Songklanakarin J. Sci. Technol.
34 (1), 23-30, Jan. - Feb. 2012

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Optimization of process parameters for enhanced biodegradation of acid red 119 by *Bacillus thuringiensis* SRDD

Shailesh R. Dave* and Riddhi H. Dave

Department of Microbiology and Biotechnology, School of Sciences, Gujarat University, Ahmedabad 380 009, India.

Received 27 May 2010; Accepted 2 December 2011

Abstract

Developed *Bacillus thuringiensis* SRDD showed degradation of C.I. Acid red 119 and growth under the extreme condition of temperature 70°C, pH 3-8, heavy metals concentration of 0.8 mM, NaCl up to 900 mM and 1000 ppm dye. Cotton seed, caster cake and corn cake powders were found to be better and cheaper nutrient supplements for the *Bacillus thuringiensis* SRDD for biodegradation as compared to molasses. After development of the culture and the process, more than 99% degradation was achieved in less than 2 hrs of contact time even on 18th cycles of addition of 100 ppm AR-119 dye. The developed process showed AR-119 biodegradation rate as high as 220 mg L^{-1} h^{-1}, which is found to be 130 times more as compared to the reported data. U.V., FTIR, TLC and HPLC analysis data confirmed biodegradation ability of the *Bacillus thuringiensis* for AR-119.

Keywords: acid red 119, Azo dye, *Bacillus thuringiensis,* enhanced biodegradation, process parameter

1. Introduction

Wastewater effluents from the textile and other dyestuff industries usually contain significant amounts of synthetic dyes. In India, an average mill discharges about 1.5 million liters of dye-contaminated effluent per day, which leads to chronic and acute toxicity (Sandhya *et al.*, 2005; Arami *et al.*, 2006). In discharged effluent, azo dyes are present in larger quantity, which can easily pass through the normal water treatment procedures (Stolz, 2001; Pearce *et al.*, 2003; Pandey *et al.*, 2007) resulting in unacceptable water. The colored wastewater treatment methods based on physical and chemical procedures are effective but suffer from shortcomings such as high expenditure, intensive energy requirements and formation of perilous by-products (Chakraborty *et al.*, 2003; Georgiou *et al.*, 2003) whereas, biological degradation of these dyes does not face such problems. Microbial methods have recently received much attention owing to its ease of application, low cost and environmental benignity (Fu and Viraraghavan, 2001; Senthilkumar *et al.*, 2006; Crini, 2006; Silva *et al.*, 2006; Batzias and Sidiras, 2007).

As dyes are designed to be stable and long-lasting colorants, they are not easily biodegraded. Nevertheless, many researchers have demonstrated partial decolorization and biodegradation of dyes by pure and mixed cultures of bacteria (Stolz, 2001; Robinson *et al.*, 2001; Dave and Dave, 2009), fungi (Fu and Viraraghavan, 2001; Mielgo *et al.*, 2002; Kadpan and Kargi, 2002; Wafaa M. Abd El-Rahim and Moawad, 2003), yeasts (Ramalho *et al.*, 2004; Ramalho *et al.*, 2005) and algae (Acuner and Dilek, 2004; Mohan *et al.*, 2002). Azo bonds are readily decolorized in anaerobic condition (Isik and Sponza, 2003). Although the phenomenon of anaerobic azo reduction is unanimously accepted, some aspects of reaction mechanism remain to be clarified. Limited data are available on biotreatment of C.I. Acid Red 119 (AR-119) acidic diazo dye and that to with an initial dye concentration of only 40 mg L^{-1} by consortium (Khehra et al., 2005). There is only one report on decolorization of AR-119 by *B.*

* Corresponding author.
Email address: shaileshrdave@yahoo.co.in

thuringiensis SRDD (Dave and Dave, 2009). In this context, biodegradation of AR-119 dye by the *B. thuringiensis* was exploited with following specific objectives: a) Optimization of bio decolorization parameters, b) Efficacy determination of biodegradation in column study, and C) confirmation of biodegradation.

2. Materials and Methods

2.1 Dye and media

The diazo dye C.I. Acid Red 119 used for the bio decolorization studies was procured from Appex dyestuff industry, Ahmedabad, India. All media components and chemicals used in the studies were of analytical grade.

2.2 Culture and Inoculum preparation

Isolation, identification and selection of *Bacillus thuringiensis* SRDD is detailed elsewhere (Dave and Dave, 2009). The 16S rRNA gene sequence of the isolate is deposited in GenBank with accession number EF063149. The culture was routinely maintained on 100 ppm AR-119 containing nutrient agar slant. For inoculum preparation actively growing 16 hrs old *Bacillus thuringiensis* SRDD from 100 ppm AR-119 containing nutrient agar slant, was inoculated in 100 mL nutrient broth (NB). Inoculated flask was incubated on environmental orbital shaker agitating at 150 rpm at 35± 2°C temperature for 18-20 hrs. From this, actively growing $1x10^8$ cells mL^{-1} in NB were used for various decolorization studies. Petroff-Hausser counting chamber was used for the cell count.

2.3 AR-119 Decolorization study and quantification

Decolorization study in aqueous medium was performed by inoculating 10% v/v (10^8 cells mL^{-1}) actively growing *B. thuringiensis* in 100 mL NB containing 300 ppm of AR-119 dye, which was incubated for 18-20 hrs at steady condition at 35±2°C. One set of the flasks without inoculation was kept as control. After incubation of 1, 2, 3, 4, 5, 6, 7 and 24 hrs, inoculated and uninoculated broths were centrifuged at 10,000 g for 15 minutes and supernatant was assayed at 530 nm wavelength and percent decolorization was calculated as mentioned by Dave and Dave (2009).

2.4 Optimization of process parameters

The present studies were undertaken to optimize various process parameters such as agitation, dye concentration, inoculum amount, incubation temperature, pH, nutrient concentration, medium supplements, metal ions, and salt concentration for decolorization and degradation of AR-119. Detail of the experimental conditions studied is given in Table 1. If otherwise mentioned all the experiments were performed using 10% v/v $1x10^8$ actively growing cells mL^{-1}, 300 ppm dye, pH 7.0±0.2 and 35±2°C incubation temperature under static condition.

2.4.1 Column study

To check process feasibility, studies were extended in glass column of 38 cm length, 7.2 cm inner diameter and 7.3 cm outer diameter. Lower end of the column was fitted with Glass wool. The column was packed with 3130 glass beads of 0.5 cm average diameter, weighing 517.6 g. The developed culture was allowed to grow for 4 days for the biofilm formation in the column. At the end of every 24 hrs used NB was drained and new sterile NB was added in the column. In each decolorization cycle 100 ppm of AR-119 dye containing 1:10 diluted NB was added continuously and allowed to react for 3.0±0.5 hrs.

2.5 Analytical methods for degradation studies of AR-119

2.5.1 UV-Visible Spectroscopy

Decolorization and degradation study was performed from the supernatant obtained by centrifugation of the broth at 10,000 g for 15 minutes. Untreated and treated samples

Table 1. Detail of the experimental parameters and their range studied for the characterization of the *B. thuringiensis* for process optimization.

Parameters	Range of the parameters studied
Reaction flask volume with shaking (150 rpm) and static conditions	100, 250, 500 and 1000 mL flask
Dye concentration	15, 25, 50, 100, 200, 300, 500 and 1000 ppm
Biomass	0.5, 1.1, 2.2 and 4.5×10^9 cells mL^{-1}
Incubation temperature	25, 35, 45, 55 and 70°C
pH	3, 5, 7 and 8
Nutrient broth concentration	1, 0.1, 0.05, 0.025 and 0.01 X
Medium supplement	Synthetic medium, caster cake, cotton seed and molasses
Heavy metals (0.8mM)	Copper, Chromium, Nickel and Zinc
Salt (NaCl) concentration	100, 300, 500 and 900 mM

were analyzed by UV-Visible spectrophotometer, V-530, Jasco, Japan. Changes in the absorption spectra were studied and compared with the control.

2.5.2 Thin layer chromatography (TLC)

The decolorized broth was centrifuged at 10,000 g for 15 minutes and MACHEREY–NAGEL, ALUGRAM^{R} SIL G/ UV_{254} 0.20 mm silica gel 60 with fluorescent indicator UV_{254} was used to confirm biodegradation of the selected dye from the collected supernatant. Propanol:butanol:water in 12:6:6 proportions were used as solvent system for TLC.

2.5.3 Fourier transform infrared spectroscopy (FTIR)

The biodegraded product of AR-119 was analyzed by Fourier transform infrared spectroscopy on Shimadzu FTIR-8400S spectrometer and compared with control dye.

2.5.4 High performance liquid chromatography (HPLC)

HPLC analysis was carried out at 530 nm for extracted metabolites from treated and untreated samples with a UV diode array detector equipped with a C-18 chromatographic column for further confirmation of biodegradation. The mobile phase used was the gradient of ammonium acetate buffer + 1% acetonitrile + 0.5% THF (tetrahydrofuran) and acetonitrile.

3. Results and Discussion

3.1 Influence of shaking and static condition

The studied *B. thuringiensis* exhibited AR-119 degradation both under stationary and agitated condition. The static condition gave as high as 88.26% degradation as oppose to 25.85% in agitated flasks. The decolorization obtained in 100 and 1000 mL flasks kept on shaker was 70 and 25.85% compare to 88.26 and 70.88% in case of flasks kept at static condition respectively (Figure 1). This could be due to the negative influence of more aerobic condition prevailed in the system, as the flask volume increased from 100-1000 mL, but had 100mL of the dye solution in all the flasks. Chang *et al.* (2001) have reported inhibition of bacterial azoreductase in the presence of oxygen. The obtained finding signifies the required ideal reactor: waste volume ratio for enhanced degradation.

3.2 Influence of dye concentration

Influence of different concentration of the AR-119 was studied, and results are shown in Figure 2. The degradation of dye increased with initial dye concentration up to 300 ppm and then it become almost constant. The percent dye removal decreased from 95.22 to 24.50 when, the dye concentration was increased from 15 to 1000 ppm. At the end of 6 hrs of incubation the degradation rate was increase from 2.5 to 43.2 mg L^{-1} h^{-1} as the initial AR-119 concentration increased from 15 ppm to 300 ppm. Under the experimental condition the obtained kinetic data were K_S 300 ppm, V_{max} 43.2 mg L^{-1} h^{-1}, R^2=0.993, and Y=5.691X+0.022 (Figure 3). The observed dye decolorization rate at initial 2 hrs of incubation was 5.05, 7.5, 13.25, 26.2, 48, 58.5, 17.5, 17.5 mg/L/h for 15, 25, 50, 100, 200, 300, 500, 1000 ppm dye respectively (Figure 2). When 500 and 1000 ppm AR-119 was present in the medium, organisms showed 2 and 3 hrs of lag phase, respectively. This indicates that as dye concentration increased, the required acclimatization time was extended. However, at the end of the 6th hour both these flasks also showed 40.8 mg L^{-1} h^{-1} degradation rate. In literature maximum degradation rate of only 5.64 mg L^{-1} h^{-1} was reported for Reactive Violet 5 by RVM 11.1 consortium (Moosvi *et al.*, 2005). Even for Acid red 119 maximum rate of 1.66 mg L^{-1} h^{-1} was reported for HM-4 consortium (Khehra *et al.*, 2005). The obtained high degradation effi-

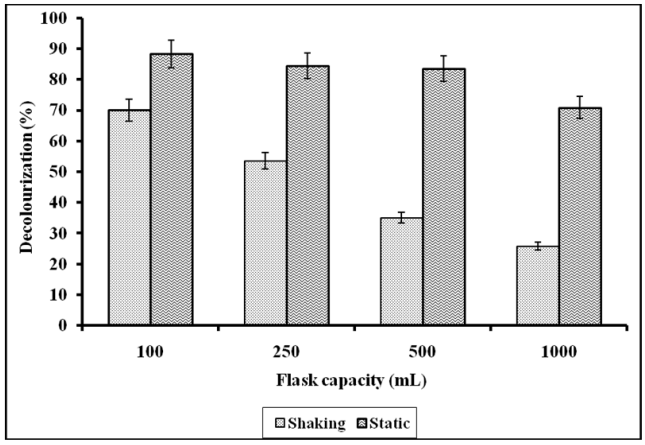


Figure 1. Influence of shaking and static condition and reaction flask volume on AR-119 bioremediation (Dye containing medium 100 mL).

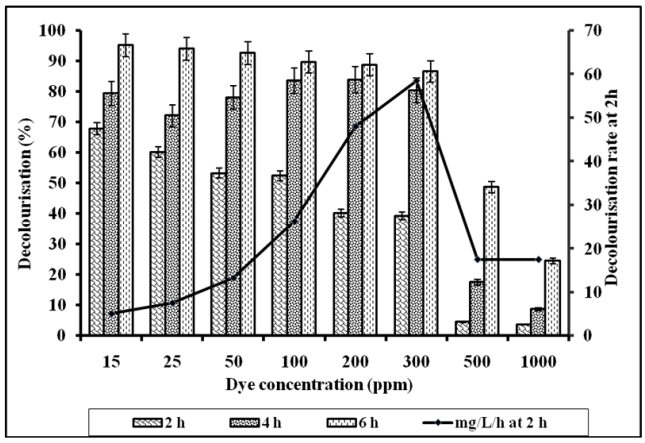


Figure 2. Influence of AR-119 concentration and reaction time on AR-119 bioremediation.

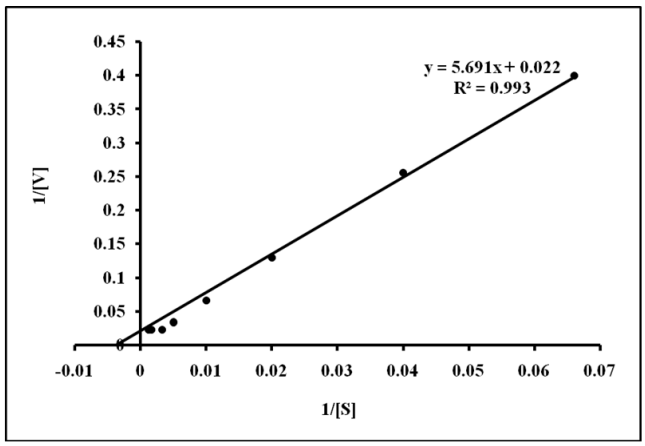


Figure 3. Double reciprocal plot of AR-119 bioremediation.

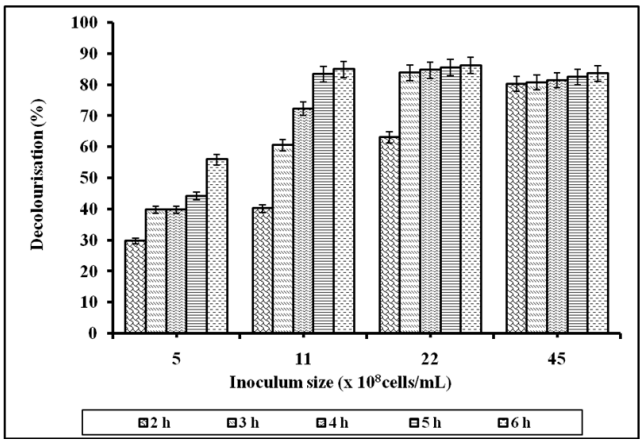


Figure 4. Influence of inoculum size and reaction time on AR-119 bioremediation.

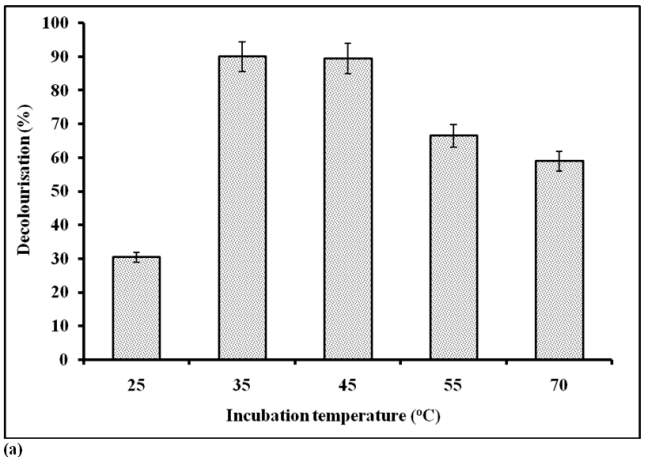
ciency was due to the selection of efficient culture (Dave and Dave, 2009).

3.3 Influence of inoculum size

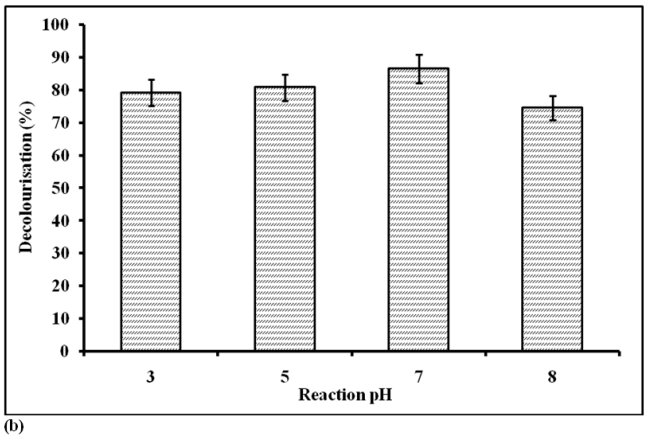
The data obtained at 2 hrs of incubation showed that the dye degradation increased as the initial inoculum biomass increased (Figure 4). However, if 4 hes incubation data are seen the removal was increased only up to 2.2×10^9 cells mL^{-1} of inoculum and then it became constant. If 5^{th} and 6^{th} hour data were considered, there was no further increase in dye removal amount beyond 1.1×10^9 cells mL^{-1} as inoculum. These observed results could be explained on the bases of the growth-linked kinetics. As reaction time increased even the flasks with 1.1×10^9 cells mL^{-1} inoculum reached to the possible maximum 9×10^9 cells mL^{-1} equivalent to the flask inoculated with the highest inoculum of 4.5×10^9 cells mL^{-1}. This indicates that beyond certain amount of inoculum there was no proportionate increase of decolorization with further increase in inoculum size. In literature similar reports have been recorded (Moosvi *et al.*, 2005; Sheth and Dave, 2009). Thus, once process is established, inoculum size is insignificant as the process is growth linked. As compare to the use of enzyme application of active culture give the benefit of growth linked activity and no stringent conditions are needed like enzyme based process.

3.4 Influence of Incubation temperature and pH

The results of incubation temperature showed no deactivation of the degradation ability of the organisms up to 45°C and 90% decolorization was achieved. Even at 70°C 59% degradation was observed (Figure 5a), which indicates the thermo tolerance ability of the organism. Therefore, the developed *B. thuringiensis* culture could be useful for the field process in a country like India, where temperatures reach up to 42-44°C in summer. Wong *et al.* (1998) have reported the inhibition of *Klebsiella pneumoniae* RS-1, and *Alcaligens liquefaciens* S-1 biodecolourisation activity at 45°C. Similarly, Moosvi *et al.* (2005) have also reported 30°C as an optimum temperature for bio decolorization of Reactive violet 5 by bacterial consortium RVM 11.1. The developed *B. thuringiensis* could be useful for treating industrial effluents having temperature as high as 55 to 70°C.



(a)



(b)

Figure 5. Influence of (a) incubation temperature and (b) pH on AR-119 bioremediation.

Under the experimental conditions pH 7.0 was found to be optimum with 87.0% dye removal. The most important observation is that there was only 7 and 12% loss in activity at pH 3.0 and 8.0 respectively (Figure 5b). The level of influence of pH observed in this study is different compare to that reported in the literature (Jirasripongpun *et al.*, 2007). Survival of the organism in the broad range of the pH could also make it as an appropriate choice; as dye waste pH is fluctuating due to, various process steps throughout the day.

3.5 Influence of nutrients concentration and medium supplements

Undiluted and 100 fold diluted NB medium showed 85 and 76% dye removal respectively (Figure 6a). Minimal salt medium and Bushnell haas medium showed less than 50% decolorization after 24 hrs of reaction time (data not shown). When the medium was diluted to 100 fold the amount of total organic substrate present in the medium was reduced to as low as 100 mg L^{-1}. Minimum nutrient requirement of the organism during degradation of AR-119 dye makes the process economical and the left out B.O.D. after the treatment also becomes low.

When 1% w/v caster cake, cotton seed and corn cake powders were used as substrate the achieved decolorization ranged between 76 to 80% (Figure 6b). Use of cotton seed powder as substrate was found to be best among the crude substrates studied, whereas molasses was found to be poor substitute from bioremediation point of view. Even the available reports also show that presence of glucose is not beneficial for the decolorization of the dye (Chen *et al.*, 2003; Sheth and Dave, 2009).

3.6 Influence of heavy metals and sodium chloride

Results of AR-119 biodegradation in the presence of 0.8 mM of chromium, copper, nickel and zinc are depicted in Figure 7a. Among the metals studied copper was found to be most inhibitory, but still it showed 60.95% degradation in 6 hrs, whereas in the presence of other metals degradation was in the range of 70 to 78.48%. On the other hand dye degradation ability was affected gradually above 100 mM NaCl and the culture showed only 40% degradation in the presence of 900 mM NaCl (Figure 7b). High concentration of salt is often used for dyes to adhere to the fiber, thus effluent normally shows presence of high salt concentration, which lead to acute toxicity problems (Zollinger, 1991). Thus, it is neces-

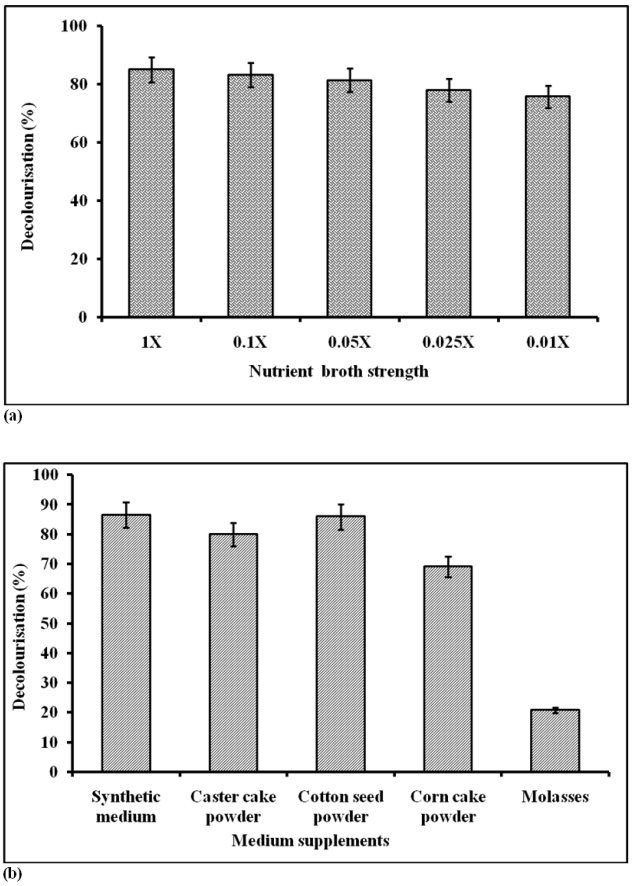


Figure 6. Influence of (a) medium strength and (b) medium supplements on AR-119 bioremediation.

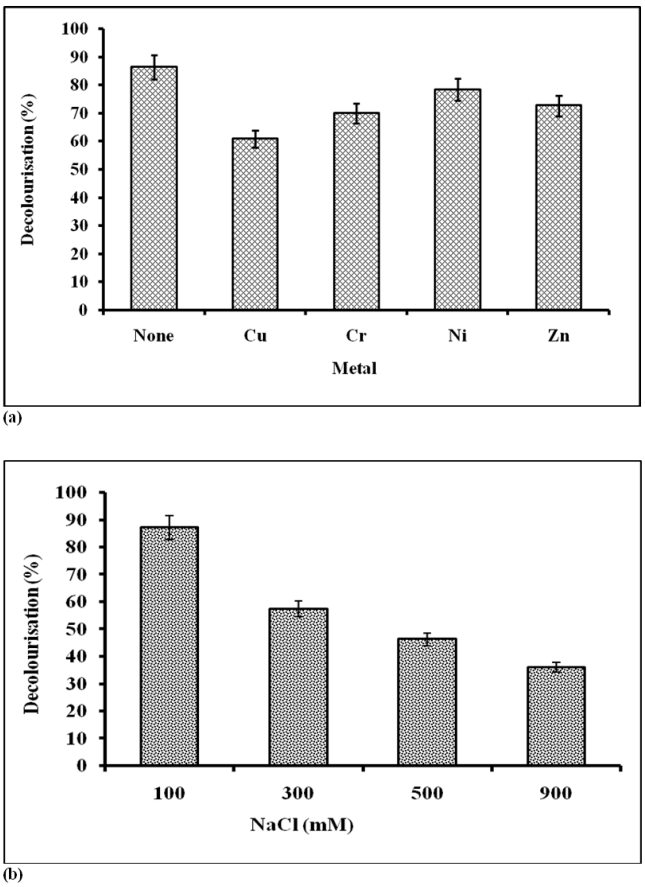


Figure 7. Influence of (a) heavy metals and (b) NaCl concentration on AR-119 bioremediation.

sary to develop the salt resistant in the culture.

3.7 Column study

The process was extended to column containing developed biofilm of *B. thuringiensis* on glass beads that resulted in more than 99% degradation in less than 2 hrs of contact time even on 18th cycles of addition of 100 ppm of AR-119 in 10 times diluted NB (data not shown).

3.8 Degradation studies of AR-119

The comparison of UV-Visible spectra of untreated and treated samples indicated complete disappearance of peak at 530 nm (Figure 8). The disappearance of the absorbance peak attributed to biodegradation of the dye (Chen *et al.*, 2003). When the supernatant from untreated and biologically treated dye solutions were centrifuged and checked by thin layer chromatography, six distinct spots for untreated dye with R_f values 0.46, 0.48, 0.52, 0.61, 0.69, and 0.72 were observed. Whereas, only one faint spot with R_f value 0.56 was observed in biologically treated sample with 95% reduction in the intensity compare to spot of untreated dye with R_f of 0.52. This indicates the degradation of the AR-119 dye. FTIR spectra of untreated dye solution showed peak position at 1611.4, 1652.66, 1695.81, 1700.00, and 1717.00 cm^{-1}. All these peaks were almost removed in the treated sample, this indicate the biodegradation of the dye (data not shown). HPLC elution profile of AR-119 and its metabolites extracted after biodegradation showed the disappearance of peak 2.849, 3.594, and 5.900 observed in untreated AR-119 solution after bacterial treatment, also confirmed the AR-119 degradation (Figure 9). Biological treatment resulted in as high as 87.0% reduction in COD. Nutrient broth containing 300 ppm AR-119 dye treated with *B. thuringiensis* for 7 hrs showed more than 99% degradation of the dye color and no dye color was extracted by desorption of dye from cell pellets in aqueous and organic solvents.

Developed *B. thuringiensis* is useful for the treatment of actual dye effluent, as it showed reduction in dye, COD, and TDS concentration from 210 to 20, 246 to 26, and 668 to 142 mg L^{-1} respectively in 13 hrs of contact time from the Gayatri Dyechem effluent without addition of any external nutrient. This shows at least a 2–fold improvement compared to our previous study (Dave and Dave, 2009).

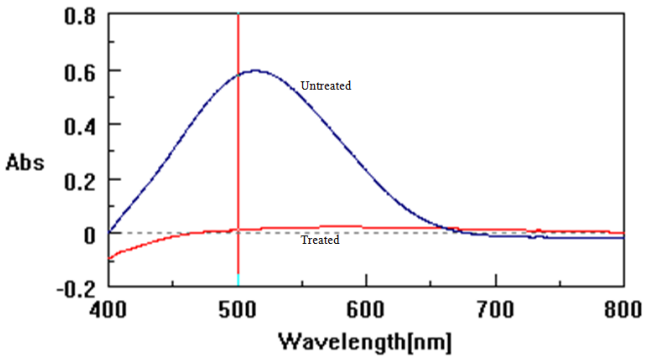


Figure 8. UV-Vis Spectra of untreated and treated sample with *B. thuringiensis* containing AR-119 dye.

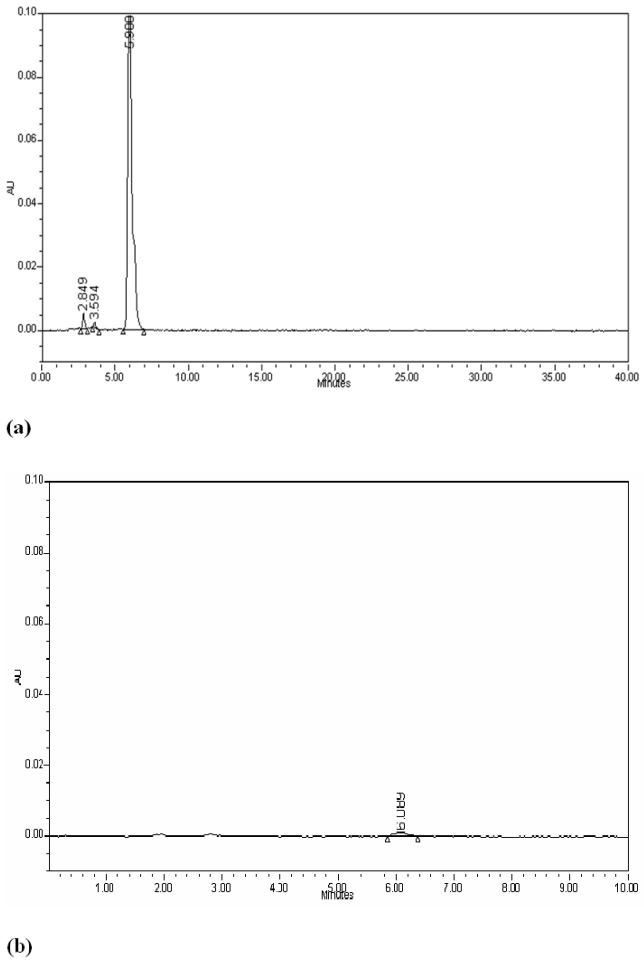


Figure 9. HPLC elution profile of (a) C.I. Acid red 119 and (b) its degradation metabolite.

4. Conclusions

- The *B. thuringiensis* SRDD culture showed degradation ability even at extreme temperature of 70°C, in the presence of different metals and broad pH range of 3-8 of the medium.
- Inoculums size 10% v/v of 1x10^8 cells mL^{-1}, incubation temperature 35±2°C, pH 7.0±0.2, NaCl 100 mM and static conditions were found to be optimum. Under the experimental conditions a degradation rate as high as 220 mg L^{-1} h^{-1} was achieved, which is found to be 130 times higher compared to the reported data. Once biofilm was developed there was no need to add inoculum even for 18 cycles.
- The ability of *B. thuringiensis* SRDD to use cheap nutrient supplements and the requirement of as low as 100 mg L^{-1} nutrients concentration to degrade AR-119 makes the organism unique and quite economical.

- Analytical methods UV-Vis, TLC, FTIR and HPLC confirm the dye biodegradation ability of *B. thuringiensis* SRDD.

Acknowledgement

We are thankful to Appex and Gayatri Dyechem dye manufacturing industries, Ahmedabad, for providing the dye samples, waste and industrial effluent. We also thank Gujarat Test House, Gujarat Laboratory, and Meghmani Organics Limited for support in the analytical work.

References

Acuner, E. and Dilek, F.B. 2004. Treatment of tectilon yellow 2G by Chlorella vulgaris. Process Biochemistry. 39(5), 623-631.

Arami, M., Limaee, N.Y., Mahmoodi, N.M. and Tabrizi, N.S. 2006. Equilibrium and kinetics studies for the adsorption of direct and acid dyes from aqueous solution by soy meal hull. Journal of Hazardous Materials. 135(1-3), 171–179.

Batzias, F.A. and Sidiras, D.K. 2007. Dye adsorption by prehydrolysed beech sawdust in batch and fixed-bed systems. Bioresource Technology. 98(6), 1208–1217.

Chakraborty, S., Purkait, M.K., DasGupta, S., De, S. and Basu, J.K. 2003. Nanofiltration of textile plant effluent for color removal and reduction in COD. Separation and Purification Technology. 31(2), 141-151.

Chang, J.S., Chou, C., Lin, Y.C., Lin, P.J., Ho, J.Y. and Lee, Hu., T. 2001. Kinetic characteristics of bacterial azo-dye decolorization by Pseudomonas luteola. Water Research. 35, 2841–2850.

Chen, K.C., Wu, J.Y., Liou, D.J. and Hwang, S.C.J. 2003. Decolorization of the textile dyes by newly isolated bacterial strains. Journal of Biotechnology. 101(1), 57-68.

Crini, G. 2006. Non-conventional low-cost adsorbents for dye removal: a review. Bioresource Technology. 97, 1061–1085.

Dave, S.R. and Dave, R.H. 2009. Isolation and characterization of *Bacillus thuringiensis* for Acid red 119 dye decolourization. Bioresource Technology. 100, 249-253.

Fu, Y., and Viraraghavan, T. 2001 Fungal decolourisation of dye wastewaters: a review. Bioresource Technology. 79, 251-262.

Georgiou, D., Aivazidis. A., Hatiras, J. and Gimouhopoulos, K. 2003. Treatment of cotton textile wastewater using lime and ferrous sulfate. Water Research. 37(9), 2248-2250.

Isik, M. and Sponza, D. T. 2003. Effect of different oxygen conditions on decolorization of azo dyes by *Escherichia coli*, Pseudomonas sp. and fate of aromatic amines. Process Biochemistry. 38, 1183-1192.

Jirasripongpun, K., Rujikan, N., Jongiira, N. and Boonsiri, C. 2007. Decolorization and Degradation of C. I. Reactive Red 195 by Enterobacter sp. Thammasat International Journal of Science and Technology. 12(4), 6-11.

Kapdan, I.K. and Kargi, F. 2002. Simultaneous biodegradation and adsorption of textile dyestuff in an activated sludge unit. Process Biochemistry. 37, 973–981.

Khehra, M.S., Saini, H.S., Sharma, D.K., Chadha, B.S. and Chimni, S.S. 2005. Decolourisation of various dyes by bacterial consortium. Dyes and Pigments. 67(1), 55-61.

Mielgo, I., Moreira, M.T., Feijoo, G. and Lema, J.M. 2002. Biodegradation of a polymeric dye in a pulsed bed bioreactor by immobilised *Phanerochaete chrysosporium*. Water Research. 36(7), 1896-1901.

Mohan, S. V., Rao, N.C., Srinivas, S., Prasad, K.K. and Karthikeyan, J. 2002. Treatment of simulated Reactive Yellow 22 (azo) dye effluents using Spirogyra species. Waste Management. 22(6), 575-582.

Moosvi, S., Keharia, H. and Madamwar, D. 2005. Decolourization of textile dye reactive violet 5 by a newly isolated bacterial consortium RVM 11.1. World Journal of Microbiology and Biotechnology. 21, 667-672.

Pandey, A., Singh, P. and Iyengar, L. 2007. Bacterial decolourisation and degradation of azo dyes. International Biodeterioration and Biodegradation. 59, 73-84.

Pearce, C.I., Lloyd, J.R. and Guthrie, J.T. 2003. The removal of colour from textile wastewater using whole bacterial cells: a review. Dyes and Pigments. 58, 179-196.

Ramalho, P.A., Cardoso, M.H., Cavaco-Paulo, A. and Ramalho, M.T. 2004. Characterization of azo reduction activity in a novel ascomycete yeast strain. Applied and Environmental and Microbiology. 70, 2279-2288.

Ramalho, P.A., Paiva, S., Cavaco-Paulo, A., Casal, M., Cardoso, M.H. and Ramalho, M.T. 2005. Azo reductase activity of intact *Saccharomyces cerevisiae* cells is dependent on the Fre1p component of the plasma membrane ferric reductase. Applied and Environmental and Microbiology. 71(7), 3882-3888.

Robinson, T., Chandran B. and Nigam, P. 2001. Studies on the production of enzymes by white-rot fungi for the decolorisation of textile dyes. Enzyme and Microbial Technology. 29, 575-579.

Sandhya, S., Padmavathy, S., Swaminathan, K., Subrahmanyam, Y.V. and Kaul, S.N. 2005. Microaerophilic-aerobic sequential batch reactor for treatment of azo dyes containing simulated wastewater. Process Biochemistry. 40(2), 885-890.

Senthilkumar, S., Kalaamani, P., Porkodi, K., Varadajan, P.R. and Subburaan, C.V. 2006. Adsorption of dissolved reactive red dye from aqueous phase on to activated carbon prepared from agricultural waste. Bioresource Technology. 97, 1618-1625.

Sheth, N.T. and Dave, S.R. 2009. Optimisation for enhanced decolourization and degradation of Reactive Red BS C.I. 111 by Pseudomonas aeruginosa NGKCTS, Biodegradation. 20, 827-836.

Silva, C.G., Wang, W. and Faria, J.L. 2006. Photocatalytic and photochemical degradation of mono-, di- and tri-azo dyes in aqueous solution under UV irradiation. Journal of Photochemistry and Photobiology. 181, 314-324.

Stolz, A. 2001. Basic and applied aspects in the microbial degradation of azo dyes. Applied Microbiology and Biotechnology. 56 (1-2), 69-80.

Wafaa, M., Abd, E.-R. and Moawad, H. 2003. Enhancing bioremoval of textile dyes by eight fungal strains from media supplemented with gelatin wastes and sucrose. Journal of Basic Microbiology. 43(5), 367-375.

Wong, M.K. M., Goh, T.K., Hodgkiss, I. J., Hyde, K.D., Ranghoo, V.M., Tsui, C.K.M., Ho, W.H., Wong, W.S.W. and Yuen, T.K. 1998. Role of fungi in freshwater ecosystem. Biodiversity and Conservation. 7(9), 1187-1206.

Zollinger, H. 1991. *In* Colour chemistry, synthesis, properties and applications of organic dyes and pigments, 2nd ed. VCH, Weinheim, Germany.