocratic method using LC solutions (Shimadzu, Japan). The mobile phase was methanol:water (70:30) with a flow rate of 1.0 mL/min and with 15 min run time.

2.9 Phytotoxicity

Phytotoxicity was carried out at ambient temperature using *Phaseolus mungo*. Ten seeds were irrigated separately by providing 5 mL of distilled water (as a control), untreated and treated DHEs. The effect of individual treatment on growth was assessed by measuring percent germination, plumule length and radical length after 10 days (Patel et al., 2015).

2.10 Microbial toxicity

The microbial toxicity of untreated DHEs and metabolites obtained after treatment was carried out using *Azotobacter* sp. and *Pseudomonas aeruginosa* (Patel et al., 2017b) and the mean of inhibition zone after 24 h of incubation at 35±2°C was recorded.

3 Results and Discussion

3.1 DHEs characterization

Characteristics of DHEs viz. E-2 and E-3 are shown in Table 1. Wide range of diversity in terms of pH, TOC, COD, BOD, TDS,

Table 1 Physico-chemical characteristics of various DHEs.

No.	Parameters	Characteristics of DHEs	
		E-2	E-3
1	pH	5.74	5.79
2	TOC (mg/L)	8322	8202
3	COD (mg/L)	23400	26000
4	BOD (mg/L)	15757	15333
5	TDS (mg/L)	231420	236850
6	TSS (mg/L)	404	412
7	VSS (mg/L)	136	280
8	NVSS (mg/L)	268	132
9	NH_3-N (mg/L)	868	854
10	Total nitrogen (mg/L)	952	882
11	Colour (cu)	1396000	1200000
12	ADMI	107066	420107
13	Metal (mg/L)		
	Cu	2.26	18.6
	Zn	2.03	12.0
	Fe	11.16	56.2
	Cd	5.43	2.82
	Cr	13.8	9.6
	Ni, Co, Pb	ND	ND

TSS, CU, ADMI and metal content was reported. The pH of the samples was found to be 5.7 alongwith narrow range of differences was observed for TOC, COD, BOD, TDS and TSS in case of both the effluents. However, samples were highly coloured and a remarkable differences was observed in case of ADMI value where ADMI value of 107066 and 420107 for E-2 and E-3, respectively. Both the effluents were orange coloured. Metal content was found as 2.26 and 18.6, 2.03 and 12.0, 11.16 and 56.2, 5.43 and 2.82, 13.8 and 9.6 for Cu, Zn, Fe, Cd and Cr metals in E-2 and E-3, respectively. The BOD:COD ratio of untreated DHEs were 0.7 and 0.6 for E-2 and E-3, respectively. As per the literature, BOD:COD indicates the biodegradability of waste water (Dhall et al., 2012, Dave et al., 2015). If the BOD:COD ratio is >0.5, the waste is considered amenable to biodegradation, whereas, the ratio <0.3 indicates the presence of toxic compounds and hence needs to be stabilized prior to treatment. The obtained characterization of DHEs indicated the presence of coloured compounds and extent of biodegradability of samples.

3.2 Identification of Bacteria from the consortium

A developed bacterial consortium showed the presence of *Pantoea ananatis* (KM502538), *Bacillus fortis* strain E4 Pb3 (KM502537), *Alcaligenes faecalis* (KM502541), *Brevibacillus parabrevis* strain GRG (KM502542) and *Bordetella trematum* (KP751929) on the basis of 16S rRNA gene sequencing of cultivable bacterial species. Sequence of the identified cultures are deposited and obtained Gen Bank accession numbers are mentioned with each organism in parenthesis (Patel et al., 2017b).

3.3 Optimization of DHEs decolourization

Influence of various parameters on decolourization is presented in Figure 1 A-D. The pH in the range of 6-10 was found to be the pH of choice for the study as the decolourization was more than 80%. The developed inoculum showed considerable activity even at pH 5 and 10 (Figure 1A). Effect of various incubation temperatures on biodecolourization of both the DHEs are shown in Figure 1B. The ADMI removal was found to be more than 96% for both the effluents at 35-40±2 °C temperature. Both the DHEs showed 4 to 26 fold higher rate of % ADMI removal at static incubation condition as compared to shaking condition (data not shown). This might be due to reduced air penetration at static condition than the shaking condition. The results

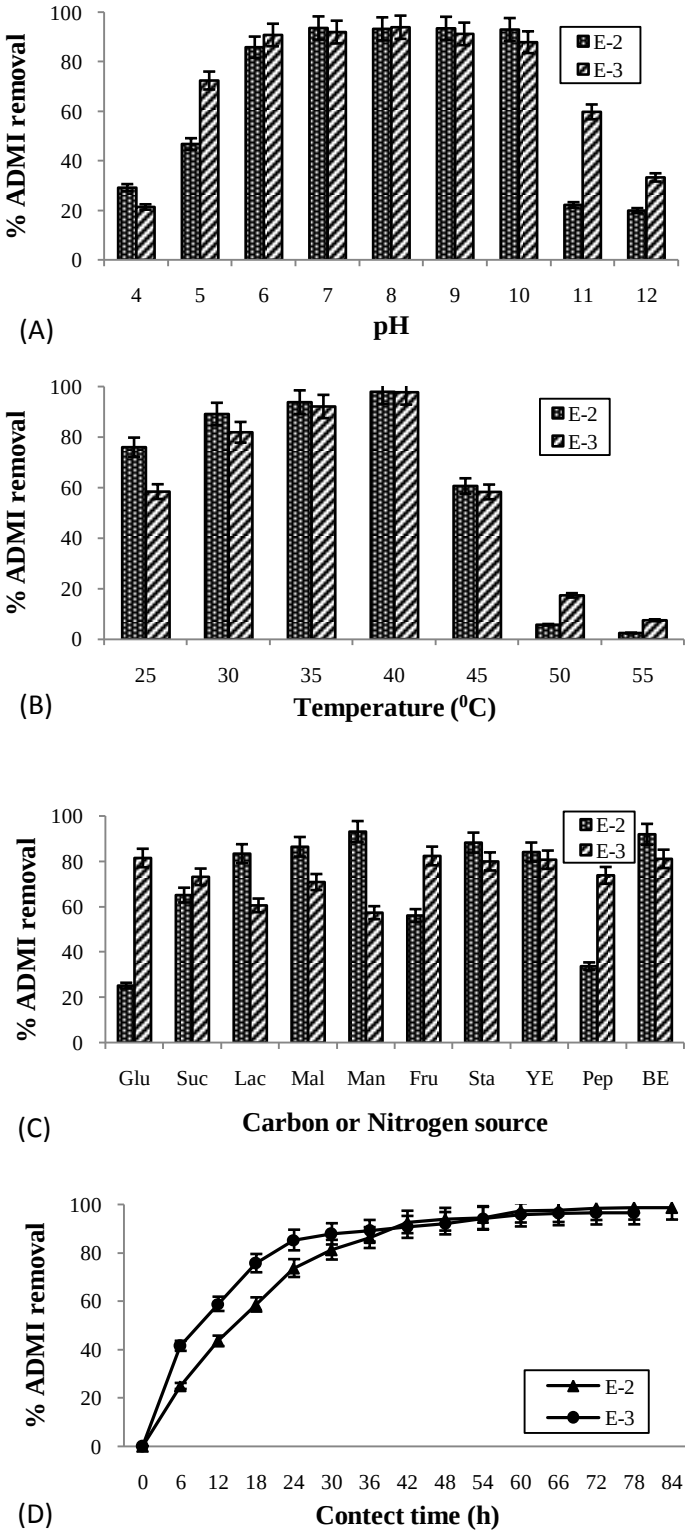

Figure 1 Effect of (A) pH (B) Temperature (C) Carbon and nitrogen source (D) Contact time on decolourization of DHEs. (90 mL waste, 10 mL consortium, incubation at static conditions at 32±2 °C)

are in coordination with Pandey et al. (2007), where nonspecific reduction of azo dyes leading to colour removal under anaerobic conditions is explained.

Influence of addition of various carbon and nitrogen sources was also investigated and results are described in Figure 1C. The influence was found to be quite distinct in terms of DHEs under study. Addition of maltose was found to be the best for E-2; whereas, glucose was favouring the decolourization of E-3. The observed variation in the influence of added sugar could be explained based on the influence of particular effluents and presence of indegenous flora in the effluents. Addition of beef extract was found to be the choice as compared to yeast extract and peptone in the medium.

Decolourization profile in terms of incubation time showed more than 90% decolourization within 42 h (Figure 1D) for both the effluents. The average decolourization rates of first 42 h of treatment for E-2 and E-3 were 3.0 and 3.9 % ADMI removal/h, respectively. Both the effluents showed fastest ADMI removal in first 6 h with 4.2 and 6.9 % ADMI removal/h. Even in case of textile effluent 48 h are required to achieve 89% ADMI removal when BL-GG consortium was used (Kurade et al., 2012; Saratale et al., 2012).

3.4 Analysis of treatment efficiency

Treatment efficiency of the consortium for both the DHEs was studied in terms of COD, BOD, TOC, TDS, TSS and ADMI value reduction and results are listed in Figure 2. The DHEs showed increase in pH, which rose from 7.4 to 8.4 after treatment. Moreover, the treatment also resulted in 87 and 62% TOC reduction along with 95 and 84% COD reduction for E-2 and E-3, respectively. More than 98% BOD removal was observed from both the DHEs. Dissolved solids reductions were 87 and 78% for E-2 and E-3, respectively. Apart from this, the consortium showed >99% metal removal as none of the metals was detected in any of the treated DHEs. In literature, reduction in high COD, ADMI and metal is reported for industrial effluent using bacterial consortium system by Patel et al. (2015). Decrease in COD, BOD, TOC, ADMI values of textile effluent was recorded after treatment using consortium AP (Lade et al., 2012) and the same parameters including reduction in solids is also documented in soil when plant and/or bacterial augmentation combinations were used. In the reported study, the presence of both plant and bacterial augmentation resulted in maximum 50-80% reduction (Khandare et al., 2013). Zhang et al. (2012) observed 40 and 84% of colour and COD reduction after anoxic-oxic treatment of a textile wastewater. Kurade et al. (2012) and Saratale et al. (2012) have observed 68 and 74% of BOD and COD reduction of a textile effluent, respectively though the initial BOD (890 mg/L) and COD (3400 mg/L) values were far less than the values in present effluents under study. More reduction in initial COD indicates the high rate of mineralization (Hassan & Hawkyard 2002).

3.5 Enzyme analysis

All the studied enzymes were significantly induced both in intracellular and extracellular fractions (Figure 3) in case of both treated and untreated DHEs. Intracellular enzyme induction was more as compared to extracellular enzymes. The two enzyme families, azoreductases and laccases, showed a great potential in

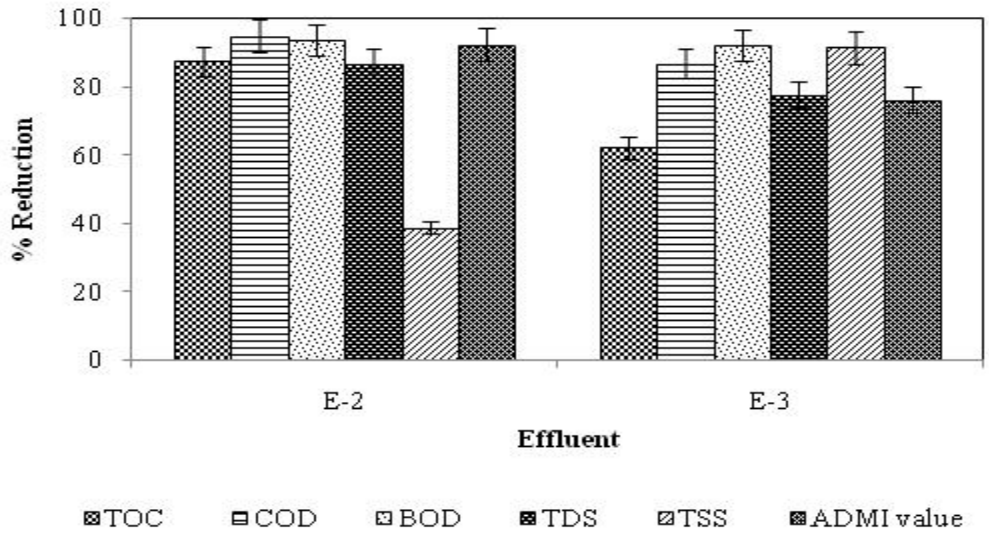

Figure 2 Percent reduction in TOC, COD, BOD, TDS, TSS and ADMI due to biological treatment of DHEs. (90 mL waste, 10 mL consortium, incubation at static conditions at 32±2 °C)

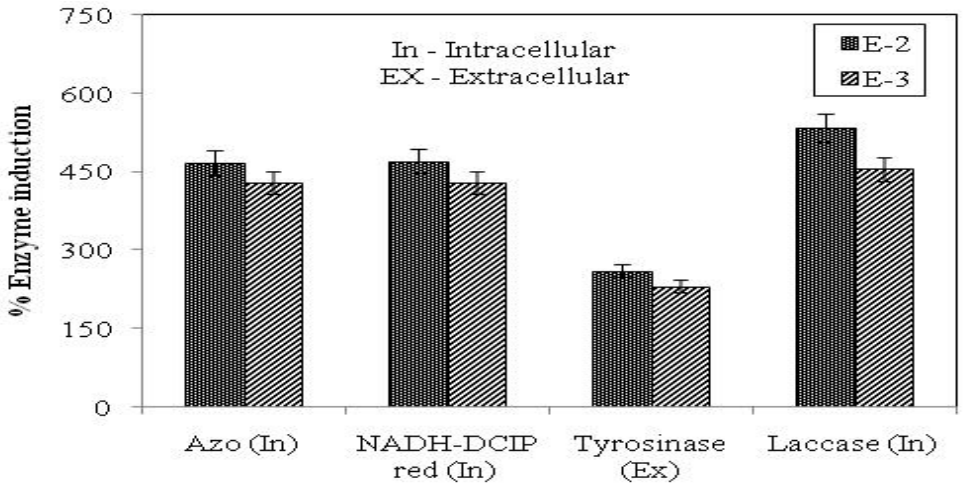

Figure 3 Induction of various enzymes by different DHEs. (90 mL waste, 10 mL consortium, incubation at static conditions at 32±2 °C)

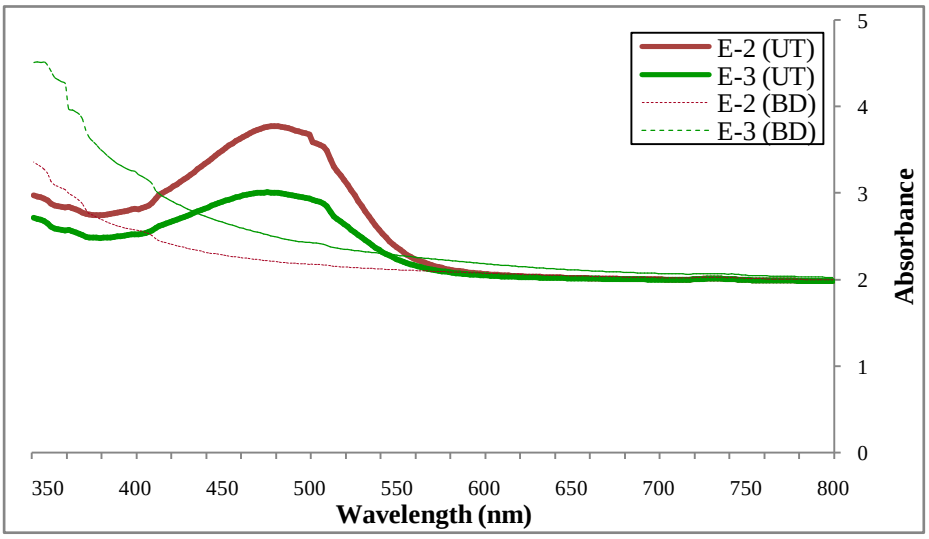

Figure 4 UV-Vis spectra of untreated (UT) and biodegraded (BD) DHEs.

decolourization and degradation of azo dyes (Singh et al., 2015). In the present study reductive enzyme activity and laccase enzyme activities were found higher as compared to other enzymes.

3.6 Biodegradation analysis

UV-Vis spectra of untreated and treated (biodegraded) DHEs showed disappearance of peaks in the visible region of the spectrum, indicated degradation of DHEs (Figure 4). Results of FTIR (Figure 5; Table 2) and HPLC (Figure 6; Table 3) analysis revealed that both the DHEs were decolourized with simultaneous degradation.

The FTIR spectra of DHEs, before and after degradation showed differences in the peaks (Figure 5; Table 2). Shifting of major peaks was found in biodegraded metabolite fractions of DHEs as compared to non degraded DHEs confirmed changes in the structural configuration of organic molecules present in the DHEs. Peaks at 3417 and 3433 cm^{-1} were found, which represent -OH stretching in untreated E-2 and E-3, respectively. This confirmed

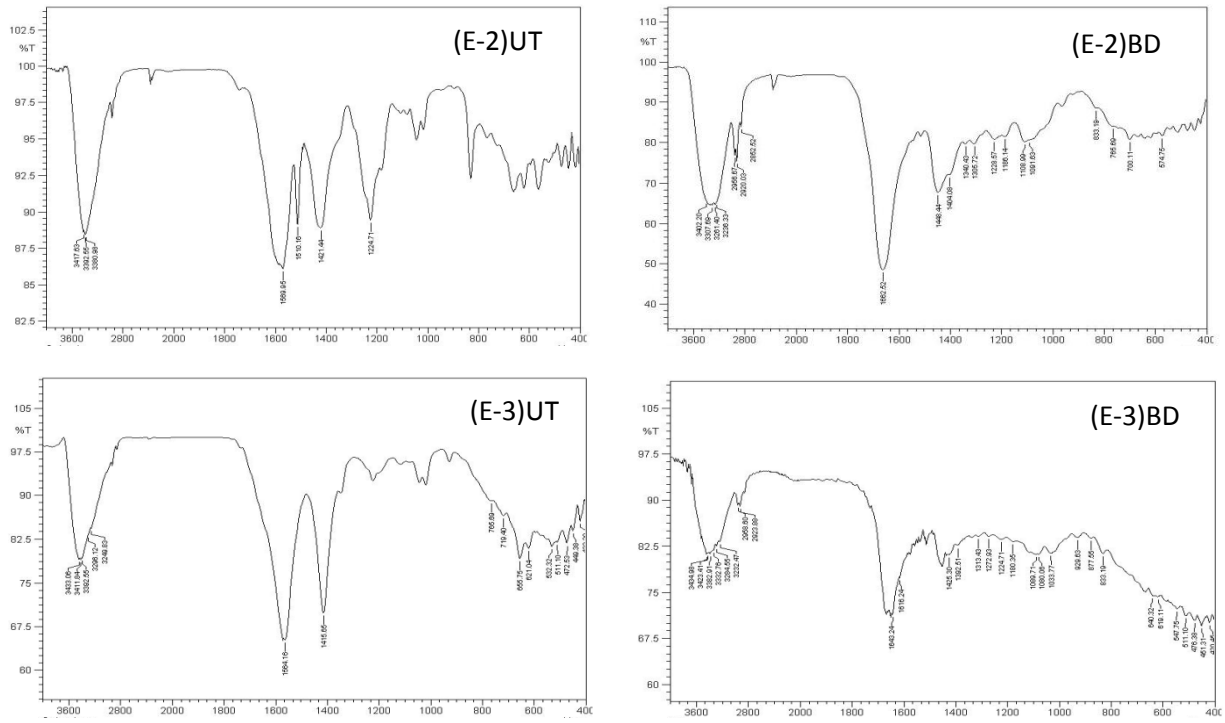

Figure 5 FTIR spectra of effluents. Untreated (UT) and Biodegraded (BD).

Table 2 FTIR spectral results of untreated (UT) and biodegraded (BD) DHEs.

E-2				E-3			
UT		BD		UT		BD	
Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond
3417.6	OH Str	3402.2	OH Str	3433.1	OH Str	3434.9	OH Str
1569.9	N=N Vib	2920.0	C-H Str	1584.2	N=N Vib	2923.9	C-H Str
1510.2	NO_2 Str	1448.4	CH_3 Def	1415.7	C-N Str	1643.2	C=O Str
1421.4	C-H Vib	1404.1	C-N Str	1041.5	S=O Str	1425.3	CH_3 Def
1224.7	C-N Str	1340.4	S=O Str	927.7	C=C Def	1392.5	C-N Vib
1041.5	S=O Str	1305.7	N=N-O Str	765.7	C-H Def	833.2	C-H Def
669.3	OH Def	1228.6	C-N Str	719.4	OH Def	547.7	Ring Def
565.1	Ring Def	1108.9	C-OH Str	511.1	NO_2 Band	511.1	NO_2 Band
493.7	NO_2 Str	833.2	C-H Def	472.5	NO_2 Str	476.4	NO_2 Str
474.5	NO_2 Str	700.1	OH Def	449.4	C-N-C Band	461.3	C-N-C bend
437.8	C-N-C Band	574.7	Ring Def				

Def: Deformation; Vib: Vibration; Str: Stretching; Bend: Bending

the presence of phenolic compounds in the DHEs. Moreover, removal of azo bond and change in spectrum profile of all five DHEs, before and after degradation confirmed the degradation of both the DHEs. Disappearances of azo bond representative peaks from 1569 and 1584 cm^{-1} confirmed the cleavage of azo bond in E-2 and E-3 after degradation, respectively. Appearance of peak at 1108.9 cm^{-1} in the degraded metabolite fraction of E-2 suggests generation of aliphatic alcohol formation. Aliphatic -CH_3

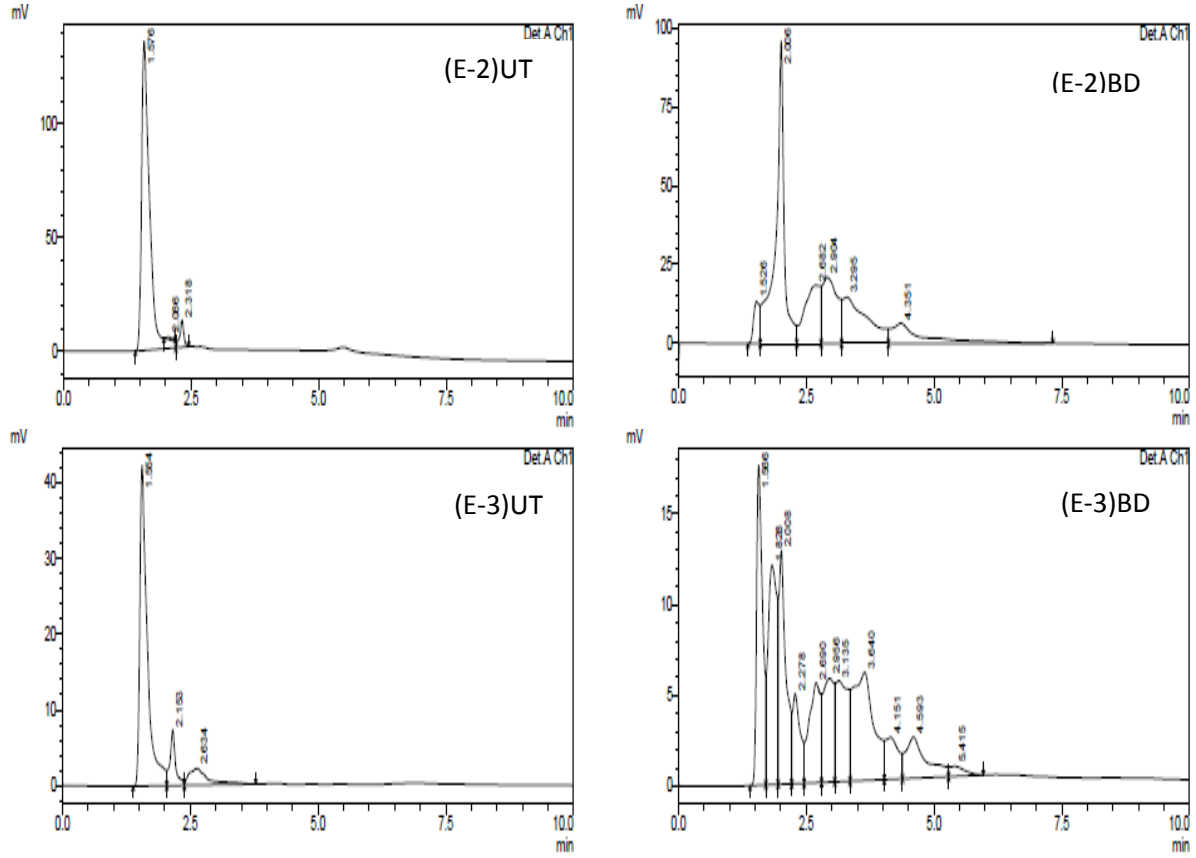

Figure 6 HPLC spectra of effluents. Untreated (UT) and Biodegraded (BD).

Table 3 HPLC elution profile of untreated (UT) and biodegraded (BD) DHEs.

Peak number	HPLC Peaks			
	E-2		E-3	
	UT	BD	UT	BD
1	1.576	1.526	1.554	1.566
2	2.066	2.006	2.153	1.828
3	2.318	2.682	2.634	2.008
4		2.904		2.278
5		3.295		2.690
6		4.351		2.956
7				3.135
8				3.640
9				4.151
10				4.593
11				5.415

Table 4 Phytotoxicity and microbial toxicity study of untreated (UT) and biodegraded (BD) DHEs.

DHEs	Treatment	Phytotoxicity *Phaseolus mungo*			Microbial toxicity	
		Germination (%)	Root length (cm)	Shoot length (cm)	*P. aeruginosa* Zone of inhibition (mm)	*Azotobacter sp.* Zone of inhibition (mm)
E-2	UT	0	-	-	19.0±0.2	18.1±0.1
	BD	70	6.9±1.0	8.6±2.4	11.0±0.2	10.0±0.1
E-3	UT	0	-	-	22.2±0.2	26.0±0.2
	BD	80	5.7±1.7	12.2±2.2	14.1±0.2	12.1±0.3
Control	-	100	6.0±1.6	13.0±3.2	NI	NI

NI: No inhibition, 10 seeds were dipped in 10 mL of UT and BD effluents and allowed to germinate at 32±2 °C temperature for 10 days. Further, 5 mL effluent was added on 4th and 7th day.

deformation peaks at 1448 to 1425 cm^{-1} and aromatic ring deformation peaks at 574 and 547 cm^{-1} frequencies were found in degraded metabolite fractions suggested aromatic ring deformation to aliphatic molecules of E-2 and E-3, respectively. Removal of metal and cleavage of azo bond followed by aromatic ring breakage to aliphatic one could be the intermittent important steps during degradation mechanism as both the DHEs were originated while manufacturing of metal complex dyes.

HPLC elution peaks of untreated and biodegraded DHEs were compared, which showed considerable differences in peak profile after degradation of DHEs (Figure 6; Table 3). Peaks of treated DHEs fractions were different in terms of number, height and area than the untreated DHE. Disappearance of major peaks with emergence of new peaks with different retention time was observed in case of both the DHEs. Major peaks were found at 1.57 and 1.55 RT in case of untreated E-2 and E- 3, respectively whereas treated DHEs showed differences in peak profile and majority of them were at higher RT than the peaks of untreated DHEs.

3.7 Toxicity study

Toxicity on plant was studied and none of the untreated DHEs showed any germination, whereas after treatment there were 70 and 80 germination with E-2 and E-3, respectively (Table 4). Reduced toxicity was observed on root and shoot lengths after treatment, irrespective of DHEs used in the study. Khandare et al. (2013) have reported reduction in phytotoxicity on *P. mungo* in terms of seed germination, length of plumule and length of radical after treatment of textile effluent.

Microbial toxicity study was carried out with *Azotobacter* and *P. aeruginosa* and results are shown in Table 4. Microbial toxicity was found to be reduced in the range of 40-42% and 44-46% in case of E-2 and E-3, respectively with both the test organisms. Reduction in phyto and microbial toxicity at a considerable extent represent the treatment efficiency by the developed consortium for such a recalcitrant industrial waste.

Conclusion

The study proved the efficiency of the developed consortium towards the biodegradation of both the dye house wastes of quite diverse and complex nature. Considerable reduction in ADMI, BOD, COD, TOC, solids and metals suggested the possible use of the developed single consortium for treatment of different dye house wastes. The probable mechanism was cleavage of azo bond, deformation of aliphatic compounds and aromatic rings which resulted in degraded, decolourized, less toxic effluents, which can be released safely. Moreover, after microbial treatment, toxicity of original DHEs was considerably decreased on plants and bacteria. It also showed reduction in organic load in the DHEs. Such single consortial system increases the applicability of bioremediation strategy as single treatment machinery and could be scaled up for the treatment of a wide range of DHEs.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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