Journal of Environmental Science and Management 24-2: 54-61 (December 2021) ISSN 0119-1144

Biodegradation of Carcinogenic Reactive Azo Dyes by Indigenous Bacterial Consortium X5RC5

ABSTRACT

Reactive azo dyes are considered as a major source of water and soil contamination. Carcinogenicity and the recalcitrant nature of these dyes is a worldwide problem. Exclusion of these dyes from the effluent is necessary for a clean and green environment. A bacterial consortium X5RC5 was developed for the effective removal of two of the primary reactive azo dyes utilized widely in textile industries (reactive orange 3R and reactive red HE7B). The consortium includes two indigenous bacterial isolates, Lysinibacillus macroides and Stenotrophomonas acidaminiphila, from textile effluent. The X5RC5 completely degraded the reactive orange 3R in 4 days and reactive red HE7B in 5 days of incubation periods. In two days, more than 50% degradation was observed for both dyes. Biodegradation of these dyes was affirmed through the UV-Vis spectra and Fourier-transform infrared spectroscopy (FTIR) analysis. This investigation advances the utilization of consortium X5RC5 as a biological tool for the bio-handling of effluent containing dyes.

Keywords: *reactive azo dyes, carcinogenicity, bacterial consortium, biodegradation, FTIR*

Ravi Kant Rahi[1*]
Varsha Gupta[1]

[1] JECRC University, Ramchandpura, Sitapura Industrial Area Extn, Vidhani Village, Jaipur, 303905, India

* corresponding author: ravikrahi.20@gmail.com

INTRODUCTION

Among other industries, it remains on top for contaminating the environment (*Awomeso et al. 2010*). Effluent released from textile industries is considered a major pollutant for the environment. Textile effluent is a blend of colors, cleansers, starch, fade, and other destructive synthetic concoctions (*Vilaseca et al. 2010*). These chemicals contribute to the elevated levels of pH, COD, BOD, TDS, TSS, turbidity, and the poisonous nature of the textile effluent (*Elango 2017*).

The fundamental segment of textile effluent is the textile dyes. Half of the dyes produced in the world are utilized in textile industries. The Indian dying market is a major producer of dyes; it produce around 6.6% of dyes utilized throughout the world (*Teli 2008*). Reactive azo dyes are the most widely recognized colorant utilized in textile industries.

During the handling and completion of fabric, around 10 to 25% of dye particles are unable to tie with the fabric because of immersion and other condition factors and therefore, are directly discharged into the textile effluent (*Ahmed et al. 2012*). Metabolites of these synthetic concoctions are noxious and adversely affect, not only the aquatic life and their habitat, but also humans.

Human health are at high risk due to the harmful effects of azo dyes being used in industries. *Rannung et al.* (*1992*) reported about the skin hypersensitivities and asthmatic problems in workers of textile industries. *Birhanli et al.* (*2005*) also revealed the teratogenic impacts of the synthetic concoctions on human embryos. In studies on benzidine (cleaved product of azo dyes) is observed as a carcinogen for the human urinary bladder (*Gičević et al. 2019*). *Feng et al.* (*2012*) also observed the carcinogenic effect of azo dyes on human intestine and the toxicological impact on gut micro biota.

As reactive azo dyes are considered human carcinogen, the removal of these dyes from textile effluent is, thus, essentially required.. Physical and chemical strategies are not satisfactory for the removal of dyes as they produce auxiliary contamination, which needs separate treatment. Biological treatment of reactive azo dyes can be the best alternate option. The present examination assesses the capability of indigenous bacterial consortium in degradation of reactive azo dyes.

According to *Shah* (*2014*) bacterial species like *Pseudomonas aeruginosa, Pseudomonas putida* and *Bacillus subtilis* have been reported effective

in degradation of textile dyes. Bacterial species, *Brevibacillus choshinensis* and *Streptococcus faecalis* have also been reported to be efficient in the degradation of dyes (*Meena et al. 2014*). In other studies, bacterial consortium also exhibited the great potential in biological removal of dyes. *Khehra et al.* (*2005*) developed a bacterial consortium of *Bacillus cereus*, *Pseudomonas putida, Pseudomonas fluorescence* and *Stenotrophomoas acidaminiphila*, which showed the high rate of biodegradation of textile dyes. This study assessed the capability of indigenous bacterial consortium in degrading reactive azo dyes.

MATERIALS AND METHODS

The Effluent samples were collected from the textile industrial cluster of Sanganer (Jaipur), India. Two different types of dyes, a a monoazo (reactive orange 3R) and a diazo (reactive red HE7B) were collected from the Sanganer textile industries. These dyes are extensively used in the Sanganer industrial area.

The enrichment culture technique was used to isolate the indigenous potent dye degrading bacterial isolates. Minimal salt media (MSM) was used to cultivate the bacterial isolate (*Shah et al. 2014*). An aliquot (5 mL) of filtered effluent sample was transferred into a 250-mL conical flask that contained pre-sterilized MSM with 100 mg L^{-1} of reactive red HE7B. Flasks were then kept for incubation on a rotary shaker at 30°C and 120 rpm. Potent bacterial isolates were screened in nutrient broth medium with 100 mg L^{-1} of reactive red HE7B. Bacterial isolates was carried out based on their cellular, morphological and biochemical properties. Potent isolates were identified at species level using the 16S rRNA technique (*Šlosarčíková et al. 2020*).

A total of 21 bacterial isolates were screened for dye degradation. Among all, two bacterial isolates coded as X5 and RC5 showed the highest degradation. These isolates were identified through their cellular characteristics, biochemical properties, and 16S rRNA sequencing techniques. Bacterial isolate X5 identified as *Lysinibacillus macroides* strain X5 (*Rahi and Gupta 2018*) and RC5 identified as *Stenotrophomonas acidaminiphila* strain RC5 (*Rahi and Gupta 2019*). Accession number of isolates NR-114920.1 and NR-025104.1 was obtained after the successful submission of the sequence into National Center for Biotechnology Information (NCBI) GenBank.

Consortium preparation was a significant step as different development rate and physiological conduct of bacterial isolates may influence the process. Pure colonies of indigenous potent bacterial isolates of *Lysinibacillus macroides* and *Stenotrophomonas acidaminiphila* were suspended into Luria Bertani (LB) broth at static conditions. After the incubation of 24 hr at 37°C, bacterial cells were harvested through centrifugation at 10,000 rpm for 15 min with controlled temperature condition (4°C). Cells were then resuspended into a 0.9% saline solution. The optical density of the culture suspensions was fixed to achieve a concentration of 1.5×10^8 cells mL^{-1} (0.5 MacFarland) for uniform cell appropriation. The consortium was then prepared by mixing the culture suspensions of both isolates in 1:1 proportion, which gives 1.5×10^8 cells mL^{-1} of each isolate in suspension. This consortium was coded as X5RC5.

For the estimation of dye degradation, % calculation method was adopted (*Adedayo et al. 2004*). The consortium was inoculated into the nutrient broth amended with 100 mg L^{-1} of reactive dyes (reactive orange 3R and reactive red HE7B), purchased from local dyeing industries in 500 mL conical flasks in static condition. After every 24 hr of incubation, 5 mL of culture suspension was extracted aseptically and centrifuged at 15,000 rpm for 5 min to exclude the bacterial slurry (*Sharma et al. 2013*). The obtained supernatant was used for spectrophotometric analysis at λ_{max} = 490 nm and 550 nm for RO 3R, λmax and RR HE7B, respectively. Un-inoculated culture of the same concentration (100 mg L^{-1}) was used for control (blank). Degradation of dyes was expressed in terms of percentage and was calculated through the given formula.

$$\text{Degradation \%} = \frac{\text{Initial Absorbance Value} - \text{Final Absorbance Value}}{\text{Initial Absorbance Value}} \times 100 \quad (1)$$

where,

Initial Absorbance Value = Absorbance value of un-inoculated (Blank) medium

Final Absorbance Value = Absorbance value of inoculated medium

UV-Vis spectra and FTIR (Fourier Transformation Infrared Spectroscopy) techniques were used to determine the biodegradation of dyes. The light absorption of each dye within the visible range i.e. 400 nm to 700 nm was recorded by the UV-Vis spectrophotometer at 10 nm interval. Spectra for the control (0 hr), 24 hr degradation and after complete degradation (~5 days) were measured and compared. For the FTIR analysis, culture suspension of the 0 hr and degradation products was centrifuged at 10,000 rpm for 10 min and supernatant was mixed into 200 mg of potassium bromide. These samples were then examined at 400-4,000 cm^{-1} wavelength with 1 cm^{-1}

resolution. FTIR spectra of the control and degradation products was analyzed for biodegradation.

RESULTS AND DISCUSSION

Isolation of dye-degrading potent bacteria from textile effluent has been reported earlier (*Roy et al. 2016*) and has indicated the regular adaption of bacteria within the harmful conditions (*Khadijah et al. 2009*). Consortium X5RC5 was prepared from *Lysinibacillus macroides* strain X5 and *Stenotrophomonas acidaminiphila* strain RC5 isolated from the textile effluent. Reactive orange 3R degrade at a quicker rate and mineralize at the fourth day while reactive red HE7B required one more day to degrade completely. Difference in the degradation rate indicated the variability in structure of the dyes. The complex structure of the dyes and the position of the azo bond affect the complete degradation rate of azo dyes (*Phugare et al. 2011*).

In this study, the consortium of potent bacterial isolates were tested against the monoazo reactive orange 3R ($C_{20}H_{17}N_3NA_20_{11}S_3$) and diazo reactive red HE7B ($C_{52}H_{26}Cl_2N_{14}Na_8O_{26}S_8$) at a final concentration of 100 mg L^{-1} of the dyes. In earlier observations, dye mass concentration of a typical textile effluent was reported as 10-50 mg L^{-1} (*Karim et al. 2018*). Thus, 100 mg L^{-1} concentration of dye was used throughout the experiment.

Consortium X5RC5 showed the complete mineralization of reactive red HE7B dye after 5 days while reactive orange was completely mineralized on day 4 (**Table 1**). Reactive orange 3R degraded 45.87% after 24 hr of incubation while reactive red HE7B was degraded 38.21% only. Both dyes were degraded by more than 50% after 48 hr of incubation with consortium X5RC5 (**Figure 1**). In the first 24 hr of incubation, reactive orange 3R has a higher degradation rate than red HE7B. On the second day, the degradation rates were comparable for reactive orange 3R and reactive red HE7B, which indicated that the consortium used the same degradation mechanism for both dyes. Effectiveness of a consortium in degrading reactive azo dyes over a monoculture was reported earlier by *Waghmode et al.* (*2012*). Consortium X5RC5 was observed to be more efficient in the degradation of reactive azo dyes compared with other consortium reported earlier. The consortium of *Enteobacter* sp. EC3 (*Wang et al. 2009*) and *Bacillus* sp. YZU1 (*Wang et al. 2013*) degraded 92.60% and 95.00% of reactive azo dyes, respectively, even after 5 days of incubation. A consortium of *Pseudomonas* sp. SKG (*Garg et al. 2012*) degraded 90% of reactive orange dye within 4 days of incubation.

Table 1. Comparative analysis of degradation percentage/ days by consortium X5RC5 for reactive red HE7B and reactive orange 3R.

	Time	Initial OD	Observed OD	% Degradation
Reactive Orange 3R	Day 1		0.25	45.87
	Day 2		0.19	58.21
	Day 3	0.464	0.12	73.12
	Day 4		0	100
	Day 5		0	100
Reactive Red HE7B	Day 1		0.69	38.21
	Day 2		0.51	53.97
	Day 3	1.124	0.23	78.81
	Day 4		0.04	96.32
	Day 5		0	100

The UV-Vis spectra of the control and the treated

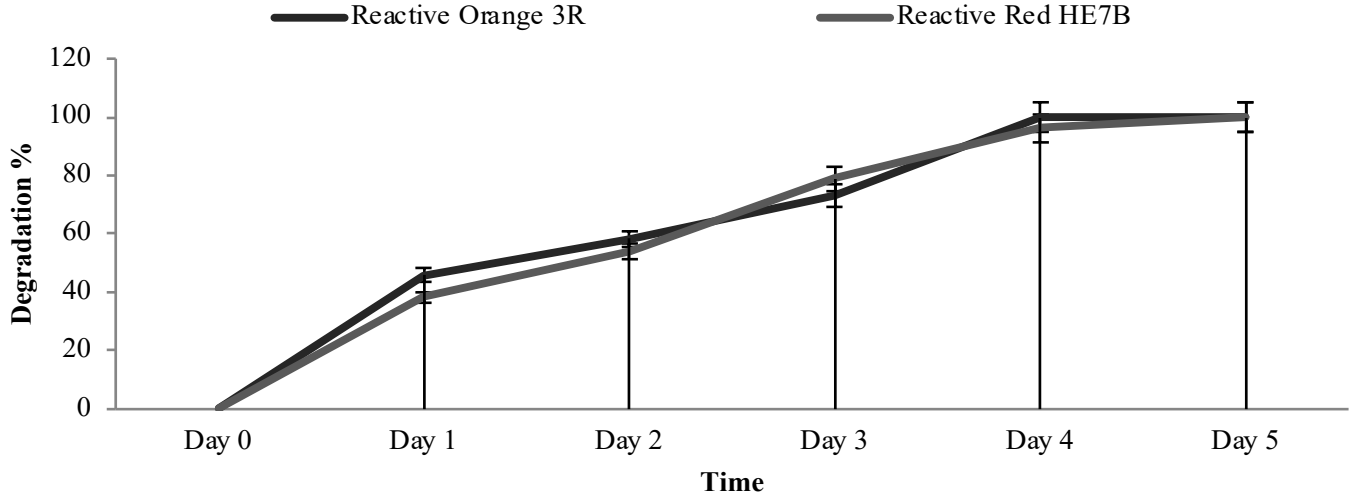


Figure 1. Degradation of reactive orange 3R and reactive red HE7B through X5RC5 consortium incubated at 37°C and initial dye concentration used was 100 mg L^{-1}.

samples were analyzed to detect the biodegradation of azo dyes. Reactive red HE7B and reactive orange 3R control dye samples were compared with decolorized broth spectra of the 1st and 5th day. Decrement of absorbance in the 1st day samples showed the initiation of biodegradation while the 5th day samples, in which the maximum absorbance peak almost disappeared, indicated the complete mineralization (i.e., into their simplest form) of the reactive azo dyes by biological activity. Therefore, it can be concluded that azo bonds were broken down during the metabolic process of the bacterial isolates, which were ultimately responsible for the conversion of primary chromophore into secondary amines (**Figure 2**). Biodegradation of reactive azo dyes was confirmed through the UV-Vis spectral analysis. UV-Vis spectral analysis of reactive red (*Jadhav et al. 2007*), reactive red 2 (*Kalyani et al. 2009*), reactive orange 3R (*Sahasrabudhe et al. 2011; Šlosarčíková et al. 2020*) has been reported earlier, which is in accordance with these findings.

The peak observed in group-specific region of the IR spectra from the control of orange 3R, particularly at 3,487.23 cm^{-1} indicated the vibration of N-H stretching and 3098.94 cm^{-1} indicated the C-H stretching in CH2 and CH3 group. The peak at 1686.21 cm^{-1} indicatesdthe N-N stretching vibration in the azo linkage (**Figure 3a**). Peak observed at 1038.32 cm^{-1} region is due to the aromatic nature of reactive orange 3R while the 910.51 cm^{-1} peak indicated the stretching vibration of S-O group. The peak at 687.82 cm^{-1} region indicated the stretching vibration of C-N group. These obtained peaks conformsedwith the nature of reactive orange 3R dye.

The spectra of the degradation product showed various peaks in the fingerprint region. Peak observed at 767.82 cm^{-1} indicated C-N stretching vibration of an aromatic ring, 1,076.15 cm^{-1} peak showed the bending vibration of C-H, 1,327.21 cm^{-1} peak indicated the C-O stretching and peak at 1,478.83 cm^{-1} showed the stretching vibration of N-O. Strong peak observed at 1,690.03 cm^{-1} region is due to the bending vibration of C-C and peak at 2,752.21 shows the stretching vibration of C-H. Disappearance of a peak at 1,656 cm^{-1} region can be attributed to the breaking of the azo linkage (**Figure 3b**).

In the FTIR spectra of the control for red HE7B, peak at 3,592.21 cm^{-1} region indicated the presence of the hydroxyl group (-OH), the 2379.47 cm^{-1} region confirmed the C-O stretching in the sample and the stretching vibration of azo group (-N=N-) was presented by the peak observed at 1,590.72 cm^{-1} region (**Figure 4a**). The aromatic ring stretching (-C=C-) is shown by the multiple peaks observed at 1520.43 to 1606.32 cm^{-1} region. Peaks observed at 1150.04 to 1270.71 cm^{-1} region indicated the stretching vibration of S-O group. A strong peak in 623.85 cm^{-1} region indicated the –O-N=O stretching vibration and peak observed at 1,054.93 cm^{-1} region indicated the C-H vibration of benzene ring.

FTIR analysis of reactive red HE7B degradation product illustrated the changes in peaks including formation of new peaks in comparison with the control (**Figure 4b**). Peak of the azo group (-N=N-) completely disappeared in the spectra of the metabolites, which confirmed the breakdown of azo group. Several peaks,

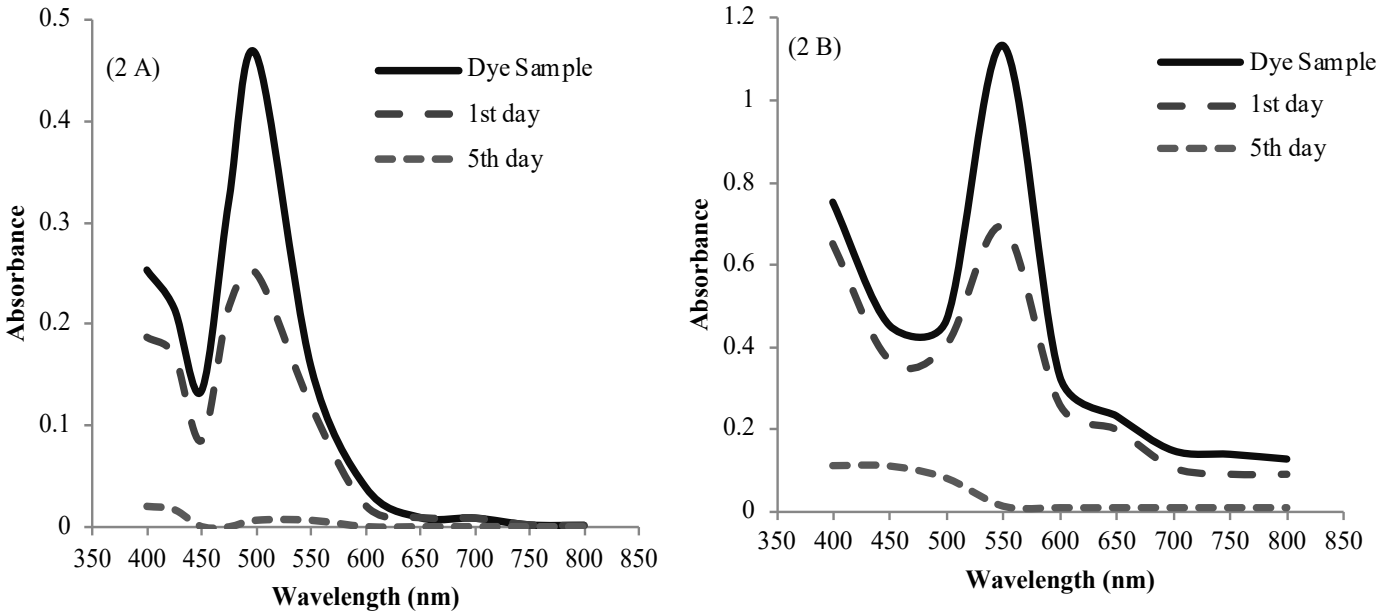
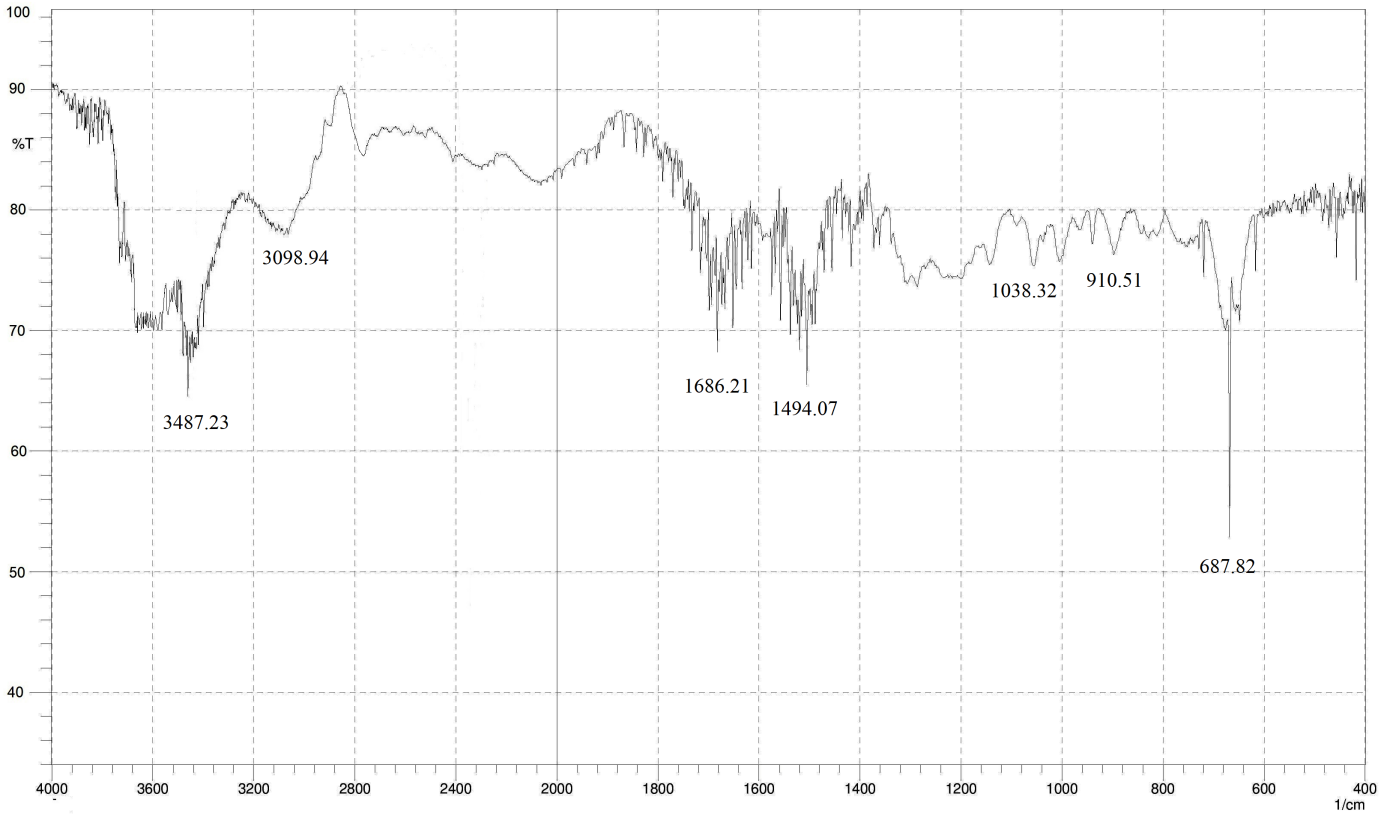


Figure 2. UV-Vis spectral analysis of reactive orange 3R dye sample, 1st day and 5th day culture (2A) and reactive red HE7B dye sample, 1st day and 5th day culture (2B).

3a



3b

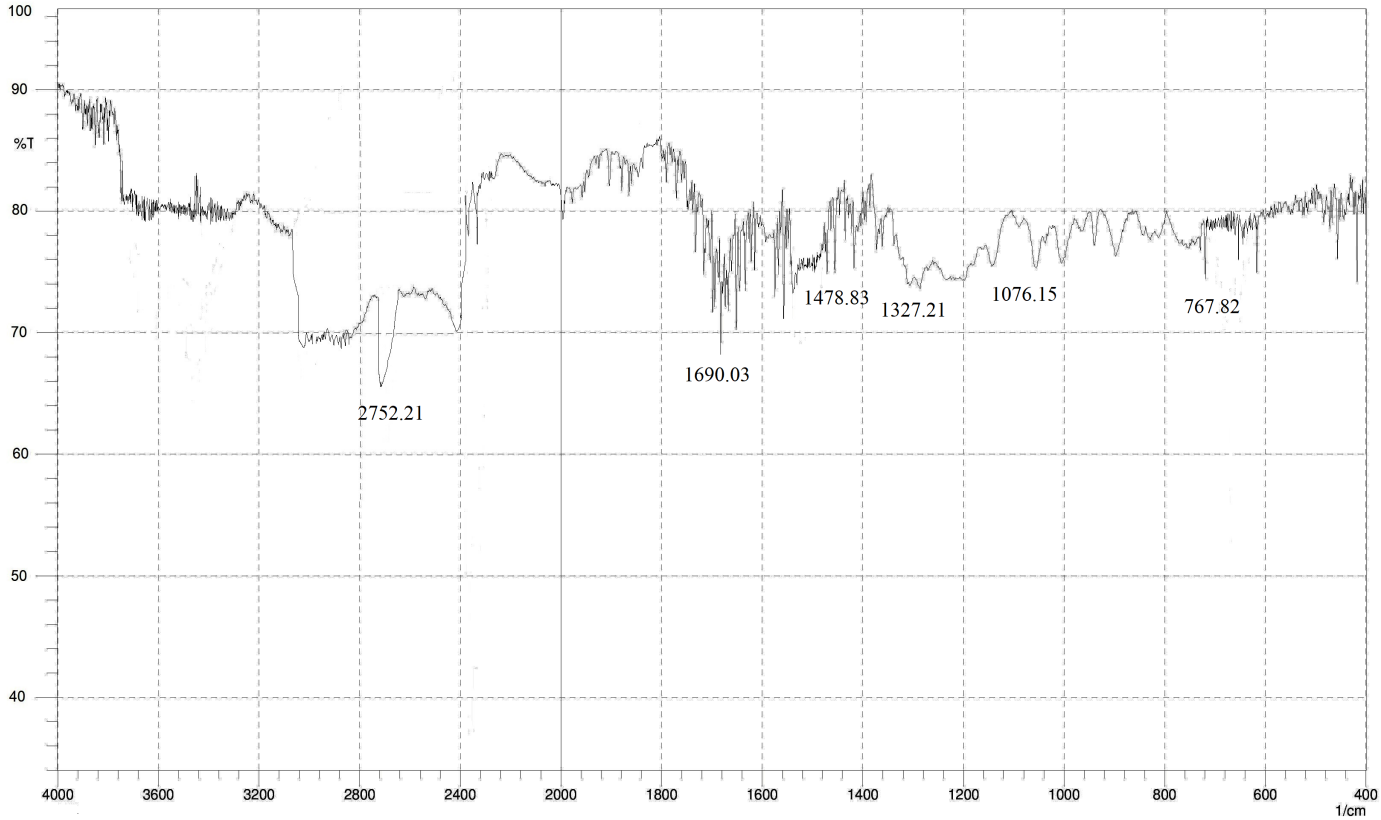


Figure 3. Fourier Transformation Infrared Spectroscopy (FTIR) analysis of control reactive orange 3R (3a) and metabolites obtained after complete degradation of reactive orange 3R (3b) by consortium X5RC5.

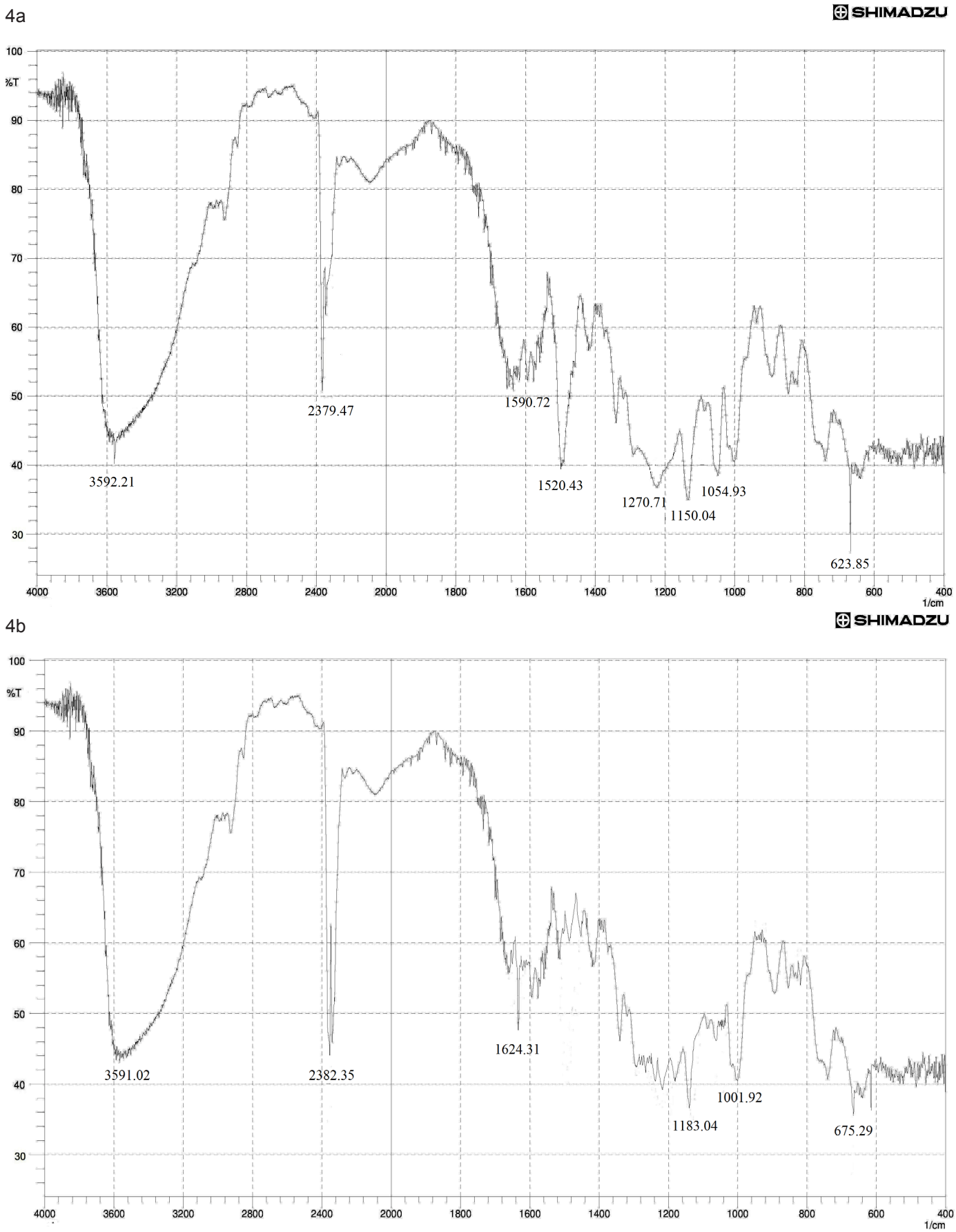


Figure 4. Fourier Transformation Infrared Spectroscopy (FTIR) analysis of control reactive red HE7B (4a) and metabolites obtained after complete degradation of reactive red HE7B (4b) by consortium X5RC5.

which were observed in the spectra of the control like the stretching vibration of S-O group disappeared and new peaks were observed at 1,150.67 to 1,270.34 cm^{-1} region. Presence of aromatic ring in the degradation product is indicated by peak observed at 1,624.31 cm^{-1}. A broad range band in 3,000 to 3,600 cm^{-1} region indicated the stretching of primary and secondary amines (-N-H). The FTIR analysis of the control and the degradation products confirmed that the degradation of reactive red HE7B was mediated through consortium X5RC5 (**Figure 4**). Additionally, it was confirmed that degradation was achieved due to the cleavage of the azo bonds.

It has been reported that breakdown of azo bond is the main cause of biodegradation (*Shobana and Hangam 2012*) Similar pattern in FTIR spectra has been reported previously for reactive red HE7B (*Bankole et al. 2017*) and for reactive orange 3R (*Ekambaram et al. 2016*). According to *Telke et al.* (*2008*), azo dyes showed the peak of azo bond in approximately 1500 cm^{-1} to 1650 cm^{-1}. Changes in peak or disappearance of a peak in this region confirmed the breakdown of azo bonds while the formation of new peaks corresponded to secondary amine formation.

Change in peak or formation of new peaks and disappearing of peaks in FTIR spectra of degraded product evident to the breakdown of azo bonds and formation of secondary amines in case of azo dyes.

CONCLUSION AND RECOMMENDATIONS

This study showed that a consortium containing indigenous bacterial isolates is useful in the treatment of effluents containing reactive azo dyes. The bacterial consortium X5RC5 effectively degraded the reactive orange 3R and reactive red HE7B dyes within five days of incubation period. This consortium can be utilized as a bioremediation tool in the degradation of other harmful synthetic substances, such as low density polyethene, fipronil, microcystin, chlorothalonil, pyrene, phenanthrene, other classes of dyes and colorant used in several industries to decrease ecological hazard.

REFERENCES

Adedayo, O., Javadpour, S., Taylor, C., Anderson, W.A. and Moo-Young. 2004. "Decolourization and Detoxification of Methyl Red by Aerobic Bacteria from a Wastewater Treatment Plant". *World Journal of Microbiology and Biotechnology* (20): 545- 550.

Ahmed, T.F., Sushil, M. and Krishna, M. 2012. "Impact of Dye Industrial Effluent on Physicochemical Characteristics of Kshipra River, Ujjain City, India". *International Research Journal of Environmental Sciences* 1(2): 41-45.

Awomeso, J.A., Taiwo, A.M., Gbadebo, A.M. and Adenowo, J.A. 2010. "Studies on the Pollution of Waterbody by Textile Industry Effluents in Lagos, Nigeria". Journal of *Applied Sciences in Environmental Sanitation* (5): 353-359.

Bankole, P.O., Adekunle, A.A. and Obidi, O.F. 2017. "Mycodecolorization of Reactive Red HE7B Dye by *Achaetomium strumarium* and *Aspergillus flavus* and Shelflife Determination". *Cogent Environmental Science* 3(1); 457-463.

Birhanli, A. and Ozmen, M. 2005. "Evaluation of Toxicity and Teratogenity of Six Commercial Textile Dyes Using the Frog Embryo Teratogenesis Assay – Xenopus". *Drug and Chemical Toxicologies* 28(1): 51-65.

Ekambaram, S.P., Perumal, S.S. and Annamalai, U. 2016. "Decolorization and Biodegradation of Remazol Reactive Dyes by *Clostridium* species". *3 Biotech* 6(1): 389- 395.

Elango, G. 2017. "Physico-Chemical Parameters of Textile Dyeing Effluent and Its Impacts with Case Study". *International Journal of Research in Chemistry and Environment* 7(1): 17-24.

Feng, J., Cerniglia, C.E. and Chen, H. 2012. "Toxicological Significance of Azo Dye Metabolism by Human Intestinal Microbiota". *Frontiers in Bioscience* (4): 568–586.

Garg, S.K., Tripathi, M., Singh, S.K. and Tiwari, J.K. 2012. "Biodecolorization of Textile Dye Effluent by *Pseudomonas putida* SKG-1 (MTCC 10510) Under the Conditions Optimized for Monoazo Dye Orange II Color Removal in Simulated Minimal Salt Medium". *International Biodeterioration and Biodegradation* (74): 24-35.

Gičević, A., Hindija, L., and Karačić, A. 2020. Toxicity of Azo Dyes in Pharmaceutical Industry. In: Badnjevic, A., Škrbić R., Gurbeta Pokvić L. (eds) CMBEBIH 2019. IFMBE Proceedings Vol 73. Springer, Cham. https://doi.org/10.1007/978-3-030-17971-7_88

Jadhav, J.P., Parshetti, G.K., Kalme, S.D. and Govindwar S.P. 2007. "Decolorization of Azo Dye Red by *Saccharomyces cerevisiae* MTCC 463". *Chemosphere* (68): 394-400.

Kalyani, D.C., Telke, A.A., Dhanve, R.S. and Jadhav, J.P. 2009. "Ecofriendly Biodegradation and Detoxification of Reactive Red 2 Textile Dye by Newly Isolated *Pseudomonas* sp. SUK1". *Journal of Hazardous Materials* (163): 735–742.

Karim, M.E., Dhar, K. and Hossain, M. T. 2018. "Decolorization of Textile Reactive Dyes by Bacterial Monoculture and Consortium Screened from Textile Dyeing Effluent".

Journal of Genetic Engineering and Biotechnology 16 (2): 375-380

Khadijah, O., Lee, K. and Mohd-Faiz, F.A. 2009. "Isolation, Screening and Development of Local Bacterial Consortia with Azo Dyes Decolourising Capability". *Malaysian Journal of Microbiology* (5): 25–32.

Khehra, M.S., Saini, H.S., Sharma, D.K., Chadha, B.S. and Chimni, S.S. 2005. "Decolorization of Various Azo Dyes by Bacterial Consortia". *Dyes Pigments* (67): 55–61.

Meena, S.G., Krishna-Mohan, M. and Ghosh, P. 2014. "Production and Purification of a Hyperthermostable Chitinase from *Brevibacillus formosus* BISR-1 Isolated from the Great Indian Desert Soils". *Extremophiles* 18 (2): 451–462.

Phugare, S.S., Kalyani, D.C., Surwase, S.N. and Jadhav, J.P. 2011. "Ecofriendly Degradation, Decolorization and Detoxification of Textile Effluent by a Developed Bacterial Consortium". *Ecotoxicology and Environmental Safety* 74 (5): 1288-1296.

Rannung, U., Bramstedt, H. and Nilsson, U. 1992. "The Presence of Genotoxic and Bioactive Components in Indigo Dyed Fabrics: A Possible Health Risk". *Mutation Research* (282): 45-53.

Roy, R., Fakhruddin, A.N., Khatun, R., Islam, M.S., Ahsan, M.A. and Neger, A.J. 2010. "Characterization of Textile Industrial Effluents and its Effects on Aquatic Macrophytes and Algae". *Bangladesh Journal of Scientific and Industrial Research* 45 (1): 79-84.

Sahasrabudhe, M. and Pathade, G. 2011. "Biodegradation of Sulphonated Azo Dye C.I. Reactive Orange 16 by *Enterococcus faecalis* Strain YZ 66". *European Journal of Experimental Biology* (1): 163-173.

Shah, M.P., Kavita, A.P., Sunu, S.N. and Darji, A.M. 2014. "Microbial Degradation and Decolorization of Reactive Dyes by *Bacillus* sp. ETL-1979". *American Journal of Microbiological Research* 2 (1):16-23.

Sharma, N., Chaterjee, S. and Bhatnagar, P. 2013. "Assessment of Physicochemical Properties of Textile Wastewater and Screening of Bacterial Strains for Dye Decolourisation". *Universal Journal of Environmental Research and Technology* (3): 345-355.

Shobana, S. and Hangam, B.T. 2012. "Biodegradation and Decolorization of Reactive Orange 16 by *Nocardiopsis alba* Soil Isolate". *Journal of Bioremediation and Biodegradation* 3 (6): 213-219.

Šlosarčíková, P., Plachá, D., Malachová, K., Rybková, Z. and Novotný, Č. 2020. "Biodegradation of Reactive Orange 16 Azo Dye by Simultaneous Action of *Pleurotus ostreatus* and the Yeast *Candida zeylanoides*". *Folia Microbiologica* (20): 139-146.

Teli, M.D. 2008. "Textile Coloration Industry in India". *Coloration Technology* 124 (1): 1-3.

Telke, A., Kalyani, D.C., Jadhav, J.P. and Govindwar, S.P. 2008. "Kinetics and Mechanism of Reactive Red 141 Degradation by a Bacterial Isolates *Rhizobium radiobacter* MTTC 8161". *Acta Chimica Slovenica* (55): 320-329.

Vilaseca, M., Gutiem, M.C., Grimau, V.L., Mesas, M.L. and Crespi, M. 2010. "Biological Treatment of a Textile Effluent After Electrochemical Oxidation of Reactive Dyes". *Water Environment Research* 82 (2): 176-181.

Waghmode, T.R., Kurade, M.B., Lade H.S. 2012. "Decolorization and Biodegradation of Rubine GFL by Microbial Consortium GG-BL in Sequential Aerobic/ Microaerophilic Process". *Applied Biochemistry and Biotechnology* 167 (6): 1578-1594.

Wang, H., Zheng, X.W., Su, J.Q., Tian, Y., Xiong, X.J. and Zheng, T.L. 2009. "Biological Decolorization of the Reactive Dyes Reactive Black 5 by a Novel Isolated Bacterial Strain *Enterobacter* sp. EC3". *Journal of Hazardous Materials* (171): 654-659.

Wang, Z.W., Liang, J.S. and Liang, Y. 2013. "Decolorization of Reactive Black 5 by a Newly Isolated Bacterium *Bacillus* sp. YZU1". *International Biodeterioration and Biodegradation* (76): 41-48.