

Exploited Application of *Bacillus* sp. ETL-A & *Pseudomonas* sp. ETL-B in Microbial Degradation of Orange 16 Dye

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Abstract Soil samples collected from common effluent treatment plant of Ankleshwar were used as a source. Two different bacterial strains are capable of decolorizing Orange 16. The individual bacterial strains *Bacillus* sp. ETL-A and *Pseudomonas* sp. ETL-B decolorized Orange 16 (200 mgL^{-1}) completely within 27 and 12 h respectively. Various parameters like pH, temperature, NaCl and initial dye concentrations were optimized. The maximum concentration of Orange 16 (1000 mgL^{-1}) was decolorized by strains ETL-A and ETL-B within 78 and 84 h respectively. These strains could decolorize Orange 16 over a broad pH range 5.5–9.0; the optimum pH was 7.2. The decolorization of Orange 16 was most efficient at 40°C . Analysis of biodegradation products carried out with UV-vis spectroscopy, HPTLC and FTIR confirmed the decolorization and degradation of Orange 16. Further, both the strains showed the involvement of azoreductase in the decolorization process. These results indicate the effectiveness of strains ETL-A and ETL-B for the treatment of textile effluents containing azo dyes.

Keywords: Orange 16, *Bacillus*, *Pseudomonas*, HPTLC, FTIR

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1. Introduction

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life (Shah et al., 2013). The textile industry is one of them, which extensively use synthetic chemicals as dyes. Waste waters from textile industries pose a threat to the environment, as large amount of chemically different dyes are used (Shah et al., 2013). Reactive dyes, including many structurally different dyes, are extensively used in the textile industry because of their wide variety of color shades, high wet fastness profiles, ease of application, brilliant colors, and minimal energy consumption. The three most common groups are azo, anthraquinone and phthalocyanine (Shah et al., 2013). Various kinds of physico-chemical methods are in use for the treatment of wastewater contaminated with dye. These methods are not environment friendly and cost-effective and hence become commercially unattractive (Karimniaee et al., 2007). Many microorganisms belonging to the different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolorize azo dyes (Asad et al., 2007). A lot of information is available on removal and degradation of Remazol Black B using pure strains of bacteria, fungi, algae and yeast such as *Shewanella* strain J18 143, *Rhizopus arrhizus*, *Phlebia*

tremellosa, *Trametes versicolor*, *Bjerkandera* sp. BOS55, *P. chrysosporium*, *Chlorella vulgaris*, *Candida tropicalis*, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus* IMB3, *P. mirabilis*, *P. luteola*, *Pseudomonas* sp. and *K. rosea* (Aksu et al., 2000., Kirby et al., 2000., Swamy et al., 1999., Meehan et al., 2000). The removal and degradation of Remazol Black B dye by various physicochemical methods have also been studied (Robinson et al., 2002., Hepel & Hazelton., 2005., Vinodgopal et al., 1998). However, maintaining the purity of single cultures in the large scale application and their inability to degrade all different dyes present in the actual effluent are the drawbacks for their commercial application (Pearce et al., 2006, Andre et al., 2007). Therefore, the use of mixed culture seems to have more potential for large scale application at field level. The syntrophic interactions present in the mixed communities lead to complete mineralization of azo dyes (Chang et al., 2004). In the present investigation, we have reported the isolation and identification of micro-organisms from textile effluent contaminated soil capable of decolorizing Remazol Black. The various parameters such as initial dye concentration, temperature, pH and NaCl concentration have been optimized to achieve the maximum dye decolorization. The intermediate metabolites formed during the degradation of Orange 16 have been characterized by using various analytical techniques. The information obtained from this study is expected to provide the basis

for the development of practical decolorization process for Orange 16 using these bacterial strains as biocatalysts.

2. Materials & Methods

2.1. Dyes

Orange 16 was purchased from local textile company of Ankleshwar, Gujarat, India. The stock solution of dye was filter sterilized and added to the growth medium in the required concentration. All the chemicals used were of analytical grade and procured from Himedia Pvt. Limited, Mumbai.

2.2. Decolorization Medium

The composition of Decolorization medium was (g L^{-1}): K_2HPO_4 6.3; KH_2PO_4 1.8; NaCl 5; NH_4NO_3 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1; MnSO_4 0.1; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1; $\text{NaMoO}_7 \cdot 7\text{H}_2\text{O}$ 0.006; pH 7.2 ± 0.1 . This medium was supplemented with yeast extract (2.5 g L^{-1}) and Metanil Yellow (200 mg L^{-1}).

2.3. Isolation and Screening

Soil samples contaminated with textile dye effluent were collected from different vicinities in Ankleshwar and were used as a source to isolate morphologically distinct bacterial colonies capable for decolorizing Orange 16. The soil sample was subjected to enrichment culture technique using nutrient broth amended with increasing concentrations of Orange 16 (100 mg/l - 500 mg/l) with incubation time of 24 hours at 37°C . The high decolourising bacteria were screened by performing a decolourisation assay with Orange 16 using UV- Vis spectrophotometer (Shimadzu UV-1800, Japan) at their respective λ_{max} (Resmi, 2004).

The percentage decolourisation (% Decolourisation) was calculated using the following formula-

$$\% \text{ Decolorization} = \frac{\left(\begin{array}{l} \text{Initial absorbance} \\ - \text{Final absorbance} \end{array} \right) \times 100}{\text{Initial absorbance}}$$

2.3. 16S rDNA Analysis

2.3.1. Isolation of DNA

Genomic DNA from each of the screened bacteria was isolated. The presence of DNA was checked by running in agarose gel (0.8%) stained with Ethidium Bromide.

2.3.2. Amplification of 16S rDNA Sequence by Polymerase chain Reaction

The reaction mixture of total volume of $30 \mu\text{l}$ consisted of $3 \mu\text{l}$ of 10 X Buffer, $1 \mu\text{l}$ of 10 mM dNTPs, $1 \mu\text{l}$ of 16S rDNA primer ($5 \text{ picomole}/\mu\text{l}$), $3 \text{ U}/\mu\text{l}$ of Taq Polymerase, $5 \mu\text{l}$ of template DNA (280 ng/ml) and $19 \mu\text{l}$ of sterile distilled water. The PCR reaction was set to initial denaturation of 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for one minute, annealing at 55°C for one minute, extension at 72°C for one minute and final extension at 72°C for 10 minutes (Govindaswami, 1993). The amplified products were stained with $0.5 \mu\text{g/ml}$

Ethidium Bromide and loaded on 0.8% agarose gel, and the DNA fragments were separated at 100V and documented using Launch Doc IFLS software.

2.3.3. Sequencing the Amplified Product Followed by BLAST

The amplified product was subjected to cycle sequencing to identify the bacterial isolate using ABI 3130 XL (Analyser, USA). The sequence was then subjected for BLAST analysis to identify the bacterial isolate.

2.4. Decolorization Studies of Orange 16 in Liquid Medium

The decolorization experiments were performed in 250 mL Erlenmeyer flasks containing decolorization medium. Orange 16 (200 mg L^{-1}) was added to Decolorization medium and inoculated with 2 mL of cultures broth ($6 \times 10^9 \text{ CFU mL}^{-1}$). The flasks were incubated at 40°C under static condition till the Decolorization was completed. The samples were withdrawn at different time intervals and analyzed for decolorization efficiency. The Decolorization was monitored spectrophotometrically (Shimadzu UV-1800, Japan) by reading the decrease in absorbance (495 nm) of the dye in culture supernatant. Uninoculated controls were used to compare abiotic color loss during the decolorization studies. Decolorizing activity is expressed in terms of percentage Decolorization (C. Yatome, 1993).

2.5. Azo Reductase Assay

Azoreductase activity was measured according to the method described previously (Vijaykumar, 2007). The bacterial cultures grown in the medium containing Orange 16 (200 mg L^{-1}) was centrifuged at 9000 rpm for 10 min at 4°C . The cell pellet was washed twice with 50 mM potassium phosphate buffer (pH 7.0) and resuspended in the same buffer. The cells were disrupted in cold condition by sonication. The sonicated cells were centrifuged at 12,000 rpm for 30 min at 4°C and the supernatant was used as the crude enzyme source. To 50 mM phosphate buffer (pH 7.0) containing 150 mM Orange 16, $100 \mu\text{L}$ of crude enzyme was added and this reaction mixture (1 mL) was pre-incubated for 3 min followed by the addition of 2 mM NADH. The azoreductase activity was determined spectrophotometrically at room temperature by monitoring the decrease in absorbance at 340 nm. One unit of enzyme activity was defined as the amount of enzyme that catalyses the $1 \mu\text{mol}$ of substrate per minute. All experiments including enzyme assay were carried out in triplicate.

2.6. Optimization Study of Physico-Chemical Parameters

Decolorization ability of strains ETL-A and ETL-B on Orange 16 was studied at different pH (4–9) and temperatures values (15 – 50°C). It was observed that pH 7.2 and temperature 37°C were found to be optimal for the decolorization activity. Based on the optimal decolorization, pH 7.2 and temperature of 37°C were selected to study the decolorization process under various physico chemical factors such as dye concentration (200 – 1000 mg L^{-1}), NaCl concentration (5 – 20 g L^{-1}) and

continuous addition of Orange 16 in the decolorization medium. Further, the decolorization of various azo dyes was studied by incubating the medium containing respective dye with individual strains ETL-A and ETL-B. Decolorization of different dyes was monitored by measuring the change in absorbance of culture supernatants at the maximum absorption wavelength (λ_{max}) of the respective dye. These experiments were performed in 250 mL Erlenmeyer flask under static condition in triplicate.

2.7. UV-Vis Spectral Analysis, HPTLC, FTIR

UV-visible spectral analysis of cell free sample, before and after dye decolorization, was carried by using Shimadzu UV spectrophotometer (Shimadzu UV 1800, Japan), and changes in its absorption spectrum in the visible range (400–800 nm) were recorded. HPTLC analysis was carried out by using HPTLC system (CAMAG, Switzerland). Samples of dye and its biodegradation metabolites (dissolved in HPLC-grade methanol) were loaded on precoated HPTLC plates (Silica gel 60F 254, Merck, Germany), by using nitrogen as a spraying gas and TLC sample loading instrument (CAMAG LINOMAT 5). TLC plate was developed in solvent system toluene to ethyl acetate to methanol (2: 4: 13). After development, the plate was observed in UV chamber (CAMAG) and scanned at 254 nm with slit dimension 5×0.45 mm by using TLC scanner (CAMAG). The results were generated by using HPTLC software

WinCATS 1.4.4.6337. For FTIR analysis, residue obtained after evaporation of solvent extracts was mixed with stereoscopically pure KBr and analysis was carried out using Shimadzu 8400S spectrophotometer in the mid-infrared region of $400\text{--}4,000\text{ cm}^{-1}$ with 16-scan speed. Identification of metabolites formed after degradation of Orange 16 was carried out using a QP2010 gas chromatography coupled with mass spectroscopy (Shimadzu, Japan). The ionization voltage was 70 eV, and helium was used as carrier gas with a flow rate 1 ml min^{-1} and 33 min run time. Gas chromatography was conducted in temperature programming mode with a Resteck column (0.25×30 mm; XTI-5). Column oven temperature was 280°C , and injection temperature was 200°C . Temperature was hold at 50°C for 1 min then rose to 280°C at 10°C rise per min.

3. Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) with the Tukey–Kramer multiple comparisons test (Jadhav JP, 2010).

4. Results and Discussion

4.1. Identification and Phylogenetic Position of Bacterial Isolates

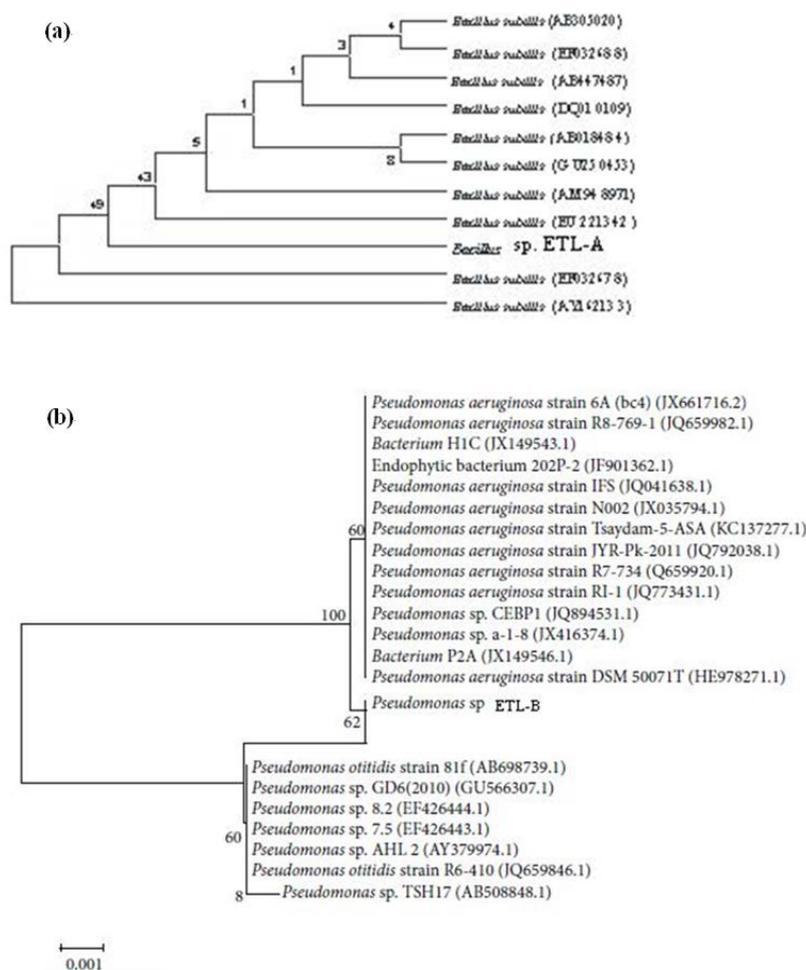


Figure 1. a Phylogenetic tree showing genetic relationship of *Bacillus* with taxonomically similar strain, species and genus based on 16s rDNA sequences; b Phylogenetic tree showing genetic relationship of *Pseudomonas* with taxonomically similar strain, species and genus based on 16s rDNA sequences

Two different bacterial strains having remarkable Orange 16 dye decolorization capacity were isolated from dye contaminated soil sample. The identification of the strains was done on the basis of morphological and biochemical characteristics (data not shown) and 16S rDNA gene sequences. Bacterial strains ETL-A and ETL-B were identified as, *Bacillus* sp. strain ETL-A and *Pseudomonas* sp. strain ETL-B respectively. To analyze the phylogenetic position, the 16S rDNA sequences of the strains ETL-A and ETL-B were determined. Figure 1a and Figure 1b shows the phylogenetic relationship between the isolated bacterial strains and other related bacteria found in the GenBank database. The homology assay results indicated that the strains ETL-A and ETL-B were in the phylogenetic branch of the genus *Bacillus* and *Pseudomonas* respectively. *Bacillus* sp. is usually dominant in the activated sludge system, decolorizing and eventually degrading azo dyes (Dwakar, 2010; Toshiaki, 1999). Chen et al. reported the phenol tolerance of indigenous phenol degraders such as *Klebsiella* sp. and *Lysinibacillus* sp. isolated from Northeast Taiwan.

4.2. Time Course for Decolorization of Orange 16 in Liquid Medium

These two bacterial cultures were subjected to further screening for their ability to decolorize Orange 16 in liquid medium. The strain ETL-B exhibited dye decolorization at a faster rate as compared to strain ETL-A when incubated under static condition. At an early 6 h of incubation 37% and 9% of decolorization was exhibited by both the strains ETL-B and ETL-A respectively. It could be seen that ETL-B exhibited high decolorization activity and could achieve 100% decolorization within 10–12 h. However, the Decolorization activity of Orange 16 by strain ETL-A was very slow and it could show only 22% decolorization within 12 h and 99% Decolorization could be achieved within 27 h. Vijaykumar et al. reported that 99% decolorization of sulfonated azo dyes by *Kerstersia* sp. under static condition (Vijaykumar, 2007). The maximum decolorization (100%) of Orange 16 by *Bacillus* sp. ETL-A and *Pseudomonas* sp. ETL-B observed in the present study was compared with those of other methods used to decolorize Orange 16 in Table 1.

Table 1. Decolorization of Orange 16 dye by various methods

Method	Decolorization	Reference
Adsorption	86.9 mg/L	17
	99%	10
Photocatalytic degradation	90%	16
Electrochemical oxidation	90%	18
<i>Bacillus</i> sp. ETL-A	100%	Present study
<i>Pseudomonas</i> sp. ETL-B	100%	Present study

4.3. Azoreductase Activity

Table 2. Decolorization of various azo dyes by *Bacillus* sp. ETL-A and *Pseudomonas* sp. ETL-B

Azo dye	λ_{max} (nm)	<i>Bacillus</i> sp. ETL-A (% Decolorization)	<i>Pseudomonas</i> sp. ETL-B (% Decolorization)
Orange 16	495	99	100
Acid Red	480	97	99
Congo Red	498	80	98
Fast Red	512	62	71
Mordant Black	385	46	58
Reactiv Black	596	31	43

Dyes of different structures were often used in the textile processing industry, and therefore, the effluents

The major mechanism involved in the microbial biodegradation of synthetic azo dyes is based on their biotransformation by enzymes (Raghukumar, 1996). The initial step involved in the biodegradation of azo dyes is the reductive cleavage of azo bond ($-N-N-$) with the azoreductase (Chang JS, 2000). The decolorization of Orange 16 by azoreductase activity was monitored over the time intervals. Figure 2 shows the decolorization of Orange 16 along with the respective azoreductase activity with time intervals for strains ETL-A and ETL-B. A significant increase in the azoreductase activity of 1615 nmol/mg protein/min and 1630 nmol/mg protein/min was observed in the strains ETL-A (Figure 2a) and ETL-B (Figure 2b) respectively. Further, a significant induction in the activity of azoreductase was observed in the cell free extracts of both the strains ETL-A and ETL-B during Orange 16 decolorization when compared to the control cells. Such inductive pattern of azoreductase was observed during Decolorization of sulfonated azo dyes by *Kerstersia* sp. strain VKY1 (Vijaykumar, 2007) and *Galactomyces geotrichum* MTCC 1360 [29]. From these results it is evident that the strains ETL-A and ETL-B degrade Orange 16 by enzymatic reduction with azoreductase.

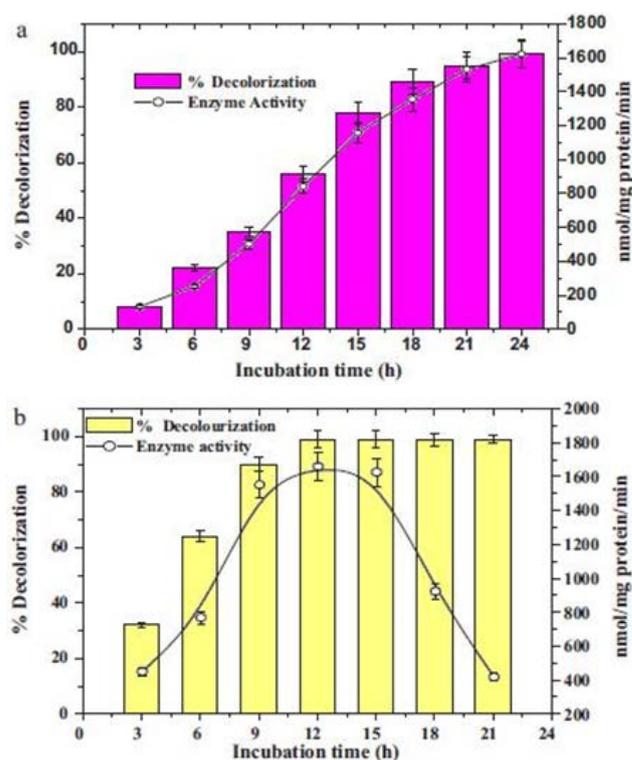


Figure 2. Azo reductase activity of (a) *Bacillus* sp. ETL-A and (b) *Pseudomonas* sp. ETL-B

4.4. Decolorization Study

from the industry are markedly variable in composition (Patil, 2008). Strains ETL-A and ETL-B were tested for

their ability to decolorize six structurally different azo dyes such as Orange 16, Acid Red, Congo Red, Fast Red, Mordant Black and Reactive Black 5. The bacterial strains ETL-A and ETL-B had shown effective decolorization of all the six structurally different azo dyes (Table 2). The rapid decolorization of all azo dyes was observed by strain ETL-B as compared to strain ETL-A. The slower decolorization with individual strains was due to structural differences, higher molecular weight and presence of inhibitory groups like $-\text{NO}_2$ and $-\text{SO}_3\text{Na}$ in the dyes (Patil, 2008). The present study confirms the ability of strains ETL-A and ETL-B to decolorize six structurally different azo dyes with decolorization efficiency of more than 50% in short time. Thus, the strains ETL-A and ETL-B could be successfully employed for the treatment of industrial wastewater containing Orange 16, Acid Red, Congo Red, Fast Red, Mordant Black and Reactive Black 5 efficiently.

Effect of initial dye concentration and repeated use of cells

The percentage decolorization of Orange 16 by strains ETL-A and ETL-B was carried out at different initial dye concentrations ($100\text{--}1000\text{ mgL}^{-1}$). It was observed that decrease in percentage of decolorization of Orange 16 with increase in concentration under static condition by both the strains. Strain ETL-B could effectively decolorize Orange 16 with decolorization percentage of 100% for 200 mgL^{-1} and 62% for 1000 mgL^{-1} dye concentration during 12 h and 78 h of incubation respectively. The Decolorization percentage of 100% for 200 mgL^{-1} and 75% for 1000 mgL^{-1} during 24 h and 84 h of incubation respectively with strain ETL-A. Lower decolorization percentage at high dye concentration was reported and was expected to be due to the inhibitory effects of high dye concentration (Güven, 2008). Similar observations have been recorded earlier for decolorization of synthetic dyes using a batch Decolorization assay (Hag, 2008). The repeated use of cells of strains ETL-A and ETL-B degrade Orange 16 (200 mgL^{-1}) up to nine cycles, but with an increase in time and reduction in percentage decolorization. The initial four cycles of dye decolorization with ETL-A and ETL-B degraded 100% dye at 12 h and 24 h respectively, but for the next remaining five cycles increase in time with reduced percentage of dye decolorization was observed for both the strains (data not shown).

4.5. Temperature Effect

The temperature effect on the decolorization of Orange 16 was significant for both the strains. When the Decolorization of the dye was tested for a wide range of temperatures from 15 to 50°C , it was observed that the increase in decolorization of Orange 16 with increase in temperature and was optimum at 37°C for both the strains ETL-A and ETL-B. Further increase in the temperature increases the decolorization of dye up to 40°C and above this temperature a decreased dye decolorization was noticed (Figure 3a). To understand the effect of low temperature, room temperature and high temperature on the decolorization of dye, the assay was carried out at different temperature range from 15 to 50°C . The decrease in dye decolorization at high temperature can be attributed to the decline in microbial activity that led to the inactivation of the enzyme and eventually the loss of cells viability (Pearce, 2003). These results further showed that

there is no thermal deactivation of decolorization activity under operational temperatures. Therefore, these strains ETL-A and ETL-B could acclimatize to broad range of temperature. Kapilkumar et al. observed that the decrease in the decolorization efficiency of mixed cultures for color removal beyond 35°C , which was predicted as thermal deactivation of the decolorization enzymes.

4.6. pH Effect

The effect of pH on the decolorization of Orange 16 by both the strains was determined over a wide range of pH (4.0–9.0). Both the bacterial strains ETL-A and ETL-B showed maximum dye Decolorization at pH 7.2 (Figure 3b). At this optimum pH, the strain ETL-A showed 98.5% and that of strain ETL-B showed 99.9% of Decolorization of Orange 16. At pH 8.0, both the strains ETL-A and ETL-B showed 80% and 84% decolorization of Orange 16 respectively. Whereas at pH 4.0, both the strains showed only 8.0% and 15% dye decolorization respectively. Similar results were also reported by Sukumar et al. [34]. Chan and Kuo reported that the neutral pH would be more favorable for decolorization of the azo dyes and is suitable for industrial applications (Chan, 2000).

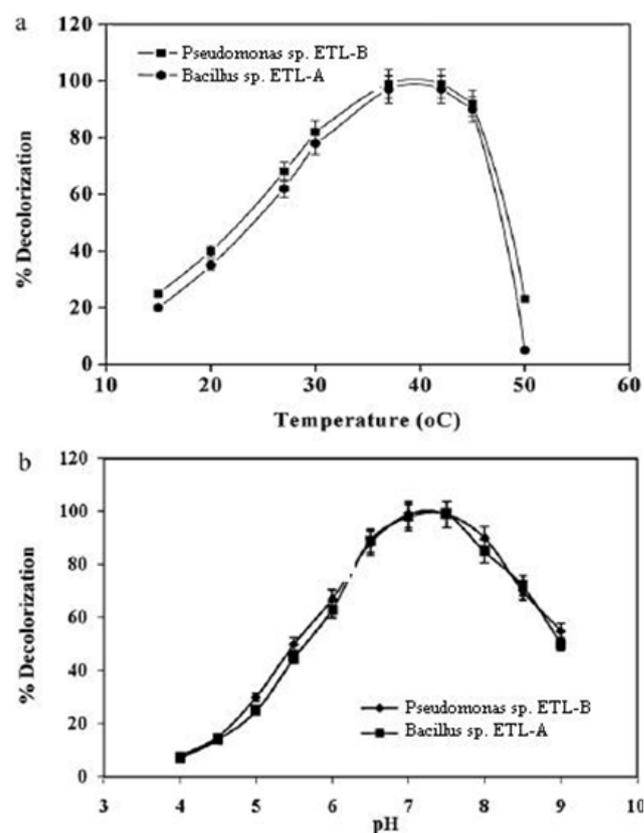


Figure 3. Effect of (a) temperature and (b) pH on the decolorization of Orange 16 by both the strains ETL-A & ETL-B

4.7. Effect of NaCl Concentration

The high salt concentration is a consequent product of batch processes in both the dye manufacturing and dye-consuming industries where the salt concentration is up to 15–20% (Manu, 2003). Generally, sodium concentration above 3 gL^{-1} can cause moderate inhibition of most bacterial activities (De Baere, 1984). In the present study, the effect of NaCl concentration on the decolorization of

Orange 16 by both the strains was examined. Both the strains ETL-A and ETL-B Exhibit 99% decolorization at 5 gL⁻¹ NaCl concentration. Further, the NaCl concentration up to 10 gL⁻¹ did not show any effect on dye decolorization by both cultures. At this concentration of NaCl, 90% and 95% of decolorization of Orange 16 was achieved by strains ETL-A and ETL-B respectively. Further, increase in concentration of NaCl results in decreased percentage of decolorization. The concentration of 20 gL⁻¹ of NaCl drops the percentage Decolorization up to 41% and 44% by strains ETL-A and ETL-B respectively.

4.8. Dye Degradation Analysis

4.8.1. HPTLC and FTIR Analyses

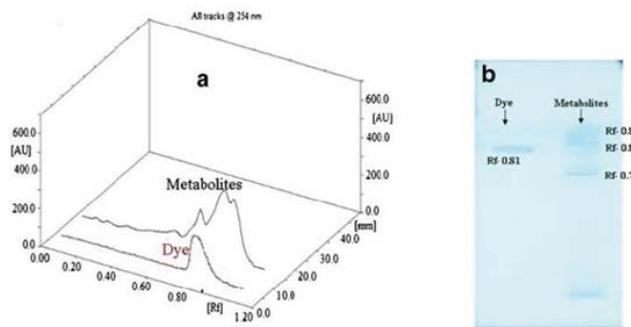


Figure 4. HPTLC analysis of Orange 16 degradation. (a) 3D graph of Rf value vs absorbance (b) Fluorescence TLC plate under UV chamber

Figure 4 gives the HPTLC analysis of parent dye compound and metabolites formed after dye degradation. Single peak was obtained for the parent dye, whereas three new and different peaks were observed for the metabolite sample in 3D graph of Rf values vs. absorbance (Figure 4a). It is also visible from fluorescent TLC plate that single band of dye at Rf value at 0.81 was almost disappeared in the metabolite sample. Three new bands with Rf values 0.89, 0.85 and 0.70 (scanned at 254 nm) appeared in the metabolite sample (Figure 4b). In FTIR analysis of dye compound (Figure 5a), presence of azo bond was revealed with the peak value 1591.66 cm⁻¹. Peak at 628.87 cm⁻¹ was responsible for C-S stretching in sulphur-containing compounds. Peaks at 1,053.86 and 1,124.19 cm⁻¹ are representing the SOO asymmetric stretching in sulphonic acid groups. These three peak values confirm the presence of -SO₃Na group in the dye structure. Trisubstituted C-H deformation was indicated by the peak at 1,224.19 cm⁻¹. Peaks at 1,374.94 and 2,922.94 cm⁻¹ reflect the alkanes C-H deformation and asymmetric stretching, respectively. Aromatic nature was exposed by the peak at COC in-plane vibrations for homocyclic compounds. Overall the structural appearance of the dye was reflected in this analysis. In analysis of metabolite sample (Figure 5b) peak at 616.20 cm⁻¹ was responsible for C-S stretching and peak at 1,339.21 cm⁻¹ for SOO symmetric stretching, suggesting that there may be an intermediate which was a sulphur-containing compound. Peaks at 1,407.84 cm⁻¹ for C-O stretching plus O-H deformation in -COOH and 1,634.17 cm⁻¹ for COO stretching in aryl carboxylic acid defines that there may be formation of carboxylic acid derivative. Peak at 2,923.98 cm⁻¹ was indicating the O-H stretching. Peak

value of 2,867.82 cm⁻¹ demonstrated symmetrical stretching C-H in alkane. Notably there was absence of the peak responsible for azo (NON) group proposing that removal of azo bond after dye decolorization. Overall both these analytical studies (HPTLC and FTIR) point out that there was structural degradation of the dye molecule which leads to the decolorization.

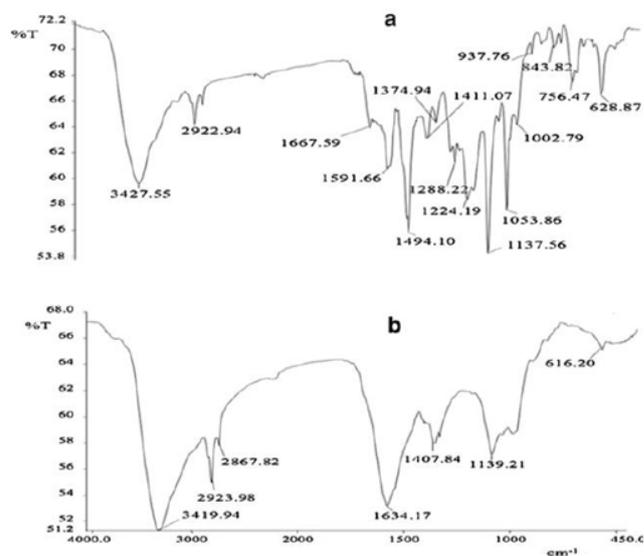


Figure 5. FTIR analysis of (a) parent dye compound and (b) metabolites obtained after dye degradation

5. Conclusion

Bacterial decolorization proves to be a very efficient method for complete decolorization of Orange 16, Acid Red, Congo Red, Fast Red, Mordant Black and Reactive Black. The two bacterial strains *Bacillus* sp. strain ETL-A and *Pseudomonas* sp. strain ETL-B are potential for decolorization and partial degradation of azo dye Orange 16 under static condition. The induction of azoreductase by Orange 16 was observed in both the strains. An enzyme azoreductase would be a good material for further research on the enzymological mechanism of dye Decolorization in bacteria. Further, the strains ETL-A and ETL-B are proved to be efficient for conventional wastewater treatment and bioremediation of recalcitrant azo dyes under normal parameters. However the complete degradation of Orange 16 is under progress from these two strains.

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