Available Online at http://www.recentscientific.com

ISSN: 0976-3031

International Journal of Recent Scientific Research
Vol. 4, Issue, 9, pp.1400- 1405, September, 2013

International Journal of Recent Scientific Research

RESEARCH ARTICLE

REMEDIATION OF TEXTILE REACTIVE DYES USING ANAEROBIC RUMEN CONSORTIUM

*Duggirala Srinivas .M[1], Sheth Niraj .T[2], Bhatt Nikhil .S[3], Vanjani Unnati .N[4]

[1,2,3,4] Department of Microbiology, Biogas Research Centre, Gujarat Vidyapith, Sadra 382320, Gujarat, India.

ARTICLE INFO

Article History:

Received 11th, August, 2013
Received in revised form 26th, August, 2013
Accepted 12th, September, 2013
Published online 30th September, 2013

Key words:

Reactive Dyes, Anaerobic consortium, Textile Waste water, Decolorization

ABSTRACT

A potent anaerobic consortium, isolated by enrichment method from cattle rumen, showed more than 85% decolorization of reactive dyes viz. Reactive Violet (RV 12), Reactive Red (RR 152) and Reactive Blue (RB 250) at concentration of 1000 mgL^{-1} each, within 48 h under strict anaerobic condition. A decolorization efficiency of 93.36–78.60% was obtained by developed anaerobic consortium, in 500 mL anaerobic sequential batch reactor (SBR) containing 500-2000 mgL^{-1} of dyes. The degradation was confirmed by TLC and FTIR. The remediation process was scaled up to 5L for RV 12 and actual textile dyeing unit's wastewater containing a mixture of dyes. The developed consortium exhibited color removal (93.4%), COD removal (68.77%), TS reduction (48.77%) and TDS reduction (65.1%) in actual textile wastewater.

INTRODUCTION

About 300,000 tones of different dyestuffs are used per year for textile dyeing operations (Bechtold et *al.*, 1999). Production of dyestuff and pigments in India is close to 80,000 tones. The textile industry, which is one of the largest water consumer in the industry, produces waste water composed of various recalcitrant agents such as dye, sizing agents, and dyeing aids (Wijetunga *et al.*, 2007). In particular, the release of colored compounds into the environment is undesirable not only because of their color, which may affect photosynthesis of aquatic plants, but also because many dyes and their breakdown products may be toxic and/or mutagenic to life (Weisburger, 2002). Dye colors are visible in water at concentration as low as 1mgL^{-1}, whereas textile processing waste water, normally contain more than 10-200 mgL^{-1} dye concentration resulting in aesthetic problems (O'Neill *et al.*, 1999). Depending on the class of dye, this loss in waste waters may range from 2% of the original concentration for basic dyes, to as high as 50% for reactive dyes, with an overall average loss of 15% (Melgoza *et al.*, 2004).

Synthetic dyes in wastewaters cannot be efficiently decolorized by traditional treatment processes because of the high cost and disposal problems for their treatment at large scale, especially in the textile and paper industries (Ghoreishi and Haghighi, 2003). The technologies for color removal can be divided into three categories: biological, chemical and physical (Robinson *et al.*, 2001). Many physicochemical decolorization methods are not ideal because they are; expensive, have restricted application areas, interfere with other wastewater components, or generate wastes that require re-treatment (Karatas *et al.*, 2009). The microbial decolorization and degradation of azo dyes has been of considerable interest since it is inexpensive, eco-friendly, and produces a less amount of sludge (Kalyani *et al.*, 2008). In the presence of specific oxygen-catalyzed enzymes called azo reductases, some aerobic bacteria are able to reduce azo compounds and produce aromatic amines (Stolz, 2001). Under anaerobic conditions, a low redox potential (< –50 mV) can be achieved, which is necessary for the effective decolorization of dyes (Bromley- Challenor *et al.*, 2000). Generally, textile wastewater is difficult to treat in activated sludge plants, due to a high organic load and the presence of dyes (Delee *et al.*, 1998). Anaerobic treatment can be a solution for both problems. It has a low sludge production, capacity to decolorize the wastewater with high organic loads (Lema and Omil, 2001). Though reduction of reactive azo dyes to simpler compounds under strict anaerobic conditions holds a tremendous advantage over the conventional and aerobic treatment methods, it has been less documented.

MATERIALS AND METHODS

Dyes and Chemicals

The Reactive azo dyes viz. Reactive Violet (RV 12), Reactive Red (RR 152) and Reactive Blue (RB 250), were obtained from Vim Dye Chem, Ganesh Dye Chem and Chemistar Industry, Vatva G.I.D.C., Ahmedabad, Gujarat. All chemicals and media used in the study were of analytical grade (AR), mainly from Hi Media, SD fine, CDH and Merck laboratories.

Enrichment of Dye Decolorizing Anaerobic Consortium

Strict anaerobic bacteria consortium used in the study, was obtained by enrichment culture technique from cattle rumen fluid. RUM 10 medium (Latham *et al.*, 1978) used for the study was distributed in roll tubes and gases [N_2:CO_2 (80:20)] were purged in the medium for 10 min. The tubes were closed with butyl rubber cork and sealed with aluminum seals to maintain strict anaerobic conditions. The medium was sterilized at 10 psi for 15 min. and allowed to cool at room temperature. 10 mL of RUM 10 medium, containing 100 mgL^{-1} dyes, was inoculated with 1 mL of 1:10th dilution of rumen fluid, in roll tubes, post-gassed with N_2:CO_2 (80:20) and incubated at 38±2°C for 6-7 d under static condition. The active culture from the decolorized broth was transferred into two mouthed anaerobic bottle containing sterile

* Corresponding author: Duggirala Srinivas .M
Department of Microbiology, Biogas Research Centre, Gujarat Vidyapith, Sadra 382320, Gujarat, India.

Glucose Phosphate Broth (GPB) comprising gL^{-1}: Glucose, 5; peptone, 10; K_2HPO_4, 5. Medium pH was adjusted to 7.2±0.2. N_2 and CO_2 (80:20) gases were purged for 15 min. The bottle was closed with butyl rubber cork and sealed with aluminum seal. For enrichment of consortium on the selected reactive dyes was repeated 4 times. The developed consortium was used for further decolorization study.

Preparation of Dye (Stock) Solution

Dye stock solution was prepared by admixing 10 g of respective dye powder in 1 L of sterile distilled water. The desired dye concentration required for different experiments was obtained by successive dilution of the respective dye stock solution.

Decolorization Study in Roll Tubes

For the decolorization study Hungate medium (Hungate, 1943) was supplemented with 1 gL^{-1} glucose as a carbon source. 20 mL medium was distributed in roll tubes and gassed with N_2 and CO_2 (80:20) for 10 min. Roll tubes were sealed with butyl rubber cork and aluminum seal. The medium was sterilized at 10 psi for 15 min. The initial concentrations 100 to 1000 mgL^{-1} for each dye was added after sterilization of medium ingredients. The tubes were inoculated with active culture using a sterile syringe. The tubes were incubated at 38±2°C for 48 h in static condition. The percent decolorization of each dye was assessed by comparing the optical density of the biotic control with the abiotic control. The rate of decolorization ($mgL^{-1}h^{-1}$) was calculated for each dye at respective dye concentrations (Jadhav *et al.,* 2008).

Sequential Batch Decolorization Experiment in Anaerobic Bottles

Laboratory scale anaerobic reactors were established in 500 mL (three mouthed) anaerobic bottles and a working volume of 400 mL (Hungate medium) was maintained. The media was purged with N_2 and CO_2 (80:20) for 10 min. and sealed with butyl rubber stoppers and aluminum seals. The incremental batch experiment (additive) were studied for different initial dye concentration (500, 750, 1000, 1250, 1500, 1750 and 2000 mgL^{-1}) for each dye. Four cycles were repeated for each dye concentration. 100 mL of the medium in bottle was replaced with new Hungate medium containing dye after two day interval thus maintaining HRT of 8 d. The dye concentration after four cycles was increased, ranging from 500-2000 mgL^{-1}. The culture of anaerobic consortium was inoculated in the medium at the beginning of each first cycle and abiotic controls of each concentration were maintained in similar conditions. The bottles were incubated at 38±2ºC. The bottles were vigorously shaken at regular intervals and the aspirated spent medium from the bottles was analyzed for percent decolorization and rate of decolorization at the end of each cycle. Biodegradation of dye was evaluated by TLC and FTIR analysis.

Thin Layer Chromatography (TLC) Analysis

The abiotic controls and decolorized broths were collected in microfuge tubes and centrifuged at 6000 rpm for 20 min. 10 µL of supernatant was spotted on TLC silica gel 60 F_{254} plate supplied by Merck, Germany, using a micro syringe. Mixture of Propanol: Ammonia: Butanol (6:6:6 v/v) was used as the solvent system. The dye chromatogram was observed under visible and ultraviolet light (254 nm). The obtained TLC bands of test and control were compared for the degradation of dye.

Fourier Transform Infrared Spectrometry (FTIR) Analysis

The FTIR analysis was carried out at the Ankleshwar Research and analytical Infrastructure limited, Gujarat. The absorbance FTIR spectra of the samples were recorded using FTIR, Model/Sr.No.FTIA-2000-104. The FTIR spectra were collected within a scanning range of 400-4000 nm.

Reactor Scale Up: Establishment of Anaerobic Reactor

To scale up the process of decolorization, continuously operating anaerobic reactor with 10 d HRT was established for treatment of dye RV 12. The bioreactor comprised of three components viz. Digester bottle (5 L capacity), Gas holder bottle (2L capacity) and Water displacement bottle (2L capacity). The Dimensions of digester bottle were as follows: Diameter of reactor; 19 cm, diameter of outlet; 1.5 cm, total volume capacity; 5 L, working volume; 4.5 L. The digester bottle had three openings one as feeding tube, other as gas outlet and one as siphon (effluent-treated waste water) outlet. Gas outlet of digester was connected to gas holder bottle. The gas holder bottle was connected with water displacement bottle through rubber tube at the bottom. The working volume of the digester was 4.5 L; which contained sterile Glucose phosphate broth (0.1X) inoculated with anaerobic consortium. The reactor was sealed to ensure strict anaerobic conditions and incubated at room temperature for 7 d to establish anaerobic condition. The system is operated at 10 d HRT by removing 450 mL of the liquid and adding 450 mL of fresh sterilized medium containing dye concentration ranging from 100-1000 mgL^{-1} at the interval of 24 h. The decolorization efficiency and gas production were measured daily.

Lab Scale remediation of Wastewater from Textile Dyeing Unit

Azo dyes containing textile wastewater was procured from Ganesh Dye Chem, Vatva, G.I.D.C., Ahmedabad, Gujarat. The approximate composition (gL^{-1}) of textile wastewater was Reactive Blue B; 17.5, Reactive Red 5B; 25.0, Reactive Violet 5; 56.0, NaCl; 60.0, $NaHCO_3$; 12.0 and Soda ash; 12.5 respectively. 1:20 diluted Textile waste water was added to roll tubes containing 20 mL of Hungate medium containing 875, 1250 and 2800 mgL^{-1} of Reactive Blue B, Reactive Red 5B and Reactive Violet 5, respectively. The active consortium (5% v/v) enriched from the scale up study was added to the roll tubes and incubated for 60 h at 38±2ºC. The decolorization percent was determined spectrophotometrically at 588 nm (λ_{max}). The experiment was further scaled up to 500 mL in anaerobic three mouthed bottles. HRT of 16 d was maintained by transferring 100 mL medium with fresh medium at an interval of 4 d and the percent decolorization was evaluated. Degradation was confirmed by TLC. The efficiency of bioremediation of textile dyeing unit's wastewater, by the developed anaerobic consortium, was checked by analyzing COD, Total Solids (TS) and Total Dissolved Solids (TDS) (APHA, 1992).

RESULTS

The decolorization experiment was carried out from 100-1000 mgL^{-1} of dye concentration for all the three selected dyes using the developed anaerobic consortium obtained by enrichment culture technique. The decolorization study of RV 12, RR 152 and RB 250 (Fig. 1) showed a dye removal efficiency of more than 85% even at dye concentration of 1000 mgL^{-1}. It was observed that as the concentration of dye increased the decolorization percent decreased. The highest decolorization percent of RV 12, RR 152 and RB 250 was 93.23%, 96.20% and 93.55%, respectively at 100 mgL^{-1} of dye concentration after 48 h of incubation (Fig. 1 and 2). As the concentration of dye increased the percent decolorization decreased though there was increase in decolorization rate. The highest decolorization rates were 18.94, 19.05 and 17.19 $mgL^{-1}h^{-1}$ for RV 12, RR 152 and RB 250 respectively at dye concentration of 1000 mgL^{-1} (Fig. 1).

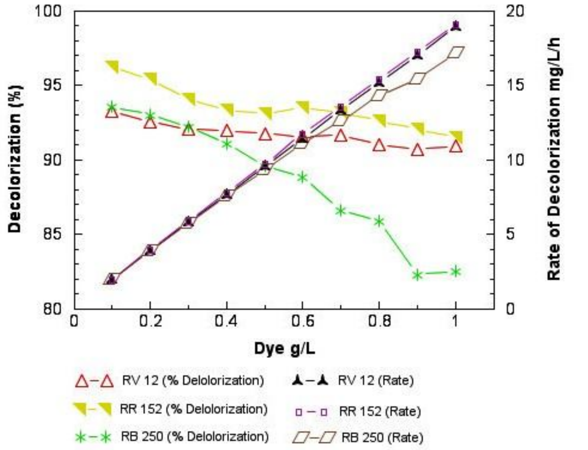

Fig. 1 Decolorization of RV 12, RR 152 and RB 250 (100-1000 mgL^{-1}) in Roll Tubes

Sequential anaerobic batch reactors operated at 8 d HRT exhibited decolorization (%) in the range of 90.53 – 80.50, 93.36 – 79.68 and 90.99 – 78.60 for RV 12, RR 152 and RB 250, respectively at 500 – 2000 mgL^{-1} of dye concentration (Fig. 3). Although the decolorization (%) decreased with the increase in the initial dye concentration, the dye removal rate was found to increase by 3.56, 3.41 and 3.46 times for RV 12, RR 152 and RB 250 respectively, even at four folds higher initial dye concentration.

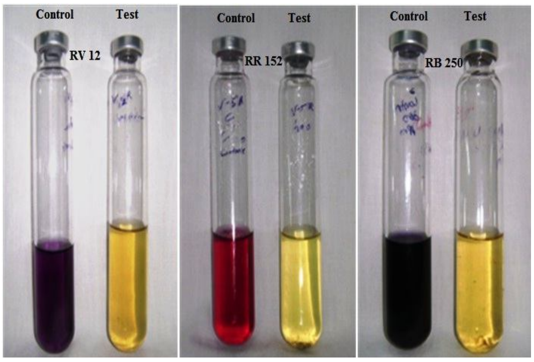

Fig. 2 Decolorization of Dyes (100 mgL^{-1}) in Roll Tubes

During the first 10 d efficient decolorization was observed upto 1000 mgL^{-1} dye concentration. The average gas production during 100 – 600 mgL^{-1}concentration of RV 12 was 168.50 mLd^{-1}, which decreased after the 6th d. During the next10 d, the dye concentration was increased up to 1500 mgL^{-1}. Upto 900 mgL^{-1} more than 80 % decolorization was observed. At 1500 mgL^{-1} initial concentration the cumulative dye concentration in the reactor was 1890 mgL^{-1} which was decolorized to 64.07%. The developed anaerobic consortium decolorized the dye efficiently (ranging from 91.38 to 64.07%) but the gas production decreased; however almost 10 fold increase was monitored in the dye removal rate with an increase in the initial dye concentration from 100 – 1000 mgL^{-1} (3.81 – 37.52 $mgL^{-1}h^{-1}$) (Fig. 4).

The decolorization and degradation of the respective dyes in this experiment were confirmed by TLC and FTIR. The prominent R_f values of RV 12 control were 0.32 and 0.36 which disappeared and new bands at 0.16, 0.53 and 0.76 were observed at 254 nm. In RR 152 prominent bands at R_f0.38, 0.46 and 0.51 disappeared and bands at R_f 0.35 and 0.48 were observed. In RB 250 prominent bands at R_f 0.35, 0.38, 0.49 and 0.66 disappeared and bands at 0.12, 0.40, 0.47 and 0.54 were observed.

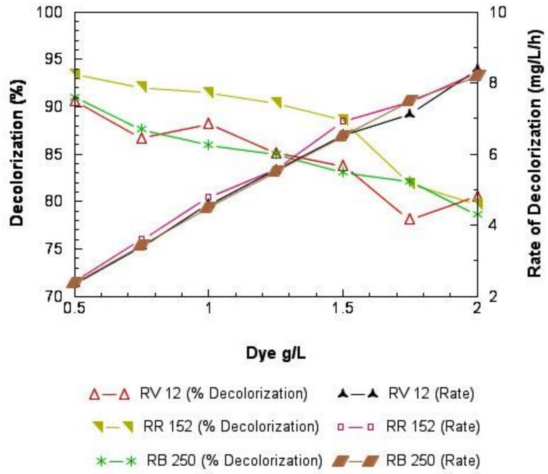

Fig. 3 Sequential Batch Decolorization of RV 12, RR 152 and RB 250, (500 – 2000 mgL^{-1}) in Anaerobic Bottles

The disappearance of prominent bands and appearance of new bands on TLC plates indicate substantial transformation or degradation of azo dyes in anaerobic conditions. These results were further confirmed by FTIR. The IR spectrum of both the dyes (RV 12; Fig.5 and 6, RR152; Fig. 7 and 8) showed changes in band intensity at 1500-1400 cm^{-1} of the azo group (-N=N-) compared to their respective controls that contributed to the chromophoric group.

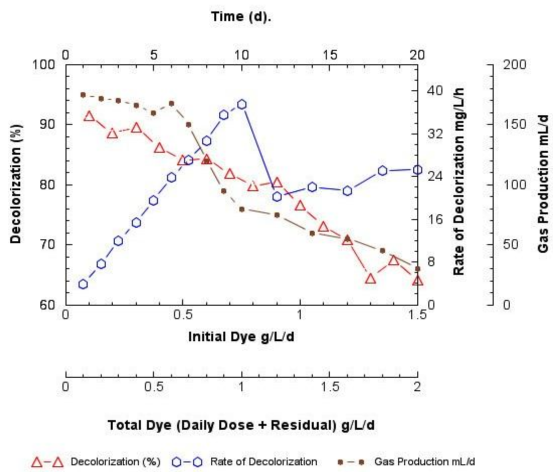

Fig. 4 Scale Up Study

Similarly change in intensities of the bands were observed around 3000-2800 $cm^{-1}$for the alkane groups, 3000-2800 cm^{-1} for aliphatic alkanes groups (-CH, -CH2-), Aryl-Cl with C-Cl stretching at 1100-1030 cm^{-1}, conjugated cyclic system of C=N with C=N stretching at 1660-1480cm^{-1} were observed between test and control.

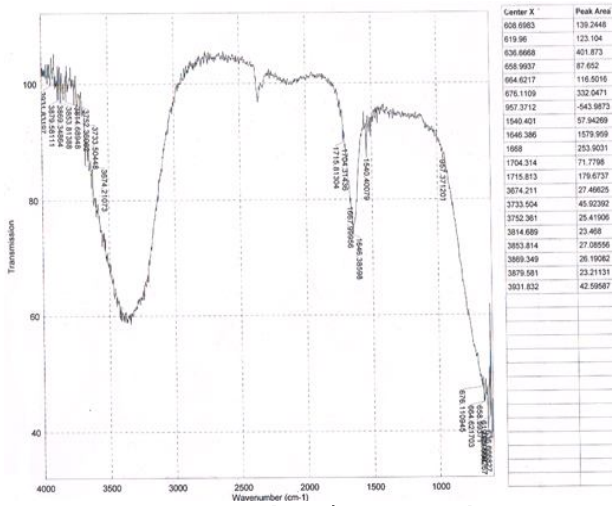

Fig. 5 FTIR Analysis of RV 12 (Control)

Treatment of Textile Waste Water

The anaerobic consortium could decolorize more than 90 % of 4925 mgL^{-1} dyes present in the textile wastewater (containing mixture of azo dyes containing Reactive Blue B, Reactive Red 5B and Reactive Violet 5) (Fig.9). Complete change in the R_f values of the anaerobically treated samples on TLC suggests the degradation of dyes in textile wastewater by the developed anaerobic consortium.

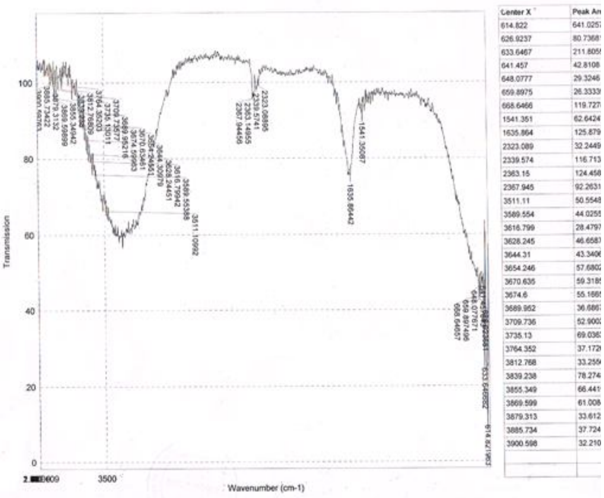

Fig. 6 FTIR Analysis of RV 12 (Test)

The degradation of the dyes in the wastewater was further supported by the reduction in the COD, TS, and TDS. After 2 cycles of 8 d HRT, color, COD, TS and TDS reductions were 93.83%, 68.77%, 48.57%, and 65.1% respectively in anaerobic reactor (Table 1)

Table 1 Treatment of Textile Wastewater

	C.O.D. mgL^{-1}	T.S. mgL^{-1}	T.D.S mgL^{-1}
Untreated wastewater	4740	630	580
Anaerobically treated wastewater	1480	324	202
Reduction (%)	68.77	48.57	65.1

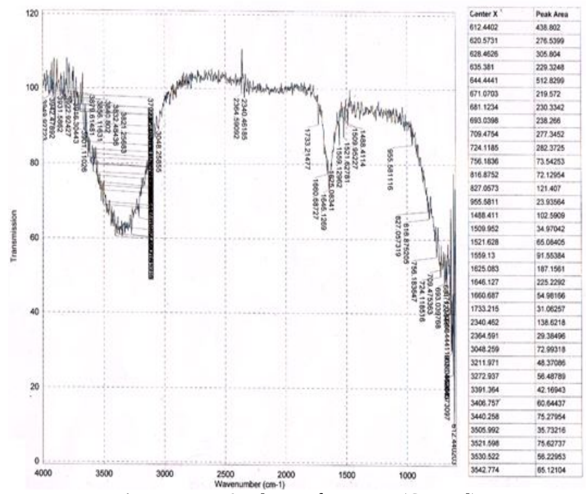

Fig. 7 FTIR Analysis of RR 152 (Control)

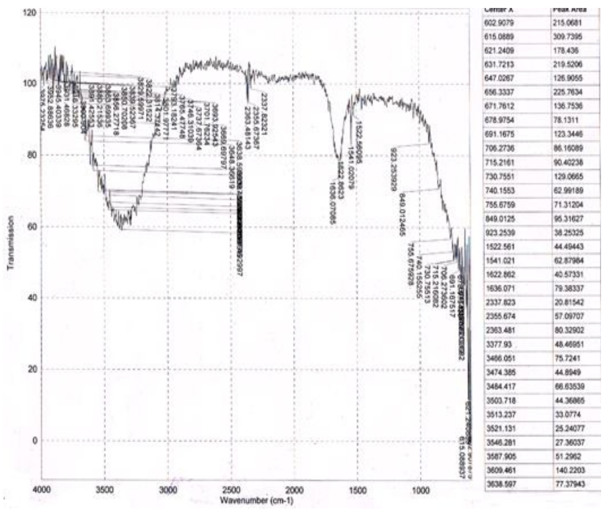

Fig. 8 FTIR Analysis of RR 152 (Test)

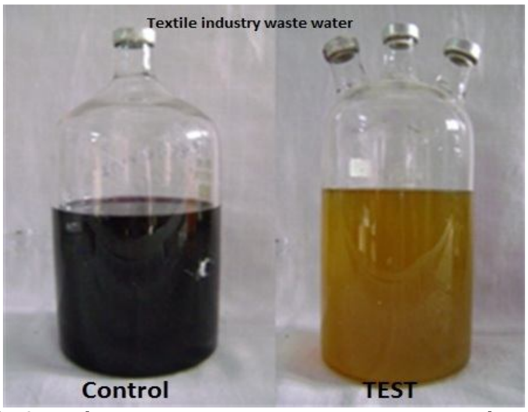

Fig. 9 Textile Wastewater treatment containing Reactive Blue B, Reactive Red 5B and Reactive Violet 5 dyes

DISCUSSION

The decrease in the decolorization (%) at higher initial concentration of the respective dyes could be due to the decrease in the available active sites present on the surface of the developed consortium; moreover a simultaneous increase in the dye removal rate clearly suggests that the developed consortium could resist as well as exponentially metabolize the respective dyes even at high concentrations of 1000 mgL^{-1}. Similar observations have been reported by Keharia *et al.,* 2004 for the decolorization of RV 5 using anaerobic sludge bacteria which exhibited a decolorization efficiency varying from 95% (100 mgL^{-1}) to 75% (1000 mgL^{-1}) during 48 h of incubation. Although the extent of decolorization decreased with higher initial dye concentration, the decolorization rate increased (1.96–15.6 $mgL^{-1}h^{-1}$), with an increase in initial dye concentration (100-1000 mgL^{-1}).

The developed consortium showed efficient biotransformation rate in log/exponential growth phase for all the selected dyes at different initial concentrations (500 – 2000 mgL^{-1}). The dye removal rate was found to be directly proportional to the increase in the initial dye concentration. These results suggest that probably the consortium may be utilizing different reactive dyes as carbon source. Microbial components of mixed microbial cultures are capable of decolorizing dyes via biotransformation and biodegradation (Banat et al. 1996) and efficiency of the decolorization process depends on the resistance to dye concentration, adaptation and enzymes activity expressed by the developed consortium (Senan and Abraham 2004). Mixed cultures are better decolorizers than individual cultures (Moosvi et al. 2005), suggesting a synergistic role of the bacterial species in mixed cultures in dye decolorization (Senan and Abraham 2004).

Aromatic compounds exits in all reactive dyes. The aromatic rings can be identified by several regions of bands in the IR spectra. Sulphonate salt, the R-SO3 - groups are essential components of the reactive dye. The main chromophoric component of the dyes under study was the azo group. Chloro aromatic compounds often exit in the structure of a reactive dye structure (Yuen, 2005). The IR spectrums of the purified dye samples were compared with the anaerobically treated samples and the distribution of the functional groups and chemical compounds indicate substantial change in the structure of these dyes. It may be presumed that the dye structure may have undergone alteration and/or degradation.

The actual textile dyeing unit's wastewater usually contains 100-500 mgL^{-1} of dyes (Sponza and Işik, 2004). Conventional method of dyeing was carried out in an open dye bath, in the dyeing of cotton, the fabric soaked in dyebath containing dyes, NaOH, NaCl and hydrose for 45 minutes at a temperature of 90^oC (Kalapriya and Gurumallesh, 2012). The wastewater used for our study contained a very high concentration containing a mixture of different reactive dyes (4925 mgL^{-1}). The developed anaerobic consortium efficiently decolorized as well as degraded the dyes present in the wastewater; these results emphasize the ability of the organisms present in the consortium to metabolize waste containing diverse types of Reactive dyes. Majority of the studies on anaerobic remediation of dye containing wastes have been reported for simulated wastes containing very low concentration of dye (100–1000 mgL^{-1}) whereas when it comes to actual wastewater, the dye types and concentration of constituents in the wastewater varies in every dyeing batch. The developed consortium exhibited the ability to degrade 2000 mgL^{-1} of three dyes namely RV 12 (monoazo), RR 152 (diazo) and RB 250 (diazo) during the batch studies; whereas it was found to be equally potent in decolorizing 2.4 fold higher concentration of dye mixture containing Reactive Blue B (diazo), Reactive Red 5B (diazo) and Reactive Violet 5R (monoazo) in actual textile wastewater used for the study. Manu and Chaudhari, 2002 conducted a laboratory scale semicontinous reactor study using simulated cotton dyeing wastewater containing orange II and Reactive black 3HN (100 mgL^{-1}) at ambient temperature (24-28°C) at HRT of 10 days. Color removal of >99% was achieved in both the dye containing reactors, COD removal upto 92% and 94% were achieved in orange and black dye containing reactors.

CONCLUSION

Biologically degradation of dyes in aerobic conditions is a real problem due to their complex cyclic structures. The reduction of dyes in anaerobic conditions is thermodynamically more suitable than oxidation in aerobic conditions. The developed anaerobic consortium was found to be efficient in decolorizing as well as degrading the three structurally different reactive dyes used for the study. Moreover the developed consortium was equally potent in decolorizing and degrading the actual dyeing unit's wastewater containing a very high concentration of reactive dyes. In anaerobic conditions, many microbial tropic groups are involved in complete degradation of complex organic compounds. Thus a better understanding of anaerobic biodegradation and treatment methods by indigenous anaerobes to transform or degrade dyes is of greater ecological significance. The developed consortium shows great promise to be used in the remediation of water pollution caused by reactive dyes.

Acknowledgement

The authors acknowledge the financial support of GUJCOST, Gandhinagar, Gujarat and Gujarat Vidyapith for the study.

References

APHA 1992. Standard methods for examination of water and wastewater. American Public Health Association/American Water Works Association/Water Environment Federation, 18th Ed. Academic Press, Washington D.C. USA.

Banat, I. M., Nigam, P., Singh, D. and Marchant, R. 1996. Microbial decolorization of textile-dye-containing effluents, a review. Bioresource Technology, 58: 217-227.

Bechtold, T., Burtscher, E. and Turcanu, A. 1999. Anthraquinones as mediators for the indirect cathodic reduction of dispersed organic dyestuffs. Journal of Electroanalytical Chemistry, 465: 80-87.

Bromley-Challenor, K. C. A., Kanapp, J. S., Zhang, Z., Gray, N. C. C., Hetheridge, M. J. and Evans, M. R. 2000. Decolorization of an azo dye by un acclimatized activated sludge under anaerobic conditions. Water Research, 34: 4410-4418.

Delee, W., O'Niell, C., Hawkers, F. R. and Pinherio, H. M. 1998. Anaerobic treatment of textile effluent: a review. Journals of Chemical Technology and Biotechnology, 73: 323-335.

Ghoreishi, S. M. and Haghighi, R. 2003. Chemical catalytic reaction and biological oxidation for treatment of non-biodegradable textile effluent. Chemical Engineering Journal, 95: 163-169.

Hungate, R. E. 1943. Further experiments on cellulose digestion by the protozoa in the rumen of cattle. Biology Bulletin, 84: 157-163.

Isık, M. and Sponza, D. T. 2004. Decolorization of azo dyes under batch anaerobic and sequential anaerobic/aerobic conditions. Journal of Environmental Science and Health, Part A: Toxic /Hazardous Substances and Environmental Engineering, 39: 1107-1127.

Jadhav, S. U., Kalme, S. D. and Govindwar, S. P. 2008. Biodegradation of methyl red by *Galactomyces geotrichum* MTCC 1360. International Journal of Biodeterioration and Biodegradation, 62: 135-142.

Kalapriya, K. and Gurumallesh Prabu, H. 2012. Greener method of coloration on conductive textile substrates. International Journal of Recent Scientific Research, 3(8): 670 -675.

Kalayani, D. C., Patil, P. S., Jadhav, J. P. and Govindwar, S. P. 2008. Biodegradation of reactive textile dye Red BL by an isolated bacterium *Pseudomonas sp*. SUK1. Bioresource Technology, 99: 4635-4641.

Karatas, M., Dursun, S. and Argun, M. E. 2009. Decolorization of reactive dyes under batch anaerobic condition by mixed microbial culture. African Journal of Biotechnology, 8(24): 6856-6862.

Keharia, H., Patel, H. and Madamwar, D. 2004. Decolorization screening of synthetic dyes by anaerobic methanogenic sludge using a batch decolorization assay. World Journal of Microbiology and Biotechnology, 20: 365–370.

Latham, M. J., Brooker, B. E., Pettipher, G. L. and Harris, P. J. (1978). *Ruminococcus flavefaciens* cell coat and adhesion to cotton cellulose and to cell walls in leaves of perennial ryegrass (Lolium perenne). Applied Environmental Microbiology. 35:156-165.

Lema, J. M. and Omil, F. 2001. Anaerobic treatment: a key technology for a sustainable management of wastes in Europe. Water Science and Technology, 44(8): 133-140.

Manu, B., and Chaudhari, S. 2002. Anaerobic decolorization of simulated textile wastewater containing azo dyes. Bioresource Technology, 82(3): 225-231.

Melgoza, R. M., Crus, A. and Bultron, G. 2004. Anaerobic/aerobic treatment of colorants present in textile effluents. Water Science and Technology, 50: 149-155.

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.

O'Neill, C., Hawkes, F. R., Hawkes, D. L., Lourenco, N. D., Pinheiro, H. M. and Delee, W. 1999. Color in textile effluents-sources, measurement, discharge consents and simulation: a review. Journal of Chemical Technology and Biotechnology, 74: 1009-1018.

Robinson, T., McMullan, G., Marchant, R. and Nigam, P. 2001. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresource Technology, 77: 247-255.

Senan, R.C. and Abraham, T.E. 2004. Bioremediation of textile azo dyes by aerobic bacteria consortium. Biodegradation, 15: 275-280.

Sponza, D.T. and Işik, M. 2004. Decolorization and inhibition kinetic of Direct Black 38 azo dye with granulated anaerobic sludge. Enzyme and Microbial Technology, 34: 147-158.

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

Weisburger, H. 2002. Comments on the history and importance of aromatic and heterocyclic amines in public health. Mutation Research, 507: 9-20.

Wijetunga, S., Xiufen, L., Wenquan, R. and Chen, J. 2007. Removal mechanisms of acid dyes of different chemical groups under anaerobic mixed culture. Ruhuna Journal of Science, 2: 96-110.

Yuen, C. W. M., Ku, S. K. A., Choi, P. S. R., Kan C. W. And Tsang, S. Y. 2005. Determining Functional Groups of Commercially Available Ink-Jet Printing Reactive Dyes Using Infrared Spectroscopy. Research Journal of Textile and Apparel, 9(2): 26-38.

****