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Treatment of dye house effluents by a developed bacterial consortium: A shake flask study

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Abstract

Bacterial consortium developed for decolourising 18 different metal complex dyes was used to investigate its efficiency towards treatment of three different dye house effluents E-1, E-4 and E-5(DHEs). Process was optimised at shake flask level for the pH, temperature, culture condition, carbon and nitrogen source. The developed consortium showed better decolourisation of the DHEs under static condition compared to shaking condition between pH 6-10 and 25-45℃ temperature. Decolourization was positively influenced by the addition of glucose, sucrose, maltose and starch in case of E-1, whereas E-5 was found to be better decolourized by supplementation of beef extract to the basal medium. However, E-4 showed variable decolourization pattern with <60% decolourization irrespective of carbon and nitrogen sources used. Decolourization and degradation profiles of all the 3DHEs were studied to optimize treatment time. Reduction in BOD, COD and American Dye Manufacturers' Institute (ADMI) values were in the range of 60-85% except for E-4 where BOD and ADMI removal was in the range of 75-90%, but rest of the other parameters were reduced in the range of 20-60% which were lost. Moreover, FTIR and HPLC spectral data analysis and enzyme induction pattern confirmed biodegradation of all the DHEs. Considerable amount of intracellular azoreductase, NADH-DCIP reductase and laccase productions were detected, which played significant role in degradation. Reduction in phyto- and microbial toxicity was found in the range of 70-100% and 40-53%, respectively which indicated detoxification of the wastes. The findings indicate that the developed bacterial consortium can be used for bio tretament of recalcitrant DHEs.

Keywords: Consortium, dye house effluent, toxicity, decolourization, biodegradation.

Introduction

Pollution by coloured effluent is one of the major areas of concern aesthetically as well as it damages the receiving water bodies and harm the life forms present in it and surrounding vicinity[1]. The major sources for the generation of coloured wastewater are effluents from dye houses and textile industries. Textile and dye stuff industries use huge amount of water resulting in generation of million liters of coloured effluents. Among all industrial sectors, coloured wastewater generating industries are found to be the most polluting[2]. Dye house effluents (DHEs) are dark coloured and may also contain heavy metals like chromium, copper, molybdenum, zinc, etc. They are also a sources of high COD, BOD, TOC, TDS and TSS which poses variety of health hazards upon disposal in to the environment[3]. Due to high cost and inefficiency of physical and chemical treatment methods, such coloured wastes remain a problem which need to be solved by alternative cost effective and ecofriendly attractive tool such as biodegradation[4,5]. Bacteria, fungi, yeast, algae and plants have been applied for their remediation potential in different studies[6-11]. Ability of consortial system to degrade more than one compound at a time along with co-metabolic activities leading to mineralization of the pollutant compound is a great benefit for the treatment of industrial wastes[12-15]. However, well optimised processes are not available for the treatment of metal containing DHEs.

Thus the present study focuses on treatment of three different DHEs, which contain high organic load, high American Dye Manufacturers' Institute (ADMI) value, dark colour and presence of toxic metals. Treatment of such wastes with a single consortium is not reported so far. Keeping these points in consideration, a consortium developed for degrading 18 different metal containing dyes was investigated for decolourization and degradation of as many as three different diverse DHEs. Analysis of effluent detoxification was carried out to check the efficiency of treatment method. The study would be the base for the development of wastewater treatment protocol for diverse group of dye containing industrial effluents.

Materials and methods

DHEs, media and chemicals: Three DHEs, E-1and E-4 were yellow and black in colour collected from Apex dye stuff industries and E-5 was black in colour and collected from Rohan Dye Chem industries, Vatva GIDC industrial estate, Ahmedabad, Gujarat, India. All the effluents contained mixture of metal complex azo dyes (MCADs), as during that period

only metal complex azo dyes were manufactured by the industry. If otherwise mentioned, treatment of all DHEs were 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 100mL Erlenmeyer flasks containing 50 mL of DHE system comprising of 10% (v/v) activated inoculum ($3x10^8$cells/mL). The static incubation was provided at 35±2°C temperature. ABTS (2,2- azinobis (3-ethylbenzothiazolin-6-sulphonic acid)), tartaric acid, NADH (nicotinamide adenine dinucleotide hydrogen), n-propane, L-tyrosine and other chemicals were of analytical grade from HiMedia Laboratories, India. Microbial cultures of *Azotobacter* sp. and *Pseudomonas aeruginosa* were obtained from the departmental culture collections.

Bacterial consortium: An indegeneous bacterial consortium was enriched from a dye polluted site and maintained in dye containing nutrient broth at 8±2°C for further use as described in the earlier reports[15-17]. Bacteria isolated on nutrient agar plate from the developed consortium were identified by analysing 16S rRNA gene sequences at Chromous Biotech, Bangaluru, India.

Optimization of DHEs decolourization: Decolourization of DHEs was optimized using various process parameters such as pH (4-12), temperature (25-55°C), incubation 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 profiles of all DHEs were studied at an interval of every 6h until decolourization remained constant. Decolourization rate was calculated in terms of percent decolourization per hour.

Physico-chemical analysis of DHEs: Analysis of DHEs for pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and total suspended solids (TSS) before and after treatment were carried out as per standard methods[18].

Analysis of colour removal: Decolourization of DHEs was measured by ADMI value calculation using Tristimulus filter method[18]. Comparison of UV-Vis spectra was made for 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 at 0 h and the final ADMI at specific reaction time.

Cell free extract preparation: Cells from activated consortium were harvested by the standard methods as reported earlier[16]. In brief the samples were centrifuged at 8000g for 10min and the obtained supernatant was used as extracellular enzyme fraction. For the intracellular enzymes, harvested cells were homogenized after suspending in 50mM potassium phosphate buffer at pH 7.4, and sonicated (Sartrious, Germany) at an amplitude of 70% with ten strokes each of 30s with a 2min interval at 4°C than centrifuged at 10000g for 20 min at 4°C for the removal of cell debris. Both the type of crude enzymes were used for enzyme assays. Reading of triplicate experiments were used for statistical calculation.

Enzyme assay: Oxidoreductive enzymes including tyrosinase, lignin peroxidase (LiP), laccase, azoreductase (Azo) and NADH-DCIP reductase (NADH-DCIP red) from intracellular as well as extracellular fractions were studied for their induction pattern while decolourization of DHEs. Azoreductase[19], NADH-DCIP reductase[20], Laccase[16,21,22] and Tyrosinase[23] enzymes activities were determined. Oxidative enzyme was defined as the amount of enzyme to increase 1.0 absorbance unit per unit of enzyme under the standard assay conditions. Reductive enzyme activity was defined as the amount of enzyme required to reduce 1µM substrate per minute per unit of enzyme.

Analysis of biodegradation: The untreated and treated DHEs centrifuged at 10000g for 10min, supernatants were extracted using twice the volume 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 600-4000cm^{-1} with 16scan speed (Bruker, Germany). For HPLC analysis, extracted metabolites were dissolved in spectroscopy grade methanol and injected in a C18 column (250mm×4.6mm,5mm) equipped with dual wavelength detector by the isocratic method using LC solutions (Shimadzu, Japan). Methanol: water (70:30) was used as mobile phase with a flow rate of 1.0mL/min and with 15min run time.

Toxicity study: Phytotoxicity study was performed at ambient temperature with *Phaseolus mungo*. Ten seeds were soaked in distilled water (control), untreated and treated DHEs. The toxic effects of individual treatment was evaluated with reference to percent germination, plumule length and radical length after 10 days[24].

Toxicity on the microorganisms of untreated DHEs and metabolites obtained after treatment was studied using *Azotobacter* sp. and *Pseudomonas aeruginosa*[16] and zone of inhibition was recorded after 24h of incubation.

Results and discussion

DHEs characterization: Characteristics of DHEs viz. E-1, E-4 and E-5 are shown in Table-1. All of them were found to be quite diverse in terms of TOC, COD, BOD, TDS, TSS, CU, ADMI and metal content. The pH of the samples were alkaline (8.34±0.15). Effluent E-4 and E-5 were black in colour but they were from two different industry thus they showed distinct diversity in terms of TOC, COD, BOD, TDS and TSS. The colour (CU) and ADMI value were 85000-564000 and 10794-

167158, respectively. Metal content in the DHEs ranged between 0.97-1.73, 2.03-13, 2.64-87.4, 2.7-2.8 and 15.9-24.3% (w/w) for Cu, Zn, Fe, Cd and Cr metals, respectively. Concentration of Cu, Zn and Fe were higher in E-4 compare to E-1 and E-5. The BOD: COD ratio of untreated DHEs were 0.7, 0.07 and 0.1 for E-1, E-2, E-3, E-4 and E-5, respectively. As per the literature, BOD: COD indicates the biodegradability of waste water[25]. 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 extensive stabilization prior to treatment. The E-4 and E-5 were found to be difficult to degrade by biological method compared to E-5, as BOD: COD ratio was 0.07 and 0.1, respectively. However, adaptation and selection of the consortium resulted in degradation of effluent E-4 and E-5. The obtained characterization of DHEs indicated the presence of coloured compounds and requirement of specific selection of consortium and its adaptation for the biodegradation of such effluents.

Table-1: Physico-chemical characteristics of various DHEs.

Parameters	Concentrations (mg/L) except pH, Colour and ADMI		
	Types of DHEs		
	E-1	E-4	E-5
pH	8.24	8.22	8.48
TOC	4731	6371	6400
COD	10500	17733	16266
BOD	7272	1212	1575
TDS	43970	109420	111090
TSS	76	240	176
VSS	44	72	56
NVSS	32	168	120
NH_3-N	58	64	53
Total nitrogen	280	476	448
Colour (cu)	85000	564000	554000
ADMI	30200	167158	10794
Metal			
Cu	1.73	7.4	0.97
Zn	2.06	13.0	2.03
Fe	2.64	87.4	ND
Cd	2.7	2.4	2.88
Cr	15.9	16.8	24.3
Ni, Co, Pb	ND	ND	ND

Identification of Bacteria from the consortium: A developed bacterial consortium showed the dominance of 5 bacteria, which represented 90% of the cultivable population. Dominant five isolates were identified as *Bacillus fortis* strain E4 Pb3 (KM502537), *Alcaligenes faecalis* (KM502541), *Pantoea ananatis* (KM502538), *Brevibacillus parabrevis* strain GRG (KM502542) and *Bordetella trematum* (KP751929) as per 16S rRNA gene sequence analysis. Sequence of the identified cultures are deposited with Gen Bank accession numbers as shown in paranthesis.

Optimization of DHEs decolourization: Influences of various parameters on decolourization are presented in Figure-1 A-E. Effect of various pH, incubation temperatures and carbon and nitrogen sources on biodecolourization of all the DHEs are shown in Figure-1A, 1B and 1C, respectively. The optimum pH was in the range of 6-9 but the consortium showed degradations even at pH 5 and 10. The temperature optima for the biodegradation was found to be 38±4ºC irrespective of the effluent under study. All the DHEs showed 4 to 26 fold higher rate of % ADMI removal at static 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 are in coordination with nonspecific reduction of azo dyes leading to colour removal under anaerobic conditions[26].

Results of effects of nitrogen and carbon sources are described in Figure-1C. As can be seen from the results, 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-1; whereas, mannitol and fructose for E-4 and E-5 respectively among the carbohydrates studied. The observed variation in the influence of added sugar could be explained based on the influence of particular effluents and presence of indigenous flora in the effluents. Addition of beef extract was found to be the choice as compared to yeast extract and peptone addition in the medium.

Decolourization profile in terms of incubation time was also studied (Figure-1D) and it was found that more than 90% decolourization was achieved within 60h in case of E-1 and 72h in case of E-5; on the other hand, E-4 showed less than 58% decolourization even after 72h. The average decolourization rate of first 42h of treatment for E-4 sample was 0.7% ADMI removal/h, while in case of E-1 and E-5 the rates were 4.0 and 1.7% ADMI removal/h, respectively. The effluent E-1 and E-5 showed fastest ADMI removal in first 6h with 8.5 and 4.3% ADMI removal/h whereas in case of E-4 the highest rate was observed between 24-30h indicated the adaptation time required for the consortium, as the rate was >1 for the first 24h. As per the available reports even in case of textile effluents which are having considerably lower COD, BOD and ADMI values, 48h are required to achieve 89% ADMI removal when BL-GG consortium was used[27,28]. The observed variation in terms of decolourization rate, optimum pH, temperature, requirement of carbohydrate and protein source as additional nutrients indicate

significant influence of the composition of the DHEs under study. The difference may be found due to the diversity of indigenous microbial flora present in particular DHEs. However, the developed consortium was found to be effective in decolourization of all the DHEs studied.

Effect of biological treatment on various parameters of DHEs: Treatment efficiency of the consortium for all the DHEs was studied in terms of reduction of COD, BOD, TOC, TDS, TSS and ADMI value and results are depicted in Figure-2. All the DHEs showed increase in pH, which rose from 7.4 to 8.4-9.2 after treatment. Moreover, the treatment also resulted in 84, 47 and 94% TOC reduction along with 90, 62 and 96% COD reduction for E-1, E-4 and E-5, respectively. In case of BOD, more than 98% BOD removal was observed in all the DHEs. Recorded dissolved solids reductions were 59, 33 and 85% for E-1, E-4 and E-5, respectively. Apart from this, the consortium showed >99% metal removal from all the three DHEs, as none of the metals were 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[24]. Decrease in COD, BOD, TOC, ADMI values of textile effluent is reported after treatment with consortium-AP[20]. Similar parameters including reduction in solids are also documented with plant and/or bacterial augmented combinations in a soil was used. In the reported study, the presence of both plant and bacterial augmentation resulted in maximum 50-80% reduction[29]. A textile effluent after anoxic-oxic treatment is described with 40 and 84% leftout of colour and COD, respectively[30]. BOD and COD reduction of a textile effluent is observed in the range of 68-74% from the initial BOD (890mg/L) and COD (3400mg/L) values, which are far less than the values in present effluents under study[27,28]. More reduction in initial COD indicates the high rate of mineralization[31].

Enzyme analysis: Treated DHEs showed better induction both in intracellular and extracellular enzyme fractions (Figure-3) as compared to untreated one. In terms of type of effluents studied E-5 showed highest induction of all the studied intracellular enzymes, whereas E-4 showed lowest induction. This can be the reason for observed lowest decolourization and lowest ADMI removal in case of E-4 sample. Intracellular enzyme induction was more as compared to extracellular enzymes. The two enzyme families, azoreductases and laccases, are described as for their great potential for decolourization and degradation of azo dyes[32]. In this study, activity of reductive enzyme and laccase was found more as compared to rest of the enzymes studied.

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 (Table-2) and HPLC (Table-3) analysis revealed that all the DHEs were decolourized with simultaneous degradation.

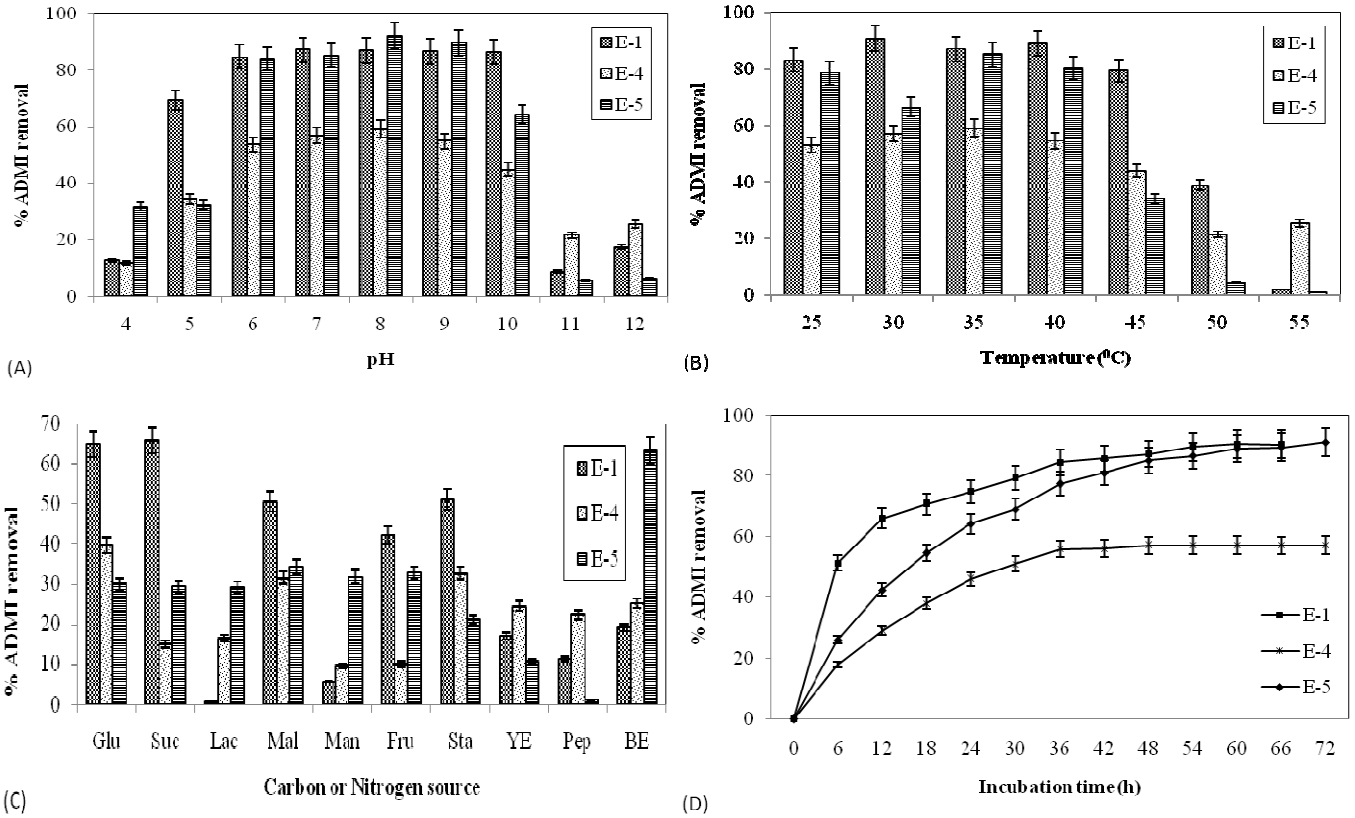

Figure-1: Influence of (A) pH, (B) Temperature, (C) Carbon and nitrogen source, (D) Contact time on decolourization of DHEs.

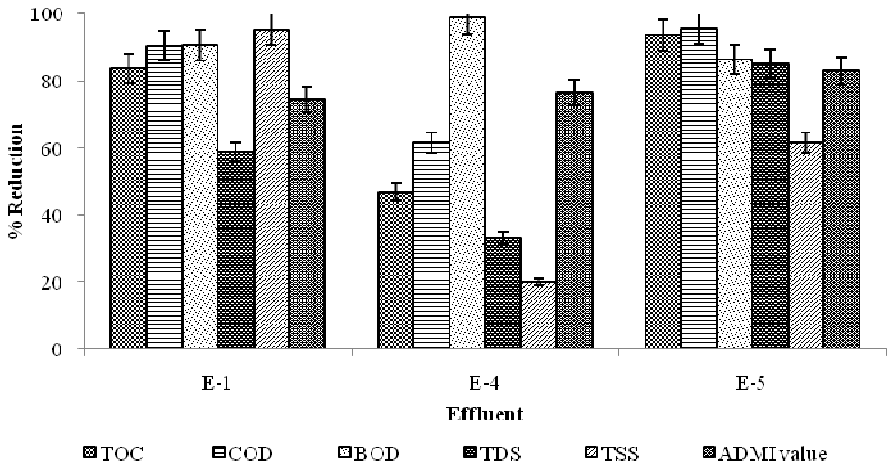

Figure-2: Percent reduction in TOC, COD, BOD, TDS, TSS and ADMI due to biological treatment of DHEs.

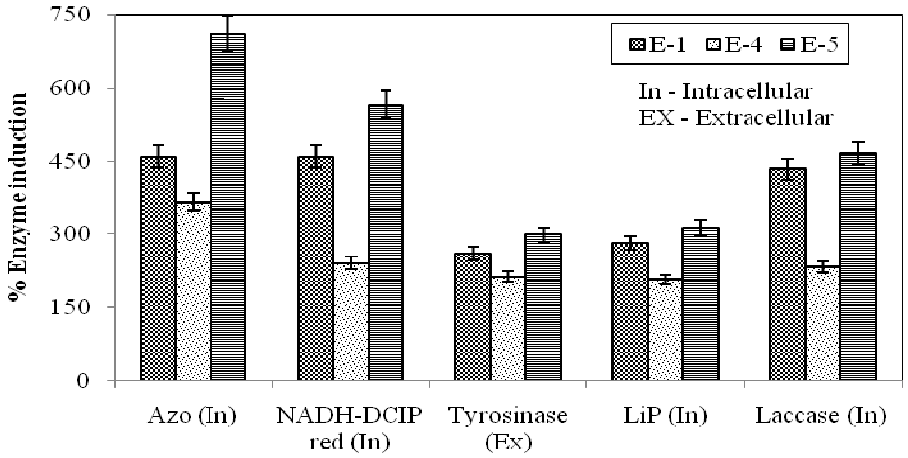

Figure-3: Induction of various enzymes by different DHEs.

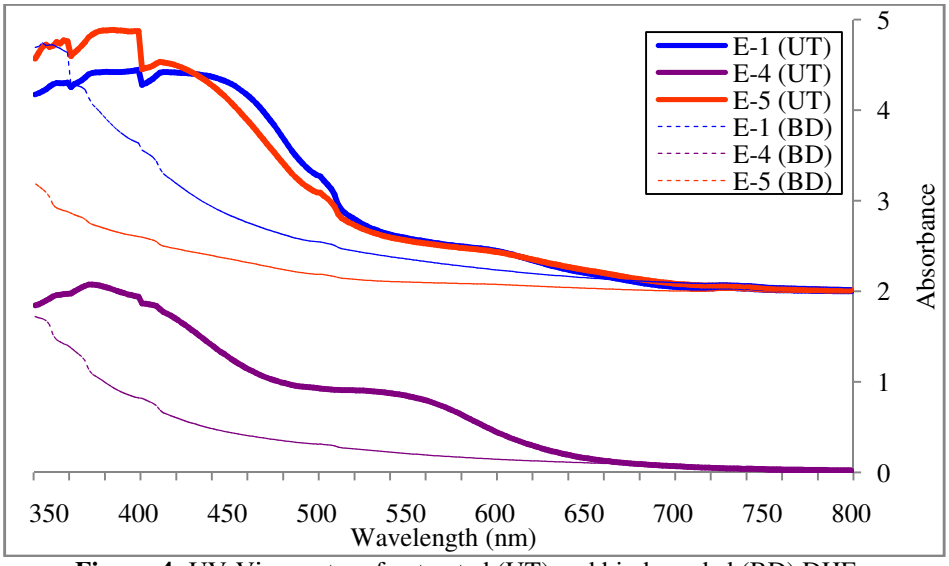

Figure-4: UV-Vis spectra of untreated (UT) and biodegraded (BD) DHEs.

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

E-1				E-4				E-5			
UT		BD		UT		BD		UT		BD	
Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond
3440.8	OH Str	3359.8	OH Str	3384.8	OH Str	3434.9	OH Str	3434.9	OH Str	3475.5	OH Str
2920.0	C-H Str	2956.7	C-H Str	2950.9	C-H Str	2958.8	C-H Str	2952.8	C-H Str	1666.4	C-N Str
1593.1	N=N Vib	1662.5	C=N Str	2358.8	NH^+ Str	1660.8	C-N Str	2426.3	OH Str	1641.3	C=O Str
1487.0	N=O Str	1541.0	NH_3^+ Def	2059.8	C=O Str	1452.3	CH_3 Def	2325.9	NH^+ Str	1427.2	CH_3 Def
1305.7	C-N Vib	1515.9	NO_2 Str	1614.3	NH_3 Def	1340.4	S=O Str	1733.9	C=O Str	1309.6	C-N Vib
1253.6	OH Def	1450.4	CH_3 Def	1556.4	N=N Vib	1303.8	C-N Vib	1512.1	N=N Vib	1089.7	C-N Str
1224.7	C-N Str	1340.4	S=O Str	1260.1	C-N Vib	1105.1	C-OH Str	1382.9	S=O Str	1033.7	S=O Str
1143.7	C-H Def	1303.8	N=N-O Str	1024.1	S=O Str	968.2	C ring Str	1269.1	C-N Vib	968.2	C ring Str
1031.9	S=O Str	1276.8	C-N Vib	977.8	C ring Str	740.4	C-H Def	1033.8	C-OH Str	921.9	C=C Def
962.4	C ring Str	1112.9	C-OH Str	929.6	C=C Def	700.1	OH Def	771.5	C-H Def	831.3	C-H Def
671.2	OH Def	968.2	C ring Str	879.4	Ring Def	590.2	C=O Band	622. 9	Ring Def	675.0	OH Def
638.5	NO_2 Def	619.1	Ring Def	829.3	C-H Def	478.4	NO_2 Str	488.7	NO_2 Str	634.5	Ring Def
590.2	C=O Band	592.1	C=O Band	669.2	OH Def	433.9	C-N-C Band	437.8	C-N-C Band	522.6	NO_2 Def
585.1	SO_2 Def	545.8	NO_2 Def	540.0	NO_2 Def					433.9	C-N-C Band
530.4	C-C=O Band	484.8	Ring bend	451.3	C-N-C Band						
445.5	C-N-C Band										

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

DHEs showed differences in FTIR spectra, before and after degradation (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 in the range of 3232 to 3475cm^{-1} were found, which represent -OH stretching in untreated DHEs. This confirmed the presence of phenolic compounds in the DHEs. Moreover, removal of azo bond and change in spectrum profile of all DHEs, before and after degradation confirmed the degradation of all the DHEs. Disappearances of azo bond representative peaks from the range of 1512 to 1592cm^{-1} in degraded DHEs confirm the cleavage of azo bond. Appearance of peaks at 1112.9 and 1105.1cm^{-1} in the degraded metabolite fraction of E-1 and E-4 suggests generation of aliphatic alcohol formation. Aliphatic -CH_3 deformation peaks in the range of 1427 to 1452cm^{-1} and aromatic ring deformation peaks in the range of 484 to 634cm^{-1} frequency were found in degraded metabolite fraction of all the DHEs suggested aromatic ring deformation to aliphatic molecules. 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 all 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 (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 all the three DHEs. Major peaks were found at 2.16, 2.43 and 2.23 RT in case of untreated DHEs E-1, E- 4 and E-5, respectively. After treatment of DHEs the profiles of the peaks changed and most of them were at higher RT than the peaks of untreated DHEs.

Toxicity study: In case of phytotoxicity study, no germination was observed in any of the plant seeds untreated DHEs, whereas after treatment there were 100, 70 and 80% germination with E-1, E-4 and E-5, respectively (Table-4). Reduction in toxicity was observed on root and shoot lengths after treatment, irrespective of DHEs used in the study. When the dilution was considered, the DHEs can be arranged in the increasing order of inhibitory effects even after the treatments, the order was E-1<E-5< E-4, respectively. In case of E-4, 1:50 dilution was required where as in case of E-1, only 1:2 dilutions was required. Reduction in phytotoxicity on *P. mungo* was recorded for seed germination and length of plumule/ radical after treatment of textile effluent is reported in study[29].

Table-3: Profile of HPLC elution peaks of untreated (UT) and biodegraded (BD) DHEs.

Peaks	DHEs					
	E-1		E-4		E-5	
	UT	BD	UT	BD	UT	BD
1	1.571	2.149	1.585	1.604	1.532	1.507
2	1.980	2.683	2.431	1.970	2.230	1.845
3	2.151	2.891	3.251	2.111		2.000
4	2.634	3.218		2.676		2.112
5	2.726	4.142		2.903		2.964
6	3.558			3.276		3.175
7	4.364			3.942		3.773
8				4.184		4.340
9				6.206		4.838

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

DHEs	Treatment	Phytotoxicity			Microbial toxicity	
		Phaseolus mungo			*P. aeruginosa*	*Azotobacter sp.*
		Germination (%)	Root length (cm)	Shoot length (cm)	Zone of inhibition (mm)	
E-1	UT	0	-	-	18.1±0.1	20.1±0.3
	BD	100	5.3±1.0	11.8±1.3	10.2±0.2	12.1±0.3
E-4	UT	0	-	-	32.1±0.1	30.0±0.1
	BD	70	8.5±2.6	13.1±2.0	16.0±0.2	14.1±0.1
E-5	UT	0	-	-	24.0±0.2	28.0±0.2
	BD	80	5.1±1.7	13.7±0.8	12.0±0.1	15.0±0.3
Control	-	100	6.0±1.6	13.0±3.2	NI	NI

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

Results of microbial toxicity study on *Azotobacter* and *P. aeruginosa* are shown in Table-4. Among the studied three effluents even after the treatment the effluent E-4 showed highest toxicity on both the organism as compared to effluent E-1 and E-5. If biodegradability is considered E-4 showed the minimum degradation which could be due to toxic effects of this effluent even on the developed consortium. The toxicity of E-4 may be attributed to its metal ions and their concentration, BOD:COD ratio (0.07) and/or presence of more recalcitrant compounds as compared to other DHEs.

Conclusion

The consortium used in the study was developed by sequential transfer in 18 MCADs individually as well as in their combinations, thus the developed consortium showed biodegradation of a variety of dye house wastes of quite diverse and complex nature. Removal of ADMI, BOD, COD, TOC, solids and metals suggested the possible use of the developed single consortium for treatment of different dye house wastes. The cleavage mechanism was atributed by breakage of azo bond, deformation of aliphatic compounds and aromatic rings which resulted in degraded, decolourized, less toxic effluents. Moreover, toxicity of original DHEs was considerably decreased on plants and bacteria after the treatment applied. Organic load in the DHEs was also reduced considerably. Such single consortium system increases the applicability of bioremediation technology as single treatment machinery could be effectively scaled up to treat variety of DHEs as well as several MCADs containing waste.

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