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Decolorization of adsorbed textile dyes by developed consortium of *Pseudomonas* sp. SUK1 and *Aspergillus ochraceus* NCIM-1146 under solid state fermentation

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ABSTRACT

The objective of this study was to develop consortium using *Pseudomonas* sp. SUK1 and *Aspergillus ochraceus* NCIM-1146 to decolorize adsorbed dyes from textile effluent wastewater under solid state fermentation. Among various agricultural wastes rice bran showed dye adsorption up to 90, 62 and 80% from textile dye reactive navy blue HE2R (RNB HE2R) solution, mixture of textile dyes and textile industry wastewater, respectively. *Pseudomonas* sp. SUK1 and *A. ochraceus* NCIM-1146 showed 62 and 38% decolorization of RNB HE2R adsorbed on rice bran in 24 h under solid state fermentation. However, the consortium of *Pseudomonas* sp. SUK1 and *A. ochraceus* NCIM-1146 (consortium-PA) showed 80% decolorization in 24 h. The consortium-PA showed effective ADMI removal ratio of adsorbed dyes from textile industry wastewater (77%), mixture of textile dyes (82%) and chemical precipitate of textile dye effluent (CPTDE) (86%). Secretion of extracellular enzymes such as laccase, azoreductase, tyrosinase and NADH-DCIP reductase and their significant induction in the presence of adsorbed dye suggests their role in the decolorization of RNB HE2R. GCMS and HPLC analysis of product suggests the different fates of biodegradation of RNB HE2R when used *Pseudomonas* sp. SUK1, *A. ochraceus* NCIM-1146 and consortium PA.

1. Introduction

More than 10,000 different synthetic dyes are widely used in textile processing industry for dyeing and printing purposes. The fixation rate of synthetic dyes is not 100%, thus they enter into the environment as wastewater. The dye concentrations in the textile processing wastewaters are in the range of 10–200 mg l^{-1}. As dyes are designed to be chemically and photolytically stable, they are highly persistent in natural environment. The release of dye containing wastewater in the natural environment may cause ecotoxic hazards [1]. Direct discharge of huge amount of industrial effluent in combination with increasingly stringent legislation; makes the search for appropriate treatment technologies an important priority [2].

Many industries have used conventional physical and chemical methods such as chemical precipitation, membrane filtration, coagulation, adsorption, and electrolysis for the treatment of textile industry wastewater [3–7]. These methods are not destructive but they only transfer the contaminants from one form to another, therefore, a new and different kind of pollution problem is being faced which intern calls for further treatment [8–10]. Out of these all techniques chemical precipitation is most common at Ichalkaranji, India and produce highly recalcitrant textile dye precipitate as chemical precipitate of textile dye effluent (CPTDE). Although, these methods are effective, they suffer from shortcomings such as high cost, formation of hazardous by-products and high energy requirements. Due to these disadvantages, many researchers have tried to develop eco-friendly biological methods for the treatment of industrial effluent.

Bacteria and fungi are widely used for decolorization of textile dyestuff and textile industry wastewater. The biodegradation ability of bacteria is associated with its intracellular and extracellular oxidoreductive enzyme system such as laccase, azoreductase and NADH-DCIP-reductase [11]. However, the biodegradation ability of fungi is associated with its extracellular oxidoreductive enzymes such as lignin peroxidase, laccase, and tyrosinase [12]. The earlier reports showed textile dyes degradation potential of bacterium *Pseudomonas* sp. SUK1 and fungi *Aspergillus ochraceus* NCIM-1146 [13–16] under submerged condition. A consortium favors the use of products formed by one organism by another organism so as to mineralize the complex dye structures in to non-toxic compo-

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nents. The complexity of microbial consortium enables them to act on variety of pollutants [17]. Bacteria and fungi are widely used for the production of industrial enzymes such as α-amylase, xylanase, lignin peroxidase, Mn-peroxidase, laccase and tyrosinase under solid state condition [18,19]. Very few reports are available on dye decolorization under solid sate fermentation [20].

Agro-industrial residues such as, wheat bran, rice bran, maize bran, gram bran, wheat straw, rice straw, rice husk, soy hull, sago hampas, grapevine trimmings dust, saw dust, corncobs and coconut coir pith are generally considered the best substrates for solid state fermentation processes [21]. Rice bran is a byproduct of the rice milling process. It is used mainly as fertilizer or fuel. Rice bran contains vitamins, carbohydrates, iron, magnesium, copper and calcium [22]. These compounds are environment friendly and are nutritious to the plants. Therefore, the use of solid-state fermentation using rice bran as a substrate for the removal of textile dyestuff from wastewater could offer some apparent economic and engineering advantages over the classical submerged fermentation.

In this work, we are reporting decolorization of the adsorbed textile dyes from textile wastewater and chemical precipitate of textile dye effluent (CPTDE) obtained in chemical treatment method by developed consortium PA under solid state fermentation. We have also studied extracellular enzyme status during decolorization, fate of metabolism of the textile dye reactive navy blue HE2R using GCMS and HPLC analysis by an individual species and in consortium.

2. Materials and methods

2.1. Dyestuff, chemicals and microbiological media

All chemicals were of highest purity and of an analytical grade. L-Tyrosine, *o*-tolidine, veratryl alcohol and microbiological media such as nutrient broth and potato dextrose broth were obtained from Hi-media Laboratory, India.

The textile dyestuff, textile industry effluent and chemical precipitate of textile dye effluent (CPTDE) were obtained from local industry of Ichalkaranji, India. The highly colored effluent of the textile processing industry contained reactive and disperse textile dyes such as reactive yellow XL, reactive red RB, reactive red ME4BL, reactive orange 3R, reactive blue ME2RL, reactive golden yellow HER, disperse blue 2BLM, disperse navy blue RE, disperse yellow 7GL and disperse yellow brown REL as raw materials. The effluents were collected in airtight plastic container and filtered through ordinary filter paper to remove large suspended particles. The pH of the filtered effluent was adjusted to 7.0 and stored at $4 \pm 1\,^{\circ}C$ until use. The mixture of textile dyes were prepared by adding various textile dyes (each $20\,mg\,l^{-1}$) such as, disperse blue 2BLM, reactive yellow XL, reactive red RB, reactive red ME4BL, reactive orange 3R, reactive blue ME2RL and reactive golden yellow HER.

2.2. Microorganism and culture conditions

A. ochraceus NCIM-1146 was obtained from National Center for Industrial Microorganisms, Pune, India. The stock culture was maintained on potato-dextrose agar slants at $4\,^{\circ}C$. *Pseudomonas* sp. SUK1 was isolated from textile dye contaminated site [13]. The stock culture was maintained on nutrient agar medium.

2.3. Agricultural waste materials

Rice bran, wheat bran, maize ran, gram bran and wood husk were obtained from local market at Kolhapur, India. It was washed with distilled water, dried at $80\,^{\circ}C$ until constant weight and preserved in desiccators for further use.

2.3.1. Acid hydrolysis of rice bran

One gram of rice bran was treated with 72% H_2SO_4 and kept for half an hour with constant stirring. After acid hydrolysis, the reaction mixture was neutralized using sodium hydroxide. The reaction mixture was centrifuged at 5000 rpm for 15 min. The clear supernatant was used for the determination of total sugar and protein content [23].

2.4. Adsorption of textile dyestuff on rice bran

The adsorption of textile dyestuff and wastewater was investigated in a batch technique. Rice bran (5 g) was agitated with 100 ml solution of textile dye RNB HE2R ($200\,mg\,l^{-1}$), for 20 min. Similar process was used for mixture of textile dyes and textile effluent. The clear supernatant was collected by centrifuging medium at 7000 rpm for 15 min. The intensity of color was measured at maximum absorbance wavelength of RNB HE2R, mixture of textile dyes and textile industry wastewater using Hitachi U-2800 spectrophotometer. pH measurement was done with a Horiba pH meter (M13, Japan). The percent of adsorbed dye was calculated from the following equation.

$$\%\,\text{Adsorbed dye} = \left(\frac{A_0 - A}{A_0}\right) \times 100,$$

where A_0 is the absorbance of sample before addition of the rice bran and A is the absorbance of sample after treatment with rice bran. The optimum pH, temperature, contact time and initial RNB HE2R concentration for adsorption of RNB HE2R on rice bran was studied as demonstrated [24]. Adsorption kinetics was studied by taking RNB HE2R concentration 40, 80, 120, 160 and $200\,mg\,l^{-1}$ in 100 ml distilled water and rice bran (5 g) as adsorbent with agitation time 5, 10, 15, 20, 25 and 30 min [25]. Adsorption kinetic models studied were *pseudo*-first order kinetic [26] and *pseudo*-second order kinetic [27]. The dye adsorbed rice bran was used for further study.

2.5. Development of inoculum for decolorization experiment

Two fungal discs (8 mm diameter) of 4-day old culture were inoculated into 250 ml Erlenmeyer flasks having 100 ml of PDB (potato-dextrose broth) medium containing ($g\,l^{-1}$) peeled potatoes 200 and yeast extract 5.0 and incubated for 96 h at $30\,^{\circ}C$ under shaking condition (120 rpm). The decolorization of textile dyes using 96 h grown mycelia of *A. ochraceus* NCIM-1146 reported in our earlier work [15].

One loop full of bacterial culture was inoculated into 3 ml nutrient broth ($g\,l^{-1}$; peptone 10, sodium chloride 10 and beef extract 2) and incubated at $30\,^{\circ}C$ under static condition.

2.6. Decolorization of adsorbed textile dyestuff from textile industry wastewater under solid state fermentation

Textile dye adsorbed rice bran (5 g) was added into 250 ml Erlenmeyer flasks. These flasks were sterilized after pH adjustment (6.5–7.0). The flasks were inoculated with 3.0 ml culture of *Pseudomonas* SUK1 (0.3 O.D. at 530 nm) for bacterial decolorization study. The flasks were inoculated with 3 g mycelium of *A. ochraceus* NCIM-1146 for fungal decolorization experiment. The flasks were inoculated with 1.5 ml culture of *Pseudomonas* SUK1 (0.3 O.D. at 530 nm) and 1.5 g mycelium of *A. ochraceus* NCIM-1146, for consortium decolorization study. Similar process was used for the mixture of textile dyes and textile effluent studies.

We have also studied the decolorization of chemical precipitate of textile dye effluent (CPTDE) using consortium-PA. The pH of CPTDE was adjusted to 7.0. CPTDE (5 g) was mixed

with 5 g of rice bran in 250 ml Erlenmeyer flasks and sterilized. These flasks were inoculated with 1.5 ml culture of *Pseudomonas* SUK1 (0.3 O.D. at 530 nm) and 1.5 g mycelium of *A. ochraceus* NCIM-1146.

The moisture content of the decolorization medium was maintained between 75 and 80% using sterile distilled water. All flasks were incubated at 30 °C under static and shaking (120 rpm) condition.

2.7. Effect of various agricultural wastes on decolorization of RNB HE2R

The 5 g of RNB HE2R (200 mg l^{-1}) adsorbed on various agricultural wastes such as rice bran, wheat bran, maize bran, gram bran and wood husk was taken in 250 ml Erlenmeyer flasks separately and it was used for decolorization by consortium-PA, *Pseudomonas* SUK1 and *A. ochraceus* NCIM-1146.

2.8. Effect of increasing initial RNB HE2R concentrations on decolorization

The RNB HE2R concentrations of 200, 400, 600, 800 and 1000 mg l^{-1} were used to adsorb on 5 g rise bran and the adsorbed RNB HE2R concentrations on rice bran were 180, 208, 276, 344 and 360 mg l^{-1}, respectively. The adsorption of dye on agricultural waste for the decolorization study and the determination of amount of adsorbed dye concentration were calculated as demonstrated in [28]. The amount of dye adsorbed on rice bran was taken as initial dye concentration to study its effect on decolorization performance by consortium-PA, *Pseudomonas* SUK1 and *A. ochraceus* NCIM-1146.

2.9. Desorption of textile dyestuff from dye adsorbed on rice bran

Desorption of dye from dye adsorbed on rice bran was done using dimethyl sulphoxide (DMSO). DMSO (100 ml) was added into 250 ml Erlenmeyer flask containing 5 g of dye adsorbed rice bran and kept under shaking condition (120 rpm) for 30 min. The solution was filtered through Whatmann filter paper and then centrifuged at 5000 rpm for 10 min. The clear supernatant was used for the color measurement. The intensity of color was measured at maximum absorbance wavelength of respective individual dyes using Hitachi U-2800 spectrophotometer. Percent decolorization was calculated as follows:

$$\text{Decolorization}(\%) = \frac{\text{initial absorbance} - \text{observed absorbance}}{\text{initial absorbance}} \times 100$$

For mixture of textile dye, textile effluent and CPTDE, the % ADMI removal values were measured. The ADMI removal ratio was calculated as follows:

$$\text{ADMI removal ratio}(\%) = \frac{\text{initial ADMI}_{(0\,h)} - \text{observed ADMI}_{(t)}}{\text{initial ADMI}_{(0\,h)}} \times 100(\%),$$

where ADMI (0 h) and ADMI (t) are the initial ADMI values (at 0 h) and the ADMI value after a particular reaction time (t), respectively. The American Dye Manufacturers' Institute (ADMI 3WL) tristimulus filter method was used to measure decolorization of mixture of dyes and textile effluent [29].

All decolorization experiments were performed in three sets. Abiotic (without microorganism) controls were always included.

2.10. Enzyme extraction

15 ml of sodium phosphate buffer (20 mM, pH 7.0) was added into culture medium and then kept under shaking condition (120 rpm) for half an hour at 30 °C. The medium was filtered using muslin cloth and then centrifuged at 7000 rpm for 10 min at 30 °C. The clear supernatant was used as a source of extracellular enzymes.

2.11. Enzyme activities

All enzyme activities were assayed in the cell free extract at room temperature (25 °C). Laccase activity was determined in a reaction mixture (2.0 ml) containing 1.70 ml sodium acetate buffer (20 mM, pH 4.0) and 100 μl *o*-tolidine (1 mM stock). The reaction was started by adding 0.2 ml of enzyme solution [11]. Tyrosinase activity was determined in a reaction mixture (3.0 ml) containing 2.50 ml of sodium acetate buffer (20 mM, pH 4.0) and 100 μM of L-tyrosine. The reaction was started by adding 0.2 ml of enzyme solution and increase in absorbance was measured at 280 nm [30]. One unit of enzyme activity was defined as the amount of enzyme required for an increase in 1.0 ABS unit min^{-1} under assay condition.

Azoreductase assay mixture (2.0 ml) contained 4.45 μM of Methyl red (MR), 50 mM potassium phosphate buffer (pH 7.5) and 0.2 ml of enzyme solution. The reaction was started by adding 100 μM of NADH and then monitored for the decrease in the color absorbance at 430 nm. The molar extinction coefficient of Methyl red was 0.023 μM^{-1} cm^{-1} at 430 nm [31]. NADH-DCIP reductase assay mixture contained 25 μM DCIP, 50 mM potassium phosphate buffer (pH 7.5) and 0.2 ml of enzyme solution in a total volume of 5.0 ml. The reaction was started by adding 100 μM of NADH. The decrease in color intensity was measured at 595 nm (19 mM^{-1} cm^{-1}) [16]. Reductase activity was defined in terms of units. One unit of enzyme activity was defined as amount of enzyme required to reduce 1 μM of substrate min^{-1}.

2.12. Analysis of metabolites obtained after decolorization of RNB HE2R

The samples collected after complete decolorization of adsorbed textile dyestuff were scanned between 400 and 800 nm using Hitachi U-2800 spectrophotometer, for qualitative analysis of decolorization. After decolorization, 100 ml of distilled water was added to the decolorized medium and kept at shaking condition 120 rpm for 1 h. The medium was centrifuged at 10,000 rpm for 20 min. The supernatant obtained was used to extract metabolites with an equal volume of ethyl acetate. HPLC analysis was carried out (Waters model no. 2690) on C_8 column (symmetry, 4.6 mm × 250 mm) using isocratic method with 10 min run time. The mobile phase used was HPLC grade methanol with a flow rate 0.50 ml min^{-1}. The GC–MS analysis of metabolites were carried out using a Shimadzu 2010 MS Engine, equipped with integrated gas chromatograph with a HP1 column (60 m long, 0.25 mm id, nonpolar). Helium was used as carrier gas at a flow rate of 1 ml $min\,l^{-1}$. The inject or temperature was maintained at 280 °C with oven conditions as: 80 °C kept constant for 2 min – increased up to 200 °C with 10 °C $min\,l^{-1}$ raised up to 280 °C with 20 °C $min\,l^{-1}$ rate. The compounds were identified on the basis of mass spectra and using the NIST library.

2.13. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparison test.

Table 1
Decolorization of adsorbed RNB HE2R (200 mg l^{-1}), mixture of textile dyes (each concentration 20 mg l^{-1}), textile industry wastewater and CPTDE by consortium-PA, *Pseudomonas* sp. SUK1 and *Aspergillus ochraceus* NCIM-1146.

Adsorbed dye on rice bran	λ max	Adsorption (%)	Decolorization (%) by consortium-PA		Decolorization (%) by *Pseudomonas* sp. SUK1		Decolorization (%) by *Aspergillus ochraceus* NCIM-1146		Time (h)
			Static	Shaking	Static	Shaking	Static	Shaking	
RNB HE2R[a]	610	90 ± 02	44 ± 03	80 ± 02	33 ± 03	62 ± 02	20 ± 02	38 ± 03	24
Mixture of textile dyes[b]	510	62 ± 03	63 ± 02	82 ± 03	49 ± 02	71 ± 03	30 ± 02	32 ± 02	36
Textile industry wastewater[b]	510	80 ± 03	55 ± 03	77 ± 03	48 ± 03	55 ± 02	34 ± 03	39 ± 02	36
CPTDE[b]	670	–	74 ± 02	86 ± 02	68 ± 02	77 ± 02	68 ± 02	68 ± 02	96

CPTDE, chemical precipitate of textile dye effluent + rice bran (1:1). Values are mean of three experiments ± SEM.
[a] % Decolorization.
[b] % ADMI removal ratio.

3. Results and discussion

3.1. Adsorption of textile dyestuff on rice bran

The rice bran showed 90, 64 and 80% adsorption for textile dye RNB HE2R, mixture of textile dyes and textile industry wastewater, respectively (Table 1). Rice bran is a cheap adsorbent for the removal of textile dyes [22]. The adsorption of navy blue HE2R on rice bran showed optimum adsorption at pH (6.0) (Fig. 1a) and temperature (50 °C) (Fig. 1b). The rate of adsorption increased with increase in initial RNB HE2R dye concentrations 40, 80, 120, 160 and 200 mg l^{-1}, but at the concentration of 240 mg l^{-1} the rate of adsorption started decreasing (Fig. 1c). The adsorption reached to equilibrium (maximum dye adsorbed) after 10 min of contact time (Fig. 1d).

To investigate possible kinetics mechanism of adsorption for initial dye concentrations of (40, 80, 120, 160 and 200 mg l^{-1}) on 5 g of rice bran with agitation time (5, 10, 15, 20, 25 and 30 min) (Fig. 2), the pseudo-first order and pseudo-second-order kinetic models were studied. The rate constants of adsorption were determined from first order rate expression given by [25].

$$\log(q_e - q) = \frac{\log q_e - k_1 t}{2.303}, \quad (1)$$

where q_e and q are amount of dye adsorbed (mg/g) at equilibrium and at time t (min), respectively, and k_1 is the rate constant of adsorption (min^{-1}). The values of k_1 calculated from plots of $\log(q_e - q)$ vs. t (fig. not shown) for different dye concentrations. The experimental q_e values shows very low correlation coefficient (R^2) with the calculated ones, obtained from linear plots (Table 2) it suggests that adsorption of RNB HE2R on rice bran does not follow pseudo-first-order kinetics.

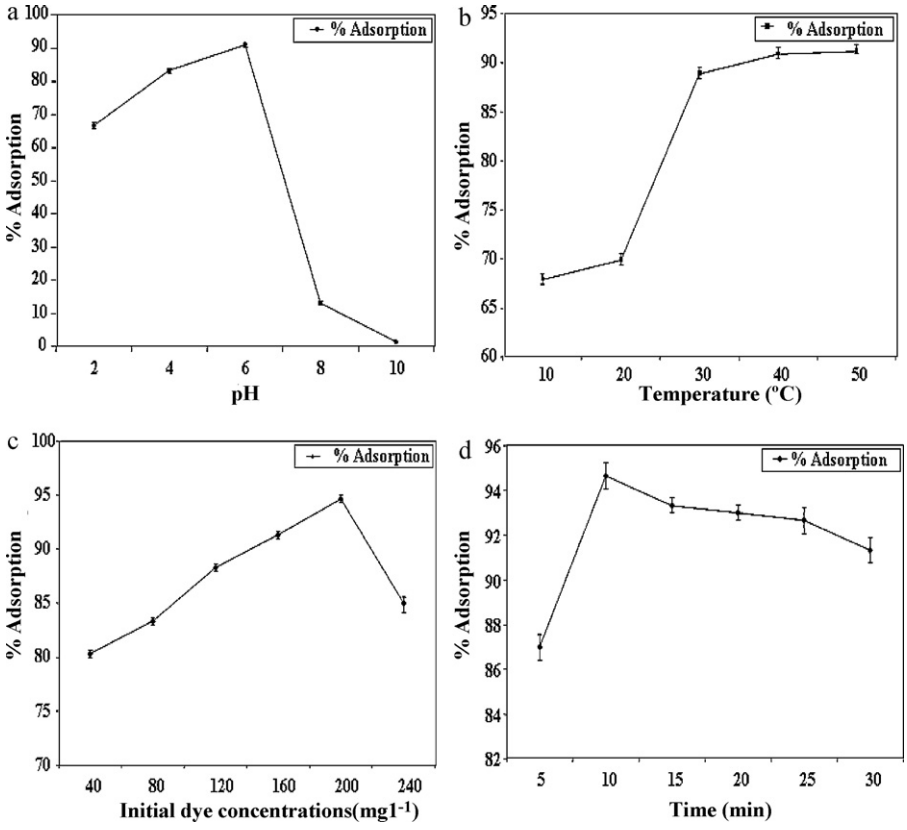

Fig. 1. (a) Effect of pH on adsorption of RNB HE2R on rice bran (initial dye concentration 200 mg l^{-1}, rice bran 5 g, temperature 30 °C and contact time 20 min); (b) effect of temperature on adsorption of RNB HE2R on rice bran (initial dye concentration 200 mg l^{-1}, rice bran 5 g, pH 5 and contact time 20 min); (c) effect of initial dye concentrations (40, 80, 120, 160 and 200 mg l^{-1}) on adsorption of RNB HE2R on rice bran (rice bran 5 g, temperature 30 °C, pH 5 and contact time 20 min); and (d) effect of contact time on adsorption of RNB HE2R on rice bran (initial dye concentration 200 mg l^{-1}, rice bran 5 g, temperature 30 °C and pH 5).

Table 2
Kinetic parameters of increasing initial RNB HE2R concentrations 40, 80, 120, 160 and 200 mg l^{-1} on 5 g of rice bran at shaking condition (120 rpm).

Initial dye concentrations (mg l^{-1})	$q_{e,exp}$ (mg g^{-1})	Pseudo-first-order kinetic model			Pseudo-second-order kinetic model		
		k_1 (min^{-1})	$q_{e,cal}$ (mg g^{-1})	R^2	k_2 (g mg^{-1} min^{-1})	$q_{e,cal}$ (mg g^{-1})	R^2
40	0.656	2.467	0.1034	0.832	0.053	0.607	0.999
80	1.47	3.109	0.1074	0.746	0.128	1.39	0.999
120	2.23	1.668	0.1126	0.850	0.040	2.26	0.995
160	2.97	1.305	0.1118	0.830	0.570	2.75	0.997
200	3.80	1.426	0.1106	0.868	0.654	3.52	0.997

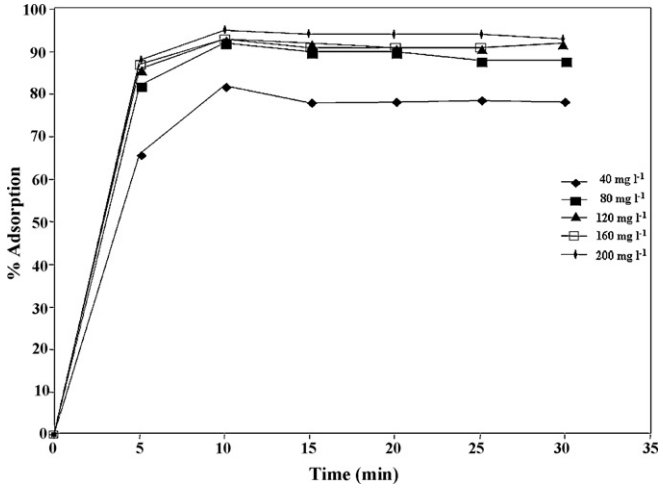

Fig. 2. Kinetics for adsorption of initial dye concentrations of (40, 80, 120, 160 and 200 mg l^{-1}) on 5 g of rice bran with agitation time (5, 10, 15, 20, 25 and 30 min).

The second order kinetics model expressed as [26]

$$\frac{t}{q} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (2)$$

where k_2 (g mg^{-1} min^{-1}) is the second order rate constant, k_2 and $q_{e,cal}$ were calculated from intercept and slopes of the plots of t/q vs. t (Fig. 3). If pseudo-second order plots are applicable, the plots of t/q vs. t should show linear relationship [32]. The experimental q_e values shows very high with correlation coefficient (R^2) with the calculated ones; obtained from linear plots (Table 2). This indicates that given adsorption mechanism was pseudo second-order.

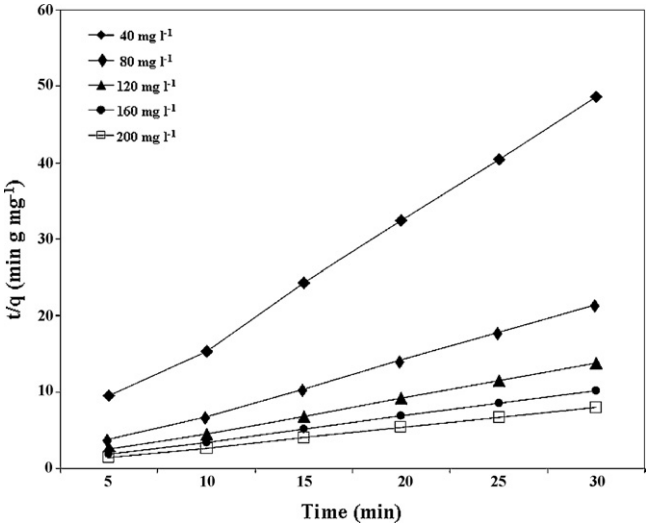

Fig. 3. Plots of pseudo second-order kinetic model of different initial dye concentrations (40, 80, 120, 160 and 200 mg l^{-1}) on 5 g of rice bran.

Similar results were obtained with adsorption of dye on coir pith [32].

3.2. Decolorization of adsorbed textile dyestuff under solid state fermentation

One gram of rice bran contained 183 mg of total sugars and 160 mg of protein content. Rice bran is low cost carbon and nitrogen rich medium for growth of microorganisms and production of industrial enzymes under solid state condition [33]. The initial dye concentration was 200 mg l^{-1} after adsorption 20 mg l^{-1} dye was retained in the solution, hence the dye adsorbed on 5 g rice bran was given as 180 mg l^{-1} as demonstrated in [28]. *Pseudomonas* sp. SUK1, *A. ochraceus* NCIM-1146 and consortium-PA showed 62, 38 and 80% decolorization of RNB HE2R adsorbed on rice bran under shaking condition (120 rpm) respectively, within 24 h (Table 1). This suggests higher efficiency of the consortium-PA compared with individual microorganisms. Similar results were also reported in case of crude oil degradation using consortium of zymogenous bacteria and fungi [34].

Rice bran showed 62 and 80% adsorption of dyes from mixture of textile dyes and textile industry wastewater, respectively (Table 1). The mixture of textile dyes, textile industry wastewater, CPTDE had the initial ADMI values as, 1643, 1723 and 2718 after the decolorization by consortium-PA the ADMI values were found to be 282, 382 and 359, respectively hence, they showed 82, 77 and 86% ADMI removal ratio at shaking condition after 36 h, 36 h and 96 h, respectively (Table 1). While, the consortium-PA showed 63, 55 and 74% ADMI removal ratio mixture of textile dyes, textile industry wastewater, CPTDE at static condition in 36 h, 36 h and 96 h, respectively (Table 1). The consortium-PA had better decolorization performance than the individual strains *Pseudomonas* sp. SUK1 and *A. ochraceus* NCIM-1146 at shaking and static condition (Table 1). It was observed that the consortium-PA showed better performance for the decolorization of various textile dyes adsorbed on rice bran than the individual *Pseudomonas* sp. SUK1 and *A. ochraceus* NCIM-1146 strains at shaking condition (120 rpm) within 48 h (Table 3). The above results indicate that the consortium-PA has more potential for the decolorization of textile dyestuff, textile wastewater and CPTDE than individual microorganisms. Chemical precipitation is commonly used technique for textile wastewater treatment. This suggests that the consortium-PA could be efficient tool for designing ecofriendly biological method for the degradation of various adsorbed textile dyes and textile wastewater.

3.3. Effect of various agricultural wastes on decolorization of RNB HE2R

Various agricultural wastes show different adsorption capacities while rice bran shows maximum 90% adsorption compared to other agricultural wastes (Table 4). The consortium-PA shows 92, 52, 60 and 28% decolorization of RNB HE2R adsorbed rice bran, wheat bran, maize bran and gram bran, respectively. While, wood husk could not showed decolorization by consor-

Table 3
Decolorization of adsorbed various textile dyes (200 mg l^{-1} each) on 5 g of rice bran by consortium-PA, *Pseudomonas* sp. SUK1 and *Aspergillus ochraceus* NCIM-114 in 48 h at shaking condition.

Dye name	λ max	Adsorption (%)	Dyes adsorbed (mg l^{-1})	Decolorization (%) by consortium-PA	Decolorization (%) by *Pseudomonas* sp. SUK1	Decolorization (%) by *Aspergillus ochraceus* NCIM-1146
Remazol red B	520	68 ± 02	136 ± 02	79 ± 01	53 ± 02	25 ± 01
Scarlet RR	604	50 ± 02	100 ± 02	73 ± 02	47 ± 01	29 ± 02
Green HE4B	510	54 ± 02	108 ± 02	59 ± 01	37 ± 01	26 ± 01
Red M5B	600	72 ± 03	144 ± 03	59 ± 02	45 ± 01	41 ± 01
Navy Blue HER	506	67 ± 01	134 ± 01	53 ± 02	42 ± 02	40 ± 02
Blue 2RNL	510	70 ± 02	140 ± 01	50 ± 03	26 ± 02	21 ± 01
Direct Red 5B	560	76 ± 01	152 ± 02	43 ± 01	22 ± 02	27 ± 01
Remazol Red	520	66 ± 02	132 ± 02	46 ± 01	30 ± 01	25 ± 02
Direct Navy Blue 3G	580	40 ± 02	80 ± 02	32 ± 02	25 ± 02	21 ± 02

Values are mean of three experiments ± SEM.

Table 4
Effect of various agriculture wastes (5 g each) on decolorization of adsorbed RNB HE2R (200 mg l^{-1}) by consortium-PA, *Pseudomonas* sp. SUK1 and *Aspergillus ochraceus* NCIM-114 in 72 h.

Various agricultural wastes	Adsorption (%)	RNB HE2R adsorbed (mg l^{-1})	Decolorization (%) by consortium-PA	Decolorization (%) by *Pseudomonas* sp. SUK1	Decolorization (%) by *Aspergillus ochraceus* NCIM-1146
Rice bran	90 ± 02	180 ± 02	92 ± 03	78 ± 02	61 ± 02
Wheat bran	70 ± 02	140 ± 02	52 ± 01	40 ± 02	42 ± 03
Maize bran	67 ± 01	134 ± 01	60 ± 02	40 ± 01	35 ± 02
Gram bran	29 ± 01	58 ± 01	28 ± 01	20 ± 02	09 ± 03
Wood husk	17 ± 03	34 ± 03	ND	ND	ND

ND, no decolorization. Values are mean of three experiments ± SEM.

tium as well as individual microorganisms (Table 4). This is due to less nutrient content in wood husk. The *Pseudomonas* SUK1 and *A. ochraceus* NCIM-1146 shows lesser decolorization of RNB HE2R adsorbed on agricultural wastes than consortium-PA (Table 4).

3.4. Effect of increasing initial dye concentration on decolorization of RNB HE2R

The adsorption rate of RNB HE2R on rice bran was decreased with increase in dye concentrations 200, 400, 600, 800 and 1000 mg l^{-1} (Table 5). With increase in initial RNB HE2R concentrations, the decolorization rate decreased by consortium-PA, *Pseudomonas* SUK1 and *A. ochraceus* NCIM-1146 (Table 5). However, the consortium-PA shows 58 and 48% decolorization of adsorbed RNB HE2R at 800 and 1000 mg l^{-1} concentrations in 72 h. While, *Pseudomonas* SUK1 and *A. ochraceus* NCIM-1146 do not showed decolorization at these concentrations (Table 5). These results suggest that consortium-PA has the ability to degrade dyes at higher concentration than individual microorganisms.

3.5. Enzyme activities

Significant induced levels of extracellular laccase (629%), NADH-DCIP reductase (441%) and azoreductase (148%) caused 62% decolorization of reactive navy blue HE2R in case of *Pseudomonas* sp. SUK1 (Table 6). When *A. ochraceus* NCIM-1146 was used alone showed significant induction of laccase (453%) and tyrosinase (122%) suggesting its role in 38% decolorization of RNB HE2R. However, consortium-PA showed significant induced levels of azoreductase (207%), tyrosinase (384%), NADH-DCIP reductase (690%) and presence of laccase suggesting their role in 80% decolorization of reactive RNB HE2R (Table 6). These results explain the synergic action of these enzymes in consortium-PA to enhance % degradation. Induction of decolorizing enzymes from bacterial and fungal cultures in these consortium leads to enhanced decolorization of azo dye [35].

3.6. Analysis of metabolites obtained after decolorization of adsorbed textile dyestuff

RNB HE2R showed maximum absorbance at 610 nm. The sample obtained after decolorization showed less absorbance suggesting its decolorization (Fig. 4). HPLC spectrum of RNB HE2R showed the peaks at retention time 2.126, 2.667, and 5.190 min (Fig. 5a) and the metabolites obtained after its degradation by: *Pseudomonas* sp. SUK1 showed the peaks at retention time 2.819, 2.91, 3.07, 3.443, 3.917 and 5.317 min (Fig. 5b), *A. ochraceus* NCIM-1146 showed the peaks at retention time 2.855, 3.478 and 5.274 min (Fig. 5c) and consortium-PA showed the peaks at retention time 2.827, 3.227 and 3.650 min (Fig. 5d). Thus, the difference in the retention times of control dye RNB HE2R and metabolites formed after its degradation by *Pseudomonas* sp. SUK1, *A. ochraceus* NCIM-1146 and consortium-PA confirms the biodegradation of RNB HE2R

Table 5
Effect of increasing RNB HE2R concentrations (200, 400, 600, 800 and 1000 mg l^{-1}) on decolorization of RNB HE2R by consortium-PA, *Pseudomonas* sp. SUK1 and *Aspergillus ochraceus* NCIM-114 in 72 h.

RNB HE2R concentrations (mg l^{-1})	Adsorption (%)	RNB HE2R adsorbed (mg l^{-1})	Decolorization (%) by consortium-PA	Decolorization (%) by *Pseudomonas* sp. SUK1	Decolorization (%) by *Aspergillus ochraceus* NCIM-1146
200	90 ± 02	180 ± 02	92 ± 01	78 ± 02	61 ± 02
400	52 ± 02	208 ± 02	70 ± 02	55 ± 01	36 ± 02
600	46 ± 01	276 ± 01	61 ± 01	48 ± 02	33 ± 02
800	43 ± 02	344 ± 02	56 ± 02	ND	ND
1000	36 ± 03	360 ± 03	48 ± 03	ND	ND

ND, no decolorization. Values are mean of three experiments ± SEM.

Table 6
Extracellular enzyme status during decolorization of RNB HE2R adsorbed on rice bran by *Pseudomonas* sp. SUK1, *Aspergillus ochraceus* NCIM-1146 and consortium-PA and % decolorization.

Enzymes	Enzyme activity					
	Pseudomonas sp. SUK1		*Aspergillus ochraceus* NCIM-1146		Consortium-PA	
	Control	Test	Control	Test	Control	Test
Laccase	0.37 ± 0.07	2.33 ± 0.90*	1.62 ± 0.50	7.35 ± 0.80*	3.19 ± 0.80	0.34 ± 0.08*
Azoreductase	0.867 ± 0.214	1.270 ± 0.127	ND	ND	1.22 ± 0.331	2.533 ± 0.629*
Tyrosinase	ND	ND	0.95 ± 0.90	1.16 ± 0.70	0.90 ± 0.09	3.46 ± 0.50*
NADH-DCIP reductase	116 ± 07	512 ± 08*	ND	ND	091 ± 08	628 ± 09*
% decolorization of RNB HE2R		62		38		80

Enzyme activity: U ml^{-1} of culture min^{-1}; Control = enzyme extracted from culture medium without dye after 24 h; Test = Enzyme extracted from dye decolorized culture medium after 24 h; ND = not detected.
Values are mean of three experiments ± SEM, significantly different from control cells at $^{*}P < 0.001$ by one way analysis of variance (ANOVA) with Tukey–Kramer multiple comparison test.

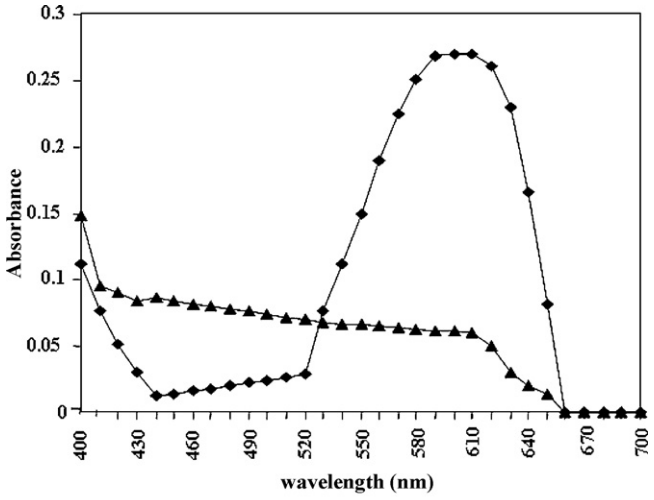

Fig. 4. Spectrophotometric analysis of decolorization of RNB HE2R by consortium-PA.

into different metabolites. We have proposed pathways for the degradation of RNB HE2R by *Pseudomonas* sp. SUK1, *A. ochraceus* NCIM-1146 and consortium-PA on the basis of GC–MS analysis. In case of, *Pseudomonas* sp. SUK1 azoreductase cleavage produced [4-(ethylsulphonyl)] aniline [R_t 23.01 min, Mw (*m*/*z*), 183 (M-2)] [A] and laccase caused asymmetric cleavage to form N-benzylacetamide [R_t 20.17 min, Mw (*m*/*z*), 149 (M)] [B] along with one unidentified compound (Fig. 6a). In case of *A. ochraceus* NCIM-1146 laccase degraded RNB HE2R using asymmetric cleavage to form [A] [4-(ethenylsulphonyl) phenyl]diazine [R_t 27.61 min, Mw (*m*/*z*), 196 (M+1)] and [B] N-benzylacetamide [R_t 20.19 min, Mw (*m*/*z*), 149 (M)] along with one unidentified compound (Fig. 6b). In case of consortium-PA azoreductase and laccase degrade RNB HE2R to form [A] N-benzylacetamide [R_t 20.18 min, Mw (*m*/*z*), 149 (M)], [I] and [II] unidentified compounds. Further degradation of [A] gave product [B] N-hydroxy-1-phenylmethanamine [R_t 5.96 min, Mw (*m*/*z*), 123 (M-3)]. Postulated unidentified compound [I] further degraded to form [C] aniline [R_t 6.33 min, Mw (*m*/*z*), 91(M+2)] (Fig. 6c). Thus, the above proposed degradation pathways suggest degrading enzyme role in the specific cleavage and advantage of

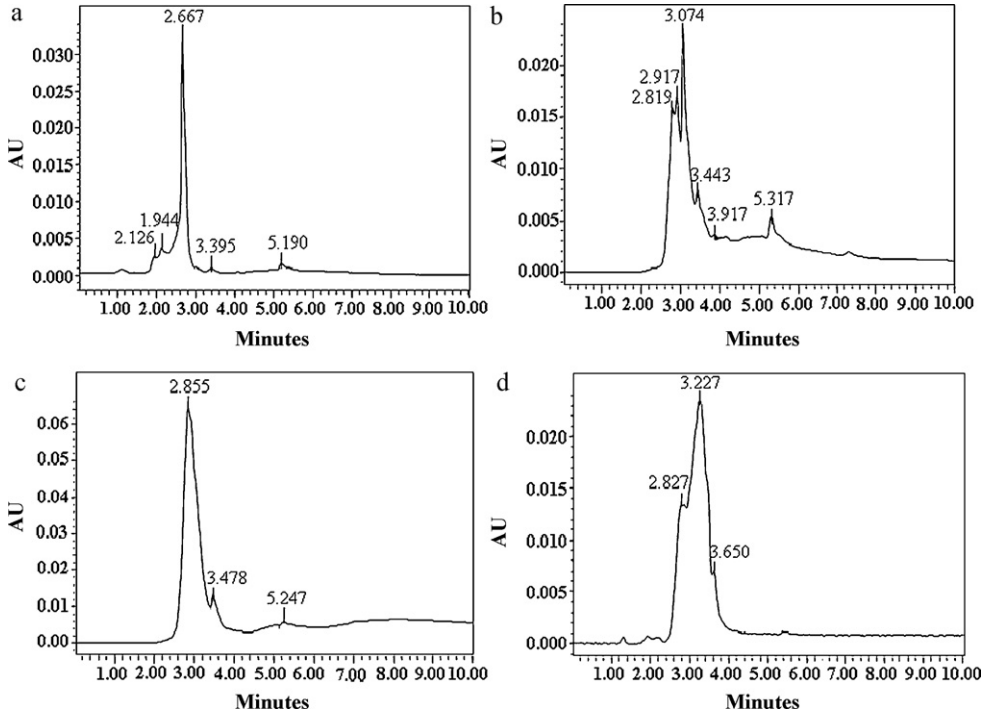

Fig. 5. HPLC pattern of (a) dye RNB HE2R before degradation, (b) metabolites obtained after degradation of RNB HE2R by *Pseudomonas* sp. SUK1, (c) metabolites obtained after degradation of RNB HE2R by *Aspergillus ochraceus* NCIM-1146, (d) metabolites obtained after degradation of RNB HE2R by consortium-PA.

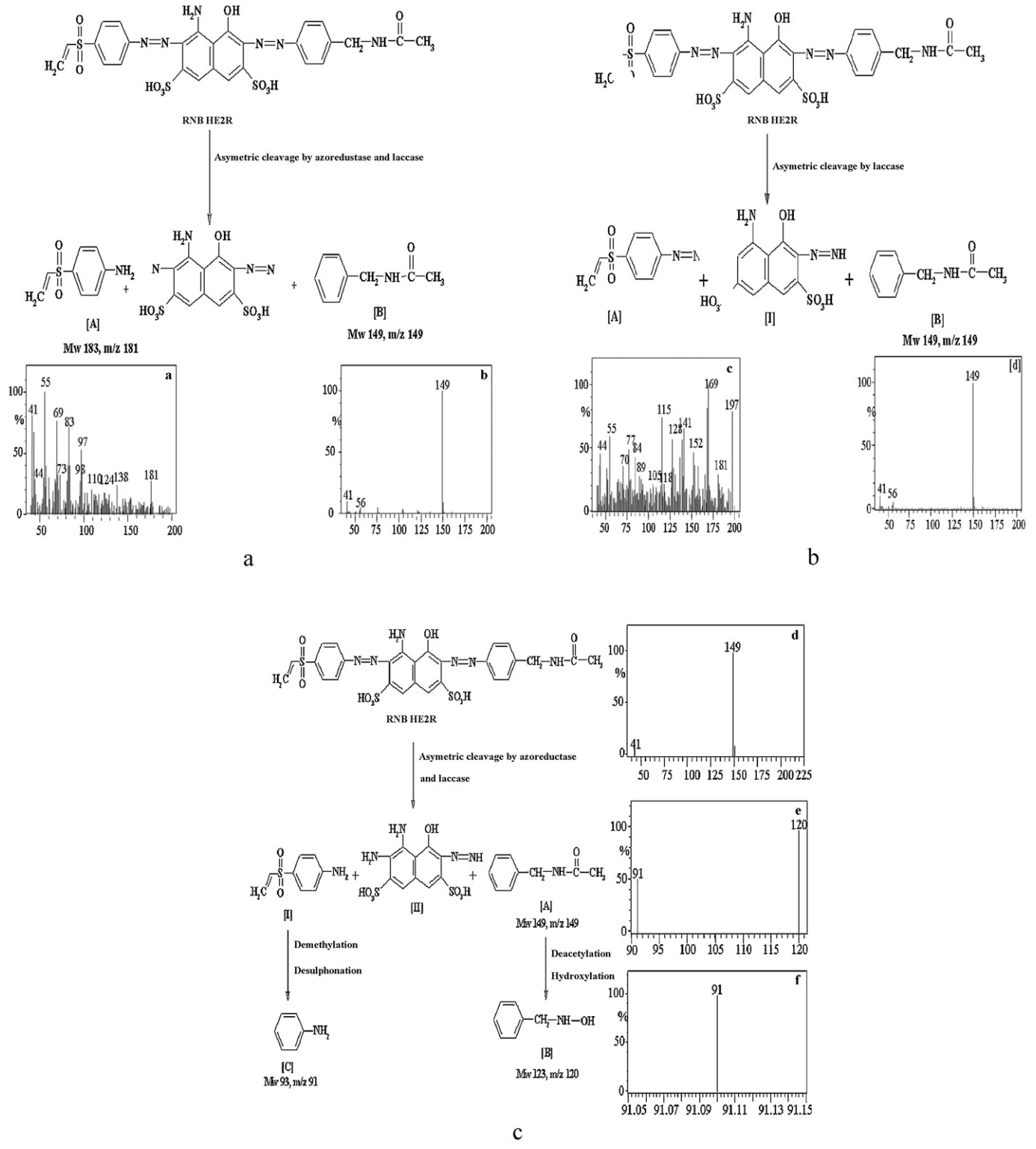

Fig. 6. (a) Proposed pathway for degradation of RNB HE2R by *Pseudomonas* sp. SUK1, [A] [4-(ethylsulphonyl)]aniline [a] GC–MS spectra for [4(ethylsulphonyl)]aniline, [I] unidentified compound and [B] N-benzylacetamide [b] GC–MS spectra for N-benzylacetamide. (b) Proposed pathway for degradation of RNB HE2R by *Aspergillus ochraceus* NCIM-1146, [A] [4-(ethenylsulphonyl)phenyl]diazine [c] GC–MS spectra for [4-(ethenylsulphonyl)phenyl]diazine, [I] unidentified compound and [B] N-benzylacetamide [d] GC–MS spectra for N-benzylacetamide. (c) Proposed pathway for degradation of RNB HE2R by consortium-PA, [A] N-benzylacetamide [d] GC–MS spectra for N-benzylacetamide, [I] and [II] unidentified postulated compound, [B] N-hydroxyl-1-phenylmethanamine [e] GC–MS spectra for N-hydroxyl-1-phenylmethanamine, [C] aniline [f] GC–MS spectra for analine.

combination of enzymes in mineralization of textile dyes. Azo dyes can be cleaved symmetrically and asymmetrically depending on the availability of enzymes [36].

4. Conclusion

Pseudomonas sp. SUK1 and *A. ochraceus* NCIM-1146 were able to degrade dye adsorbed on rice bran under solid state fermentation; however consortium-PA was more efficient than individual microorganisms. Consortium-PA had potential to decolorize CPTDE (highly recalcitrant) when added with rice bran for their growth. Degradation of adsorbed dyes on rice bran could be strongly attributable to the synergistic effect of excreted extracellular enzymes such as, azoreductase, laccase, tyrosinase and NADH-DCIP reductase secreted by the fungi and bacteria. This approach will be effective to reduce effluent volume by adsorbing textile dye on agricultural waste residues available at low cost and its bioremediation/disposal to nontoxic by solid state fermentation.

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