Research Article

# Combined biological and advanced oxidation process for decolorization of textile dyes

**Muruganandham Thanavel[1] · Suhas K. Kadam[2] · Shivtej P. Biradar[2] · Sanjay P. Govindwar[3] · Byong-Hun Jeon[3] · Senthil Kumar Sadasivam[4]**



**Abstract**

The present study evaluated the efficiency of combined biological and AOPs treatment (Bio–AOP) using *Aeromonas hydrophila* SK16 and AOPs-$H_2O_2$ (4%) for the remediation of the textile dyes. Bio–AOP treatment showed 100% decolorization of Reactive Red 180 (RR 180), Reactive Black 5 (RB 5) and Remazol Red (RR), while 72% decolorization was observed in individual treatments. Combined treatment significantly reduced BOD and COD of RR 180–78 and 68%, RB 5–52 and 83% and RR—42 and 47%, respectively as compare to individual treatment. Significant increased levels of tyrosinase, laccase, lignin peroxidase, riboflavin reductase and azoreductase were observed in *A. hydrophila* SK16. Fourier-transform infrared spectroscopy and high-performance liquid chromatography analysis showed noteworthy biotransformation of textile dyes. Possible metabolic pathway of degradation of dyes were predicted based on GC–MS analysis. This study indicates that the Bio–AOP treatment is more efficient than an individual treatment of textile wastewater.

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✉ Muruganandham Thanavel, murugannct@gmail.com | [1]Post Graduate and Research Department of Biotechnology, National College (Autonomous), Dindigul Road, Tiruchirappalli, Tamil Nadu 620 001, India. [2]Department of Biochemistry, Shivaji University, Kolhapur, Maharashtra 416 004, India. [3]Department of Earth Resources and Environmental Engineering, Hanyang University, Seoul 04763, South Korea. [4]Post Graduate and Research Department of Botany, National College (Autonomous), Dindigul Road, Tiruchirappalli, Tamil Nadu 620 001, India.

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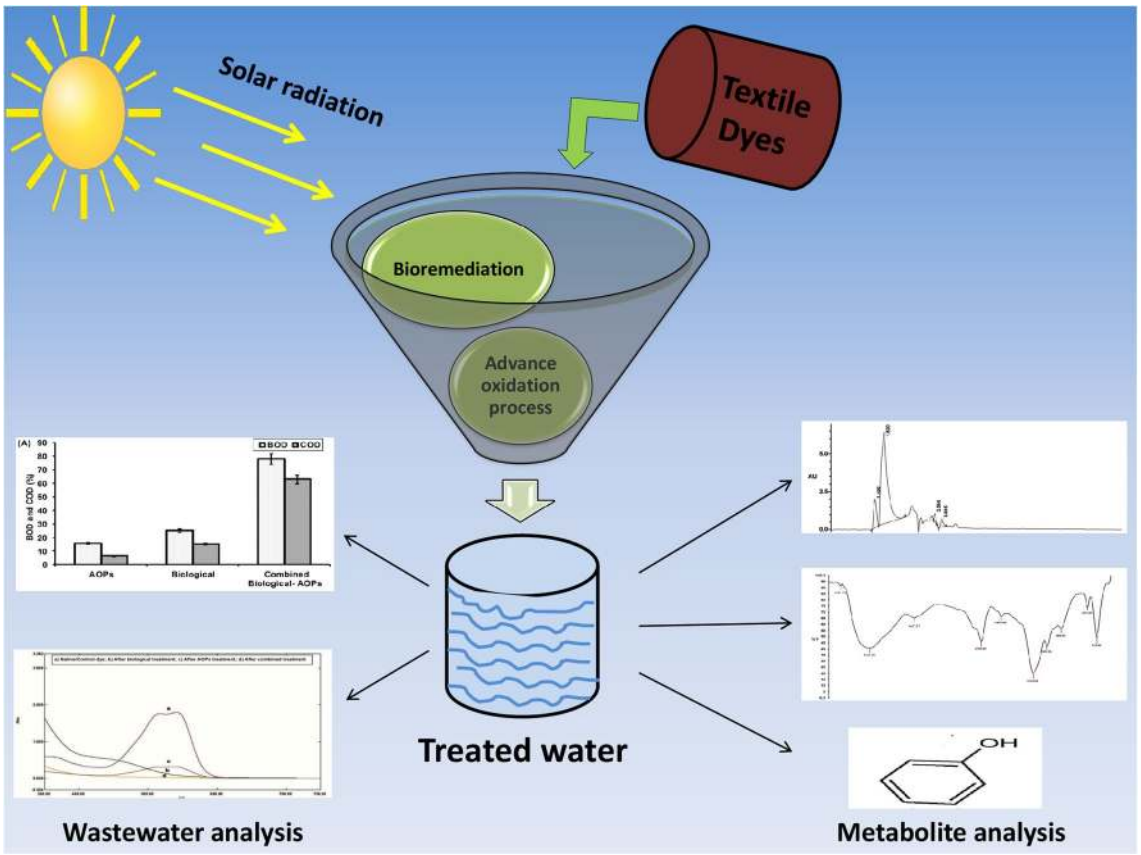
**Graphical abstract**




**Keywords** Reactive dyes · *Aeromonas hydrophila* SK16 · Decolorization · Desulfonation · Biological–AOPs · $H_2O_2$

## 1 Introduction

Textile industries are using structurally different varieties of reactive dyes for accomplishment of many shades of color, stronghold profiles, comfort to use, less energy utilization and excellent colors. The more commonly used dyes are anthraquinone, azo dyes (N=N) and phthalocyanine. These coloring agents are become serious threats to all life form as they are carcinogenic and mutagenic in nature [28]. More than 100,000 commercial dyes are available in market whose annual production is more than one million tons. Out of all the dyes used for dying and printing, around 20% of dyes are being discharged into the environmental sink because of its low level of affinity with the fabrics [20]. The aromatic amines produced after Azo dyes biotransformation has mutagenic and carcinogenic effects [12]. The discharge of these effluent containing dyes into the water resources results in alteration of pH, reduction in light penetration and gas solubility, as well as raise in the COD, BOD, TOC of water resources that causes inimical effects on all life form including animals and plants [34, 53]. So, the management of textile effluent is inevitable before final discharge to the ecosystem.

Many studies have been conducted on the consequence of biological, chemical and physical treatments of textile wastewater. Treatment of textile wastewater with already existing physicochemical methods is inefficient due to its inability to reduce COD, TOC, BOD, color, pH and metals. These techniques are costly and generate huge quantity of toxicants and sludge [7]. Textile wastewater treatment using biological method is an ecofriendly and low cost treatment methods [5]. Many biological agents such as bacteria, yeasts, fungi, algae and actimonycetes are capable of degrading azo dyes, among which bacterial cells represent an inexpensive and promising tool for the removal of different azo dyes from textile effuluent [42]. Different taxanomic group of bacteria repored for dye degradation [6] (*Aeromonas hydrophila*); [54] (*Bacillus cereus*); [17] (*Halomonas sp.*) and [18] (*Serratia liquefaciens*). All biological treatments involve use of biocatalysts to oxidize

recalcitrant pollutants. But, they have some drawbacks like low biodegradability [50] and no efficient removal of refractory compounds [44].

AOPs are set of chemical treatment planned to remove organic and some inorganic waste from water by hydroxyl radical (·OH) oxidation [32]. The ·OH production from $H_2O_2$ catalyzes with the help of UV light by hemolytic O–O bond cleavage. The most current developments in AOPs is the use of solar radiation, as UV photon source, decreasing artificial energy requirement for the application of these methods [51]. These reactive species (·OH) are potent oxidant can react with any kind of pollutant dissolved in water matrix. Initially they attack on larger molecules to breakdown into fragments then subsequently attack until the ultimate mineralization.

$$H_2O_2 + UV \rightarrow 2{\cdot}OH$$

At an initial stage, AOPs aimed to improve biological treatment [8]. In the later stage, the oxidation process is used for the removal of those contaminants which are not completely degraded in the biological treatment process [39]. The prebiological treatment responsible for breakdown of strong bonds like azo and utilize biodegradable compounds. Later advance oxidation treatment deal with toxic aromatic amines as well as other recalcitrant metabolites produced by bioremediation process [35]. In addition to these the use of AOPs as pretreatment could results stable intermediates formation that are more toxic to biological system as well as less biodegradable than the original molecule [33]. Moreover, high quantity use of oxidants used for oxidation also lead to creation of condition that are toxic to microorganisms [21]. Pretreatment of AOPs is become expensive as they required lots of chemicals, therefore it was suggested that prebiological treated wastewater is beneficial to use for AOPs treatment [1, 40, 43]. Other oxidation techniques like Fenton oxidation process and sonolysis are also available. But, sonolysis require high energy inputs and use of Fenton oxidation technique combined with biological process is limited due to low pH 2 and 3. At the same condition use of UV/$H_2O_2$ is best option instead of these techniques for wastewater treatment [52].

The combinatorial approach executed as bioremediation couple with advance oxidation process with *Aeromonas hydrophila* SK16 was thought to have additional benefit of faster treatment efficiency. Both bioremediation and AOP involve oxidation process to degrade waste. The several studies have been reported about successive oxidative treatment with chemicals and organisms [45]. The pilot scale wastewater treatment by Photo-Fenton oxidation coupled with bioremediation showed 94% mineralization of effluent as well as found to reduced 96% toxicity [55]. The study carried out by Mandal et al. [31] showed combined AOP and biological treatment help out in reduction of COD and BOD. Several studies have been reported about successive oxidative treatment with chemicals and organisms [15]. These reports are precisely on the use of combination of AOPs and biological treatment for textile wastewater management.

In present study, we have focused on an evaluation of efficiency of combined biological (*Aeromonas hydrophila* SK16 bioremediation) and AOPs treatment using $H_2O_2$ for treating various textile dyes. This combined biological and AOPs approach is useful for removing contaminant from environment and reducing health associated problems. This combined process is easily adaptable in large scale for significant decontamination.

## 2 Materials and methods

### 2.1 Chemicals and dyes

The dyes Remazol Red (RR), Reactive Red 180 (RR 180) and Reactive Black 5 (RB 5) (Table 1) were obtained from Jamara Textile Industry SIPCOT Perundurai, Tamil Nadu, India. Decolorization studies were performed in Nutrient broth (Himedia, India). Hydrogen peroxide was procured from Merck, India. All reagents and chemicals were highly pure.

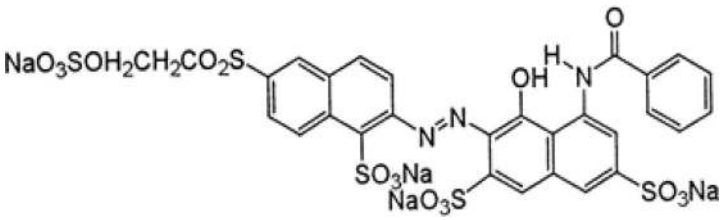
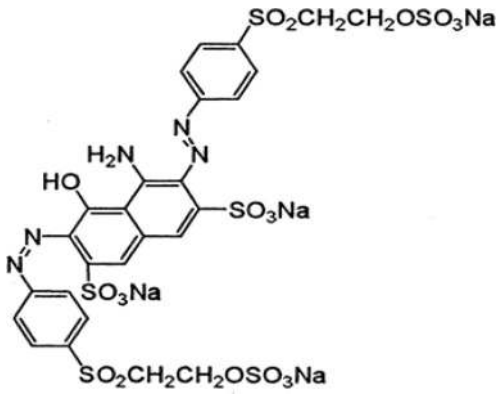
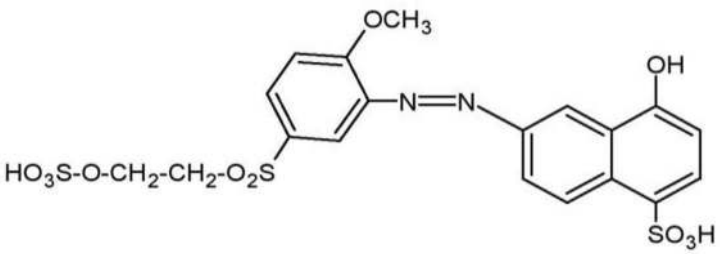
### 2.2 Culture condition and decolorization studies

Bacterial strain *A. hydrophila* SK16 was isolated earlier from the textile dye polluted soil [26]. The pure strain was maintained at 4 °C on nutrient agar. *A. hydrophila* SK16 was revived and used for the present study after examining its purity.

Decolorization studies of RR 180, RB 5 and RR were carried out in 100 ml nutrient broth (Beef extract—1.5 g, Yeast extract—1.5 g, Peptone—5 g and NaCl—5 g for 1 L). The concentration of 100 mg $L^{-1}$ dyes was added in log phase culture after acquiring the bacterial count at $10^4$–$10^5$ CFU. The experiments were executed in both static and shaking states at 37 °C. The $H_2$ ion concentration of the pregrown culture was optimized and adjusted to 8. Aliquot (5 ml) was withdrawn at every 2 h intervals, centrifuged (10,000×*g*, 15 min) and color removal was determined by computing $\lambda_{max}$ of the dyes. Abiotic control (without microorganisms) was kept along with every sets. All tests were executed in three sets and % decolorization was determined using following Eq. 1,

$$Decolorization(\%) = \frac{Initial\ absorbance - Final\ absorbance}{Initial\ absorbance} \times 100 \quad (1)$$

**Table 1** Structure of three dyes used for decolorization by *Aeromonas hydrophila* SK16

| Sr. no. | Name of the dye | Structure | Wavelength (nm) |
|---|---|---|---|
| 1 | Reactive Red 180 |  | 520 |
| 2 | Reactive Black 5 |  | 570 |
| 3 | Remazol Red RR |  | 520 |

## 2.3 Advanced oxidation process

The advanced oxidation process was performed in 250 ml conical flask containing dye (100 mg $L^{-1}$) and $H_2O_2$ (4%, based on the optimization from 1 to 10%). The flask was irradiated under solar light for 6 h. The same procedure was followed for dark conditions. Control was used without $H_2O_2$. The light intensity was measured by using Lux Meter (Lutron LX-101, Taiwan).

## 2.4 Combined biological and advanced oxidation process

The biomass was removed after biodegradation with the help of centrifugation. The remaining dye solution was exposed to AOPs treatment using 4% $H_2O_2$ (based on the optimization from 1 to 10%). The sample was exposed under sunlight for 3–6 h and then analyzed using UV–Vis Spectrophotometer. The intensity of light was measured using Lux Meter (Lutron LX-101, Taiwan).

## 2.5 BOD and COD determination of dye solution

BOD-Biological oxygen demand and COD-Chemical oxygen demand were determined using standard procedures according to APHA [3].

## 2.6 Sample preparation for enzyme analysis

*Aeromonas hydrophila* SK16 was grown in 100 ml nutrient medium for 24 h at 37 °C. The centrifuged (10,000 rpm, 15 min) pellet was resuspended in potassium phosphate

buffer for sonication (40 A, 8 strokes of 40 s using sonics-vibracell). The prepared sample was used as source of enzyme. The same method of enzyme preparation was carried out for control as well as treated cell (dye exposed).

## 2.7 Enzyme studies

The assays of enzyme lignin peroxidase, tyrosinase, riboflavin reductase, NADH-DCIP reductase, azoreductase and laccase were carried out spectrophotometrically using crude source of enzyme. Lignin peroxidase assay was performed in a total 2.5 ml volume comprising tartaric acid (250 mM) and n-propanol (100 mM). The propanaldehyde production was estimated at 300 nm as reported by [24]. The assay of laccase was carried out in a reaction mixture of 2 mL containing 0.1 M acetate buffer (pH 4.9) with 10% ABTS and an increase in the absorbance was measured at 420 nm [19]. Tyrosinase activity was calculated in a reaction mixture of 2 mL, containing in 0.1 M phosphate buffer (pH 7.4) with 0.01% catechol at 495 nm [56]. Azoreductase assay was carried out using Methyl red as substrate as reported by Kurade et al. [27]. Riboflavin reductase activity was determined using riboflavin as substrate as mentioned in previously reported method [41]. Protein estimation was done by using Lowry et al. [30].

## 2.8 Extraction and analytical studies of metabolites obtained after degradation

The decolorization study was evaluated by observing the change in spectra before and after biotransformation of dye with the help of spectrophotometer (Shimadzu 1800, Japan). The supernatant was obtained from culture by centrifugation after complete decolorization of RR 180, RB 5 and RR. The metabolites were extracted using ethyl acetate (1:1, v/v). The evaporation of extracts was done using $Na_2SO_4$ in a rotary evaporator. The residual solids were mixed in methanol (grade one) and subjected to different analytical systems. FTIR analysis was carried out to find out the functional groups of control and biodegraded sample. The analysis was carried out with 20 scan at infrared region (4000–400 $cm^{-1}$). The samples of FTIR were prepared in high grade potassium bromide (5:95). The Shimadzu LC 40102010 instrument was used for HPLC study connected with C18 column. The solvent methanol was used in mobile phase with 1 ml $min^{-1}$ flow rate and analysis was done at 470 nm.

Identification of degraded product after biotransformation was performed with the help of 45XGC-44 GC–MS (Bruker). The helium gas (mobile phase) was used for 26 min with 1 ml $min^{-1}$ flow rate. The oven temperature conditions was primarily maintain for 120 s at 80 °C then progressively increased by 10 °C for each 60 s up to 250 °C

**Table 2** Characterrization of textile dyes before and after treatment

| | Untreated dye | | | Biological treatment | | | AOPs treatment | | | Bio–AOPs treatment | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Color (OD) | BOD Mg/L | COD Mg/L | Color (OD) | BOD Mg/L | COD Mg/L | Color (OD) | BOD Mg/L | COD Mg/L | Color (OD) | BOD Mg/L | COD Mg/L |
| Remazol Red | 2.0 | 945 | 239 | 0.2 | 741 | 176 | 0.6451 | 841 | 219 | 0.00 | 548 | 132 |
| Reactive Red 180 | 1.1 | 612 | 213 | 0.1 | 458 | 179 | 0.1867 | 516 | 200 | 0.00 | 136 | 79 |
| Reactive Black 5 | 2.0 | 1455 | 879 | 0.2 | 1152 | 425 | 1.2621 | 1259 | 572 | 0.00 | 659 | 129 |

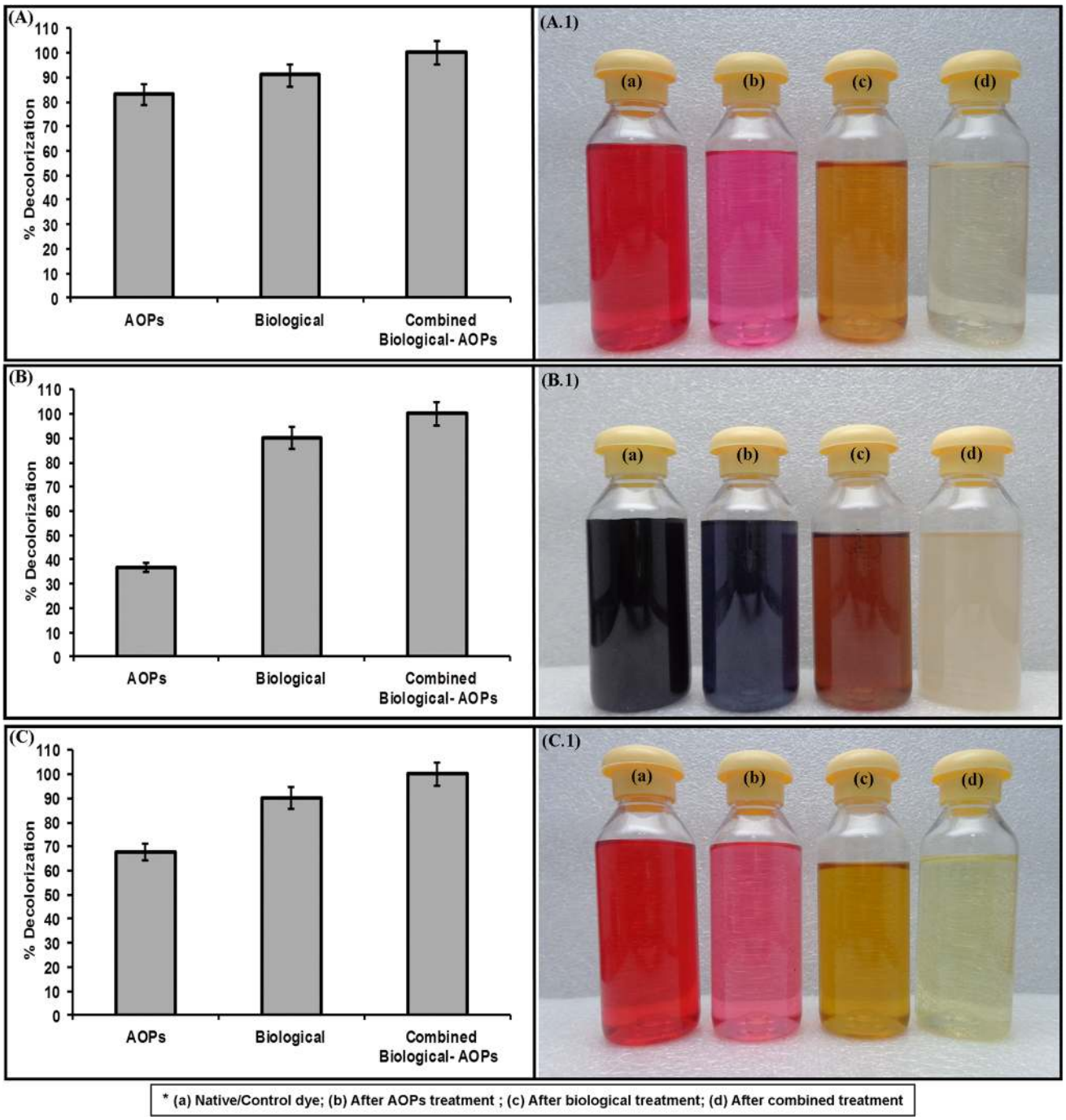



**Fig. 1** Decolorization of textile dye by advanced oxidation process (AOPs-4% $H_2O_2$) and combined biological (*A. hydrophila* SK16)-AOPs-4% $H_2O_2$ of **A** Reactive Red 180, **B** Reactive Black 5 and **C** Remazol Red RR. Data presented are mean ± SD of three parallel experiments.*$p \leq 0.05$

and maintain for 26 min. The formed products after transformation were recognized with the help of mass spectra using the NIST library. The elucidation of intermediate product produced, and degradative pathway of dyes was proposed using a tool Chemsketch 2.0.

## 3 Results and discussion

### 3.1 Biological degradation of RR 180, RB 5 and RR by *A. hydrophila* SK16

The potential of *A. hydrophila* SK16 to decolorize various dyes such as RR 180, RB 5 and RR were studied in both

static and shaking conditions. Present study represented the color removal capability of pure culture with respect to several textile dyes. The static condition showed noteworthy decolorization of RR 180, RB 5 and RR than shaking (91%; 50%), (90%; 72%) and (90%; 26%), respectively in 9 h (Fig. S1) (Table 2). Similarly, *Pseudomonas* sp. SUK1 also showed significant increased growth in shaking condition than static, but was not found decolorization even up to 48 h [23]. The dye Crystal violet was also removed by 100% at static condition [11]. The race held among dye and $O_2$ aimed for reduced $e^-$ carrier could be possible reason for reduced decolorization in aerobic condition [22] or azo bond reduction activity was inhibited by the existence of $O_2$ under shaking, because the aerobic respiration predominates usefulness of nicotinamide adenine dinucleotide (NADH), thus retard transferring of $e^-$ towards azo bonds [10]. As the decolorization efficiency was achieved at pH 8 and it is most appropriate for treatment of textile wastewater due to its alkaline condition [13]. Thus, these results concluded significant decolorization of RR 180, RB 5 and RR by *A. hydrophila* SK16 in static condition than shaking.

### 3.2 Decolorization of RR 180, RB 5 and RR by advanced oxidation process (AOPs)

The $H_2O_2$ concentration influences the efficiency of decolorization. AOPs showed 83, 37 and 68% decolorization of RR 180 (Fig. 1A, A.1.b), RB 5 (Fig. 1B, B.1.b) and RR (Fig. 1C, C.1.b), respectively, at 4% $H_2O_2$ and solar light exposure of 6 h (Table 2). Under AOPs, 30% color removal was observed by using $TiO_2$ alone [1], thus, the single treatment of AOPs results incomplete degradation [36]. No color change was observed in the dark condition without using $H_2O_2$ [38].

The result of several researchers such as Almomani et al. [2], Foteinis et al. [14] and Rani and Shanker [37] reveals contribution of solar light is more in mineralization of wastewater and textile dyes. When the photon energy (hv from UV range of sunlight) of solar light is absorbed by $H_2O_2$ which leads to generation of hydroxyl radicals ($OH^{\cdot}$). The hydroxyl radicals destroys the organic pollutants. All the dyes are not degraded under AOPs especially structurally and chemically complex dyes. So, that combined treatment could overcome all the limitations which are occurred from other treatments.

### 3.3 Decolorization of RR 180, RB 5 and RR by combined biological–AOPs treatment

The culture broth was centrifuged after biological treatment to separate biomass and used to optimize the concentration of $H_2O_2$. The supernatant was subjected for AOPs with various concentrations of $H_2O_2$ (1–10%) in 250 ml conical flask having 100 ml of supernatant. This mixture was subjected for treatment under sunlight. The decolorization of RR180 (Fig. 1A, A.1.d), RB 5 (Fig. 1B, B.1.d) and RR (Fig. 1C, C.1.d) was increased to 100% using 4% of $H_2O_2$ within 180 min (Table 2). This decoloization efficiency was better than the combined treatment (biological and AOPs) for RB5, RB13 and AO7 reported by Tantak and Chaudhari [[47]]. In another study, combined AOPs and biological treatment showed 99% color reduction [29]. The color removal was not effectual in the concentration range of 1–3% of $H_2O_2$. The decolorization efficiency was improved from 4% of $H_2O_2$. Combined treatment method was found to produce satisfactory results [4]. As per our

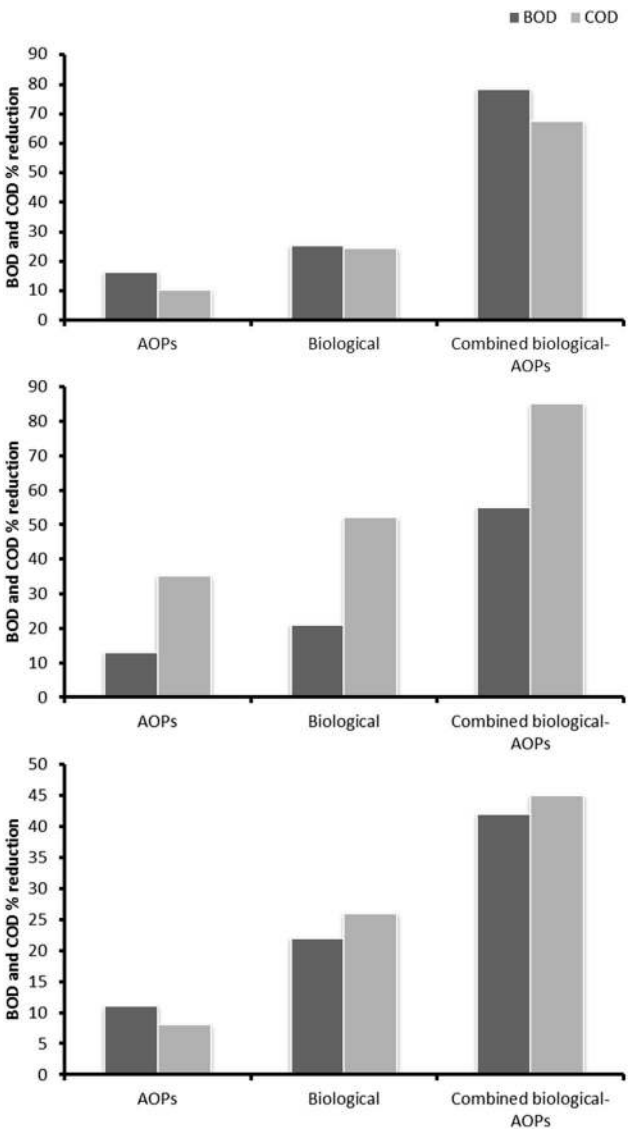



**Fig. 2** Effect of combined biological–AOPs treatment on BOD and COD of textile dye **a** Reactive Red 180, **b** Reactive Black 5 and **c** Remazol Red RR. Data presented are mean ± SD of three parallel experiments.*$p \leq 0.05$

finding, the combined Bio–AOPs treatment was more significant than single biological or AOPs processes for decolorization purposes.

### 3.4 Effect of combined Bio–AOPs treatment on BOD and COD of RR 180, RB 5 and RR after degradation

The sample was tested for BOD and COD reduction to assess the efficiency of Bio–AOPs system after decolorization. The BOD and COD of RR 180 were found to be reduced by 16; 10, 25; 24 and 78; 67% by the AOPs-$H_2O_2$, Biological-*A. hydrophila* SK 16 and Bio–AOPs, respectively (Fig. 2a). In case of RB 5, the BOD and COD were found to be reduced by 13; 35, 21; 52 and 55; 85% by the AOPs-$H_2O_2$, Biological-*A. hydrophila* SK 16 and Bio–AOPs, respectively (Fig. 2b). Also, BOD and COD of RR were found to be reduced by 11; 8, 22; 26 and 42; 45% by the AOPs-$H_2O_2$, Biological-*A. hydrophila* SK 16 and Bio–AOPs, respectively (Fig. 2b) (Table 2).

The above all results were showed that the individual AOPs and biological treatment is inadequate for textile dyes remediation, while combined Bio–AOPs treatment showed significant remediation. Both methods have some different limitation in waste treatment therefore, individually they unable to treat the dyes at maximum extent. In case of combined Bio–AOPs initially dye was degrade by *A. hydrophila* at some extent further AOPs was deal with the bio-recalcitrant compounds and showed maximum mineralization of all textile dyes. The significant reduction of BOD, COD and color in wastewater up to 98, 93 and 100%, respectively were observed during combined treatment process by *Thiobaccilus ferrooxidans* ATCC19859 [31]. The treatment with combined Fenton oxidation and biological treatment showed 98% COD removal of wastewater [48]. The COD (83%), BOD (88%), and total suspended solids (98%) were significantly reduced by combined treatment in non-biodegradable textile effluent [16]. The combined treatment not only achieve decolorization efficiency, but also contribute for the reduction of the treatment cost [9, 25].

### 3.5 Induction of enzyme activities after decolorization of RR 180, RB 5 and RR by *A. hydrophila* SK16

Enzyme studies exhibited the participation of number dye degrading biocatalyst like lignin peroxidase, tyrosinase, azo reductase, riboflavin reductase and laccase. The participation of these biocatalyst during color removal was documented by Telke et al. [49]. The levels of enzyme were meaningfully increased during dye transformation indicates complex mechanism of enzymes of dye degradation. In this study, activity of enzymes before and after decolorization of RR 180, RB 5 and RR were measured and found to be induced after decolorization. The decolorization of RR 180, RB 5 and RR showed induced activities of lignin peroxidase from 237.70, 121.84 and 82.59 to 536.99, 298.58 and 634.91 U $ml^{-1}min^{-1}$ (130, 145 and 669%)respectively. Laccase showed induction from 0.13, 0.03 and 0.06 to 0.22, 0.13 and 0.08 U $ml^{-1}$ $min^{-1}$ (69, 303 and 33%) after exposed to RR 180, RB 5 and RR, respectively. The activity of tyrosinase was elevated from 4.70, 1.33 and 0.30 to 28.86, 4.19 and 2.05 U $ml^{-1}$ $min^{-1}$ (514, 214 and 583%) by RR 180, RB 5 and RR exposure, respectively. The RR 180, RB 5 and RR dyes exposure was found to increase in the azo reductase from 91.12, 11.35 and 15.88 to 211.24, 19.47 and 78.05 µmol NADH reduced $min^{-1}$ mg $protein^{-1}$ (132, 72

**Table 3** Activities of enzyme involved in before and after degradation of Reactive Red 180, Reactive Black 5 and Remazol Red RR by using *A. hydrophila*

| Sets | Reactive Red 180 | | Reactive Black 5 | | Remazol Red RR | |
|---|---|---|---|---|---|---|
| | Before decolorization | After decolorization | Before decolorization | After decolorization | Before decolorization | After decolorization |
| Lignin peroxidase[a] | 237.70 ± 0.01 | 536.99 ± 0.01* | 121.84 ± 0.01 | 298.58 ± 0.01* | 82.59 ± 0.01 | 634.91 ± 0.01* |
| Laccase[a] | 0.13 ± 0.002 | 0.22 ± 0.005* | 0.03 ± 0.002 | 0.13 ± 0.001* | 0.06 ± 0.002 | 0.08 ± 0.003 |
| Tyrosinase[a] | 4.70 ± 0.021 | 28.86 ± 0.002* | 1.33 ± 0.032 | 4.19 ± 0.046* | 0.30 ± 0.003 | 2.05 ± 0.037* |
| Azo reductase[b] | 91.12 ± 0.03 | 211.24 ± 0.13* | 11.35 ± 0.03 | 19.47 ± 0.15* | 15.88 ± 0.09 | 78.05 ± 0.03* |
| Riboflavin reductase[c] | 38.34 ± 0.04 | 151.09 ± 0.12* | 23.32 ± 0.05 | 98.24 ± 0.07* | 35.82 ± 0.06 | 40.28 ± 0.09 |

Data presented are mean ± SD of three parallel experiments

*$p \leq 0.05$

[a]Units $ml^{-1}$ $min^{-1}$

[b]µmol NADH reduced $min^{-1}$ mg $protein^{-1}$

[c]µg of riboflavin reduced $min^{-1}$ mg $protein^{-1}$

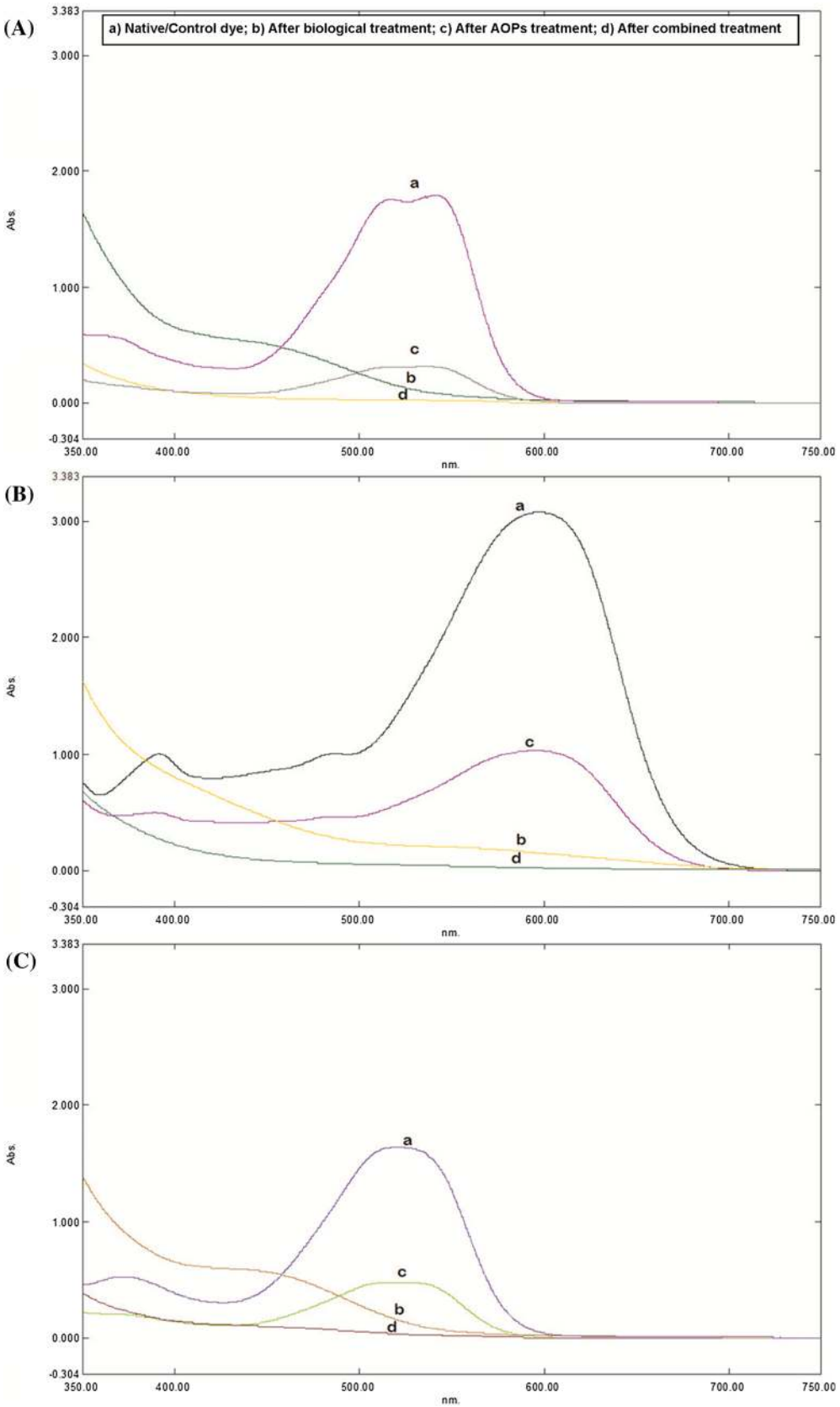



**Fig. 3** UV–Vis spectrophotomentric analysis of native/contro dye, after biological treatment, after AOPs treatment and after combined biological–AOPs treatment of textile dyes **A** Reactive Red 180, **B** Reactive Black 5 and **C** Remazol Red RR

and 391%) and riboflavin reductase from 38.34, 23.32 and 35.82 to 151.09, 98.24 and 40.28 µg of riboflavin reduced $min^{-1}$ mg protein $^{-1}$ (294, 321 and 12%), respectively (Table 3). Thus, our finding stated that, RR 180, RB 5 and RR significantly degraded by enhancing dye degrading enzymes activity in *A. hydrophila* SK16 at static condition.

## 3.6 Effect of combined Bio–AOPs treatment on RR 180, RB 5 and RR

The spectral scrutiny (350–750 nm) was done to confirm transformation of RB 5, RR 180 and RR. The absorption spectra after decolorization of RR 180, RB 5 and RR by AOPs ($H_2O_2$), biological and combined Bio–AOPs along with control showed complete reduction of RR 180 (Fig. 3A), RB 5 (Fig. 3B) and RR (Fig. 3C) due to combined Bio–AOPs. These UV–Vis spectral results stated that combined Bio–AOPs method is more noteworthy than individual one for the removal of dye contaminant from wastewater.

## 3.7 Spectroscopic and chromatographic analysis of decolorized product after combined Bio–AOPs treatment

### 3.7.1 FTIR analysis

The transformation of textile dye into different degraded product was evaluated by Fourier-transform infrared spectroscopy (FTIR) spectral analysis via comparison between RR 180, RB 5 and RR and its degraded products produced by *A. hydrophila* SK16 at static condition within 24 h. The FTIR spectra of RR 180 before biodegradation and after biodegradation differed with number of peaks and their position. The FTIR spectrum of untreated RR 180 indicated number peak at 3413.22 $cm^{-1}$ representing N–H stretching (str) of secondary amides and 1758.41 $cm^{-1}$ representing C=O str. of amide. The peak at 1621.24 $cm^{-1}$ confirmed the presence of azo (N=N) bond by a while, a peak at 1553.09 $cm^{-1}$ represent N–H deformation (def). The peak at 1462.22 $cm^{-1}$ represented N=O str. of nitrosamine, and peak at 1131.86 $cm^{-1}$ C–H def. in tri-substituted benzenes. The presence of sulfur group is represented by a peak at

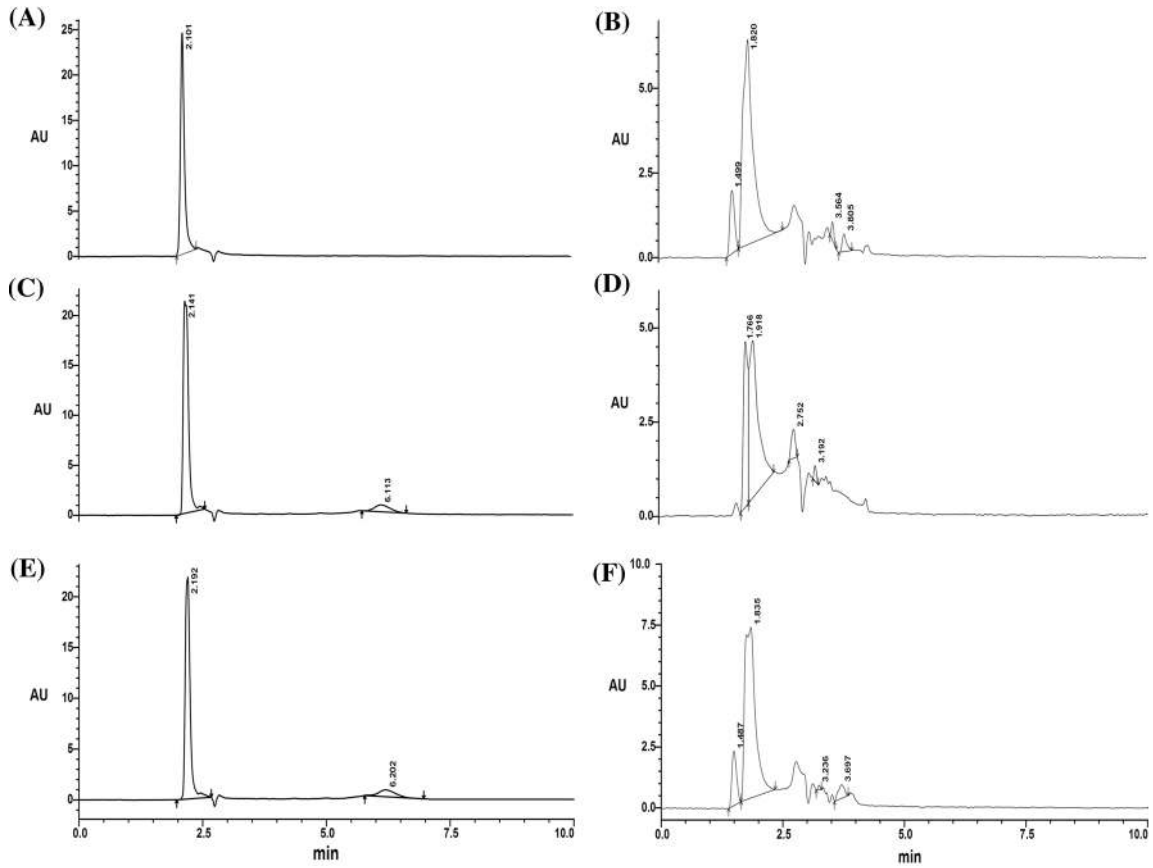



**Fig. 4** Profile of HPLC elution at 470 nm of dyes **a** Reactive Red 180 and **b** extracted metabolic products of Reactive Red 180 after decolorization by *A. hydrophila* SK16; **c** Reactive Black 5 and **d** extracted metabolites of Reactive Black 5 after decolorization by *A. hydrophila* SK16; **e** Remazol Red RR and **f** extracted metabolites of Remazol Red RR after decolorization by *A. hydrophila* SK16

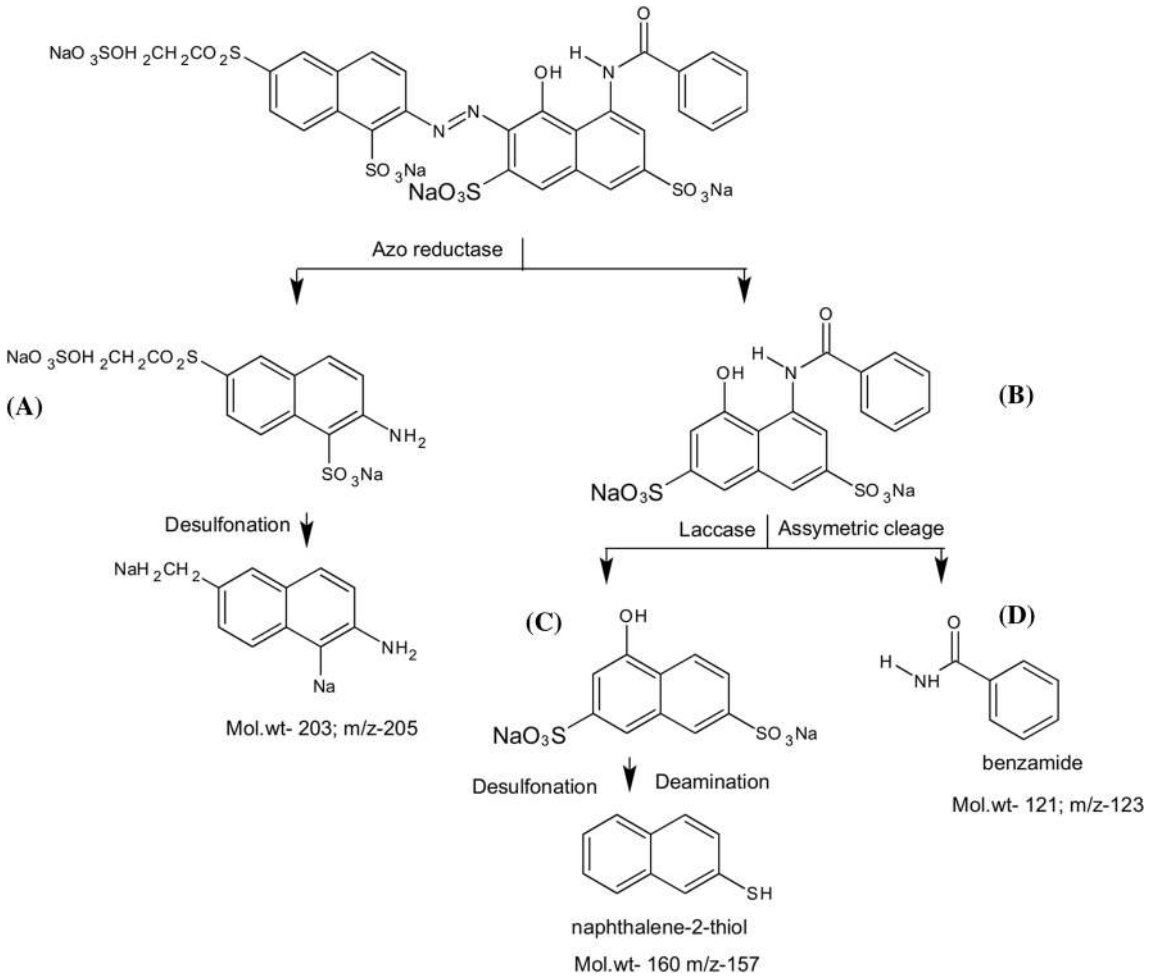



**Fig. 5** Pathways proposed for the degradation of Reactive Red 180 by *A. hydrophila* SK16

1043.36 $cm^{-1}$ for S=O str. of sulfonic acid, while a peak at 836.71 $cm^{-1}$ representing C–H def. of benzene ring possessing 2 adjacent $H_2$ atoms (Fig. S2A). Whereas, the products of the dye formed after decolorization by *A. hydrophila* SK16 showed a peak at 3244.65 $cm^{-1}$ revealed O–H str. and 2417.27 $cm^{-1}$ representing $NH^+$ str. of charged amines. Although a peak at 1595.42 $cm^{-1}$ representing $NH^{3+}$ def. of charged amine derivatives, while a peak at 1410.66 $cm^{-1}$ representing S=O str. of sulfites. The peak at 1115.42 $cm^{-1}$ revealed C–OH str. of 2° alcohols and at 989.28 $cm^{-1}$ revealed C–H def. of mono-substituted alkanes (Fig. S2B). The arrival several new peaks in biotransformed sample and disappearing of peaks in untreated spectra confirmed the biodegradation of dye RR 180 by *A. hydrophila* SK1.

Similarly, FTIR spectral analysis of RB 5 before biodegradation showed peaks at 3435.71, 2418.55, 1813.31, 1591.10, 1492.15 and 1418.68 $cm^{-1}$ which represents the presence of N–H str., O–H def., C=O symmetric str., –N=N– azo bond, aromatic compound, C–H def. of alkanes, respectively. Peaks at 1050.27 and 631.90 $cm^{-1}$ showed –C=O str. and –C=S str. indicates the presence of sulfur containing groups. Peaks at 1130.59 and 839.92 $cm^{-1}$ represents C–H def., a peak at 895.28 $cm^{-1}$ for aromatic and a peak at 631.90 $cm^{-1}$ showed C–S sulfur group presents in control dye (Fig. S3A). After biodegraded product showed various peaks at 3273.37 $cm^{-1}$ for O–H str., C=O str. at 1658.46 $cm^{-1}$, a peak at 1110.14 $cm^{-1}$ for C–OH secondary alcohol. The disappearance of 1591.10 $cm^{-1}$ in degraded metabolites confirms azo bond cleavage. Peaks at 983.15, 866.13 and 619.09 $cm^{-1}$ represent C–OS stretching, C–HS stretching and C–H deformation (Fig. S3B). Finally, disappearance of peaks and formation of new peaks in metabolites confirms biodegradation of RB 5.

Difference between parent dye and metabolite produced after 9 h of RR revealed biodegradation. Parent dye represents NH group at 3415.31 $cm^{-1}$, a peak at 2925.76 $cm^{-1}$ for C–H alkane stretching, C–H stretching at 1756.05 $cm^{-1}$, a peak at 1597.78 $cm^{-1}$ for –N=N– azo bond, a peak at 1489.79 $cm^{-1}$ for C=C stretching (aromatic compound). Peaks at 1407.47, 1319.41, 1291.04, 1126.64,

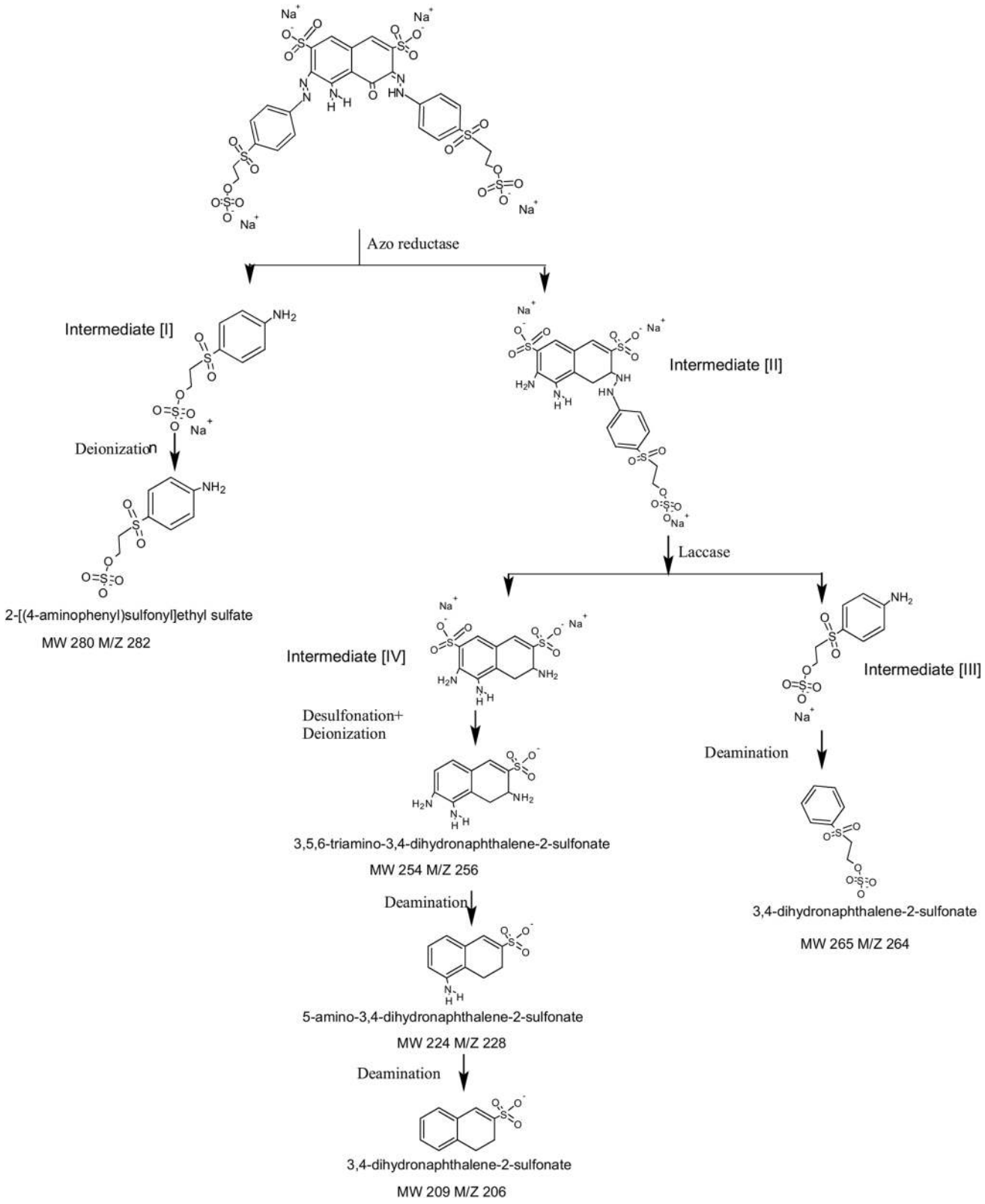



**Fig. 6** Pathways proposed for the degradation of Reactive Black 5 by *A. hydrophila* SK16

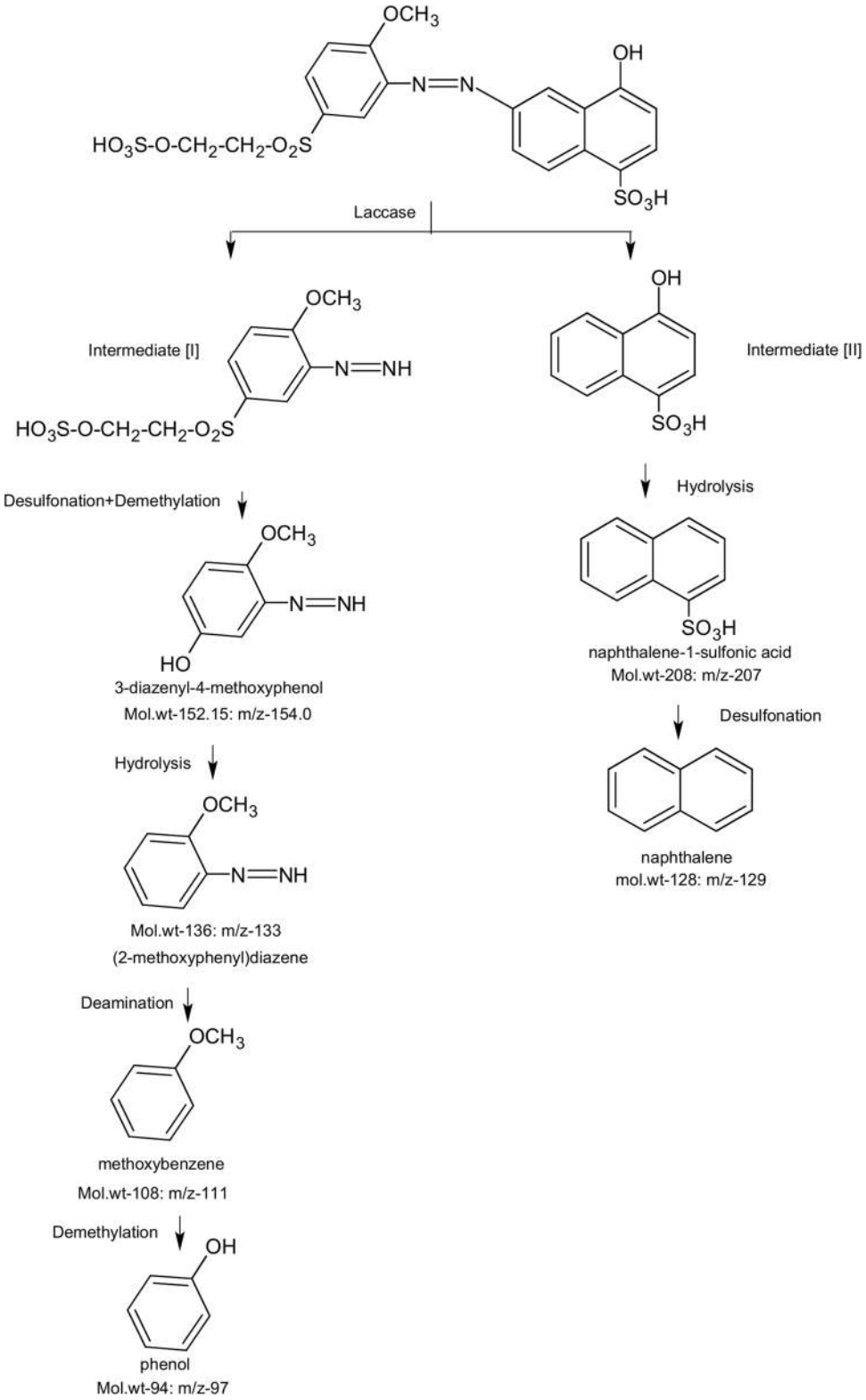



**Fig. 7** Pathways proposed for the degradation of Remazol Red RR by *A. hydrophila* SK16

1042.82, 843.49 and 622.21 $cm^{-1}$ represents O–H str. vibration (vib), C–N vib., O–$NO_2$ vib., C–O str., C–S sulfonated (aromatic compound), and C–H def., respectively. Peaks at 793.08 and 743.51 $cm^{-1}$ represented for C–H bending (Fig. S4A). biotransformed RR showed vanishing of major peaks and appearance of new peaks 3294.46 $cm^{-1}$ for O–H str., a peak at 1648.22 $cm^{-1}$ for C=N str., a peak at 1458.19 $cm^{-1}$ for C–H def. (alkanes), respectively (Fig. S4B).

#### 3.7.2 HPLC analysis

HPLC profile of the parent dye represented major peak with at retention time (Rt) of 2.101 min (Fig. 4a), while biotransformed dye by *A. hydrophila* SK16 represent 4 new peaks with the Rt of 1.499, 1.820, 3.564 and 3.805 min (Fig. 4b). It is the confirmation of degradation of RR 180 by *A. hydrophila* SK16 by appearing of some new peaks at different retention time of some new metabolites. Although, analysis of RB 5 before degradation represent main peak at 2.141 min and a slight peak at 6.113 min Rt (Fig. 4c), however in biodegraded sample represent 4 new peaks at 1.766, 1.918, 2.752 and 3.192 min Rt (Fig. 4d) confirmed biodegradation of RB 5. Similarly, analysis of RR represents a main peak at 2.192 min and a slight peak at 6.202 min Rt (Fig. 4e). Biodegraded sample represents 4 different peaks at 1.487, 1.835, 3.236 and 3.697 min Rt suggested biodegradation of RR (Fig. 4f).

### 3.8 The proposed pathways of dyes transformation

Fate of metabolism of RR 180 by *A. hydrophila* SK16 is proposed on the basis of increased level of enzyme *A. hydrophila* SK16 and the GC–MS profiling. Initially, by the action of azo reductase on azo bond, it leads to the production of transitional compound [A] and [B]. Desulfonation of compound [A] provided an unidentified product. Asymmetric cleavage of laccase on Intermediate [B] gave compound [C] and [D] which was identified as benzamide [Rt 21.093 min; Mol.wt-121; m/z-123]. Further, desulphonation and deamination of Intermediate [C] led to formation of naphthalene-2-thiol [Rt 20.673 min; Mol.wt-160; m/z-157] (Fig. 5). The asymmetrical cleavage of azo dyes is well studied action of laccase [46].

In case of RB 5, initially azo reductase activity breaks azo linkage leads the production of Intermediate [I] and [II]. Deionization of Intermediate [I] resulted in the formation of 2-[(4-aminophenyl)sulfonyl]ethyl sulfate. Cleavage of Intermediate [II] by laccase formed Intermediate [III] and [IV]. The Intermediate [III] delaminated to the formation of 4-dihydronaphthalene-2-sulfonate. Further, breakdown of Intermediate [IV] in the formation of 3,5,6-triamino-3,4-dihydronaphthalene-2-sulfonate was through desulfonation and deionization. Sequential deamination produced 5-amino-3,4-dihydronaphthalene-2-sulfonate and 3,4-dihydronaphthalene-2-sulfonate as final product (Fig. 6). Similarly, GCMS profiling was done to analyze the biotransformed product during degradation of RR and identified the metabolites as 3-diazenyl-4-methoxyphenol, (2-methoxyphenyl)diazene, methoxybenzene, phenol, naphthalene-1-sulfonic acid and naphthalene (Fig. 7).

## 4 Conclusion

This combined Bio–AOPs treatment not only achieves significant decolorization, but also contributes towards reduction in BOD and COD of wastewater. It is more efficient than an individual treatment. It is reliable, efficient, passive, economical, feasible solar energy dependent and requires less maintenance for treatment of textile dyes and wastewater as compared to individual biological and advanced oxidation process.

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### Compliance with ethical standards

**Conflict of interest** The authors declares that they have no conflict of interest.

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