

Evaluation of the efficacy of a fungal consortium for degradation of azo dyes and simulated textile dye effluents

Samta Saroj¹ · Swati Dubey¹ · Pragati Agarwal¹ · R. Prasad¹ · R. P. Singh¹

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Abstract Azo dyes are widely prevalent environmental contaminants that are recalcitrant to biodegradation processes and have detrimental biological effects. Hence, exploring the novel microbial agents and to develop eco-friendly cost-effective process is pertinent for treatment of textile effluents. A fungal consortium comprising three strains *Penicillium oxalicum* SAR-3, *Aspergillus niger* SAR-6 and *Aspergillus flavus* SAB-3 has been developed. The consortium exhibited remarkably high potential to degrade azo dyes (Acid Red 183, Direct Blue 15 and Direct Red 75) at various initial concentrations. The consortium used was found to decolorize all the three dyes almost completely at lower initial concentrations (200–400 mg L⁻¹). The consortium was able to effectively decolorize simulated textile wastewater. UV–Visible and FTIR spectroscopic analysis had denoted the degradation of dyes. Furthermore, toxicity analysis of metabolites generated following degradation had shown significant reduction in the toxicity of dyes.

Keywords Azo dyes · Consortium · Decolorization · *Aspergillus* sp. · *Penicillium oxalicum* · Bioremediation

Introduction

Contamination of water caused by discharge of residual dyes, dispersing agents, salts and heavy metals by textile industries creates a serious environmental problem due to their inhibitory effect on aquatic photosynthesis, ability to deplete dissolved oxygen, toxicity to flora and fauna and their potential human toxicity (Noroozi et al. 2007). These wastewaters have adverse impacts in terms of Total Organic Carbon (TOC), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), suspended solids, salinity, colour, a wide range of pH (5–12) and the recalcitrance of organic compounds, such as azo dyes (Savin and Butnaru 2008). A number of physicochemical methods for the efficient removal of residual azo dyes from industrial effluents have been employed by various industries but, the effectiveness of these methods is limited due to the low efficiency, incomplete (20–30 %) colour removal, limited versatility, high cost, production of large amounts of sludge and handling of the effluents generated (Saroj et al. 2014).

Several reports are available on the use of microbial consortium for treatment of dye-contaminated wastewaters, and it has been observed that efficiency of the treatment depends on the competitive abilities of the individual strains to indigenous populations that are often well acclimatized to the existing environmental conditions (Banat et al. 1996). The treatment systems composed of mixed microbial populations possess higher degree of biodegradation and mineralization due to synergistic metabolic activities of microbial community and offers considerable advantages over the use of pure cultures in the degradation of synthetic dyes (Khehra et al. 2005). Individual strains of microbial consortium may attack the dye molecules at different positions or may have the capability

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✉ R. P. Singh
rpsbsfbs@iitr.ac.in

¹ Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee 247667, India

to utilize metabolites produced by the co-existing strains to promote decomposition (Forgacs et al. 2004). Anastasi et al. (2009) used consortium of *Trametes versicolor*, *Bjerkandera adusta*, *Bostra fumosa* using straw as solid substrate, to detoxify Poly R-478. Highly efficient fungal mixed culture systems for degradation of azo dyes and textile wastewater treatment have been reported by Yang et al. (2009). Fungal consortium has been considered as better treatment for textile wastewater as compared to conventional biological methods or system using single fungal strains (Yang et al. 2009). However, up to date, very few studies are available on use of fungal consortium for textile wastewater treatment.

Present study describes the isolation and screening of the fungal strains from dye-contaminated soil and the dye degradation potential of the consortium comprising *Penicillium oxalicum* SAR-3, *Aspergillus niger* SAR-6, and *Aspergillus flavus* SAB-3 for the azo dyes viz. Acid Red 183 (AR 183), Direct Blue 15 (DB 15) and Direct Red 75 (DR 75). Efficacy of consortium to decolorize simulated textile effluent has also been evaluated. The degradation of the dyes was further analysed by UV–visible and FTIR spectroscopy. In addition, toxicity of the biodegradation products of azo dyes generated following degradation by the fungal consortium was also evaluated.

Materials and methods

Dyes, chemicals and strain

The direct and reactive dyes viz. Acid Red 183 (AR183), Direct Red 75 (DR75), Acid Blue 161 (AB161), Acid Red 88 (AR88), Acid Blue 45 (AB45), Reactive Black 5 (RB5) (Supplementary Table 1), having chromophoric azo group were purchased from MP Biomedicals (USA). *S. cerevisiae* BY4741 strain was procured from Microbial Type Culture Collection bank (MTCC), India. Hydrogen peroxide was obtained from Sigma Chemical Company (St. Louis, MO, USA). Veratryl alcohol, tween-80 and other fine chemicals were obtained from HiMedia (Mumbai, India).

Culture media

Fungal strains were isolated and screened using potato dextrose agar medium. Dye decolorization and degradation experiments were carried out using modified Kirk's basal salt medium as described earlier (Saroj et al. 2014).

Isolation and screening of microorganisms

Isolation and screening of fungal sp. was performed as described earlier (Saroj et al. 2014).

Morphological and molecular identification of isolates

Fungal isolates were identified by sequencing of partial 18S and complete internal transcribed spacer (ITS) regions of rDNA gene. Further, analysis of the sequence data was done following the procedure described by Saroj et al. (2014).

Fungal compatibility assay

Compatibility among the selected fungi viz. *P. oxalicum* SAR-3, *A. niger* SAR-6 and *A. flavus* SAB-3 was analysed by inoculating all the three strains aseptically on potato dextrose agar plate followed by incubation at 30 °C. Fungal strains were point inoculated on to PDA plate in the middle of each partitions and growth was observed for successive 9 days. The parallel controls of all three fungi were used for the comparative growth pattern.

Estimation of enzyme activity

Manganese peroxidase activity was estimated as described by Paszczynski et al. (1988). Lignin peroxidase activity was determined by following the procedure of Arora and Gill (2001). Laccase was assayed using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) as substrate (de Souza-Cruz et al. 2004).

Evaluation of effect dye concentrations on azo dyes degradation by consortium

To develop a consortium, two discs of each fungal mycelia (*P. oxalicum* SAR-3, *A. niger* SAR-6 and *A. flavus* SAB-3) were taken with the help of cork borer from the edge of rapidly growing fungal strains and were inoculated in 50 ml modified kirk's medium and incubated at 30 °C under shaking conditions (200 rpm). To analyse the effect of higher concentrations of AR183, DB15 and DR75 on decolorization potential by consortium, after 48 h of growth at 30 °C under shaking conditions (200 rpm), the flasks were supplemented with 200–1000 mg L⁻¹ of dyes. Aliquots (3 ml) of culture supernatant were withdrawn at different time intervals from each flask, centrifuged at (5000 rpm, 10 min) to separate the mycelial cell mass and thereafter was used for determining the decolorization level of respective dyes. All experiments were run in triplicates.

Analysis of fungal consortium to degrade simulated textile wastewater

The simulated textile wastewaters were prepared in 30 g L⁻¹ NaCl, 5 g L⁻¹ Na₂CO₃ and 1.5 mL L⁻¹ of 32.5 % (w/v) NaOH in deionised distilled water supplemented with

different concentrations 100–900 mg L⁻¹ of mixture of azo dyes viz. AR183, DB15 and DR75 (Osma et al. 2010). To analyse the decolorization potential of fungal consortium to degrade simulated wastewaters, the cultures were grown as stated earlier in Sect. 2.3 in parallel and following 48 h of incubation at 30 °C under shaking conditions (200 rpm), the flasks were supplemented with simulated wastewaters consisting of different dye concentrations. Unsupplemented flasks were used as controls. Further, the determination of decolorization level of azo dyes was performed as described in Sect. 2.7.

Analytical procedure

UV–VIS spectral analysis and FTIR

The metabolites produced during the biodegradation of azo dyes were extracted and analysed through UV–VIS and Fourier Transform Infrared Spectroscopy (FTIR).

Toxicity analysis of degradation metabolites of azo dyes

Toxicity assays were performed based on the inhibitory effects of azo dyes and their metabolites obtained following degradation on the growth of the yeast *S. cerevisiae* BY4741 (Mendes et al. 2011). Initially the yeast cells were grown up to a mid-exponential phase and then inoculum was prepared from the above culture, which was centrifuged and suspended to OD_{640nm} = 0.15 in a triple strength minimal growth medium. The toxicity analysis was carried out following the procedure described by Mendes et al. (2011).

Results

Screening of isolates SAR-3, SAR-6 and SAB-3 for enzymatic profiles

The fungal isolates viz. SAR-3, SAR-6 and SAB-3, showing notable levels of dye degradation were subsequently subjected to elucidation for the enzymatic profile possibly involved in the azo dye degradation process. Among all the three strains selected, SAR-3 had higher levels of manganese peroxidase ($367.4 \pm 4.9 \text{ UL}^{-1}$) after 168 h of incubation, followed by SAR-6 ($240.6 \pm 1.8 \text{ UL}^{-1}$) and SAB-3 with ($211.5 \pm 1.6 \text{ UL}^{-1}$) following 144 h of incubation (Fig. 1). However, negligible amount of lignin peroxidase and no laccase activity was obtained among all the three strains. These results indicated that manganese peroxidase is probably the major enzyme involved in dye degradation.

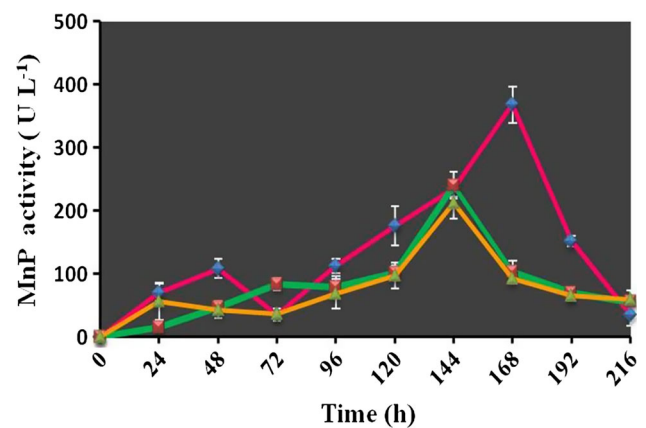


Fig. 1 Levels of MnP, from SAR-3 (filled diamond), SAR-6 (filled square) and SAB-3 (filled triangle), at different time intervals

Morphological and molecular identification of fungal isolates

The strains SAR-3, SAR-6 and SAB-3 were morphologically identified as *P. oxalicum*, *A. niger* and *A. flavus* by Indian Type Culture Collection Bank, Indian Agricultural Research Institute, New Delhi, India. Further, sequencing of internal transcribed spacer (ITS1–5.8S–ITS2) rDNA domains had denoted SAR-3 as *P. oxalicum*, SAR-6 as *A. niger* and SAB-3 as *A. flavus*, respectively. The phylogenetic tree was constructed using MEGA 5.05 using the top hits obtained against ITS gene sequences of all three strains after performing BLAST-n (Fig. 2a–c). The internal transcribed spacer sequences for the strains have been deposited in the GenBank under the accession numbers JQ349067, KJ184541 and KJ501092, respectively.

Growth compatibility of fungal strains in consortium

The three fungal strains viz. *P. oxalicum* SAR-3, *A. niger* SAR-6 and *A. flavus* SAB-3 that initially had denoted higher dye decolorization ability were chosen for developing the fungal consortium. These strains have similar growth pattern consecutively for seven days (Fig. 3a–e). The growth compatibility of the three strains suggests that none of these were competing with each other in the consortium. Thus, a fungal consortium consisting of these strains appeared suitable for analysing its potential for the dye degradation studies.

Decolorization at different dye concentrations

Decolorization of the dyes by the consortium can be attributed to the cumulative and catalytic effect of all the three strains. AR183 appeared recalcitrant as compared to the other two dyes DB15 and DR75. The consortium used was found to decolorize all the three dyes almost

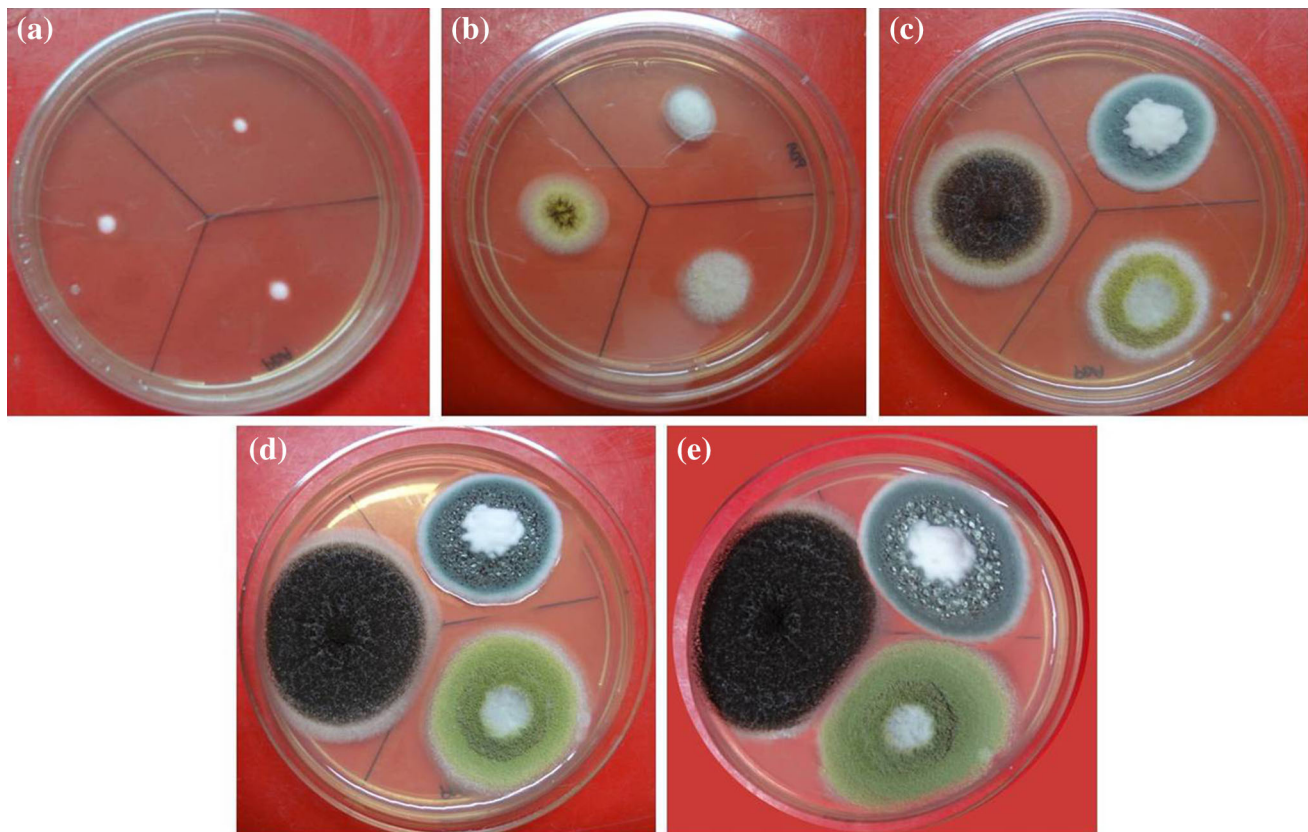


Fig. 2 Growth pattern and compatibility of *P. oxalicum* SAR-3, *A. niger* SAR-6 and *A. flavus* SAB-3 after **a** 24 h, **b** 76 h, **c** 120 h, **d** 168 h and **e** 216 h of incubation at 30 °C for suitability of strains as fungal consortium

completely at lower initial concentrations (200–400 mg L⁻¹). Higher concentrations (600–1000 mg L⁻¹) of AR183 had effected into lower biomass yields and concomitantly lower (26.1 %) decolorization levels (Fig. 4a). Consortium was able to decolorize DB15 and DR75 within 24–72 h (Fig. 4b, c) when the dyes were used up to a concentration of 600 mg L⁻¹; however, decolorization decreased to 87.8–47.5 % in relatively longer period of time (72–168 h) when the dye concentration was raised to 1000 mg L⁻¹. Thus, the level of decolorization at higher dye concentrations observed with consortium reached to maximal within 24–72 h of duration.

Biomass yield remained almost unchanged at varying concentration of DB15 and DR75 suggesting the adaptability of the consortium to increasing concentration of dyes (Fig. 4b, c). Thus, consortium appears to be productive and promising system as compared to single culture for decolorization of dyes.

Degradation product analysis

UV-visible analysis of degradation products

UV-Vis spectrum (350–750 nm) of supernatants withdrawn at different time intervals denoted decreased

absorbance for AR 183, DB 15 and DR 75 dyes within 48 h indicating the notable levels of decolorization by the consortium. Consortium was rapid in decolorization as evident from the (Supplementary Fig. 1), that denotes complete disappearance of major peaks of respective dyes following 48 h of incubation. Mycelial biomass of fungal strains, i.e. *Aspergillus* sp. and *P. oxalicum* SAR-3 appeared coloured at initial stages as the dyes probably get adsorbed on to fungal biomass followed by degradation by fungal extracellular enzymes.

FTIR analysis of degradation products

Comparison of the FTIR spectrum of dye and the degradation products following incubation of dye along with consortium had indicated the biodegradation of the AR 183, DB 15 and DR 75 dye by the consortium (Supplementary Fig. 2a–f). The spectrum of AR 183 (Supplementary Fig. 2a) shows the characteristic peaks at 3440.46, 1631.36 cm⁻¹ corresponding to –OH stretching vibration, –N=N– stretching in azo group. The peaks between 523.63 to 614.88 cm⁻¹ could be assigned to substituted benzene indicating the aromatic nature of dye. Peak between 1095.88–1035.81 cm⁻¹ corresponds to asymmetric stretching vibration of the –SO₃Na group and peak at

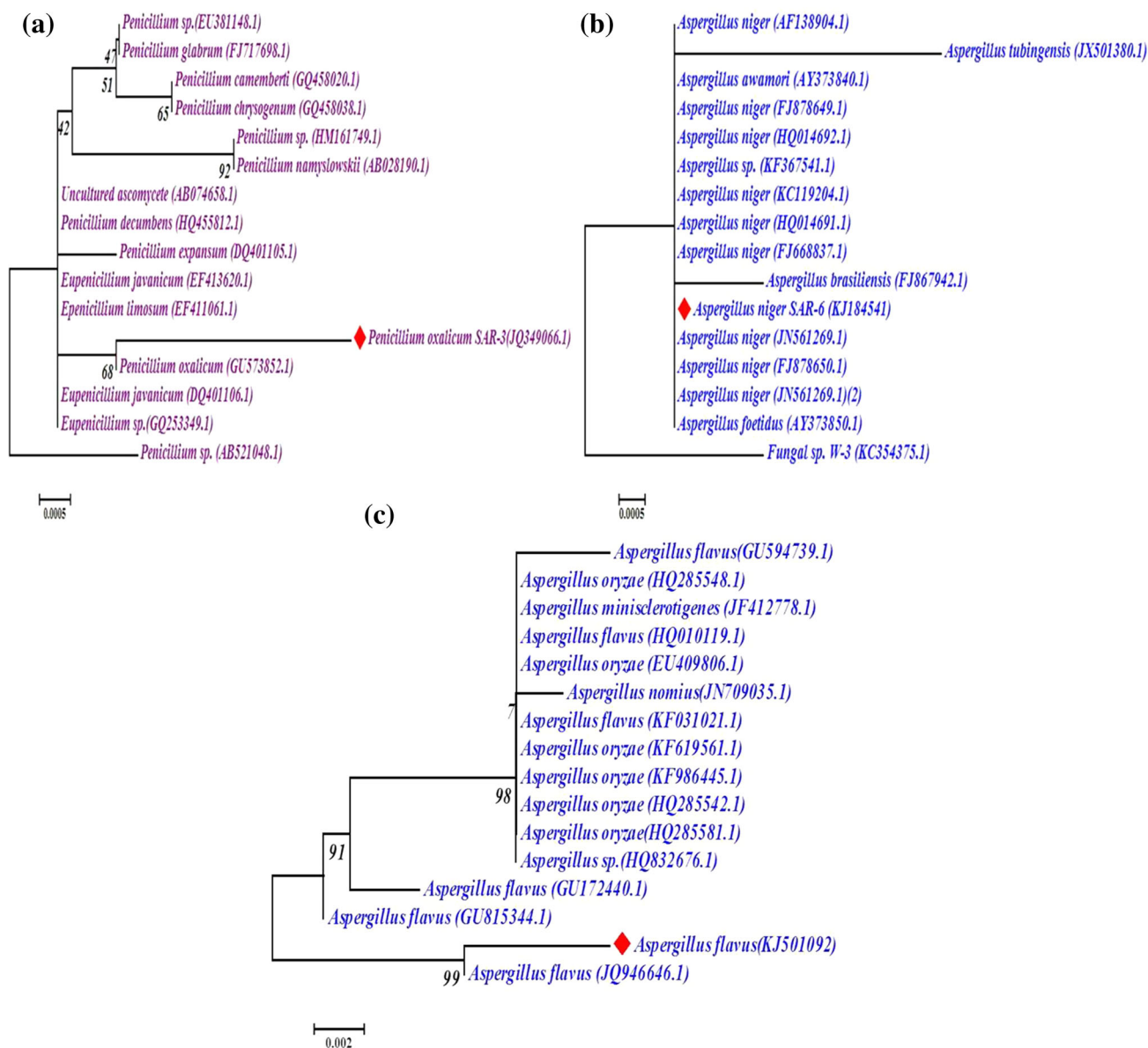


Fig. 3 Phylogenetic tree based on the ITS sequence of **a** *P. oxalicum* SAR-3, **b** *A. niger* SAR-6 and **c** *A. flavus* SAB-3 and the homologous ITS sequences available at GenBank (neighbour joining tree). Numerical values the bootstrap percentiles from 1000 replicates

1410.43 cm^{-1} represents bending frequencies of methyl group on aromatic ring. The FTIR spectrum of the extracted metabolites after degradation of AR 183 denoted absence of peaks at 523.63, 614 cm^{-1} that denotes the loss of aromaticity and appearance of new peaks indicates the production of intermediates in the degradation process (Supplementary Fig. 2b). Broadening of peak at 3429.51 cm^{-1} shows $-\text{OH}$ stretching and appearance of peaks at 1401.35, 1577.43 and 1634.21 cm^{-1} were indicative of C–H deformation of methyl group, $-\text{NO}_2$ stretching in aliphatic nitro compound and stretching of secondary amides. Furthermore, the appearance of new peaks at 2922.65 cm^{-1} indicates C–H stretching of alkanes.

The spectrum of DB 15 (Supplementary Fig. 2c) had shown characteristic peaks at 3437.49, 1630.43 cm^{-1} corresponding to $-\text{OH}$ stretching vibration, and $-\text{N}=\text{N}-$ stretching in azo group. The peaks at 482.42 and 835.65 cm^{-1} indicated the aromatic nature of the dye. While peak near 1403 cm^{-1} denoted C–H deformation of methyl group and peak at 2922.65 cm^{-1} denotes alkane C–H stretching. Peaks at 1118.37–620.77 cm^{-1} for S=O stretching indicate sulphoxide nature of the dye. The FTIR spectrum of the degradation products of DB15 (Supplementary Fig. 2d), showed appearance of peaks at 2926.50 and 2863.78 cm^{-1} indicating alkanes C–H stretching. Absence of peaks at 482.42, 620.77 and 482.42 cm^{-1} denotes loss of aromaticity or benzene ring and broadening

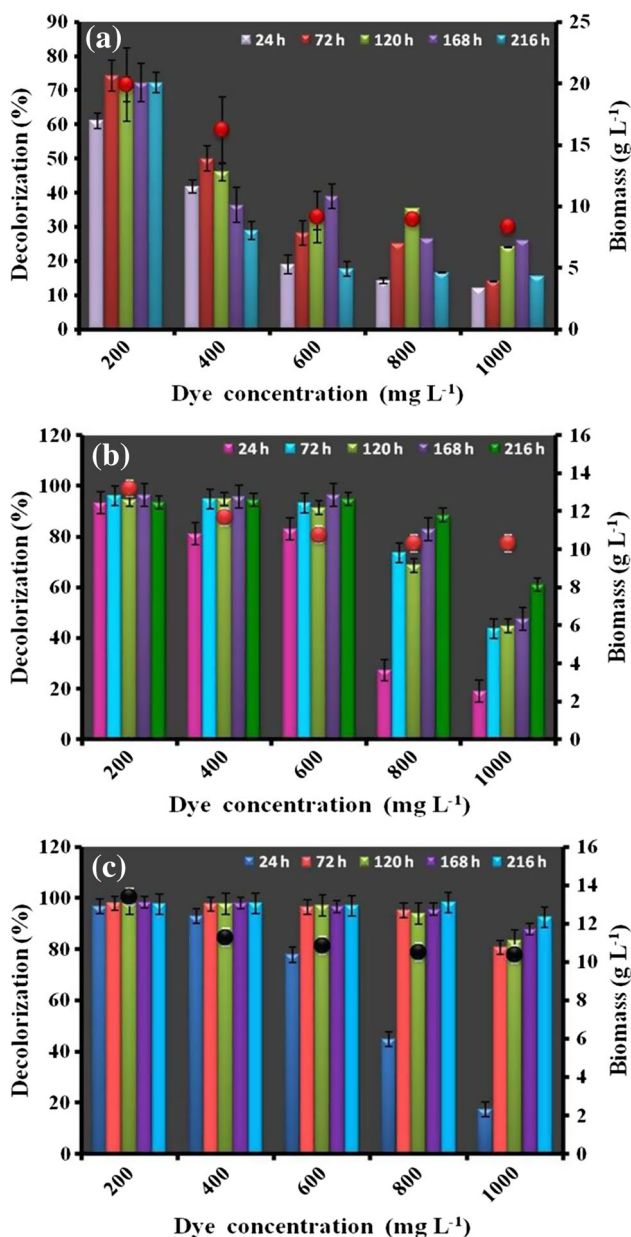


Fig. 4 Decolourization of **a** AR183, **b** DB15, **c** DR75 by consortium at various initial concentrations

of peak at 3429.89 cm^{-1} shows $-\text{OH}$ stretching. Peaks at 1634.31 and 1401.42 cm^{-1} correspond to stretching of secondary amides and $\text{C}-\text{H}$ deformation of methyl group.

Spectrum of DR 75 represented characteristic peaks at 3443.66 , 1630.92 cm^{-1} corresponding to $-\text{OH}$ stretching vibration and $-\text{N}=\text{N}-$ stretching in azo group. Peaks at 1041.70 and 1115.29 cm^{-1} show $\text{S}=\text{O}$ stretching vibration and sulphoxide nature of the dye. Aromatic nature of the dye is displayed by peaks from 523.63 to 820.92 cm^{-1} . The peaks at 1203.6 cm^{-1} denote $\text{C}-\text{N}$ vibrations and 1400.82 cm^{-1} for ketone CH deformation (Supplementary Fig. 2e). Metabolites extracted following degradation of

DR 75 showed peak at 3434.99 cm^{-1} for $-\text{OH}$ stretching. Appearance of peaks at 2926.58 cm^{-1} indicated the $\text{C}-\text{H}$ stretching of alkanes (Supplementary Fig. 2f). Peak at 1400.36 cm^{-1} and appearance of new peak at 1574.49 cm^{-1} denote ketone $\text{C}-\text{H}$ deformation.

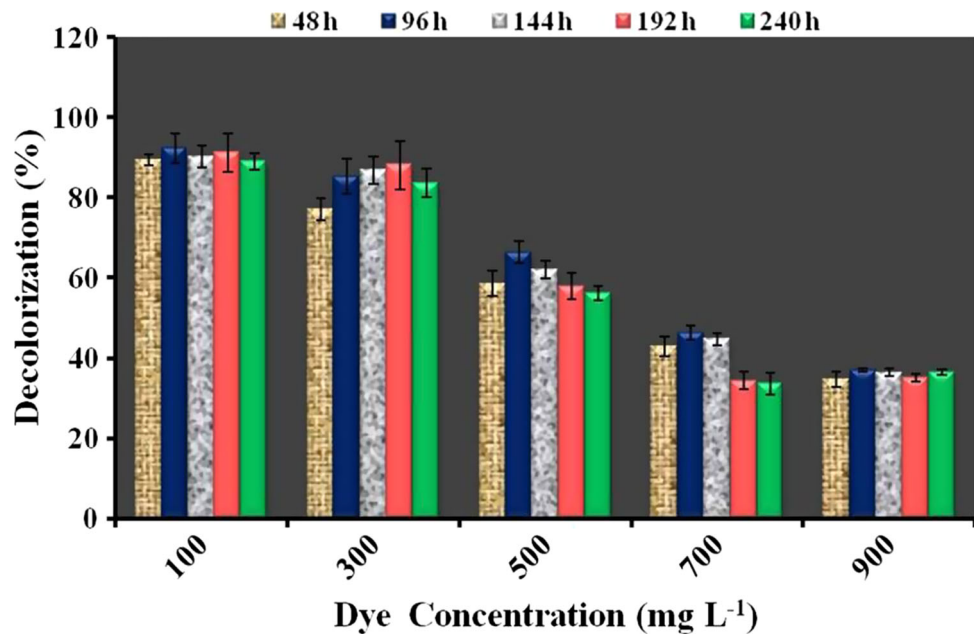
Analysis for decolorization of simulated textile wastewater by consortium

The decolorization of simulated textile wastewaters using fungal consortium had resulted into higher levels of decolorization, i.e. $(92.38 \pm 3.7$ to $66.4 \pm 2.24\%)$ in relatively shorter period of time (96 h) at the dye concentration from 100 to 500 mg L^{-1} , respectively (Fig. 5). Dye mixture at higher concentrations (700 to 900 mg L^{-1}) was decolorized to notable levels (46.2 ± 1.78 to $36.3 \pm 0.98\%$) by the consortium used. Thus, the fungal consortium used was observed to be a promising and productive system for treatment of textile wastewaters.

FTIR analysis of degradation products of simulated wastewaters

Comparison of FTIR spectrum of simulated wastewaters with that of degradation products obtained had indicated the biodegradation of the dye components of the simulated wastewater by *P. oxalicum* SAR-3 and consortium (Supplementary Fig. 3a–c). The spectrum of simulated wastewater (Supplementary Fig. 3a) had shown the characteristic peaks at 3440.72 , 1630.79 cm^{-1} corresponding to the $-\text{OH}$ stretching vibration and $-\text{N}=\text{N}-$ stretching in azo group. The peaks between 626.66 to 514.80 cm^{-1} could be assigned to substituted benzene indicating the aromatic nature of dye; whereas the peak between 1035.81 cm^{-1} corresponds to asymmetric stretching vibration of the $-\text{SO}_3\text{Na}$ group and the peak at 1409.65 cm^{-1} represents bending frequencies of methyl group on aromatic ring similar to peak observed in AR183 spectrum. Peak at 1191.82 cm^{-1} corresponds to $\text{C}-\text{N}$ vibrations. The FTIR spectrum of the extracted metabolites following degradation by *P. oxalicum* SAR-3 had indicated the production of intermediates in the degradation process (Supplementary Fig. 3b). Peaks at 2919.71 and 2854.95 cm^{-1} denote alkane $\text{C}-\text{H}$ stretching and 1106.46 cm^{-1} for $\text{S}=\text{O}$ stretching. Appearance of peaks at 1633.74 and 1580.38 cm^{-1} was indicative of NO_2 stretching in aliphatic nitro compound. Peak at 1401.55 cm^{-1} denotes $\text{C}-\text{H}$ deformation of methyl groups. However, the FTIR spectrum of the extracted metabolites after degradation of wastewater by fungal consortium denoted appearance of new peaks (Supplementary Fig. 3c). Sharpening of peak at 2925.73 and 2857.06 cm^{-1} had denoted increased alkane $\text{C}-\text{H}$ stretching and peak at 1147.67 cm^{-1} for $\text{S}=\text{O}$

Fig. 5 Decolorization profile for simulated textile wastewater at various initial dye concentrations by consortium



stretching. The appearance of peaks at 1742.27 cm^{-1} indicated C=O stretching, whereas peak at 1401.08 cm^{-1} denotes C–H deformation of methyl groups. Absence of peaks between 626.66 to 514.80 cm^{-1} denotes loss of aromaticity or benzene ring. Thus, loss of aromaticity of extracted metabolites and appearance of new peaks denoted the degradation of simulated wastewaters by the fungal consortium.

Toxicity analysis of degradation products over *S. cerevisiae* BY4741 growth

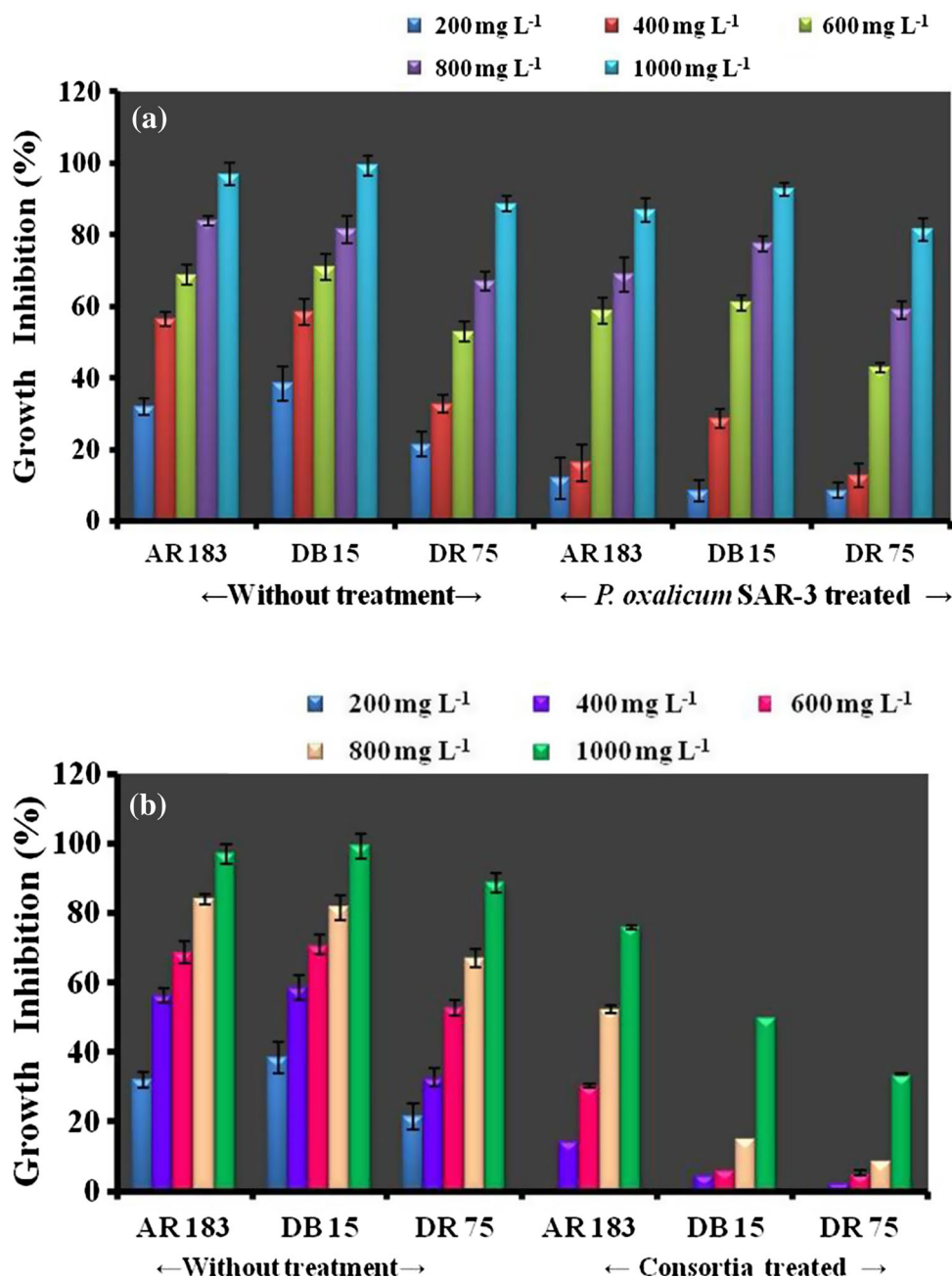
Azo dyes The toxicity of the azo dye and residual metabolites following degradation of azo dye by *P. oxalicum* SAR-3 and consortium was enumerated based on the inhibitory effects on the growth of *S. cerevisiae* BY4741 (Fig. 6a). It has been observed that dyes as compared to their degradation products were nearly lethal to the yeast cells. Dyes when used at 200 – 1000 mg L^{-1} had adversely affected the growth of the yeast cells and inhibition of growth from 21.4 ± 3.6 to $99.3 \pm 2.76\%$, respectively, was observed. Metabolites generated following incubation of dyes (200 – 400 mg L^{-1}) with *P. oxalicum* SAR-3 alone had effected into reduced inhibition to about 78 – 30% of yeast growth indicating therefore a notable decrease in the toxicity level of the dyes following degradation of dyes. Higher concentration of the dyes 600 – 1000 mg L^{-1} used was inhibitory (77.4 to 92.7%) probably as the dyes remained largely undegraded at these concentrations. On the other hand, remarkably reduced toxicity

was observed following degradation of dyes by consortium (Fig. 6b). Lower concentration of dyes (200 – 400 mg L^{-1}) when added to consortia had resulted into no or remarkably reduced levels of inhibition (Fig. 6b). However, higher concentration of dyes (600 – 1000 mg L^{-1}) mainly AR 183 had effected into 30.4 – 75.9% inhibition of growth. However, higher concentrations (600 – 1000 mg L^{-1}) of DB 15 and DR 75 had resulted into considerably reduced inhibition of growth (5.3 – 15.4%), further higher concentration (1000 mg L^{-1}) of these dyes was more inhibitory (33.5 – 50%) for yeast growth (Fig. 6c).

Simulated textile wastewater

The toxicity of the simulated wastewaters and residual metabolites following degradation by consortium was analysed by assessing their inhibitory effects on the growth of *S. cerevisiae* BY4741 (Fig. 7). It has been observed the untreated effluents were nearly lethal to the yeast strain as compared to the degradation products. Simulated wastewaters exhibited growth inhibition of 40 ± 2.3 to $99.4 \pm 2.56\%$ at dye concentrations of 100 – 900 mg L^{-1} . A remarkably reduced toxicity (74.9 – 86.7%) was observed when the simulated wastewaters consisting 100 – 300 mg L^{-1} of the dyes were subjected to degradation by consortium (Fig. 7). A further increase in the concentration of the dyes to 500 – 900 mg L^{-1} resulted into 26.2 – 49.6% inhibition in the growth. These observations denote that the fungal consortium appears to be a viable and promising system for treatment of the textile wastewaters.

Fig. 6 Inhibitory effects of intact azo dyes AR 183, DB15, DR75 and their residual metabolites obtained after degradation by **a** *P. oxalicum* SAR-3 and **b** consortium over *S. cerevisiae* BY4741 growth



Discussion

Single microbial strains are capable of decolourizing dyes; however, sometimes the metabolites obtained following degradation are much more difficult to biodegrade than the parent dye. In addition, these microorganisms seem specific to a category of dyes, and due to the variable composition of wastewater from the textile industry, several research groups have attempted to develop more efficient microbial processes. The complete degradation of aromatic compounds such as azo dyes is possible with the

cumulative effect of dye degrading enzymes, which implies the necessity of building microbial consortia (Asgher et al. 2008; Waghmode et al. 2012; Phugare et al. 2011a). There are certain advantages of using microbial consortia over the use of single strains in the degradation process of azo dyes, as communally the different strains can attack the dye molecules at different positions and can use the metabolites produced by another strain for further decomposition, in some cases attaining the mineralisation of azo dyes (Jadhav et al. 2010). Degradation potential of *P. oxalicum* SAR-3 for various azo dyes investigated here (AR183, DB15 and DR75) was effective only at lower concentration

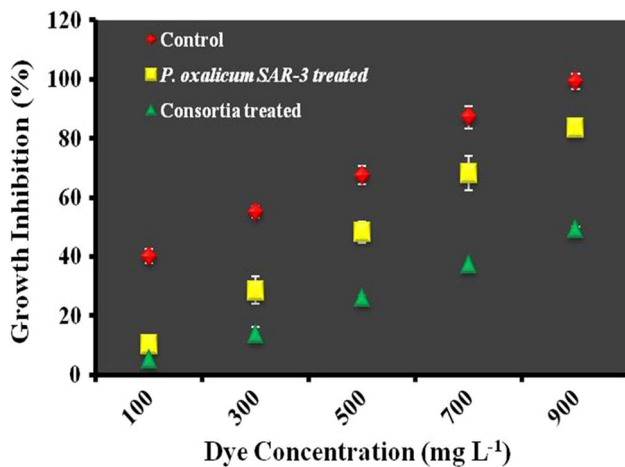


Fig. 7 Inhibitory effects of simulated textile wastewaters and their residual metabolites obtained after degradation by *P. oxalicum* SAR-3 and consortium over *S. cerevisiae* BY4741 growth

(100–200 mg L⁻¹). In contrast, the consortia developed using *P. oxalicum* SAR-3, *A. niger* SAR-3 and *A. flavus* SAB-3 achieved almost complete decolourization of DB15 and DR75 at the concentration ranging from 200 mg L⁻¹ to 1000 mg L⁻¹ within 24–72 h. However, 74.3 % decolourization was observed for AR183 at 200 mg L⁻¹, which decreased to about 5 % when the concentration was increased to 400 mg L⁻¹. It has been described by Khehra et al. (2005) that the higher decolorization efficiency of consortium might be attributed to the combined activities of the constituent strains. The individual strain(s) may transform the dye to intermediates which can act as redox mediators for efficient transfer of reducing equivalents from other strains, leading to an enhanced decolorization potential of the consortium (Khehra et al. 2005).

Penicillium oxalicum is the most abundant genus of fungi in soils characterized by its spore bearing hypha having dense-brush-like appearance. It seems to be a potential strain producing spectra of catabolic enzymes which can effectively be employed for biodegradation/detoxification of a broad range of environmental pollutants (Opasols and Adewoye 2010).

Aspergillus sp. have the ability to tolerate extreme cultivation conditions (Raper and Fennel 1965; Kis-Papo et al. 2003; Machida and Gomi 2010) as it can be cultivated over a wide range of temperatures (10–50 °C), pH (2–11), salinity (0–34 %) and under nutrient-deficient conditions. Therefore, *Aspergillus* can be used for solid-state or submerged fermentations and for large-scale industrial processes. Important feature of *Aspergillus* includes its capability to degrade and utilize diverse biopolymers such as starch, (hemi-) cellulose, pectin, xylan and proteins, allowing it to be cultivated on renewable resources such as plant biomass.

Further, results obtained after screening of *P. oxalicum* SAR-3, *A. niger* SAR-6 and *A. flavus* SAB-3 for their enzymatic profile had indicated that MnP is possibly the key enzyme involved in azo dye decolorization.

Analysis of degradation of azo dyes

The UV visible and FTIR spectral analysis had ascertained the degradation of azo dyes during the treatment by fungal consortium. Similar results for FTIR analysis were obtained by Patil et al. (2008) with a bacterial consortium PMB11 comprising *Bacillus odyseey* SUK3, *Morganella morgani* SUK5 and *Proteus* sp. SUK7. Phugare et al. (2011a) had also shown FTIR analysis of degradation products by bacterial consortium of *Providencia* sp. and *Pseudomonas aeruginosa* BCH, with similar set of peaks as observed for the present study. Toxicity analysis of metabolites generated after degradation with consortium over *S. cerevisiae* BY4741 had shown almost complete reduction in the toxicity of dyes following degradation. Decrease in toxicity of the metabolites generated had also been shown after conducting phytotoxicity studies with the degraded metabolites of Reactive Blue 59 indicating effectiveness of bacterial consortium PMB11 for the treatment of textile effluent containing Reactive Blue 59 (Patil et al. 2008).

Analysis for degradation of simulated textile wastewater

Coloured industrial effluents from textile, paper and pulp contain compounds with complex aromatic structures that make them quite difficult to be degraded. Besides containing dyes, textile effluents contain salts often at very high ionic strength and have alkaline pH values as well. The fungal consortium was able to effectively decolorize simulated textile wastewater from 100 mg L⁻¹ (92 %) to 900 mg L⁻¹ (36 %). At higher dye concentrations, it is likely that apart from enzymatic catalysis, biosorption process may also be involved in the removal of dyes from the simulated wastewater, since there is almost negligible difference in percentage level of decolorization of wastewater at increasing time intervals (Fig. 5). Besides, the major mechanism involved in dye removal by *Aspergillus* sp. has also been reported through biosorption process (Fu and Viraraghavan 2002; Ali et al. 2008; Wang and Hu 2008).

Similar results were obtained for *P. aeruginosa* that achieved 92 % decolouration of a textile effluent within 30 h, and *Providencia* sp. achieves 84 % decolouration within 48 h, while the consortium of both microorganisms is responsible for complete effluent decolorization in 20 h (Phugare et al. 2011b). Furthermore, Reactive Navy Blue HE2R was more rapidly decolorized by consortium of

Pseudomonas sp. SUK1 and *Aspergillus ochraceus* NCIM-1146 up to 92 % within 72 h (Kadam et al. 2011). Similarly, *Penicillium* sp. QQ was observed to be more efficient in the decoloration of azo dyes than the bacterial strain *Exiguobacterium* sp. TL, but the consortium comprising both had higher extent of decolorization levels (Qu et al. 2010).

Toxicity evaluation of degradation metabolites

Reduction in toxicity of metabolites generated after degradation is a prerequisite to the development of effective ecofriendly bioremediation technology. In the current investigation, substantial reduction in toxicity of the degradation metabolites generated following treatment with consortium was observed and these results are comparable with the earlier observations of Mendes et al. (2011) that reported more than 50 % reduction in azo dyes toxicity following treatment with laccase enzyme. Furthermore, Pereira et al. (2009) had shown threefold reduction in the toxicity of Sudan Orange G after treatment using bacterial CotA-laccase. Present study describes almost complete reduction in the toxicity of dyes following degradation at lower concentration of dyes (200–400 mg L⁻¹). Higher concentrations (600–1000 mg L⁻¹) of DB15 and DR75 had resulted into considerably reduced inhibition of growth (5.3–15.4 %). Therefore, it is likely that the fungal consortium is a viable and promising system for treatment of the textile wastewaters.

Conclusion

A fungal consortium evaluated consisting *P. oxalicum* SAR-3 and the other two isolates, i.e. *A. niger* SAR-6 and *A. flavus* SAB-3 had led into rapid and higher levels of decolorization and degradation potential of the azo dyes. The consortium used was found to decolorize all the three dyes almost completely at lower initial concentrations. Furthermore, the consortium was able to effectively degrade simulated textile wastewater consisting of dyes at the concentration from 100 to 500 mg L⁻¹. Toxicity analysis of dye degradation metabolites generated over *S. cerevisiae* BY4741 had shown almost complete reduction in the toxicity of dyes following degradation. Hence, the developed consortium with faster and effective dye degradation profile will be a productive, promising and ecofriendly approach for the treatment of the textile industry wastewaters.

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