Appl Biochem Biotechnol (2010) 160:1241–1253
DOI 10.1007/s12010-009-8592-1

Aerobic Biodegradation Pathway for Remazol Orange by *Pseudomonas aeruginosa*

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Received: 28 December 2008 / Accepted: 23 February 2009 /
Published online: 10 March 2009

Abstract Removal of azo dyes from effluent generated by textile industries is rather difficult. Azo dyes represent a major class of synthetic colorants that are mutagenic and carcinogenic. *Pseudomonas aeruginosa* grew well in the presence of Remazol Orange (RO) and was able to decolorize and degrade it. In the present study, the decolorization and degradation efficiency using single culture *P. aeruginosa* with RO and textile wastewaters is studied. The elucidation of decolorization pathway for *P. aeruginosa* is of special interest. The degradation pathway and the metabolic products formed during the degradation were also predicted with the help of high performance liquid chromatography, Fourier transform infrared spectroscopy, and nuclear magnetic resonance spectroscopy analysis. The data show the cleavage of the azo dye RO to form both methyl metanilic acid and 4-aminobenzoic acid after decolorization and finally to oxidation forms benzoic acid, alkenes, aldehydes, and alkynes. The organism was able to decolorize the dye RO and wastewater effectively to the maximum of 82.4% and 62%, respectively.

Keywords *Pseudomonas aeruginosa* · Decolorization · Biodegradation · Metabolic pathway · Textile wastewater treatment · Growth kinetics

Introduction

Azo dyes are widely used in printing, paper making, and cosmetic industries for their versatility. Industrial effluents often contain residual dyes, which not only affect water quality, but also become threats to public health because certain azo dyes or their metabolites are highly toxic and carcinogenic. Besides, azo dyes are generally considered to be recalcitrant in conventional wastewater treatment processes [1, 2].

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It has been found that some microorganisms can transform azo dyes into colorless products. Bacterial degradation of azo dyes is often initiated by an enzymatic step that involves cleavage of azo linkages with the aid of an azoreductase and an electron donor [3]. As the azoreductase in some microorganisms can catalyze the reductive cleavage of azo groups, they have potential advantages in developing bio-treatment methods of wastewater containing azo compounds [4]. Azoreductase activity has been identified in several bacteria, such as *Xenophilus azovorans* KF46 [5], *Pseudomonas luteola* [6], *Rhodococcus* [7], *Shigella dysenteriae* type I [8], *Klebsiella pnumoniae* RS-13 [9], and *Clostridium perfringens* [10].

Generally, it is assumed that the first step in the biodegradation of azo compounds is the reduction to the corresponding amines, a reaction catalyzed by azoreductase. Then, the resulting aromatic amines are further degraded aerobically [11]. Moreover, conventional aerobic wastewater treatment processes, such as activated sludge, cannot usually efficiently remove the color of azo dyes since these compounds are often recalcitrant aerobically [12]. So, there is still a need to develop a novel biological process leading to a more effective cleanup of azo dye contamination.

The present study demonstrates the efficiency of aerobic bacterial culture *P. aeruginosa* to decolorize and degrade commercial azo dye Remazol Orange (RO) and textile wastewater. Metabolic pathway for decolorization and degradation of RO was proposed based on identification of the metabolites by high performance liquid chromatography (HPLC), Fourier transform infrared spectroscopy (FTIR), and nuclear magnetic resonance spectroscopy (NMR). The combination of major and minor metabolic pathways for RO will indicate probable intermediate metabolites of dye. This potential of the microorganism will definitely prove cost effective in wastewater treatment.

Materials and Methods

Bacterial Strain and Culture Conditions

RO decolorizing bacterium was isolated from a sewage treatment plant facility at Chennai by enrichment culture technique. A basal salt medium (containing the following in g/L: NH_4Cl 0.5 g, KH_2PO_4 0.4 g, K_2HPO_4 1.2 g, glucose, and peptone 0.2 g each, pH 7.0) at 30°C was used in the study. A mixed culture that showed quick and stable decolorization activity was transferred to the newly prepared synthetic medium. After five successful transfers, it was plated on synthetic medium containing 50 mg/L of RO. The bacterial colonies forming a clear zone were collected for further studies.

Identification of Strain

Pseudomonas strain was identified according to morphological and biochemical criteria such as the gram reaction, flagella type, catalase activity, oxidase activity, oxidation fermentation test, and the production of diffusible fluorescent pigment. The identified strain is deposited with IMTECH, Chandigarh, India (no. 6463) as *Pseudomonas aeruginosa.*

Azo Dyes

Commercial grade azo dye was obtained from Color Chem India Ltd and used without any purification.

Analysis

Chemical oxygen demand (COD) and ammonia are measured as per standard methods [13] and amines by colorimetric method [14].

Decolorization Studies

Cells of *P. aeruginosa* were harvested from early stationary phase cultures by centrifugation (10,000×*g*, 10 min) and transferred to 100 mL basal medium containing a known concentration of dye to give a cell concentration of 1.5 g dry weight per liter. Batch decolorization experiment was performed in static condition (without agitation), where typical dissolved oxygen levels were in the range of 0.1–0.8 mg/L. Samples were collected at regular intervals and analyzed for residual color.

Color Measurement

The residual color in the original and treated samples was analyzed by measuring the absorbance of the samples at 494 nm wavelength (absorbance maxima of RO) using a UV–visible spectrophotometer (Shimadzu 160A, Japan). Dye concentration in experimental samples was calculated from a calibration graph of RO.

Nuclear Magnetic Resonance Spectroscopy

The samples containing RO after decolorization were extracted with ethyl acetate and dried using a rotary evaporator. The dried samples were dissolved in $CdCl_3$ and transferred to NMR tubes. The ^1H spectrum of the dried samples was recorded using a 200-MHz Thermos electron corporate UV2 spectrometer to observe the structural transformation in the dye molecules present in the synthetic dye wastewater during treatment.

Fourier Transform Infrared Spectroscopy

FTIR (Perkin-Elmer) was used for investigating the changes in surface functional groups of the samples, before and after microbial decolorization. The dried sample was mixed with KBr of spectroscopic grade and made into pellets at a pressure of about 1 MPa of 10 mm diameter and 1 mm thickness. The prepared pellets were scanned by exposing it to mid-infrared light (400–4,000 cm^{-1}). The spectra were then subjected to baseline correction and the bands were studied to quantify the changes in the chemical structure of the dye RO.

High Performance Liquid Chromatography

Samples were extracted with hexane and the intermediates formed due to bacterial decolorization, and degradation of a dye RO was analyzed by HPLC. The HPLC (Jasco PU2089 plus) was equipped with a quaternary gradient pump system and a multi-wavelength detector. The samples were eluted in gradient mode using a C18 column (Agilent ZORBAX ODS 5 μm). The mobile phase consisted of water and acetonitrile in the ratio of 70:30, 60:40, and 70:30, respectively (all HPLC grade). The flow rate of mobile phase was 0.5 mL/min. The samples were filtered with a 0.2-μm membrane filter and about 20 μL of the filtered samples was injected into the HPLC.

Results and Discussion

Decolorization Profile of *P. aeruginosa*

Decolorization profile of *P. aeruginosa* was studied by growing the organism in a medium containing 50 mg/L of RO under static condition for 24 h after which it was switched to shaking mode. The decolorization profile as shown in Fig. 1 clearly proves that *P. aeruginosa* was able to decolorize the dye RO very effectively. It may also be observed that decolorization occurred essentially in the first 24 h after which there was little change in color. Chen [15] has studied a bacterial decolorization of azo dyes and considered it as nongrowth-associated entity. Under static condition, *P. aeruginosa* effectively decolorized 82.4% of dye RO within 24 h. Biodegradation of dyes was monitored by the removal of organics (COD). The maximum organic removal was 93% within 144 h.

Effect of Dye Concentration

The decolorization by *P. aeruginosa* was after 24 h of static incubation, resulting in only 90% of color removal in the presence of 50 mg/L of RO (Table 1). During static decolorization, no essential growth was observed due to insufficient dissolved oxygen. Azo dyes serve as alternative energy source as electron acceptor results in dye reduction. Bacterial decolorization required an oxygen-deficient condition for enzymatic transformation. As the dye concentration increases, there is no significant reduction in decolorization as well as in COD removal. The higher concentration of the dye up to 200 mg/L was easily decolorized and degraded.

Kinetic Parameter for Decolorization of RO

To evidently reveal the difference of decolorization capability of *P. aeruginosa* to RO, time courses of dye concentration in batch cultures with various initial dye concentrations were used for determination of kinetic parameters. Considering the whole cells of

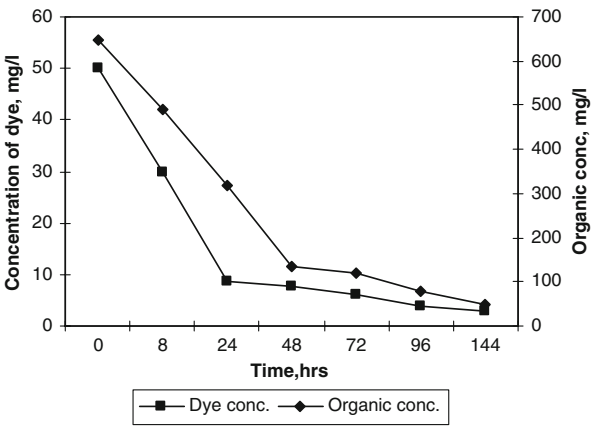


Fig. 1 Decolorization and organic removal profiles of *P. aeruginosa*

Table 1 Effect of dye concentration on decolorization and degradation of RO by *P. aeruginosa.*

Dye concentration, mg/L	Dye decolorization, %	Organic removal, %
50	90	93
100	93	93
200	94	92

P. aeruginosa as biocatalysts, the Monod kinetic model was applied to depict this decolorization. The decolorization rate V can be expressed as follows:

$$V = \frac{-\mathrm{d}S}{\mathrm{d}t} = \frac{V_{\mathrm{m}}S}{K_{\mathrm{S}} + S'} \tag{1}$$

where S and t are the dye concentration and elapsed time, respectively. Parameters V_{m} and K_{s} are the maximal decolorization rate and system coefficient, respectively. Then Eq. 1 can be integrated to obtain:

$$V_{\mathrm{m}}t = (S_0 - S) + K_{\mathrm{s}} \ln\left(\frac{S_0}{S}\right) \tag{2}$$

where S_0 is initial concentration of the dye solution.

Kinetic parameters K_{s} and V_{m} can then be evaluated from the intercept and slope of the least-squared linear plot $\ln(S_0/S)/(S_0-S)$ versus $t/(S_0-S)$ (or similar to Hanes–Woolf plot; Shuler and Kargi [16]. Using these kinetic parameters, time courses of residual dye concentrations were then predicted from Eq. 2 (i.e., predicted curves in Fig. 2).

The Monod kinetic model was applied to the RO decolorization data when no appreciable amount of product (i.e., decolorization intermediate) has accumulated. Gumport et al. [17] have shown that the simple Monod kinetic model was applied only to the initial velocity of the biocatalyzed reaction. As mentioned earlier, the RO decolorization was not possible beyond 200 mg/L using *P. aeruginosa*. The K_{s} value for RO as given in Table 2 indicates a parallel series of binding preference with intracellular azoreductase in *P. aeruginosa*.

Decolorization of Textile Wastewater Using *P. aeruginosa*

To verify that the *P. aeruginosa* is able to express the azoreductase enzyme, experiments were conducted by supplementing nutrients along with textile wastewater under static

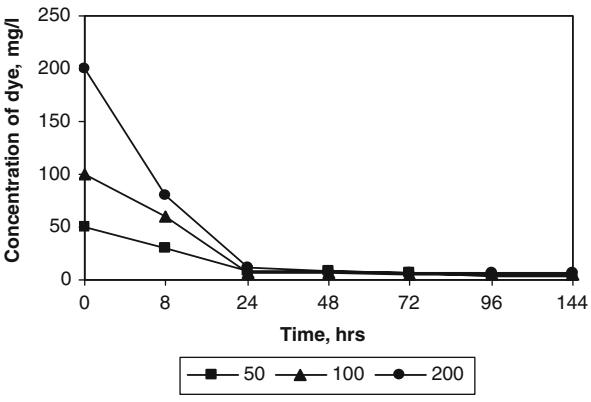


Fig. 2 Time course of residual concentration of RO during decolorization by *P. aeruginosa*

Table 2 Kinetics parameters for decolorization of RO by *P. aeruginosa.*

Parameters	RO
Slope	0.004
Intercept	0.006
r^2	0.996
V_m, mg/L/min	4.5
K_s, mg/L	1.65

condition for 24–48 h after which it was switched to shaking mode. The decolorization profile of the strain is shown in Fig. 3. The results clearly prove that *P. aeruginosa* was able to decolorize the wastewater effectively both in sterile and unsterile conditions. The unsterile wastewater showed the highest decolorization (76%) than with sterile (62%) which needed longer incubation time for decolorization. This shows that the presence of co-cultures in the wastewater enhances the decolorization and *P. aeruginosa* gains a survival advantage via decolorization to compensate the cost of competition exclusion due to the expression of the intracellular azoreductase activity. This is the reason why co-existence of co-cultures enhanced the decolorization [18]. *P. aeruginosa* was able to remove organics from the wastewaters (80–90%) as depicted in Fig. 3.

During the wastewater treatment using *P. aeruginosa*, the pH of the treated sample containing azo dyes was alkaline due to the accumulation of amines and ammonia (Table 3). When textile wastewater was treated using *P. aeruginosa* both under sterile and unsterile conditions, about 75 to 150 mg/L of amines is formed with 7–35 mg/L of ammonia. The wastewater under unsterile condition was more efficiently decolorized in the presence of *P. aeruginosa*.

Analysis of Products Formed

FTIR Analysis

Comparison of FTIR spectra of dye with extracted metabolites clearly indicated the biodegradation of the parent dye by *P. aeruginosa*. Peaks at 0 h of RO (Fig. 4) showed the

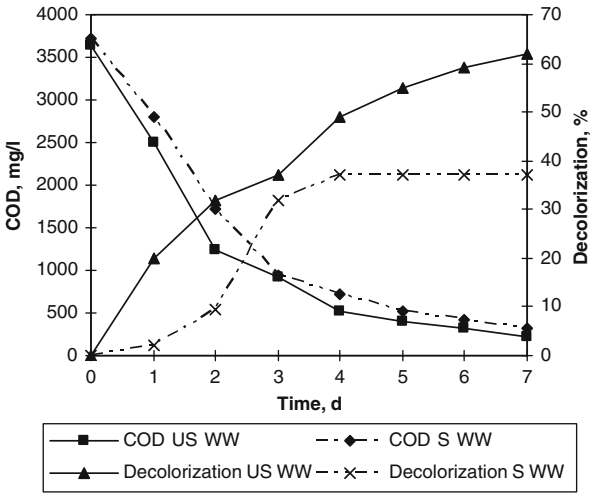


Fig. 3 Decolorization and treatment of textile wastewater by *P. aeruginosa*

Table 3 Characterization of wastewater after treatment.

Samples	Condition	pH	Amines, mg/L	Ammonia, mg/L
Textile wastewater (sterile)	Initial	7.0	75	7
	Experiment	8.2	150	15
Textile wastewater (unsterile)	Initial	7.0	75	35
	Experiment	8.4	200	23

presence of aromatic compounds in association with –C=O, –C=C, –C–O–C, and –C–O–O–C. Majority of absorption peaks observed prior to decolorization were either shifted or disappeared during decolorization. The bands characteristic of azo bond in the region of 1,500–1,550 cm^{-1} of RO (a) was absent in the decolorized samples (b, c) indicating that –N=N– may be oxidized with the disappearance of the parent dye. Hu et al. [19] reported

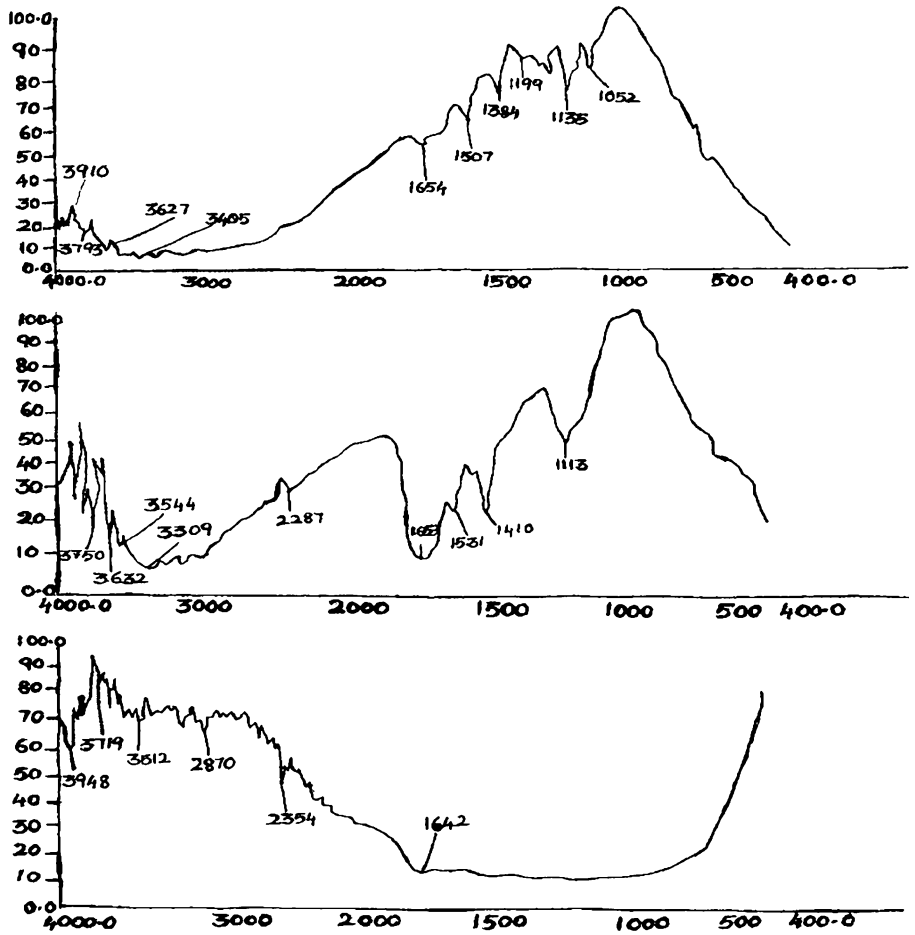


Fig. 4 FTIR data for Remazol Orange decolorization by *P. aeruginosa*. Broad range of peak at 1,653.60 cm^{-1} (aliphatic, aromatics, and aldehydes); peak at 2,287.06 cm^{-1} (C≡N); peak at 2,870.84 cm^{-1} (amines, alkynes, and alkenes); broad range of peak at 3,309.05 cm^{-1} (amides and amines)

that the peak observed at 1,500 cm^{-1} characteristic due to azo bond (–N=N–) and the benzene ring at 1,400 to 1,600 cm^{-1} decreased with time. Peaks represented the symmetric stretching at 1,199 cm^{-1} and asymmetric stretching at 1,050 cm^{-1} for CN. The stretching vibration between C–N showed a bond at 1,384 cm^{-1} representing the nature of the aromatic amine group present in the parent dye compound, while 1,507 and 3,405 cm^{-1} represent the presence of free NH group in RO. Bands observed after decolorization in the range of 3,379 to 3,437 cm^{-1} may be attributed to carboxylic acids. Stretching between N=N of the azo group was represented at 1,654 cm^{-1}.

The FTIR spectrum after decolorization followed by aerobic degradation showed significant change in position of peaks when compared to parental RO. After decolorization, the broth was extracted and the metabolites were analyzed by FTIR. A new peak at 1,653 cm^{-1} pointed towards the formation of substituted naphthalene products with C=O stretching. A new peak at 1,410 cm^{-1} represents C–H deformation of alicyclic CH_2, whereas peaks at 3,544 and 3,309 cm^{-1} were for substituted amines. Samples after aerobic treatment showed a peak at 1,642 cm^{-1} for C=O stretching. A peak at 2,870 cm^{-1} showed the presence of compounds like alkynes and alkenes. This confirms that oxidation has occurred effectively to degrade the aromatic amines to simpler compounds.

NMR Analysis

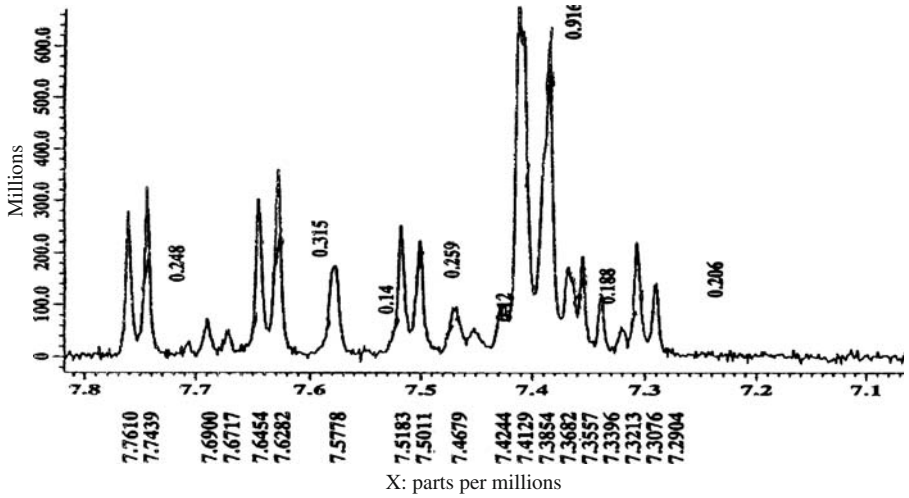
The ^1H NMR spectrum of the dye RO showed doublet signals centered at δ 7.76–δ 7.35, singlet signals centered at δ 7.33, and triplet signals centered at δ 7.32, δ 7.30, and δ 7.29 from hydrogen of the aromatic rings, $H_2C{=}CH{-}CH_2$ groups, aliphatic, and side chain compounds like –S, –N, –C=O, and –O present in the dye (Fig. 5a).

The ^1H spectrum of the sample after aerobic treatment showed no aromatic signals observed in the final treated extract (Fig. 5b) in comparison with the ^1H spectrum of the RO dye, indicating a complete biotransformation of the dye molecules into metabolites with the signals completely different from the RO dye as describe above. The multiplet centered at δ 6.89, δ 6.79, δ 6.77, and δ 6.76 were formed from the hydrogen of the compound $H_2C{=}CH{-}CH_2$. The doublet signals centered at δ 6.36 and δ 6.32 and at δ 6.13 and δ 6.11 and the triplet signals centered at δ 4.25, δ 4.24, and δ 4.23 show the presence of aliphatic and side chain compounds like –S, –N, –C=O, and –O.

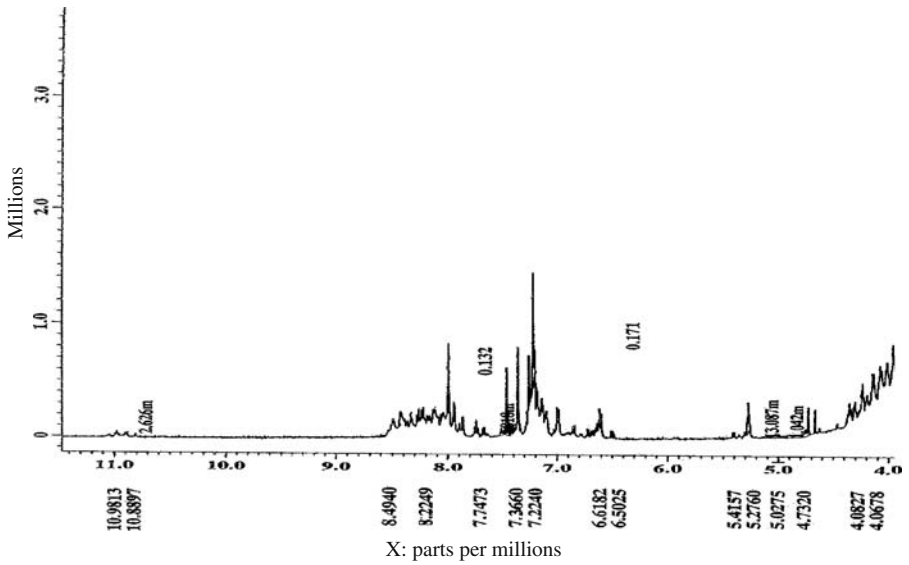
HPLC Analysis

The HPLC analysis of the sample before treatment (Fig. 6) shows major peak at retention time of 2 and 4 min (0.4 and 2 units), and after microaerophilic treatment, the peaks at retention time of 2 and 4 min have reduced considerably (0.1 and 1 units), showing the structural change in the original compound and a new peak is seen at the retention time of 7 min (Fig. 6) and identified as 4-aminobenzoic acid by matching with the standards. From this, we can predict that the dye RO under microaerophilic treatment by *P. aeruginosa* could be probably broken down to 4-aminobenzoic acids and another unknown compound after decolorization (breakage of N=N).

This upon further degradation at aerobic condition forms simple aromatics like benzoic acid and aliphatic and other simpler compounds, which are known to be recalcitrant. This is supported by the new peaks formed at the retention time of 11 and 13 min and the change in initial peak height at the retention time of 2 and 4 min (0.1 and 0.9 units) in the HPLC data, whereas the peak at the retention time of 7 min has increased considerably to about 1.2 units (Fig. 6). This indicates that after aerobic treatment with the same organism the



a NMR of Remazol Orange dye



b NMR after decolorization

Fig. 5 **a** NMR of Remazol Orange dye. **b** NMR after decolorization. ^{1}H NMR data for RO decolorization by *P. aeruginosa*. Peak at δ=3.2078–4.0827 (multiplet; CH_2–N, CH_2=O, CH_2–S); δ=5.2760 (singlet; C=CH); δ=7.2240–7.3660 and 8.2249–8.4940 (multiplet; aromatic compounds); δ=10.8869–10.9813 (doublet; –COOH)

compounds were further oxidized to simpler compounds like benzoic acid, alkenes, aldehydes, and alkynes (Fig. 6).

Probable Pathway of Degradation

The data obtained from HPLC, FTIR, and NMR analysis of RO show the presence of aromatic compounds. After decolorization with microaerophilic treatment, the predominant

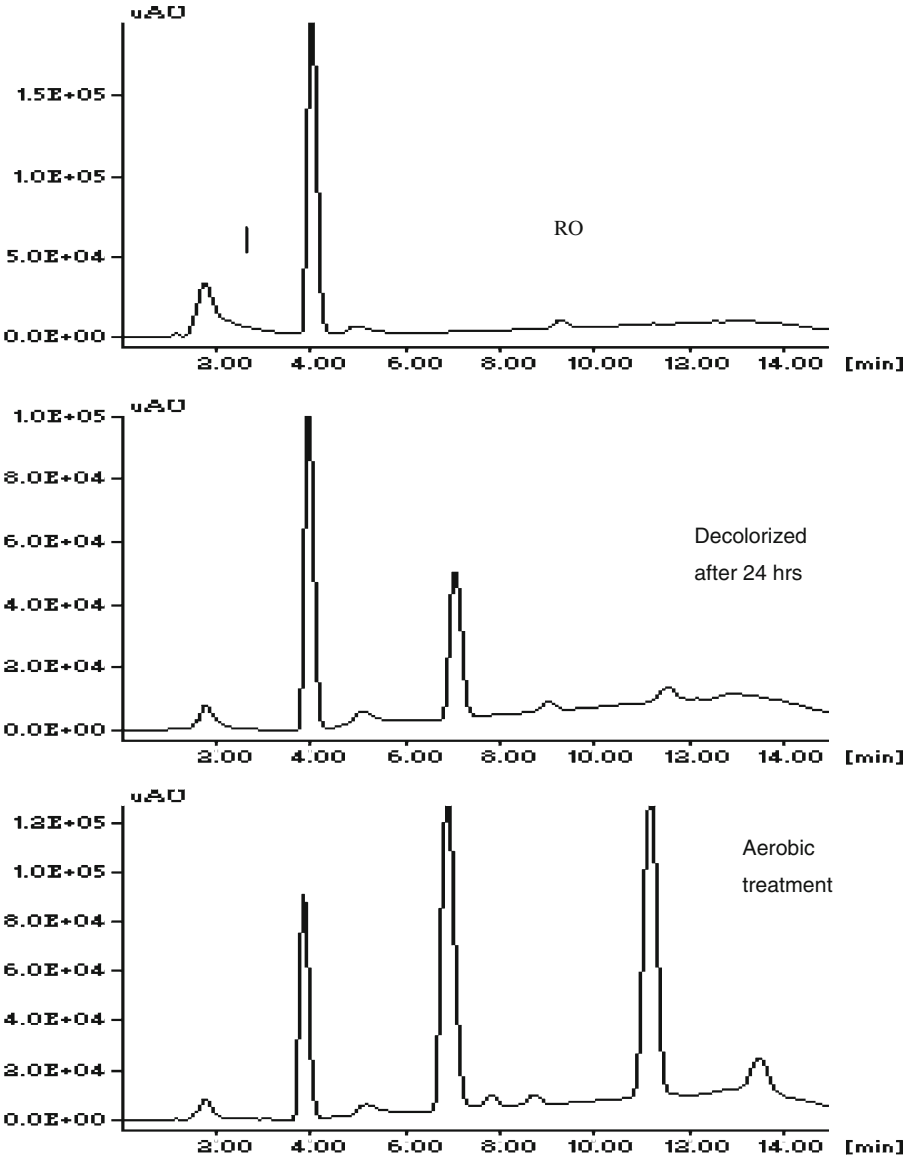


Fig. 6 HPLC chromatogram for the untreated, decolorized, and the aerobically degraded dye Remazol Orange using *P. aeruginosa*

peak at the retention time of 2 and 4 min in HPLC chromatogram showed a considerable reduction and led to the formation of a new peak at the retention time 7 min, which was 4-aminobenzoic acid. The FTIR data also revealed the presence of aromatic rings (1,500 cm^{-1}) and amines (3,000 cm^{-1}) in the sample, which was further confirmed from the information obtained from the NMR data that support the presence of aromatic compounds and amine groups along with other breakdown products. From the above details, we could say that the cleavage of –N=N– in the dye during decolorization has led to

the formation of aromatic amines that could possibly be 4-aminobenzoic acid and another aromatic amine (methyl metanilic acid).

This upon further degradation at aerobic condition forms simple aromatic, aliphatic, and other compounds. Furthermore, the peak patterns in FTIR (Fig. 4) show the presence of amines, alkynes, and alkenes which is confirmed by the NMR (Fig. 5) report. From this, we predict that the dye RO is broken under microaerophilic treatment by *P. aeruginosa* either into methyl metanilic acid and 4-aminobenzoic acid after decolorization. Bin et al. [20] have obtained *N N* dimethyl-*p*-phenylenediamine (retention time of 2.51–3.01 min) and 2-aminobenzoic acid (retention time of 8.45–9.21 min) after complete reduction of Methyl Orange. After aerobic treatment with the same organism, it is further oxidized to simpler compounds like benzoic acid, alkenes, aldehydes, and alkynes (Fig. 6). Based upon the FTIR, NMR, and HPLC results, a possible mechanism for the decolorization and biodegradation of RO by *P. aeruginosa* is proposed as presented in Fig. 7, which shows that complete reduction of the azo bond may occur in sequential microaerophilic–aerobic system.

Discussion

P. aeruginosa isolated from a sewage treatment plant was used for the decolorization of RO as well as for the elucidation of metabolic pathway of biodegradation. The microorganism was able to decolorize RO in sequential static–aerobic condition. The ^{1}H NMR and FTIR spectra after aerobic degradation of RO by *P. aeruginosa* showed that the dye was broken down to intermediates. The probable biodegradation products of RO were identified as methyl metanilic acid and 4-aminobenzoic acid. Nortemann et al. [21] characterized and

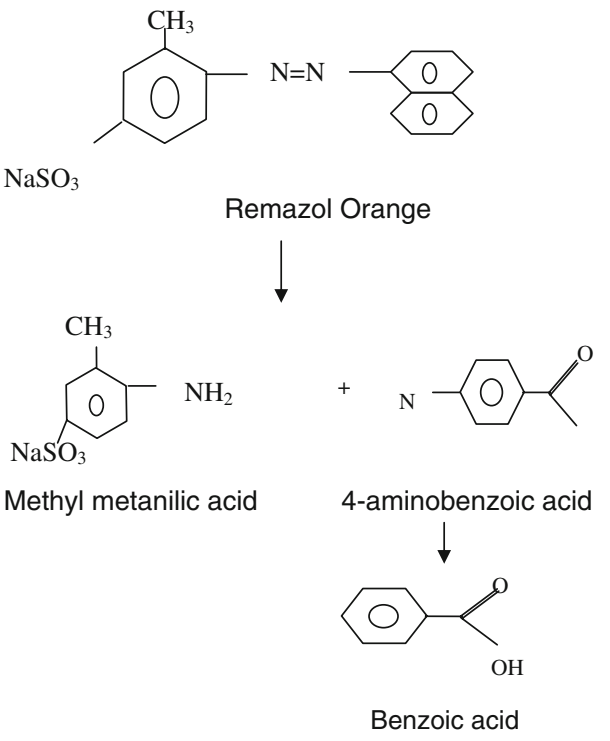


Fig. 7 Proposed pathway of degradation of the dye Remazol Orange by *P. aeruginosa*

identified the metabolic product of acid yellow 9 biodegraded by *Pseudomanas fluorescence* and reported 2,4 dihydroxy benzene sulfonic acid as the product of biodegradation. The HPLC analyses of the sample after aerobic treatment showed that the initial peak (2 min RT) in RO is removed totally and another peak (4 min RT) is reduced. Biodegradation in the aerobic stage may result in the formation of polar derivaties (e.g., aldehydes, carboxylic acids) having lower aromaticity as suggested by Nortemann et al. [21] in biodegradation of 6-aminonaphthalene-2sulfonate and Supaka et al. [22] in Remazol brilliant orange dye.

Conclusions

Thus, *P. aeruginosa* can grow under both static as well as aerobic conditions resulting in complete breakdown of aromatic moieties of dye structure. The decolorization efficiency needed peptone and glucose, which are vital to the decolorization triggered by enzymatic reduction of azo bonds. Based on the FTIR, NMR, and HPLC data, decolorization of RO by *P. aeruginosa* seems to involve a complete breakdown of azo bond to form two aromatic amines, as well as further degradation of the azo bond.

Acknowledgments We thank the director of the National Environmental Engineering Research Institute, Nagpur, India for allowing us to publish this work. We are grateful to DBT, New Delhi for funding the project.

References

1. Bisschops, A. E., & Spanjers, H. (2003). *Environmental Technology, 24*, 1399–1411. doi:10.1080/09593330309385684.
2. Weisburger, J. H. (2002). *Mutation Research, 506*, 9–20.
3. Yeh, M. S., & Chang, J. S. (2004). *Journal of Chemical Technology, 79*, 1354–1360.
4. Nachiyar, C. V., & Rajakumar, G. S. (2005). *Enzyme and Microbial Technology, 36*, 503–509. doi:10.1016/j.enzmictec.2004.11.015.
5. Blumel, S., Knackmuss, H. J., & Stolz, A. (2002). *Applied and Environmental Microbiology, 68*, 3948–3955. doi:10.1128/AEM.68.8.3948-3955.2002.
6. Hu, T. L. (2001). *Water Science and Technology, 43*, 261–269.
7. Chang, J. S., & Lin, C. Y. (2001). *Biotechnology Letters, 23*, 631–636. doi:10.1023/A:1010306114286.
8. Ghosh, D. K., Mandal, A., & Chaudhuri, J. (1992). *FEMS Microbiology Letters, 98*, 229–234. doi:10.1111/j.1574-6968.1992.tb05519.x.
9. Wong, P. K., & Yuen, P. Y. (1996). *Water Research, 30*, 1736–1744. doi:10.1016/0043-1354(96) 00067-X.
10. Rafii, F., & Coleman, T. (1999). *Journal of Basic Microbiology, 39*, 29–35. doi:10.1002/(SICI) 1521-4028(199903) 39:1<29::AID-JOBM29>3.0.CO;2-W.
11. Sandhya, S., Padmavathy, S., Swaminathan, K., Subrahmanyam, Y. V., & Kaul, S. N. (2005). *Process Biochemistry, 40*, 885–890. doi:10.1016/j.procbio.2004.02.015.
12. Chang, J., Chou, C., Lin, P., Ho, J., & Hu, T. L. (2001). *Water Research, 35*, 2841–2850. doi:10.1016/S0043-1354(00) 00581-9.
13. APHA. (1998). *Standard methods of water and wastewater* (19th ed.). Washington D.C.: American Public Health Association.
14. Jeffery, G. A., Bassett, D., Mendhad, J., & Denney, R. C. (1991). *Vogel's text book of quantitative chemical analysis*. New York: ELBS with Logman.
15. Chen, B. Y. (2002). *Process Biochemistry, 38*, 437–446. doi:10.1016/S0032-9592(02) 00151-6.
16. Shuler, M. L., & Kargi, F. (1992). *Bioprocess Engineering Basic Concepts*. New Jersey, USA: Prentice Hall.
17. Gumport, R. I., Jones, A., Mintel, R., Rhodes, C., & Koeppe, R. E. (1990). *Student's Comparison of Stryer's Biochemistry*. New York: W.H. Freeman.

18. Chen, B. Y., Chen, S. Y., Lin, M. Y., & Chang, J. S. (2006). *Process Biochemistry, 38*, 437–446. doi:10.1016/S0032-9592(02) 00151-6.
19. Hu, C., Yu, J. C., Hao, Z., & Wong, P. K. (2003). *Applied Catalysis. B, Environmental, 42*, 47–55. doi:10.1016/S0926-3373(02) 00214-X.
20. Bin, Y., Jiti, Z., Jing, W., Cuihong, D., Hongman, H., Zhiyong, S., et al. (2004). *FEMS Microbiology Letters, 236*, 129–136. doi:10.1111/j.1574-6968.2004.tb09638.x.
21. Nortemann, D. B., Baumyanten, J., Rast, H. G., & Knackmuss, H. J. (1986). *Applied and Environmental Microbiology, 52*, 1195–1202.
22. Supaka, N., Juntongjin, K., Damronglerd, S., Delia, M. L., & Strehaiano, P. (2004). *Chemical Engineering Journal, 99*, 169–176. doi:10.1016/j.cej.2003.09.010.