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Decolorization of various azo dyes by bacterial consortium

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Abstract

This study was taken up to enrich and isolate bacterial strains capable of decolorizing azo dyes present in soil/sludge samples collected from waste disposal sites of local textile industries. Four bacterial isolates identified as *Bacillus cereus* (BN-7), *Pseudomonas putida* (BN-4), *Pseudomonas fluorescence* (BN-5) and *Stenotrophomonas acidaminiphila* (BN-3) capable of completely decolorizing C.I. Acid Red 88 (AR-88), were used to develop consortium designated HM-4. The concerted metabolic activity of these isolates led to complete decolorization of AR-88 (20 mg L^{-1}) in 24 h, whereas individual cultures took more than 60 h to achieve complete decolorization of the added dye. The consortium was screened for its ability to decolorize different concentrations of other commonly used azo dyes in addition to AR-88. It was able to decolorize 78% of C.I. Acid Red 88, 99% of C.I. Acid Red 119, 94% of C.I. Acid Red 97, 99% of C.I. Acid Blue 113 and 82% of C.I. Reactive Red 120 dyes at an initial concentration of 60 mg L^{-1} of mineral salts medium (MSM) in 24 h. This consortium will be used to develop bioreactor for achieving effective decolorization of textile industry effluent containing mixture of azo dyes.

Keywords: Azo dyes; Decolorization; Bacterial consortium; Anoxic; Static incubation

1. Introduction

The textile dyeing and finishing industry use wide variety of dyestuffs due to the rapid changes in the customer's demands. The world annual production of the dyestuffs amounts to more than 7×10^5 tonnes [1]. Azo dyes, containing one or more azo bond (−N=N−), account for 60−70% of all textile dyestuffs used [2]. It is estimated that about 10−15% of the total production of colorants is lost during their synthesis and dyeing processes [3,4]. Colored industrial effluent is the most obvious indicator of water pollution and the discharge of highly colored synthetic dye effluents is aesthetically displeasing and cause considerable damage to the receiving water bodies by impeding the penetration of light. Many microorganisms belonging to different taxonomic groups of bacteria [5], fungi [6], actinomycetes [7] and algae [8] have been reported for their ability to decolorize azo dyes. Although many reports are available in literature regarding capability of pure cultures to decolorize dyes [9−11] but they do not find much applications in treatment system for industrial effluent because of heterogeneity of the components in effluents depending upon production schedule. The treatment systems having mixed microbial populations are more effective due to concerted metabolic activities of microbial community [12−14]. The azo bond is known to undergo reductive cleavage whereas the resultant aromatic amines are metabolized under aerobic conditions [15]. Thus, the microbial populations forming part of treatment system should be able to work under both anaerobic/anoxic and aerobic conditions so

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as to achieve complete mineralization of the dye molecule. In view of these problems, adapted microorganisms were isolated from samples collected in the vicinity of textile dying units located in and around the city of Amritsar. These isolates were used to develop a consortium, which can decolorize azo dyes at faster rate and can be used further to develop a continuous process for the treatment of textile processing industry wastewater containing a wide variety of textile dyes.

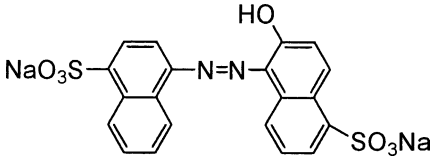
2. Experimental

2.1. Materials

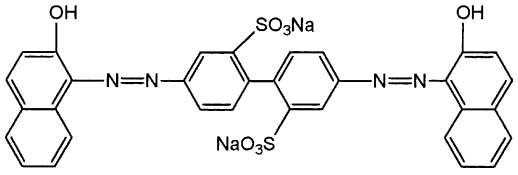
2.1.1. Dyes and chemicals

The azo dyes C.I. Acid Red 88 (AR-88), C.I. Acid Red 119 (AR-119), C.I. Acid Red 97 (AR-97), C.I. Acid Blue 113 (AB-113) were obtained from Punjab Rang Udyog, a dye-manufacturing unit, Amritsar, Punjab (India). C.I. Reactive Red 120 (RR-120) with formulation designated as Procion Red H-E3B dye (Zeneca Colours, Manchester, UK) was a generous gift from Prof. John Taylor, UMIST, UK. The media components and chemicals were purchased from Himedia Labs, Bombay (India). All chemicals used were of analytical grade. The complexity of the chemical structures of some of the dyes is evident from the following structures:

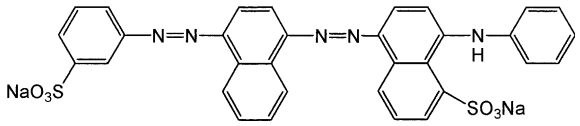
(1) C.I. Acid Red 88 (AR-88), C.I. 15620, $\lambda_{max} = 505$ nm



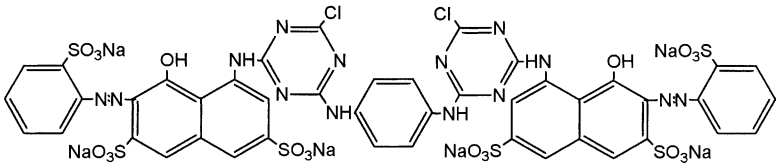
(2) C.I. Acid Red 97 (AR-97), C.I. 22890, $\lambda_{max} = 494$ nm



(3) C.I. Acid Blue 113 (AB-113), C.I. 26360, $\lambda_{max} = 554$ nm



(4) C.I. Reactive Red 120 (RR-120), C.I. 25810, $\lambda_{max} = 530$ nm



2.1.2. Growth medium

Mineral salts medium (MSM) of following composition (g L^{-1}): Na_2HPO_4 (3.6), $(NH_4)_2SO_4$ (1.0), KH_2PO_4 (1.0), $MgSO_4$ (1.0), Fe(NH_4) citrate (0.01), $CaCl_2{\cdot}2H_2O$ (0.10) and 10.0 mL of trace element solution per liter was used for all the studies. The trace element solution used was of following composition (mg L^{-1}): $ZnSO_4{\cdot}7H_2O$ (10.0), $MnCl_2{\cdot}4H_2O$ (3.0), $CoCl_2{\cdot}6H_2O$ (1.0), $NiCl_2{\cdot}6H_2O$ (2.0), $Na_2MoO_4{\cdot}2H_2O$ (3.0), H_3BO_3 (30.0), $CuCl_2{\cdot}2H_2O$ (1.0). The final pH of the medium was adjusted to 7.0. The MSM was supplemented with 0.1% (w/v) of each of yeast extract and glucose unless otherwise mentioned. The yeast extract, glucose and dyes were added to sterilized MSM from their respective filter sterilized stock solutions.

2.2. Isolation and screening of dye-decolorizing microorganisms

The MSM broth was inoculated with 10% (w/v) of sludge/soil samples collected from waste disposal sites of textile processing and dye-manufacturing units in and around Amritsar (India). The flasks were incubated at 35 °C under shaking conditions (100 rpm). After 48 h of incubation, 1.0 mL of the culture broth was appropriately diluted and plated on MSM-agar containing 20 mg L^{-1} AR-88. The morphologically distinct bacterial isolates showing clear zones around their colonies due to decolorization of dye were selected for further studies. The pure culture stocks of these isolates were stored at 4 °C on MSM-agar slopes containing 20 mg L^{-1} of AR-88. These isolates were screened for their ability to decolorize AR-88 in liquid culture. A loopful of growth form stock culture slope was inoculated into MSM broth and the flasks were incubated

at 35 °C under shaking conditions (100 rpm). After 24 h of incubation under shaking conditions, the culture broth was transferred to serum vials of 50 mL volume containing 20 mg L^{-1} of AR-88 supplement. The vials were filled completely and sealed with screw caps so as to achieve anoxic conditions. The number of vials incubated corresponded to the number of samples withdrawn so that each vial was opened only once. The uninoculated control was also incubated to check the abiotic decolorization of dye. The decolorization efficiency of the isolates was determined as described below.

2.3. Decolorization assay

The samples from serum vials were centrifuged at 10,000 rpm for 15 min. The supernatant of the centrifuged samples was read at absorbance maximum (λ_{max}) of the dyes used i.e. 505 nm for AR-88, 520 nm for AR-119, 494 nm for AR-97, 554 nm for AB-113 and 530 nm for RR-120 using Shimadzu UV-1601 model (Kyoto, Japan) spectrophotometer. The uninoculated dye free medium was used as blank. All assays were performed in duplicate and compared with uninoculated controls. The decolorization efficiency of different isolates was expressed as per the following Eq. (1):

$$\text{Decolorization}\ (\%) = \frac{(I-F)}{I} \times 100 \quad (1)$$

where I = initial absorbance and F = absorbance of decolorized medium.

2.4. Development of consortium

Four laboratory isolates designated BN-3, BN-4, BN-5 and BN-7 having best decolorization efficiency were used for consortium development. The isolates were submitted to Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India for identification. These isolates were used in different combinations to determine the effect of their concerted metabolism on decolorization efficiency. Axenic cultures of the individual isolates were grown to their respective mid-log phase in MSM under shaking conditions and mixed in 1:1 ratio by volume so that each isolate contributed optical density (O.D.) at 540 nm of 1.0. The decolorization efficiency of different consortia thus developed (Table 3) was determined as per procedure mentioned above. The consortium having all the four isolates i.e. BN-3, BN-4, BN-5 and BN-7 was designated HM-4 and was selected for further studies as it achieved maximum decolorization efficiency as compared to all other consortia.

2.5. Optimization of medium supplements

The effect of different initial concentrations of glucose (0–7.0 mM) and yeast extract (0–0.15%w/v) in MSM on the decolorization ability of consortium HM-4 was studied.

2.6. Decolorization of different azo dyes

The consortium HM-4 was checked for its ability to decolorize other azo dyes viz. AR-97, AR-119, AB-113 and RR-120. The effect of different initial concentrations of each dye ranging from 20–100 mg L^{-1} in MSM broth on decolorization potential of consortium HM-4 was also tested.

3. Results and discussion

3.1. Isolation and identification of dye-decolorizing bacteria

The screening of microbial populations of the collected samples led to the isolation of 15 morphologically distinct isolates showing decolorization of dye on MSM dye agar plates. The selected isolates were checked for their ability to decolorize AR-88 (20 mg L^{-1}) in MSM broth (Table 1). The unidentified isolates designated BN-5, BN-4, BN-3 and BN-7 decolorized 78%, 80%, 85% and 89%, respectively, of the added dye in 24 h with no accumulation of dye on cells and complete decolorization of the dye was achieved in 60 h. The dye accumulation was checked by the extraction of dye from the cell pellets in methanol. The isolates designated CS-7, BR-1, VB-2, 31-B, CS-5, and C-97 showing 26%, 25%, 37%, 31%, 15% and 18% decolorization of AR-88 (20 mg L^{-1}),

Table 1
Percent decolorization of AR-88 (20 mg L^{-1}) by isolated bacterial strains

Isolates	Decolorization (%) after					
	12 h	24 h	36 h	48 h	60 h	72 h
BN-7[a]	40	89	95	99	100	100
CS-7	25	26	26	27	27	28
41B	08	09	12	12	12	12
BR-1	24	25	25	26	26	27
BN-4[a]	35	80	94	99	100	100
VB-2	37	37	37	37	38	38
31B	28	31	39	42	43	43
NS-1	08	09	09	10	10	10
7A	05	10	12	12	13	14
BN-3[a]	50	85	99	99	99	99
31C	02	06	06	07	07	07
CS-5	14	15	19	19	20	20
VB25	04	11	15	15	15	15
BN-5[a]	31	78	88	94	100	100
C-97	18	18	20	20	21	21

[a] Selected strains.

Table 2
Biochemical and physiological characterization of selected strains

Character	*Stenotrophomonas acidaminiphila* (BN-3)	*Pseudomonas putida* (BN-4)	*Pseudomonas fluorescence* (BN-5)	*Bacillus cereus* (BN-7)
Gram's staining test	Gram negative	Gram negative	Gram negative	Gram positive
Spore staining test	–	+	–	+
Morphology	rods	rods	cocci	rods
H_2S production	–	–	+	–
MacConkey agar growth	+	–	+	–
Fluorescence	–	–	+	–
Motility	–	–	+	+
Catalase	+	+	+	+
Oxidase	+	+	+	+
Methyl red test	–	–	–	–
Voges Proskauer test	–	–	–	–
Indole production	–	–	–	–
Citrate utilization	+	+	+	+
Starch hydrolysis	–	–	–	+
Casein hydrolysis	–	–	–	+
Gelatin hydrolysis	–	–	–	+
Oxidation–Fermentation	–	O	O	O
Nitrate reduction	+	–	+	+
Anaerobic growth	–	–	–	+
Urease	–	–	–	–
Acid/Gas production from glucose	–	+	+/G	+
Arabinose	–	–	–	–
Mannitol	–	+	+	–
Xylose	–	+	+	–
Meso-inositol	–	–	–	+
Raffinose	–	–	–	–
Rhamnose	–	–	–	–
Salicin	–	–	–	–
Sucrose	–	–	–	–
Galactose	–	+	+	+
Lactose	–	–	–	–

respectively, after 24 h were dye accumulators. No other isolate was capable of decolorizing more than 15% of the added dye after 72 h incubation (Table 1). The strains BN-3, BN-4, BN-5 and BN-7 were selected for further studies due to their higher decolorization potential. The results of routine morphological and physiological characteristics of the isolated strains are summarized in Table 2. These strains were identified as *Stenotrophomonas acidaminiphila* (BN-3), *Pseudomonas putida* (BN-4), *Pseudomonas fluorescence* (BN-5), *Bacillus cereus* (BN-7) by MTCC, IMTECH, Chandigarh, India. The isolation of efficient dye decolorizers from the samples collected from the waste disposal sites indicates the natural adaptation of these microorganisms to survive in the presence of toxic dyes. Abd El-Rahim et al. [16] reported isolation of microorganisms adapted to high dye concentrations from sites near textile industry complex. Similarly, Chen et al. [5] reported isolation and screening of microorganisms capable of decolorizing different azo dyes from sludge samples collected from lake-mud and wastewater treatment plant.

3.2. Development of consortium

The consortia based on different combinations of the four isolates were screened for their decolorization efficiency. The results presented in Table 3 reveal that consortium, designated HM-4, based on all the four selected isolates showed significant increase in decolorization efficiency as 20 mg of dye AR-88 per liter was decolorized in 20 h whereas the individual isolate took 60 h to achieve complete decolorization (Table 1). Thus, concerted metabolic activities of the isolates comprising the consortium HM-4 resulted in three-fold increase in decolorization efficiency. The concerted metabolic potential of the microbial populations to decolorize colored wastewater of textile processing industry has been reported by Coughlin et al. [13] wherein two uncharacterized bacterial strains were used to mineralize up to 90% of the added dye C.I. Acid Orange 7. Similarly, Yoo et al. [17] used an anaerobic mixed culture consisting of sulfate reducing, methane producing and fermentive bacteria for decolorization of hydrolyzed C.I. Reactive Orange 96.

3.3. Effect of glucose

Different initial concentrations of glucose (0–7.0 mM) in MSM were used to determine their effect on decolorization rate of AR-88 (20 mg L^{-1}). The results presented in Fig. 1 indicate that glucose is

Table 3
Decolorization of azo dye AR-88 (20 mg L^{-1}) by various combinations of the cultures BN-3, BN-4, BN-5 and BN-7

Culture combinations	Decolorization (%) after					
	4 h	8 h	12 h	16 h	20 h	24 h
BN-3+BN-4	35	50	64	80	92	94
BN-3+BN-5	37	43	52	74	83	95
BN-3+BN-7	28	31	39	52	73	84
BN-4+BN-5	18	24	34	54	70	77
BN-4+BN-7	32	55	72	86	95	95
BN-5+BN-7	38	53	68	80	87	92
BN-3+BN-4+BN-5	39	43	52	61	71	77
BN-3+BN-4+BN-7	38	54	68	82	89	89
BN-3+BN-5+BN-7	34	51	75	89	94	98
BN-4+BN-5+BN-7	31	48	61	74	84	93
BN-3+BN-4+BN-5+BN-7 (HM-4)	48	86	90	98	99	100

Individual isolates were mixed in equal proportion to achieve a final $O.D._{540\ nm}$ of 4.0 at the start of the experiment.

essential for decolorization of AR-88, as in the absence of glucose only 20% decolorization of the added dye was achieved in 24 h. However, at increasing glucose supplement in MSM, decolorization efficiency of the consortium increased proportionally and complete decolorization was achieved with 4.2 mM glucose in 24 h. Further increase in glucose concentration in MSM up to 7.0 mM had no effect on decolorization rate of AR-88. Thus, 4.2 mM glucose supplement in MSM was optimum to achieve complete decolorization of AR-88 dye. The metabolism of the glucose resulting in production of reduced nucleotides (NADH, FADH) lead to enhanced decolorization efficiency. These reduced nucleotides are reported to be redox mediators involved in reduction of azo bond [2]. Similarly, Sponza and Isik [18] reported that glucose equivalent to 3000 mg COD L^{-1} provided reduced environment to enhance decolorization rate of azo dye C.I. Direct Black 38 by granulated anaerobic sludge obtained from the UASB reactor treating the wastewaters of yeast factory. However, Chung et al. [19] reported that glucose inhibited the reduction of azo dye tetrazine by *Bacteriodes thetaiotaumicron* at concentration as low as 1 μM.

3.4. Effect of yeast extract

Different nitrogen sources such as yeast extract, beef extract, peptone, tryptone, soybean meal were used at an initial concentration of 0.1% (w/v) in MSM broth to study their effect on the decolorization efficiency of consortium HM-4. Yeast extract was found to be the most effective supplement for supporting higher decolorization efficiency of HM-4 (Data not shown). The organic nitrogen sources are considered essential medium supplements for the regeneration of NADH that act as electron donor for the reduction of azo dyes by microorganisms [20]. Effect of different concentrations of yeast extract in the range of 0–0.15% (w/v) in MSM broth on the decolorization efficiency of HM-4 was evaluated (Fig. 2). In the absence of any yeast extract supplement in MSM, only 34% of colour removal was observed whereas complete decolorization of the dye was achieved at 0.1% (w/v) of yeast extract in medium in 12 h. Further increase in yeast extract concentration has no effect on overall decolorization potential. Therefore, for further studies 0.1% (w/v) of yeast extract in MSM was selected. Chen et al. [5] reported yeast extract as the best nitrogen source supplement among different sources viz. peptone, tryptone, monosodium glutamate, meat extract, beef extract, yeast extract and urea checked for enhancing the decolorization rate of azo dye C.I. Reactive Red 198 (Red RBN; 50 mg L^{-1}). It was further reported that optimum concentration of yeast extract for achieving maximum decolorization was 0.8% (w/v) while higher concentration has no significant effect on decolorization efficiency of *Aeromonas hydrophila* whereas, HM-4 achieved optimum working at 0.1% (w/v) yeast extract supplement in MSM. Effect of yeast extract concentration

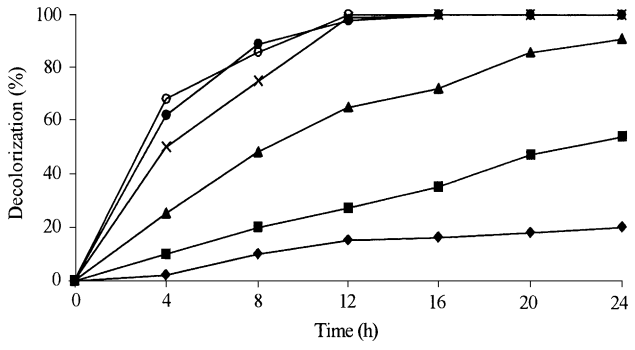


Fig. 1. Effect of initial glucose concentration on decolorization of AR-88 (20 mg L^{-1}) by consortium HM-4 in MSM supplemented with 0.1% (w/v) of yeast extract (◆, 0.0 mM; ■, 1.4 mM; ▲, 2.8 mM; ×, 4.2 mM; ○, 5.6 mM and ●, 7.0 mM).

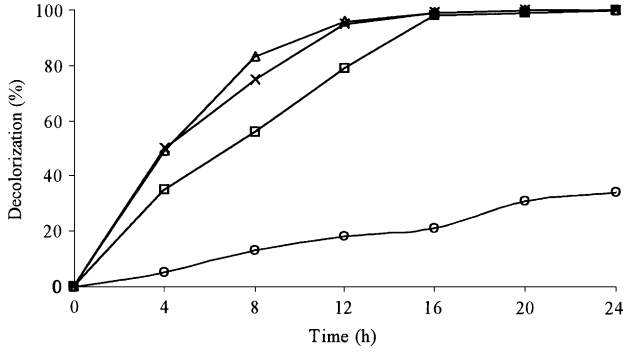


Fig. 2. Effect of different yeast extract concentrations on decolorization of AR-88 (20 mg L^{-1}) by consortium HM-4 in MSM supplemented with 4.2 mM glucose (○, 0.0%; □, 0.05%; △, 0.1% and ×, 0.15%).

on decolorization of dye in bioreactor studies is also reported in literature [21].

3.5. Decolorization of different azo dyes by HM-4

The consortium was tested for its ability to decolorize four other azo dyes viz. AR-97, AR-119, AB-113 and RR-120 in addition to AR-88. The overall decolorization of 95% to 100% was achieved up to an initial dye concentration of 40 mg L^{-1} in 24 h (Fig. 3), whereas, with increasing concentration of respective dyes the decolorization efficiency of the consortium decreased as 69%, 73% and 86% decolorization of AR-88, RR-120 and AR-97, respectively, was observed at an initial dye concentration of 100 mg L^{-1} in MSM. Dyes AB-113 and AR-119 were decolorized almost completely upto 100 mg L^{-1} of initial dye concentration. The decrease in decolorization efficiency might be due to the toxic effect of dyes or the blockage of active sites of azoreductase enzymes by dye molecule with different structures [18]. Similarly, Sani and Banerjee [22] found that dyes were easily decolorized at concentration $\leq$ 10 μM by *Kurthia* sp, but colour removal was reduced when dye concentration was increased to 30 μM. Similarly, Nigam et al. [23] reported a mixed bacterial culture-PDW capable of decolorizing various textile dyes but at a comparatively slower decolorization rate as it took minimum of 48 h to achieve complete decolorization of the added dye. The individual isolates of the consortium-PDW were unable to decolorize any of the dyes. A dye-decolorizing strain *A. hydrophila* achieved complete decolorization of eight out of 24 dyes checked at an initial concentration of 50 mg L^{-1} after 7 days of incubation [5]. The consortium HM-4 was able to completely decolorize the tested dyes in 24 h up to a concentration of 40 mg L^{-1}. This ability of consortium HM-4 to decolorize different azo dyes at comparatively faster rate will be exploited in a continuous process for treatment of textile effluent containing a wide variety of azo dyes. Enhanced rate of decolorization of various TPM dyes by bacterial consortium has already been reported in our previous study [14].

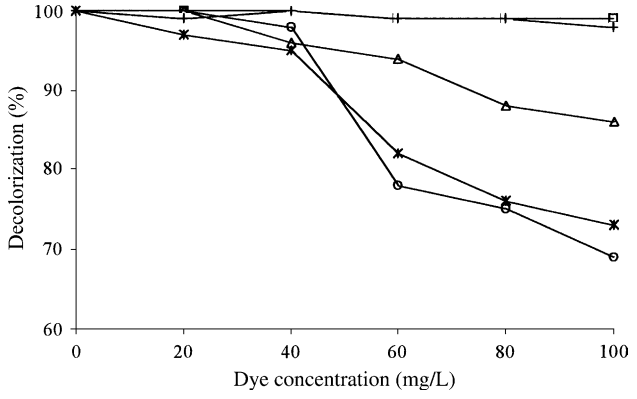


Fig. 3. Decolorization of different azo dyes by consortium HM-4. MSM broth was supplemented with 4.2 mM glucose and 0.05% (w/v) yeast extract (○, AR-88; □, AR-119; △, AR-97; ×, AB-113 and ●, RR-120).

4. Conclusions

1. The concerted metabolic activities of the consortium HM-4 consisting of four bacterial strains viz. *B. cereus* (BN-7), *P. putida* (BN-4), *P. fluorescence* (BN-5) and *S. acidaminiphila* (BN-3) resulted in about three-fold increase in decolorization efficiency as compared to that shown by individual strains. The consortium was able to decolorize various azo dyes viz. AR-88, AR-119, AB-113, AR-97 and RR-120 having diverse and complex structures at faster rate than individual axenic cultures.
2. The consortium HM-4 will be further used for development of continuous treatment system for textile effluents. The potential of the consortium HM-4 to decolorize and degrade a variety of dyes used in textile processing industries will be evaluated, with a special emphasis on complete metabolism of aromatic amines formed after reduction of azo dyes.

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