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ORIGINAL ARTICLE

Accelerated biodecolorization and detoxification of synthetic textile dye Acid Maroon V by bacterial consortium under redox mediator system

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Abstract
The treatment of textile industrial wastewater is an important concern owing to its negative impact on the biosphere. The present study highlighted dye decolorization potential of bacterial consortium EDPA containing *Enterobacter dissolvens* AGYP1 and *Pseudomonas aeruginosa* AGYP2 in the presence of redox mediators. Rapid decolorization of Acid Maroon V (100 mg l^{-1}) was achieved in the presence of lawsone compared to other redox mediators. The dye decolorization was best fitted with first order kinetics with higher reaction kinetics ($k1=0.328\ h^{-1}$) and regression coefficient ($R^2 = 0.979$). The removal of dye by the consortium was 1.47 times faster in 8 h with 0.01 mM lawsone. The consortium EDPA was able to decolorize 1200 mg l^{-1} concentration of dye with apparent R_{max}, K_m and R_{max}/K_m values 1000 mg $l^{-1}\ h^{-1}$, 5000 mg l^{-1} and 0.2 h^{-1}, respectively. The lawsone-mediated system could decolorize the dye 80.44% in 10 h at the end of 11 dye spiking cycle. The superior biodecolorization of 14 different textile dyes was obtained in the presence of lawsone-mediated system. The intracellular enzyme activities of azoreductase, NADH-DCIP reductase, laccase, manganese peroxidase and lignin peroxidase increased significantly. The sequential microaerophilic-aerobic incubation resulted into 89.31% reduction of total aromatic amines. The microbial toxicity, phytotoxicity and genotoxicity measurements revealed biotransformation of toxic nature of dye Acid Maroon V into non-toxic metabolites by the action of consortium EDPA, and thus its suitability for biotreatment of dye containing industrial effluents.

Keywords Consortium EDPA · Acid Maroon V · Decolorization · Lawsone · Toxicity

Introduction

Color is considered as an integral part of human perception and life. In very early life, natural colors were frequently used by people for staining their skins and clothes, for decoration of their hides, shells and feathers. Nevertheless, modern colorful life seems to be dependent on the utilization of several synthetic colorants the dyes. The utility of synthetic dyes is spanned in several fields; textile industry occupies major share in the consumption of such dyes. As a result, the dye containing wastewaters pose several eco-toxicological hazards adversely affecting aquatic and terrestrial lives (Tungare et al. 2022; Nanjani et al. 2021). Utilization of such wastewater for irrigation in agriculture reduces soil integrity and fertility, and thereby overall crop yield (Patel et al. 2021; Ambika et al. 2022). Therefore, several treatment strategies have been adopted by researchers to achieve fate of dyes from the wastewaters with minimal or no detrimental effects to the environment (Patel and Gupte 2015). Bioremediation is viewed as promising option owing to number of advantages over conventional dye removal technologies (Barathi et al. 2022). The mechanisms for bio-based removal of dyes include bioaccumulation, bioadsorption and enzymatic degradation (Patel and Chhaya 2019; Tanyol et al. 2022). The fungi including both white rot and nonwhite rot types are widely explored for the treatment of dye containing waste waters owing to production of array of degradative enzymes

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(Shah et al. 2013; Dharajiya et al. 2016; Yildirim and Erguven 2020).

The application of bacteria-based system for the treatment of industrial wastewaters is the most common approach because of several reasons, including production of large biomass with limited nutrient supply, easy acclimatization with diverse environmental conditions, prevalence of different mechanisms (anaerobic, facultative anaerobic and aerobic) resulting into complete fate of dye, and eco-friendly strategy with less volume of harmful sludge generation (Banat et al. 1996; Meng et al. 2012). The removal of different textile dyes by several species of bacteria either pure culture or in the form of consortium is well established in previous literatures (Rafii et al. 1990; Bafana et al. 2007; Patel and Chhaya 2019; Kamal et al. 2022). In fact, mixture of bacterial population in comparison to pure cultures provides superior level of biodegradation of dyes. This could be correlated with synergistic metabolic activities of different members of microbial consortium to achieve an effective breakdown and complete mineralization of complex molecular structures of dyes (Forgacs et al. 2004). However, supplementation of redox mediators offers faster rate of dye removal due to their inherent characteristics (Rau et al. 2002; Rau and Stolz 2003). The literature survey reports use of flavin-based compounds such as flavin adenide mononucleotide (FMN) and flavin adenide dinucleotide FAD), as well as quinone-based compounds like anthraquinone-2-sulfonate (AQS), anthraquinone-2,6-disulfonate (AQDS), cyanocobalamin (vitamin B12), lawsone (2-hydroxy-1,4-naphthoquinone) and riboflavin (vitamin B2) for decolorization of several dyes (Ikram et al. 2022; Nanjani et al. 2021; Olukanni et al. 2019; Gupte 2012; Cui et al. 2015). The biodegradation of dye is achieved by several oxido-reductive enzymes. The role of reductive enzymes is principally focused toward reduction of azo linkage of dye chromophore and generation of aromatic amines under oxygen limiting condition. The generated aromatic amines are further degraded via oxidative enzymes in the presence of oxygen resulting into complete mineralization of parent dye molecule (Franciscon et al. 2009; Isik and Sponza 2008; Mohanty and Kumar 2021; Kamal et al. 2022).

There are limited reports pertaining to utilization of lawsone for the accelerated biodecolorization of dyes compared to other quinone-based redox mediators (Liu et al. 2009, 2013; Cui et al. 2015). Therefore, present study is focused on exploration of lawsone-based system to achieve faster biodecolorization of several textile dyes. In our study, we could achieve significant decolorization of dye Acid Maroon V with minimal quantity of lawsone. Moreover, same lawsone-bacterial consortium combination had considerable capability to decolorize quite higher concentrations of dye. The decolorization of dye upon repetitive addition showed the reusability potential of lawsone-mediated system. In the present study, not merely decolorization, but degradation of dye has been studied. The involvement of oxido-reductive enzymes of the consortium has resulted into formation of degraded metabolites, with minimal microbial, phyto and genotoxicity. Therefore, present study exhibits the viability of lawsone-mediated system to be applied for faster decolorization and degradation of textile dyes.

Materials and methods

Dye and culture media

Commercially available dye Acid Maroon V (C.I. Acid Red 119) was used as model dye and procured from textile industry at Ankleshwar, Gujarat, India. Lawsone was used as model redox mediator. The characteristics of dye and redox mediator are depicted in Table 1. The bacterial members of the consortium EDPA (*Enterobacter dissolvens* AGYP1—GenBank accession number HQ336043, and *Pseudomonas aeruginosa* AGYP2—GenBank accession number HQ336042) were isolated from dye contaminated soil samples (Patel et al. 2012). The dye decolorization experiments were performed in Mineral Salt Medium (MSM) containing g l^{-1}: sucrose, 4; $NH_4H_2PO_4$, 0.3; K_2HPO_4, 6; KH_2PO_4, 1; $MgSO_4.7H_2O$, 0.1; NaCl, 5; and pH 7 ± 0.2. The bacterial inoculum was prepared in Luria Bertani (LB) broth containing g l^{-1}: casein enzymatic hydrolysates, 10; yeast extract, 5; NaCl, 5; and pH, 7 ± 0.2. Microbial toxicity analysis was performed using following media: Nutrient agar (NA) containing g l^{-1}: peptone, 10; meat extract, 3; NaCl, 5; and agar, 30, pH, 7.2 ± 0.2; Ashby's Mannitol Agar (AMA) containing g l^{-1}: mannitol, 20; K_2HPO_4, 0.2; $MgSO_4.7H_2O$, 0.2; NaCl, 0.2; K_2SO_4, 0.1; $CaCO_3$, 5; agar, 30; pH 7.4 ± 0.2, and Yeast Extract Mannitol Agar (YEMA) containing g l^{-1}: yeast extract, 1; mannitol, 10; NaCl, 0.1; $MgSO_4.7H_2O$, 0.2; K_2HPO_4, 0.5; agar, 30; pH 6.8 ± 0.2.

Dye decolorization experiment in liquid medium

The 250 ml Erlenmeyer flasks containing 150 ml MSM and Acid Maroon V (100 mg l^{-1}) were inoculated with the equal quantity of actively grown culture of *Enterobacter dissolvens* AGYP1 and *Pseudomonas aeruginosa* AGYP2 (0.5 OD at 600 nm) to achieve 10% (v/v) inoculum size. All the flasks were incubated at 30 °C for 16 h under static condition for color removal. To monitor sequential microaerophilic-aerobic degradation of dye, same flasks after decolorization were further incubated under shaking condition (120 rpm) for 112 h. Aliquots were withdrawn from different time intervals and analyzed for dye decolorization and consortium growth.

Table 1 Characteristics of dye Acid Maroon V (A) and redox mediator lawsone (B) used in present study

A

C.I. Name: Acid Red 119

C.I. No: 262085

Synonym: 3-[[Ethyl[4-[2-[4-[2-(3-sulfophenyl) diazenyl]-1-naphthalenyl] diazenyl]phenyl]amino] methyl] benzenesulfonic acid sodium salt (1:2)

Molecular formula: $C_{31}H_{25}N_5Na_2O_6S_2$

Molecular weight: 629

CAS No: 12220-20-1/70210-06-9

Maximum absorbance (λmax): 523

Application: For dyeing and printing polyamide, silk and leather

Standard	Light Fastness	Soaping		Perspiration Fastness	Oxygen Bleaching		Fastness to Seawater	
		Fading	Stain		Fading	Stain	Fading	Stain
AATCC[a]	4	5	5	5	4	3	3-4	-
ISO[b]	5-6	4-5	5	4	-	-	4-5	-

[a] = American Association of Textile Chemists and Colorists
[b] = International Organization for Standardization

B

C.I. Name: Natural Orange 6

C.I. No: 75480

Chemical name: 2-hydroxy 1,4-naphthoquinone

Molecular formula: $C_{10}H_6O_3$

Molecular weight: 174.15

CAS No: 83-72-7

Decolorization of Acid Maroon V by consortium EDPA in the presence of redox mediator

The rapid biodecolorization of dye was evaluated in the presence of four redox mediators like AQS, AQDS, lawsone and pyrocatechol (1, 2-dihydroxybenzene). The redox mediators were supplemented at 1 mM concentration separately into the flasks containing MSM, Acid Maroon V (100 mg l^{-1}) and the consortium EDPA. All the flasks were incubated at 30 °C under static condition. The flasks devoid of redox mediator served as control. At different time intervals, samples were taken and processed for decolorization. The

color removal of Acid Maroon V was assessed with initial lawsone and Acid Maroon V concentrations in the range of 0.01–1 mM and 100–1200 mg l^{-1}, respectively, to establish their effect on decolorization.

Long-term decolorization ability of lawsone-mediated system upon repetitive dye addition was evaluated at flask level. The flasks containing MSM, dye (100 mg l^{-1}), lawsone (0.01 mM) and consortium EDPA were incubated under static condition for decolorization of dye. After decolorization of Acid Maroon V, the same flasks were again supplemented with 100 mg l^{-1} dye and further incubated for next cycle for decolorization. This procedure was repeated for 11 dye spiking cycles.

The decolorization ability of lawsone-consortium EDPA combination was screened using 14 different textile dyes. The individual dye at 100 mg l^{-1} concentration was emended into the flasks containing MSM, lawsone (0.01 mM) and consortium EDPA. The flasks were incubated at 30 °C under static condition and the extent of color removal was monitored spectrophotometrically.

Kinetic modelling

The decolorization of dye in the presence of redox mediators was evaluated by kinetic study using zero, first, and second order models according to following equations, respectively (Fareed et al. 2022; Du et al. 2011):

$$\frac{dCt}{dt} = -k0 \tag{1}$$

$$\frac{dCt}{dt} = -k1 \cdot Ct \tag{2}$$

$$\frac{dCt}{dt} = -k2 \cdot (Ct)^2 \tag{3}$$

where *k0*, *k1*, and *k2* are apparent kinetic rate constants of zero, first, and second order models, respectively.

Linearized form of the equations can be obtained as follows, respectively

$$Ct = Co - k0 \cdot t \tag{4}$$

$$Ct = Co.\exp(-k1 \cdot t) \tag{5}$$

$$\frac{1}{Ct} = \frac{1}{Co} + k2.t \tag{6}$$

Equation (5) can be further simplified as follows

$$\ln\ Ct\ =\ \ln\ Co - k1 \cdot t \tag{7}$$

where t is reaction time (h), Co (mg l^{-1}) and Ct (mg l^{-1}) is Acid Maroon V concentrations at initial and given time t.

Intracellular enzymes detection

Preparation of cell-free extract for enzyme assay

The consortium EDPA was inoculated in MSM containing 100 mg l^{-1}Acid Maroon V. After decolorization, the culture medium was centrifuged at 10,000 rpm for 15 min at 4 °C in the refrigerated centrifuge (Kubota 6200, Japan). The cell pellet was suspended in 50 mM potassium phosphate buffer (pH 7) and sonicated giving 8 strokes, each of 60% amplitude for 30 s with 2 min interval at 4 °C (Sonics Vibracell VCX 130, USA). Cell debris was removed by centrifugation at 10,000 rpm for 15 min at 4 °C, and clear supernatant was used as source of crude enzyme. Similar procedure was performed to determine enzyme activities in cells obtained before decolorization. All enzymatic activities were carried out at 30 °C. The reference blank contained all components of assay system except enzyme. All assays were performed in triplicate and average rates were calculated.

Intracellular enzymes assay

Oxido-reductive enzyme activities of the consortium EDPA were assayed spectrophotometrically before and after decolorization of Acid Maroon V. The Laccase, Manganese Peroxidase (MnP) and Lignin Peroxidase (LiP) activities were performed as reported earlier with minor modifications (Niku-Paavola et al. 1990; Martinez et al. 1996). For laccase assay, the reaction mixture containing 50 mM ABTS in 0.1 M sodium acetate buffer (pH 5) and suitably diluted cell-free supernatant was incubated for 1 min. The development of dark color was monitored at 420 nm, and laccase activity was calculated using molar extinction coefficient of oxidized ABTS (36,000 M^{-1} cm^{-1}). The activity of MnP was determined using assay mixture containing 1 mM dimethoxyphenol (DMP), 1 mM $MnSO_4$, 1 mM H_2O_2 in 0.2 M sodium tartrate buffer (pH 4.5) and suitably diluted enzyme solution, and incubated for 3 min. The color development was monitored at 469 nm, and MnP activity was calculated using molar extinction coefficient of DMP (27,500 M^{-1} cm^{-1}). The activity of LiP was monitored using reaction mixture containing 10 mM guiacol, 1 mM H_2O_2 in 0.33 M sodium tartrate buffer (pH 4) and suitably diluted supernatant. The reaction mixture was incubated for 2 min, and development of color was monitored at 436 nm. The LiP activity was calculated using molar extinction coefficient of guiacol (6,740 M^{-1} cm^{-1}). The activity of azoreductase

was determined using Zimmermann et al. (1982) with minor modification. The assay mixture containing 24 µM Methyl Red, 1 mM NADH in 0.1 M potassium phosphate buffer (pH 7) and appropriately diluted enzyme solution. The reaction was started by addition of NADH after 4 min of pre incubation at 30 °C. The decrease in color was measured at 437 nm, and azoreductase activity was calculated using molar extinction coefficient of Methyl Red (23,360 M^{-1} cm^{-1}). For determination of NADH-DCIP reductase activity, the reaction mixture containing 50 µM DCIP, 50 µM NADH in 50 mM potassium phosphate buffer (pH 7.4) and suitably diluted cell-free supernatant. The reduction of DCIP was monitored at 590 nm and molar extinction coefficient 19 mM^{-1} cm^{-1} was used to calculate enzyme activity (Salokhe and Govindwar 1999).

Decolorization and degradation analysis of Acid Maroon V

The aliquots (2 ml) were withdrawn periodically and centrifuged at 8000 rpm for 10 min. The cell-free supernatants were scanned in the range of 200–800 nm using UV–Visible spectrophotometer (SHIMADZU UV-1800, Japan). Based on an absorbance of standard and decolorized samples at maximum absorbance wavelength of dye, the decolorization percentage was calculated using following formula:

$$\text{Decolorization } (\%) = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100 \quad (8)$$

After centrifugation, the cell pellet obtained was re-suspended in 2 ml distilled water and its absorbance was measured at 600 nm. The cell growth was expressed in terms of cell dry weight using following equation:

$$\text{Cell dry weight}(g/l) = 0.06 \times \text{OD at 600nm} \quad (9)$$

Total aromatic amines (TAA) were measured spectrophotometrically according to the method described by Oren et al. (1991). The reaction mixture containing cell-free supernatant (0.2 ml), distilled water (0.8 ml) and 1 M HCl (0.05 ml) was mixed with sequential addition of ethanol (3 ml), 5% *p*-dimethylaminobenzaldehyde in ethanol (0.5 ml) and 15.7% citric acid in 6% NaOH (0.5 ml). After 10 min incubation at room temperature, distilled water (2.5 ml) was added, and the absorbance of reaction mixture was recorded at 440 nm. Benzidine (100 mg l^{-1}) was used as standard aromatic amine.

Toxicological studies of Acid Maroon V and biodegradation metabolites

Microbial toxicity of dye and its metabolites was assessed using some important microorganisms like *Bacillus subtilis,*

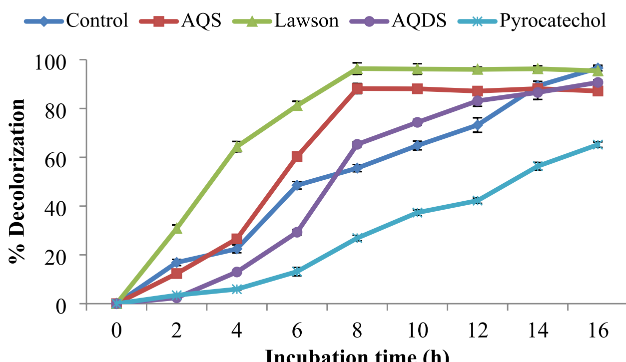


Fig. 1 Time profile of biodecolorization of Acid Maroon V by consortium EDPA in the presence of redox mediators

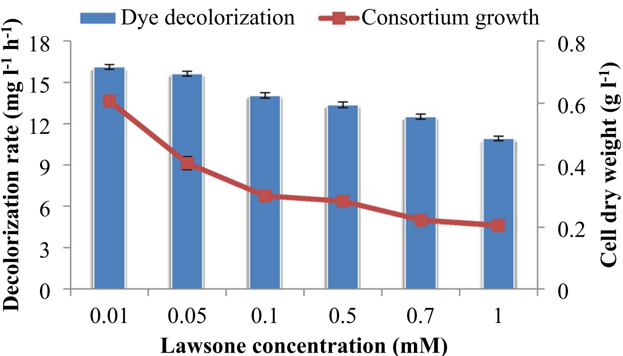


Fig. 2 Effect of lawsone concentration on decolorization of Acid Maroon V by consortium EDPA. Values are mean of three experiments ± SEM, compared by One-Way ANOVA with $p \leq 0.05$

Rhizobium sp.*, Bacillus megaterium, Azotobacter* sp.*, Bacillus cereus, Staphylococcus aureus, Micrococcus luteus, Proteus vulgaris, Salmonella typhi* and *Escherichia coli*. *Rhizobium* sp. was cultivated on YEMA; *Azotobacter* sp. on AMA and other bacteria were cultured on NA. Pure colony of bacterial culture was grown in nutrient broth at 30 °C till the optical density reached 0.08–0.1 at 620 nm. After spreading the culture evenly on the respective agar plates, two wells of 8 mm diameter were made with sterile gel puncture. One well was filled with 0.1 ml of untreated dye and other with the extracted metabolites dissolved in water. All the plates were kept at 4 °C for 45 min for pre-diffusion, and then incubated at 30 °C for 24–28 h. After incubation, the zone size of inhibition surrounding the well was measured and represented as the index of toxicity (Fareed et al. 2022; Saratale et al. 2011).

The phytotoxicity was tested according to the method described earlier (Kalme et al. 2009; Patel et al. 2012). The seeds of *Triticum aestivum* were surface sterilized by treating with 0.2% $HgCl_2$ and 95% ethanol three times, and then thoroughly washed with sterile distilled water three times.

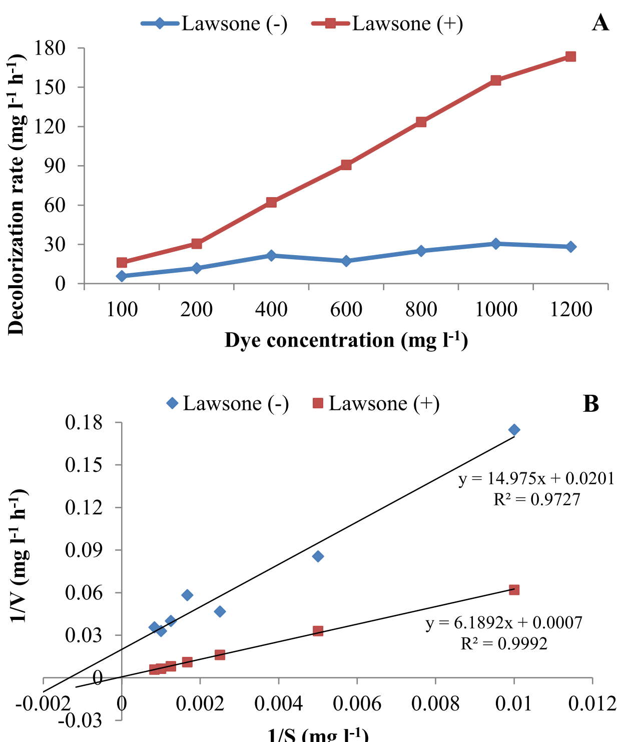


Fig. 3 Effect of dye concentration on Acid Maroon V decolorization by consortium EDPA. Comparison of dye removal in the absence and presence of under lawsone (**A**), double reciprocal plot for kinetic analysis of Acid Maroon V decolorization by consortium EDPA (**B**). Values are mean of three experiments ± SEM, compared by Two-Way ANOVA with $p \leq 0.05$

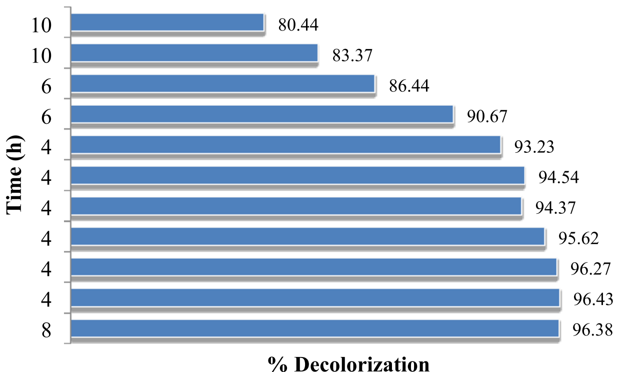
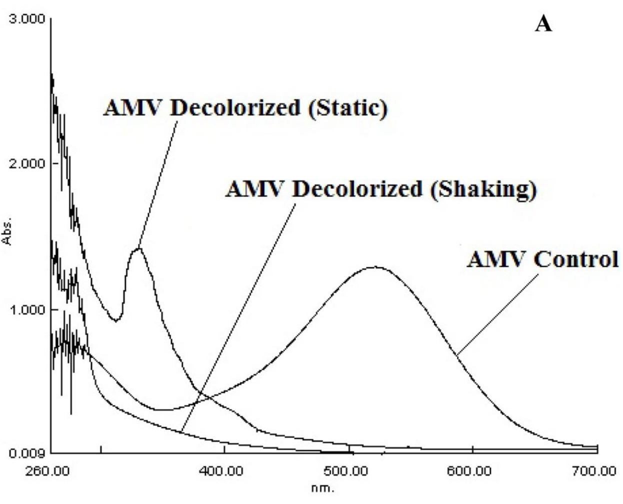


Fig. 4 Repeated decolorization of Acid Maroon V by consortium EDPA under lawsone-mediated system. Values are mean of three experiments ± SEM, compared by One-Way ANOVA with $p \leq 0.05$

A total of ten seeds were sown in sterile field soil in different plastic pots. The seed was irrigated daily with 10 ml dye solution, degradation metabolites dissolved in water, and distilled water. All the pots were maintained at proper light and temperature (30 °C). After 15 days, the toxicity



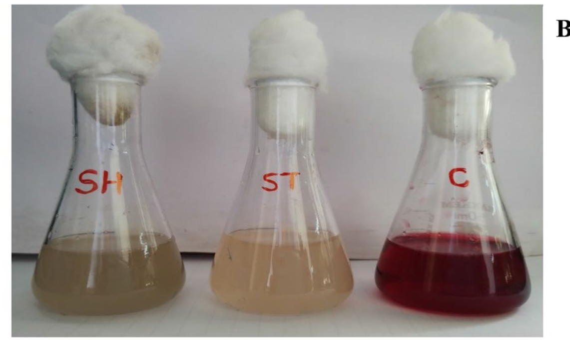


Fig. 5 Biodecolorization of Acid Maroon V by consortium EDPA: UV–visible spectrum of dye decolorization (**A**); real image of decolorization of dye—C is control, ST is static condition, SH is shaking condition (**B**)

effect was assessed in terms of percent germination, length of plumule and radical of the plants.

Genotoxicity was performed by alkaline comet assay using human peripheral blood lymphocytes exposed to control dye and degradation metabolites (Singh et al. 1988). Peripheral blood from healthy volunteers was collected in heparinized container and lymphocytes were isolated using lymphocyte separation medium (Himedia, Mumbai, India). Cells were washed with phosphate buffered saline (PBS) and suspended in RPMI 1640 medium (Hyclone, Thermo Scientific) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U ml^{-1} penicillin, 100 µg ml^{-1} streptomycin (Sigma, St. Louis, USA). Cells (1×10^5 cells ml^{-1}) were incubated with different samples for 2 h at 20 °C. The cells incubated in RPMI (2 h, 20 °C) and H_2O_2 (100 µM, 5 min, 4 °C) were used as the negative and positive control, respectively. After incubation, the cells were centrifuged at 5000 rpm for 5 min and re-suspended in PBS. The cell suspension was mixed with low-melting agarose (0.55%), and added to slides pre-coated with normal-melting agarose (1%) and kept on ice. After solidification of agarose, slides were

Table 2 Decolorization of Acid maroon V by consortium EDPA in the presence of redox mediators with apparent kinetic rate constant (*k*) and regression coefficient (R^2)

Mediator	Zero order		First order		Second order	
	k0 (mg l^{-1} h^{-1})	R^2	*k1*(h^{-1})	R^2	*k2* (l mg^{-1} h^{-1})	R^2
Control	6.010	0.982	0.184	0.857	0.004	0.654
AQS	6.398	0.895	0.186	0.957	0.008	0.935
Lawsone	9.507	0.931	0.328	0.979	0.023	0.676
Pyrocatechol	4.301	0.968	0.065	0.922	0.001	0.844
AQDS	6.670	0.940	0.164	0.960	0.005	0.860

covered with another layer of low-melting agarose (0.5%) and immersed in lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, and 10% DMSO) for 2 h at 4 °C. The slides were placed into an electrophoresis tank containing freshly prepared, chilled alkali buffer (300 mM NaOH, 10 mM Na_2EDTA, pH > 13) for 25 min. Electrophoresis was carried out at 25 V and 300 mA for 25 min at 4 °C. Slides were washed thrice with neutralizing buffer (0.4 M Tris–HCl, pH 7.5), stained with ethidium bromide (75 µl, 20 µg ml^{-1}) and observed under the fluorescence microscope (Olympus CX41, Japan) equipped with CCD camera. The DNA damage (in arbitrary units) was calculated using the visual scoring method as described by Collins (2004). For each treatment 100 comets per slide and three slides per treatment were scored and classified into five classes (0, 1, 2, 3, and 4), i.e., from undamaged (0) to maximum damaged (4) according to tail intensity. The arbitrary units were calculated by multiplying comet class with number of cells in that class and then summing up the total.

Data analysis

All the experiments were performed in triplicates, and the data presented are in the form of mean ± standard deviation (SD). Analysis of variance (ANOVA) was performed for each parameter, and statistical significance at $p \leq 0.05$ was considered.

Results and discussion

Decolorization of Acid Maroon V in the presence of redox mediators

The presence of redox mediator in dye decolorization system has profound effect on color removal. In our study, the consortium EDPA-mediated decolorization of Acid Maroon V was evaluated in the absence and presence of redox mediators. The overall dye removal performance of the consortium linearly increased with an increment in the incubation time (Fig. 1). The decolorization in control was 16.88 ± 1.29% at 2 h incubation, and reached to 96.56 ± 0.8% after 16 h. Nevertheless, the presence of lawsone had strong stimulatory effect; 96.33 ± 2.36% decolorization of Acid Maroon V was achieved within 8 h static incubation. This resulted into 1.99 times faster color removal compared to control flask. After 8 h incubation, the decolorization of dye in the presence of AQS and AQDS was 88.15 ± 2.11 and 65.31 ± 1.02% with 1.83 and 1.35 times faster decolorization, respectively, in comparison to control. In contrast to above findings, the fate of dye was quite lesser (26.88 ± 1.12%) with pyrocatechol. The kinetic study of consortium EDPA-mediated removal of Acid Maroon V was carried out in the presence of redox mediators (S Fig. 1, 2, 3, 4, 5). The overall rate constants (*k*) and regression coefficient (R^2) are depicted in Table 2. It was observed that rate constant values of zero (*k0*), first (*k1*), and second (*k2*) order reactions were quite higher with lawsone suggesting its suitability for dye decolorization. Moreover, the regression coefficient (R^2) was higher with first order compared to zero and second order kinetic reactions, which suggested that removal of Acid Maroon V in the presence of lawsone was best fitted with first order kinetics. The reaction kinetics for decolorization of Reactive Orange 16 and Reactive Black 5 by *Bacillus cereus* strain ROC (Fareed et al. 2022), and Acid Black 172 by *Pseudomonas* sp. strain DY1 (Du et al. 2011) was well established. The faster rate of dye decolorization could be correlated with an ability of redox mediator to lower the reaction's activation energy by speeding up the transfer of reducing equivalents to the terminal electron acceptor (azo dye). Moreover, redox mediators also minimize the steric hindrance of the dye molecule (Rau and Stolz 2003). An oxidation–reduction potential (*Eo*') of the redox mediator also determines the color removal rate; the *Eo*' of lawsone is −139 mV, which is between −320 and −50 mV of many quinine compounds used for biological azo dye reduction (Rau et al. 2002). The application of diverse type of redox mediators for accelerated decolorization of several dyes is depicted in Table 3. In accordance to our results, the presence of lawsone exhibited accelerated biodecolorization of different dyes by *Geobacter metallireducens* (Liu et al. 2013), *Escherichia coli* (Liu et al. 2009) and *Escherichia coli* strain CD-2 (Cui et al. 2015). In another

Table 3 Biodecolorization of textile dyes by bacterial cultures/enzyme system in the presence of redox mediators

Microorganism/enzyme system	Dye(s)	Redox mediator	Dye removal	Reference
Escherichia coli	Basic Orange 2	Sodium benzoate (66 mg/l)	89.88% in 3 days	Ikram et al. 2022
Klebsiella spp. K1	Reactive Violet 5R	Menadione (2 mM)	91.82% in 15 h	Rathod and Archana 2022
Klebsiella spp. E2			87.89% in 15 h	
Acinetobacter sp. L1			74.46% in 15 h	
Klebsiella spp. K1		Lawsone (1%)	70% in 15 h	
Pseudomonas aeruginosa	Methyl Red	Uric acid	48.63% in 5 h	Ikram et al. 2022
		Hydroquinone	62.08% in 5 h	
		EDTA	61.23% in 5 h	
		Sodium benzoate	46.72% in 5 h	
Streptomyces ipomoea Laccase	Indigo carmine	PhCOOH (50 µM)	> 80% in 1 h	Coria-Oriundo et al. 2020
	Xylidine Ponceau Remazol Brilliant Blue R Malachite Green		66% in 24 h	
			51% in 24 h	
			93% in 3 h	
Providencia rettgeri	Methyl Red	Quinol	94.41% in 5 h	Olukanni et al. 2019
		NAD^+	26.86% in 5 h	
		NADH $NADP^+$	19.44% in 5 h	
		NADPH (1 mM each)	82.30% in 5 h	
Iodidimonas sp. Q-1	Orange G	Iodide (0.1 mM)	80–100% in 5 h	Taguchi et al. 2018
	Indigo Carmine		80–100% in 5 h	
	Amido Black		80–100% in 5 h	
	Remazol Brilliant Blue R		78% in 8 h	
	Industrial wastewater	Iodide (1 mM)	46% in 2 h	
Citrus limon peroxidase	Direct Yellow	p-coumaric acid	81.1% in 30 min 84.2% in 30 min	Nouren et al. 2017
		HOBT		
		Syringaldehyde	86.3% in 30 min	
		Vanillin	82.05% in 30 min	
		Syringic acid	80.3% in 30 min	
		Veratryl alcohol Pyrocatechol	81.5% in 30 min, 77.3% in 30 min	
Escherichia coli strain CD-2	Methyl Orange	Bromamine Acid	40% in 6 h	Shen et al. 2016
		AQDS	60% in 6 h	
		Menadione	90% in 6 h	
		Lawsone (0.1 mM each)	85% in 3 h	
Anaerobic sludge	Remazol Yellow Gold	Residual yeast lysate (350 mg/l)	90% in 48 h	Victral et al. 2016
Shewanella algae	Acid Red 27	AQDS (0.1 mM)	> 95% in 4 h	Meng et al. 2014
	Methyl Orange		> 95% in 9 h	
	Acid Orange 7		> 95% in 9 h	
	Reactive Red 120		43.7% in 12 h	
	Direct Blue 71		83.7% in 12 h	
Bacterial consortium-PES containing *Pseudomonas aeruginosa*,	Acid Red 2	AQS	98% in 12 h	Gupte 2012
Escherichia hermanii and *Stenotrophomonas maltophilia*		AQDS	90% in 12 h	

Table 3 (continued)

Microorganism/enzyme system	Dye(s)	Redox mediator	Dye removal	Reference
		Lawsone	60% in 12 h	
		Ethyl viologen	62% in 12 h	
		Alizarin	50% in 12 h	
		Catechol (1 mM each)	60% in 12 h	
Trichosanthes dioica Peroxidase	Congo Red	Riboflavin, AQDS (1 mM each)	97.5%, 92.5% in 2 h	Jamal et al. 2011
	Reactive Red 2		74.6%, 74.7% in 2 h	
	Reactive Red 120		93.2%, 89.3% in 2 h	
	Reactive Black 5		77.8%, 74.2% in 2 h	
	Reactive Orange 16		89.7%, 90.9% in 2 h	
	Remazol Brilliant Blue R		92.4%, 92.3% in 2 h	
	Reactive Blue 4		62.5%, 59.3% in 2 h	

EDTA ethylenediamine tetraacetic acid, *PhCOOH* β-(10-phenothiazyl)-propionic acid, NAD^+ nicotinamide adenine dinucleotide, *NADH* nicotinamide adenine dinucleotide with hydrogen, $NADP^+$ nicotinamide adenine dinucleotide phosphate, *NADPH* nicotinamide adenine dinucleotide phosphate with hydrogen, *HOBT* 1, hydroxybenzotriazole, *AQDS* Anthraquinone 2,6-Disulphonic acid, *AQS* Anthraquinone 2-Sulphonic acid

study, an incorporation of menadione and lawsone had positive effect on decolorization of Reactive Violet 5R by *Klebsiella* spp. K1, *Klebsiella* spp. E2 and *Acinetobacter* sp. L1 (Rathod and Archana 2022). Redox mediators like AQDS and AQS on the other hand were found to be effective for the removal of Reactive Blue 28 by the bacterial consortium SCP (Nanjani et al. 2021).

Effect of lawsone concentration on decolorization of dye

Considering an accelerating effect of lawsone on azo dye reduction, the decolorization of Acid Maroon V was tested against lawsone gradient (0.01–1 mM). The results in Fig. 2 revealed that the consortium EDPA could remove dye effectively in the presence of 0.01 mM lawsone with the highest decolorization rate of 16.11 ± 0.18 mg l^{-1} h^{-1}. The color removal performance of the consortium EDPA did not improve with further increment in the lawsone concentration. For instance, at 0.1 and 1 mM concentrations, the dye decolorization rates were 14.06 ± 0.19 and 10.94 ± 0.16 mg l^{-1} h^{-1}, respectively. The color removal rate was 1.47 times superior with 0.01 mM lawsone compared to 1 mM. In addition to this, the consortium cell biomass was comparatively higher at 0.01 mM concentration (0.607 ± 0.011 g l^{-1}) compared to 1 mM concentration of lawsone (0.205 ± 0.016 g l^{-1}). Lesser color removal performance at higher concentration of lawsone may be owing to its cellular toxicity or dearth of electron source (Liu et al. 2009; Sauriasari et al. 2007). Therefore, in our study, quite lower concentration of lawsone was enough to support considerable biomass production with concomitant decolorization of Acid Maroon V. Another thing to be kept in consideration is that an addition of higher concentrations of quinine redox mediators may influence the cost-effectiveness of the biological treatment process at large scale (Blánquez et al. 2019; Victral et al. 2016). Our results are quite satisfactory than Liu et al. (2009), wherein they demonstrated 70% decolorization of Acid Red 27 and Acid Red 73 by *Escherichia coli* YB with 0.2 mM lawsone. In another study 0.1 mM lawsone concentration was found to be optimum for decolorization of Methyl Orange by *Escherichia coli* strain CD-2 (Cui et al. 2015). The decolorization of Acid Red 27 by *Geobacter metallireducens* increased with rise in lawsone concentration from 0.5 to 10 μM; the dye decolorization did not influence upon further increase in lawsone concentration (Liu et al. 2013).

Effect of dye concentration on lawsone-mediated decolorization of Acid Maroon V

The ability of consortium EDPA to decolorize different concentrations of Acid Maroon V was investigated in the presence and absence of lawsone (Fig. 3A). When the lawsone was not supplemented, the decolorization rate was 5.72 ± 0.04 mg l^{-1} h^{-1} at 100 mg l^{-1} concentration of Acid Maroon V; the color removal rate reached to 30.42 ± 0.36 mg l^{-1} h^{-1} at 1000 mg l^{-1} concentration. The consortium EDPA on the other hand decolorized dye very efficiently with 16.13 ± 0.9 mg l^{-1} h^{-1} decolorization rate in the presence of lawsone at 100 mg l^{-1} dye. Furthermore, the lawsone-mediated system was able to decolorize 1200 mg l^{-1} dye with maximum color removal rate 173.49 ± 1.23 mg l^{-1} h^{-1}. Therefore, the presence of

Table 4 Lawsone-mediated decolorization of different dyes by bacterial consortium EDPA

Dye	C.I. name	λ max (nm)	Molecular weight	CAS no	Decolorization rate ($mg\ l^{-1}\ h^{-1}$)	
					Absence of lawsone	Presence of Lawsone
Acid Orange II	Acid Orange 7	486	350.33	633–96-5	2.49 ± 0.01	3.55 ± 0.07
Reactive Purple H3R	Reactive Violet 1	560	926.54	12,239–45-1	3.80 ± 0.02	4.28 ± 0.08
Reactive Green HE4BD	Reactive Green 19	622	1418.94	61,931–49-5	2.50 ± 0.01	3.78 ± 0.01
Reactive Red M5B	Reactive Red 2	538	615.34	12,226–03-8	1.21 ± 0.01	2.62 ± 0.02
Direct Black CA	Direct Black 22	496	1083.97	6473–13-8	1.85 ± 0.03	3.43 ± 0.01
Acid Black RM	Acid Black 194	564	461.38	61,931–02-0	0.54 ± 0.007	0.98 ± 0.005
Reactive Brown GR	Reactive Brown 18	474	NA	12,225–73-9	1.85 ± 0.02	3.53 ± 0.01
Acid Fast Red A	Acid Red 88	497	400.38	1658–56-6	1.63 ± 0.01	3.64 ± 0.01
Reactive Orange HER	Reactive Orange 84	491	1850.29	91,261–29-9	0.61 ± 0.004	1.57 ± 0.006
Reactive Violet 5R	Reactive Violet 5	560	735.59	12,226–38-9	3.96 ± 0.03	4.53 ± 0.02
Reactive Red ME4BL	Reactive Red 195	541	1136.32	93,050–79-4	2.44 ± 0.01	5.94 ± 0.03
Acid Red F2R	Acid Red 151	510	454.43	6406–56-0	2.23 ± 0.02	9.23 ± 0.03
Reactive Green ME4BL	Reactive Green 27	621	NA	NA	2.31 ± 0.01	2.91 ± 0.01
Reactive Red C2G	Reactive Red 106	502	NA	105,635–66-3	3.94 ± 0.03	9.44 ± 0.03

NA not available, values are mean of three experiments ± SEM, compared by Two-Way ANOVA with $p \leq 0.05$

lawsone displayed 5.70 times higher decolorization of Acid Maroon V, which was quite considerable. Under experimental condition, a relationship between decolorization rate (R) and initial dye concentration ($[AMV]$) was established by Michaelis–Menten double reciprocal model ($R_{max}[AMV]/(K_m + [AMV])$). In the absence of lawsone, the calculated values of an apparent maximum decolorization rate (R_{max}), Michaelis constant (K_m) and specificity constant (R_{max}/K_m) were 50 $mg\ l^{-1}\ h^{-1}$, 769.23 $mg\ l^{-1}$ and 0.065 h^{-1}, respectively. The values of R_{max}, K_m and R_{max}/K_m for lawsone-mediated system were 1000 $mg\ l^{-1}\ h^{-1}$, 5000 $mg\ l^{-1}$ and 0.2 h^{-1}, respectively (Fig. 3B). The specificity constant of lawsone-mediated system was 3.08 times higher, suggesting better decolorization performance of consortium EDPA-lawsone system. The Michaelis–Menten kinetics was established in order to evaluate the relationship between initial decolorization rate and dye concentration (Liu et al. 2013; Meng et al. 2012). Additionally, at 1200 $mg\ l^{-1}$ Acid Maroon V, the molar ratio of lawsone/dye was 0.0052, which was 12 times lower compared to 100 $mg\ l^{-1}$. The decolorization of dye is better at lower mediator/dye molar ratios, and the color removal performance declines at high ratios (Kudlich et al. 1996). Therefore, overall results depicted significant color removal of dye at higher concentration in the presence of lawsone.

Repeated decolorization of dye with lawsone-mediated system by consortium EDPA

The reusability of lawsone-mediated system was evaluated for repeated decolorization of Acid Maroon V. In the presence of 0.01 mM lawsone, the consortium EDPA exhibited 96.38 ± 2.3% decolorization of the dye in 8 h (Fig. 4). After color removal, the flasks were again amended with another 100 $mg\ l^{-1}$ dye for next round of decolorization. Surprisingly, faster decolorization of the dye was obtained within 4 h in 2nd and 3rd dye spiking cycles compared to 1st cycle. The decolorization of the dye was between 95 and 93%

Table 5 Enzyme profile of consortium EDPA during decolorization of Acid Maroon V (100 $mg\ l^{-1}$)

Treatment	Enzyme activity (U mg of protein^{-1} min^{-1})				
	Azoreductase	NAD-DCIP reductase	Laccase	Manganese peroxidase	Lignin peroxidase
Before decolorization*	0.55 ± 0.001^{c}	20.57 ± 1.24^{c}	ND^{c}	1.08 ± 0.021^{c}	0.03 ± 0.002^{c}
After decolorization (Static)$^{\#}$	11.55 ± 1.13^{a}	222.10 ± 4.65^{a}	2.37 ± 0.12^{b}	4.58 ± 0.23^{b}	1.09 ± 0.012^{b}
After decolorization (Shaking)$^{\#\#}$	2.69 ± 0.021^{b}	165.26 ± 5.32^{b}	9.57 ± 1.04^{a}	8.29 ± 0.95^{a}	2.71 ± 0.011^{a}

Cells harvested *at 0 h, #after 16 h, ##after 116 h, *ND* not detected, values are mean of three experiments ± SEM, compared by Pairwise Mean Comparison of Treatment with $p \leq 0.05$; means with the same letter are not significantly different

during 4 to 7 cycles in 4 h. The enhanced decolorization activity may be correlated with acclimatization of the consortium EDPA with subsequent increase in dye concentration. Furthermore, the dye degradation metabolites could be optimally utilized for higher biomass production, resulting into rapid decolorization performance of the consortium EDPA. Nevertheless, the color removal efficiency of the consortium declined and time required for decolorization also increased upon subsequent dye spiking cycles. This may be owing to accumulation of toxic dye metabolites as well as scarcity of nutrients, which adversely affected the overall dye removal potential of the consortium (Patel et al. 2012). However, the consortium EDPA exhibited 80.44 ± 2.6% decolorization in 10 h at the end of 11th cycle, which was quite considerable. Therefore, our results depicted good persistence and stability of consortium EDPA for repetitive dye decolorization compared to previous report. For instance, Liu et al. (2009) achieved only four-repeated batch decolorization of Acid Red using *E. coli* YB strain with quite higher concentration (0.2 mM) of lawsone. The decolorization of Indanthrene Blue RS dye by individual cultures *Bacillus fexus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* was limited to 3rd cycle; the bacterial consortium retained decolorization capability up to 11th cycle (Mohanty and Kumar 2021).

Decolorization of different dyes by consortium EDPA in the presence of lawsone

The textile industry releases large volume of effluent containing mixture of various dyes in varying concentration. Therefore, any biological system which exhibits decolorization potential for diverse textile dyes is quite significant (Ikram et al. 2022; Meng et al. 2014). In our study, the consortium EDPA lawsone-mediated system was assessed for decolorization of 14 different textile dyes. The result in Table 4 depicted that dye removal rates were improved with lawsone than non-lawsone system. The decolorization of Acid Red F2R was 4.14 times superior with lawsone as compared to the absence of it. Additionally, an increment in decolorization rate of Reactive Orange HER, Reactive Red ME4BL, Reactive Red C2G, Acid Fast Red A and Reactive Red M5B was between 2.57 and 2.17 times with lawsone-mediated system. However, comparatively minor enhancement in the decolorization rate of other dyes was evident. For instance, lawsone-mediated system improved color removal of Reactive Green ME4BL, Reactive Violet 5R and Reactive Purple H3R by 1.26, 1.14 and 1.13 times, respectively, in comparison to absence of lawsone. The observed variation in decolorization rates may be owing to structural complexity, number of azo bonds, and the presence of sulfonate groups in different

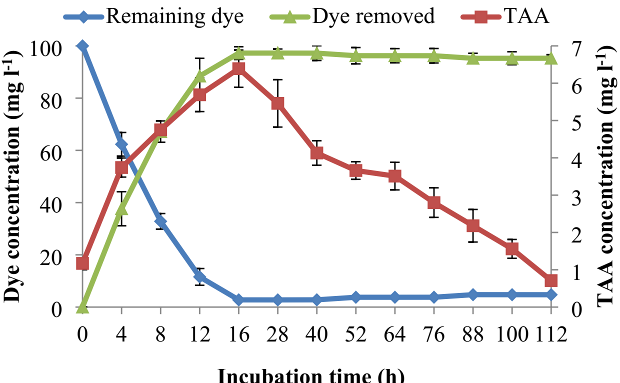


Fig. 6 Fate of total aromatic amines (TAA) and dye Acid Maroon V by bacterial consortium EDPA under sequential microaerophilic-aerobic condition. Values are mean of three experiments ± SEM, compared by One-Way ANOVA with $p \leq 0.05$

textile dyes (Zimmermann et al. 1982). Moreover limited cell membrane permeability as well as microbial toxicity negatively influences the decolorization rate. Therefore, an extracellular reduction of such azo dyes can be significantly enhanced by redox mediators (Encinas-Yocupicio et al. 2006). The results of our study demonstrated utility of consortium lawsone system for the treatment of industrial effluent containing mixture of dyes concurrently.

Intracellular enzymes activity

The biodegradation of dye is multistep process, and it involves several enzyme catalyzed reactions. The role of different intracellular enzymes in dye degradation process is well documented (Patel and Chhaya 2019). Therefore, the measurement of various oxido-reductive enzyme activities provides narrative picture of their contribution in the dye decolorization and degradation mechanism. The results depicted in Table 5 revealed that the activity of azoreductase increased 21 times under static incubation than control. Similarly, 10.80 times higher activity of NAD-DCIP reductase was evident under static condition. Nevertheless, the activities of both the enzymes were comparatively lower under shaking incubation suggesting their involvement in the reductive decolorization of azo dye. Bacterial biodegradation of azo dyes is often initiated with the cleavage of azo bond by azoreductase resulting into the formation of amines. The azoreductase-mediated cleavage of azo linkage requires four electron transfer reaction in two stages; in each stage two electrons are transferred to the azo linkage of the dye (Chang and Kuo 2000). Several species of bacteria (aerobic and anaerobic) are known to possess azoreductase activity (Rafii et al. 1990; Bafana et al. 2007). On the other hand, the activities of laccase,

Table 6 Microbial and phytotoxicity studies of Acid Maroon V and its degraded metabolites formed after biodegradation by consortium EDPA

Microbial toxicity study			
Microflora		Zone of inhibition (mm*)	
		Untreated AMV*	Treated AMV*
Rhizobium sp.		13 ± 1	NI
Azotobacter sp.		14 ± 3	NI
Bacillus subtilis		13 ± 2	NI
Proteus vulgaris		10 ± 2	NI
Bacillus cereus		15 ± 4	NI
Bacillus megaterium		16 ± 3	NI
Salmonella typhi		10 ± 2	NI
Staphylococcus aureus		14 ± 1	NI
Micrococcus luteus		15 ± 2	NI
E. coli		11 ± 3	NI
Phytotoxicity study			
Parameter	Germination (%)	Plumule (cm)	Radical (cm)
Control (DW)	100^{a}	16.24 ± 1.4^{a}	6.23 ± 0.7^{a}
Untreated AMV*	50^{b}	7.67 ± 0.8^{c}	1.43 ± 0.2^{c}
Treated AMV*	90^{a}	13.56 ± 1.8^{b}	4.45 ± 1.1^{b}

NI no inhibition, *DW* distilled water, *AMV concentration 700 mg l^{-1}; values are mean of three experiments ± SEM, compared by Pairwise Mean Comparison of Treatment with $p \leq 0.05$; means with the same letter are not significantly different

MnP and LiP were quite higher under shaking condition with 9.57, 7.68 and 90.33 times increment compared to control. The results thus suggested that biodegradation of Acid Maroon V by the consortium EDPA is multi enzyme process. The production of laccase is frequent characteristic of fungi (Yanto et al. 2021; Gahlout et al. 2013); certain bacteria like *Bacillus licheniformis* LS04 (Lu et al. 2012), *Pseudomonas stutzeri* AK6 (Joshi et al. 2020), and *Pseudomonas stutzeri* MN1 and *Acinetobacter baumannii* MN3 (Kuppusamy et al. 2017) produced it during dye degradation process. The oxido-reductive enzymes like azoreductase, laccase, LiP, tyrosinase, NADH–DCIP reductase and veratryl alcohol oxidase were detected in mixed bacterial cultures during degradation of Direct Red 81 (Kamal et al. 2022) and Indanthrene Blue RS (Mohanty and Kumar 2021). The bacterial cultures *Micrococcus glutamicus* NCIM 2168 (Saratale et al. 2009) and *Pseudomonas desmolyticum* 2112 (Kalme et al. 2009) produced LiP for biodegradation of Reactive Green 19A and Direct Blue 6 dyes.

Decolorization and degradation analysis of Acid Maroon V

UV–visible spectrophotometric analysis

The UV–visible spectral analysis revealed maximum absorption of Acid Maroon V at 523 nm. The same absorption peak in the treated dye samples was gradually decreased with the time and disappeared completely (Fig. 5A). Moreover, the pH of the decolorized samples was around 6.8, indicating that the color removal was a true consortium activity, not because of the change in pH. In our study, the significant decrease in color intensity at 523 nm wavelength explained that chromophore group of Acid Maroon V dye was completely reduced by the consortium EDPA. Furthermore, in the static samples, new peak was generated in UV region, but it was completely disappeared in the shaking sample. This revealed biodegradation or biotransformation of native dye molecule by the action of consortium EDPA. Moreover, the cell pellet after decolorization of dye was colorless, suggesting absence of bioadsorption process for the removal of Acid Maroon V. Figure 5B depicts original picture of dye decolorization under static and shaking condition. Therefore, above results thus signify that the mechanism of color removal was merely due to biodegradation capability of the consortium EDPA. The use of UV–visible spectral analysis

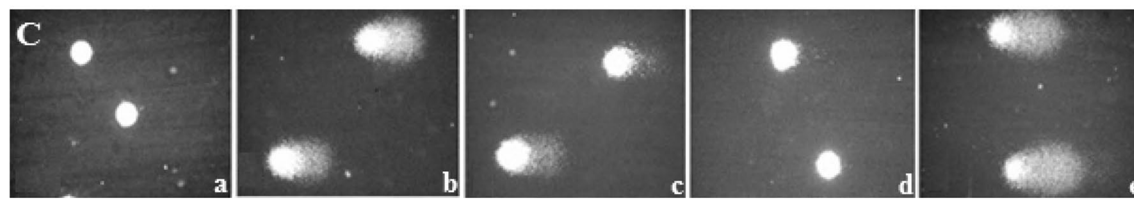


Fig. 7 Genotoxicity analysis of untreated and treated dye samples on human peripheral blood lymphocytes: Extent of DNA damage in arbitrary units (**A**), percent cell distribution in various DNA damage classes (**B**), and representative comet images (**C**): control (**a**), dye (**b**), intermediate (static) (**c**), product (shaking) (**d**) and H_2O_2 (**e**); Values are mean of three experiments ± SEM, compared by One-Way ANOVA with ** $p \leq 0.01$, *** $p \leq 0.001$

for the prediction of dye degradation pattern was described in the previous reports (Baena-Baldiris et al. 2020; Sinha et al. 2018).

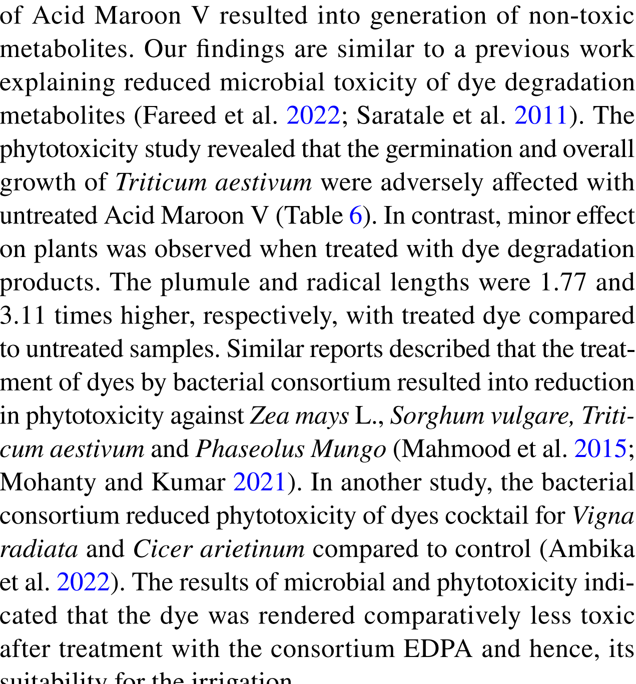
Determination of aromatic amines

The fate of aromatic amines during sequential microaerophilic-aerobic biodegradation of Acid Maroon V was evaluated. During first 16 h of incubation, the level of aromatic amines increased from 1.25 ± 0.04 to 7.58 ± 0.06 mg l^{-1} (Fig. 6). At the same stage, the consortium EDPA decolorized dye 96.62 ± 1.1%. It has been reported that the generated aromatic amines are liable to auto-oxidation forming highly colored polymers with low solubility (Kudlich et al. 1996). In contrast to the above expectation, our study showed no significant increment in color in the visible region. The aromatic amines generated in static condition were gradually degraded under shaking condition from 7.58 ± 0.06 to 0.81 ± 0.11 mg l^{-1} during the period of 16–112 h. This corresponded to 89.31% removal of TAA. Therefore, above results revealed the capability of the consortium EDPA to render Acid Maroon V degradation into less toxic metabolites under sequential microaerophilic-aerobic condition. The reductive cleavage of azo bond is mediated by azoreductase in microaerophilic condition; aromatic amines generated are subsequently oxidized and degraded by oxidative enzyme laccase (Zimmermann et al. 1982). In support to this, the activities of oxido-reductive enzymes were enhanced during such event, which also pointed out biodegradation of parent dye into simpler compounds. It is observed that presence of oxygen favors hydroxylation and ring opening of aromatic amines by non-specific enzymes resulting into its complete mineralization under aerobic condition (Pandey et al. 2007). The effectiveness of sequential microaerophilic-aerobic condition for the removal of aromatic amines was reported by researchers (Ogugbue et al. 2012; Lade et al. 2015; Franciscon et al. 2009; Isik and Sponza 2008).

Toxicity study of control and degraded Acid Maroon V

Microbial and phytotoxicity analysis

The direct use of untreated industrial effluents for agriculture may adversely affect soil microbial community and soil fertility. Thus, it is of concern to assess the toxicity of the dye before and after treatment (Ambika et al. 2022; Kalme et al. 2009). In the present study, the control dye and extracted metabolites (700 mg l^{-1}) were tested against different bacterial species. The results in Table 6 showed that the growth of all bacterial cultures was inhibited in the presence of control dye. The extracted metabolites on the other hand did not show any growth inhibition at similar concentration. This suggested that consortium EDPA-mediated biodegradation



of Acid Maroon V resulted into generation of non-toxic metabolites. Our findings are similar to a previous work explaining reduced microbial toxicity of dye degradation metabolites (Fareed et al. 2022; Saratale et al. 2011). The phytotoxicity study revealed that the germination and overall growth of *Triticum aestivum* were adversely affected with untreated Acid Maroon V (Table 6). In contrast, minor effect on plants was observed when treated with dye degradation products. The plumule and radical lengths were 1.77 and 3.11 times higher, respectively, with treated dye compared to untreated samples. Similar reports described that the treatment of dyes by bacterial consortium resulted into reduction in phytotoxicity against *Zea mays* L., *Sorghum vulgare, Triticum aestivum* and *Phaseolus Mungo* (Mahmood et al. 2015; Mohanty and Kumar 2021). In another study, the bacterial consortium reduced phytotoxicity of dyes cocktail for *Vigna radiata* and *Cicer arietinum* compared to control (Ambika et al. 2022). The results of microbial and phytotoxicity indicated that the dye was rendered comparatively less toxic after treatment with the consortium EDPA and hence, its suitability for the irrigation.

Genotoxicity study by comet assay

The genotoxicity was performed to evaluate the effect of control and degraded dye samples on DNA damage using alkaline comet assay. The extent of DNA damage was expressed in terms of arbitrary units and DNA damage class. In our study, the treatment of human peripheral blood lymphocytes with dye for 2 h resulted into a significantly higher DNA damage with greater arbitrary value. In contrast, the DNA damage effect was moderately lower with biodegraded intermediate (static). Moreover, with final degraded product (shaking), the pattern of DNA damage and arbitrary value were quite similar to the control (Fig. 7A). The comparison of cells in the various DNA damage categories revealed that the majority of the cells in the control group belonged to undamaged category 0, with very few cells in the other categories (Fig. 7B). Whereas, following treatment with dye and intermediate (static), the cells shifted from category 0 to other damage categories, confirming dye-induced DNA damage in the lymphocytes. On the other hand, the cells retained in category 0 and 1 when exposed to degradation product (shaking) suggesting consortium EDPA-mediated biodegradation of azo dyes resulted into the formation of non-toxic products. Figure 7C depicts the comparative comet images of lymphocytes exposed to controls, dye and degradation products. Above findings suggest that the dye degradation by bacterial consortium EDPA is thus an environmental benign method. Our results are quite consistent to Barathi et al. (2022), explaining quite lower genotoxicity effect on *Allium cepa* root cells when Reactive Red 170 dye was

treated with bacterial consortium consisting of *Bacillus subtilis*, *Brevibacillus borstelensis* and *Bacillus firmus*. Treatment of Reactive Blue 4 by hairy roots of *Helianthus annuus* reduced genotoxicity in *Allium cepa* root cells (Tungare et al. 2022).

Conclusion

The present study reports feasibility of redox mediator system for enhanced decolorization of di azo dye Acid Maroon V by the bacterial consortium EDPA. An accelerated color removal of higher concentrations of dye was achieved with quite lower concentration of lawsone. The consortium-lawsone combination was able to decolorize Acid Maroon V repeatedly as well as decolorization of several textile dyes. An induction of intracellular oxidoreductive enzyme activities in relation to reduction in aromatic amines pointed true biodegradation of the dye by the consortium EDPA. The microbial, phyto and genotoxicity study revealed that degraded dye metabolites were less toxic.

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Author contributions YP contributed to conceptualization, experiment, and original draft. AG contributed to formal analysis, supervision, and review draft.

Data availability Data and required material will be provided on request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest in the publication.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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