Vol 7 (1), January 2016; 28-34

Microbial Decolorization of Textile Effluent

Talya Khalid[1], Aziz Fatima[1], Amna Shafiq[1], Sumaira Javed[1], Naheed Afshan[1], Sayyada Ghufrana Nadeem[1]

[1]Department of Microbiology, Jinnah University for Women, 5C Nazimabad, Karachi -74600.

ABSTRACT

Textile industries can produce huge amount of waste during disposal and directly discharge into the environment. This study is aimed to screen and identify dye degrading bacteria. Experimentation was performed by collecting waste water and soil sample from textile dumping sites. Six bacterial strains were isolate by using NAM. The identified microorganisms were *Staphylococcus aureus*, *Bacillus spp*, *Pseudomonas aeruginosa*, *Streptococcus spp*, *Bacillus spp*, and *Pseudomonas aeruginosa* (S1, S2, S3, S4, S5 and S6) respectively. These isolates were inoculated in Nutrient Broth with five different dyes viz. Red (3BS), Orange (R.R), Yellow (3RS), Blue(R), and Turquoise (V.S.C). Decolorizing ability of dyes was observed by dye decolorization assay. Result showed that these isolates degrade almost all dyes, (S1) 50-80 %, (S2, S6) 61-84% to 5-84%, (S3, S5)75-87% to 71-86% and (S4)70-85%. Almost all dyes except turquoise were decolorized after 4 days of incubation. This approach is promising for detoxification of textile effluent.

Keywords: *Bacteria, Textile Dyes, Effluent, Biodegradation.*

INTRODUCTION

The textile dying industry release enormous amount of waste waters every next dyeing process. The dyeing practice has in usual a small yield and the relative amount of the invisible dye in the effluents capability up to 50% (Sudha *et al.*, 2014; Pierce, 1994). Reactive and azo compounds are vulnerable to biological degradation under equally aerobic and anaerobic circumstances (Field *et al.*, 1993). Further More, the dyes without a relevant treatment can remain in the environment for comprehensive periods of time and are injurious not only for the photosynthetic processes of the sea plants They are generally used to color the substratum like textile fiber, paper, leather, hair, fur, plastic material, wax, a cosmetic base and food stuff. The textile dye industries directly discharge effluent into the neighboring canal, undeveloped field, irrigation channels, plane stream and these lastly go in to the river. Azo dyes have been second-hand increasingly in industries as of their simplicity and rate efficiency in production compared to normal dyes. Nevertheless, the majority azo dyes are poisonous, carcinogenic and mutagenic. (Shah *et al.*, 2013). Azo compounds are vulnerable to biological degradation under equally aerobic and anaerobic circumstances (Field *et al.*, 1993). Waste water from textile industry is a complex mixture of lots of pollutes matter such as organochlorine based pesticides, heavy metals, pigments and dyes. Treatment of such wastewaters is consequently, necessary but difficult. Biological treatment based on microbial alteration of textile dyes hold promise in only if a lower treatment cost and a further resourceful mean of effluent treatment (Mahbub *et al.*, 2012) Many microorganisms have been explored to have capability of degrading unlike harmful chemical of textile effluent. (Blánquez *et al.*, 2006). Up to 70% color exclusion was notice with different micro flora (Balakrishnan *et al.*, 2008). The technologies for dye subtraction have been separated into three major categories: physical, chemical and biological methods (Lokesh and

Sivakiran, 2014; Joshi *et al.*, 2013). Bacteria engage in biodegradation of dyes are *Bacillus firmus*, *Bacillus laterosporus*, Legionella, Chryseobacterium, Flavobacterium, *Pseudomonas*, *Enterobacter sp*, *Serratia sp*, *Staphylococcus sp*, *Yersinia sp*, *Erwinia sp*, and *Bacillus subtilis* etc. A number of funguses such as *Bjerkandera sp*, *Schizophyllum commune*, *Penicillium oxalicum*, *Rhizopus arrhizus*, *Phanerochaete chrysosporium*, *Fusarium moniliforme*, *Aspergillus terreus*, *Aspergillus niger*, *Mucor racemosus*, *Cladosporium cladosporioides*, *Trichoderma viride*, *A. ochraceus*, *Thermotolerant yeast*, Kluyveromyces marxianus etc, are concerned in dye degradation (Mahbub *et al.*, 2012).

This study is aimed to screen and identify dye degrading bacteria from natural environment and which have the capability to degrade commercial dyes used for textile dyeing industries and developed much easier method for the biodegradation of textile effluent and to control the environmental pollution which contribute to change different level of ecosystem.

MATERIALS AND METHODS

Samples Collection: Wastewater sample (n=5) from textile industry effluent and soil sample (n=5) contaminated with dyes were collected from the surrounding of textile factory from Karachi (Pakistan). The samples were collected in a sterile plastic container. Then the samples were transfer to laboratory as early as possible and kept at 4°C.

Dyes and Media: Five different reactive dyes were used for studying bioremediation process including Red (3BS), Orange (R.R), Yellow (3RS), Blue(R), and Turquoise (V.S.C) which were used by textile industries. Nutrient broth and Nutrient Agar media were used for isolation and preservation of bacterial isolates. The entire chemicals used in the study were purchased from Synozol Company (KISCO).

Isolation of Decolorizing Bacteria: Bacterial isolate were carried out by serially diluting 1 ml of each effluent sample and 1.0 gm of each soil sample was inoculated in 100 ml sterile distilled water in 250 ml flask. Inoculated flask was stir gently for 10 min. Then obtained suspension were serially diluted (10 fold) with sterile saline solution and from last dilution take 0.1 ml of each dilution was streak on nutrient agar plate and Incubated these plates at 35+ 2°C for 24 hrs. (Bayoumi *et al.*, 2014). Colonies were picked up with different morphology and purified by further sub culturing. The isolated colonies were maintained on nutrient agar slant for further identification.

Identification of Decolorizing Bacteria: The bacterial isolates were identified and characterized on the basis of morphological, cultural and biochemical characteristics as per Bergey's Manual of Determinative Bacteriology. The tests performed were Gram staining, IMVIC, H2S Production Catalase, Oxidase and Citrate utilization.

Isolated Microorganism Screened For Dye Removal: The bacterial isolates were tested for their ability to decolorize textile dye. The dyes were tested with pure isolates of bacteria isolated previously on Nutrient Agar Medium (NAM). The color removal efficiency of the isolate were measure in Nutrient Broth (NB). The experiment was done in 10ml inoculated and un inoculated test tubes tube containing 300 ppm of each study dyes .These inoculated tubes were inoculated with a loop full of growth from the slant and incubated at 37 °C for 4 day's. After 4 day's of incubation the test tube were observed for decolorization.

Measurement of Decolorization: The degradation of dyes was evaluated by decolorization efficiency of the solution, by measuring the absorbance (OD) of the culture filtrates at 4th day of incubation. 10ml of the uninoculated and inoculated broths

were centrifuge at 10,00rpm for 15 min then supernatant check at different wavelength related to studied dye (520nm for Red; 460nm for Blue; 575nm for Yellow; 490 for Turquoise; 590nm for Orange) with the help of UV-Visible Spectrophotometer. The values of decolorization were predicted as percentage of change of color intensity (OD value) in conjunction with control that hold the original dye concentration and no microbial inoculants (Abd El–Rahim *et al.*, 2003). Percent of decolorization was calculated as:

Decolorization (%) = I-F÷ I X 100

Where I = initial absorbance and F = absorbance of decolorized medium.

RESULTS & DISCUSSION

Soil and effluent sample collected from the surrounding of textile industry dumping side. Bacteria were isolate and screened by making 10

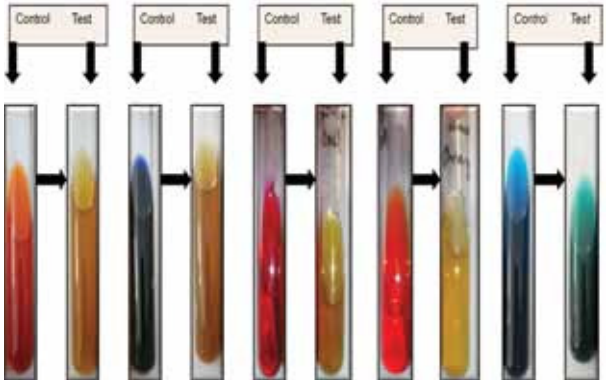

Figure 1. Uninoculated at right and inoculated tube at left containing study dyes.

Table I. Colony Characters and Morphology of the Selected Isolate

Sample	*Shape*	*Color*	*Surface*	*Pigmentation*	*Morphology*	*Gram staining*
S1	Irregular	Yellow	Smooth	Yes	Cocci in bunches, or scattered	Gram+ve
S2	Irregular	Cream	Rough	No	Rods, long chains	Gram+ve
S3	Irregular	Greenish	Smooth	Yes	Short rods ,single	Gram-ve
S4	Irregular	White	Smooth	No	Cocci, diploid, tetrad or in chains	Gram+ve
S5	Irregular	Cream	Smooth	No	Short rods, single or in pairs	Gram-ve
S6	Irregular	Cream	Rough	No	Short rods , single	Gram+ve

Table II. Results of Biochemical Characterization of Selected Bacterial Strains.

Bacterial strain	*Coagulase*	*Catalase*	*Indole*	*Citrate*	*Triple iron sugar/TSI*
Staphylococcus aureus	Positive	Positive	_	_	_
Bacillus spp.	_	Positive	_	_	_
Pseudomonas aeruginosa	_	Positive	Negative	Positive	Alkaline/ Alkaline
Enterococcus spp.	Negative	Negative	_	_	_
Pseudomonas aeruginosa	_	Positive	Negative	Positive	Alkaline/ Alkaline
Staphylococcus aureus	_	Positive	_	_	_

Table III. Decolorization Percentage of Each Isolate Average

S.No	Isolate	Dye's used in experiment (%)				
		Red	Blue	Turquoise	Yellow	Orange
1.	*Staphylococcus aureus*	54%	75%	50%	80%	77%
2.	*Bacillus spp.*	67.80%	80.5%	0%	84.80%	61%
3.	*Pseudomonas aeruginosa*	77.03%	74%	0%	74.85%	87%
4.	*Streptococcus spp.*	73.20%	70.30%	0%	85%	70.90%
5.	*Pseudomonas aeruginosa*	71%	86.80%	0%	81.70%	77.50%
6.	*Bacillus spp.*	70.30%	5.70%	0%	75.40%	84%

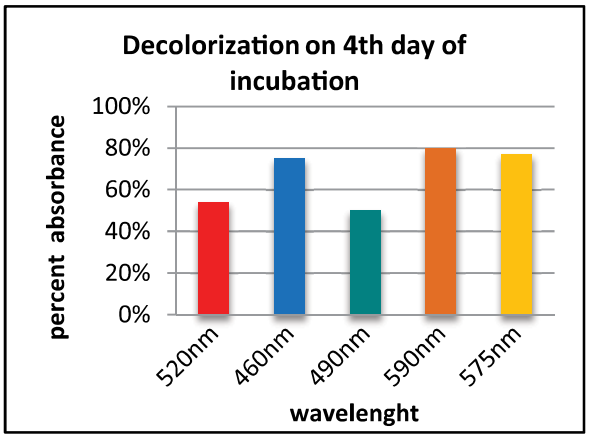

Figure 2(a): Bar Diagram Show's Degradation by *Staphylococcus aureus (S1)*

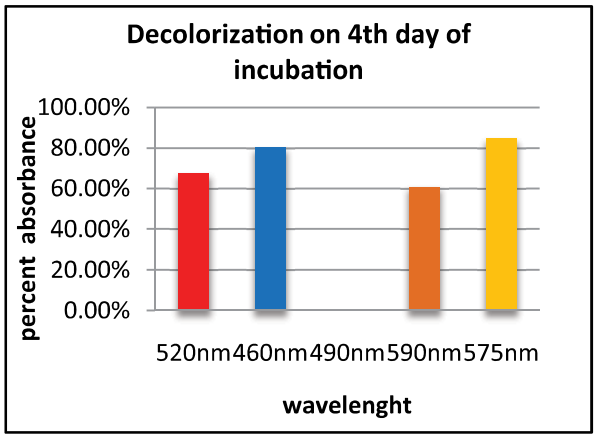

Figure 2(b): Bar Diagram Show's Degradation By *Bacillus spp. (S2)*

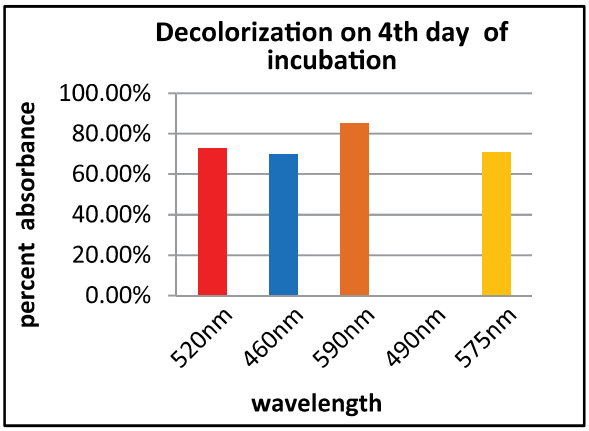

Figure 2(c): Bar Diagram Show's Degradation by *Pseudomonas.aeruginosa (S3)*

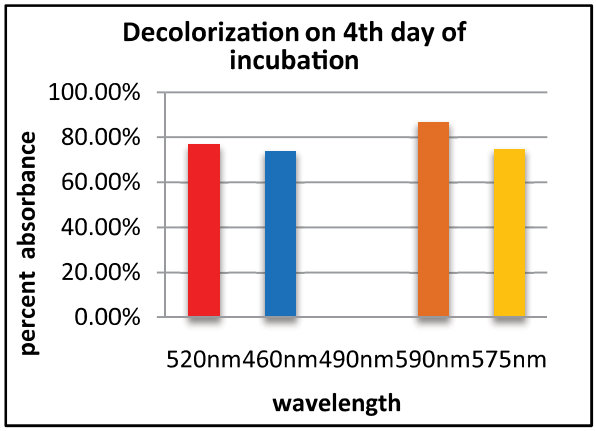

Figure 2(d): Bar Diagram Show's Degradation by *Streptococcus spp. (S4)*

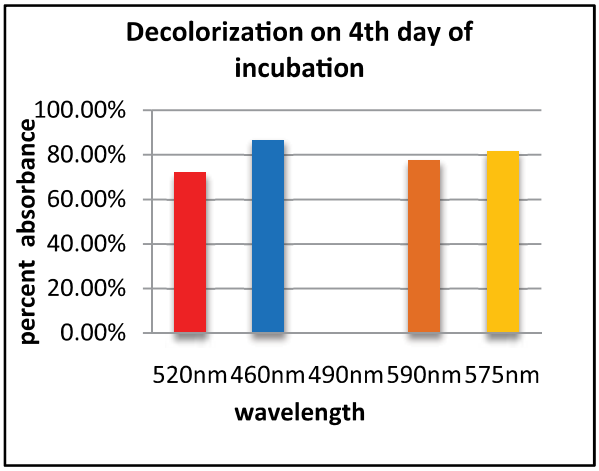

Figure 2(e): Bar Diagram Show's Degradation by *Pseudomonas sp. (S5)*

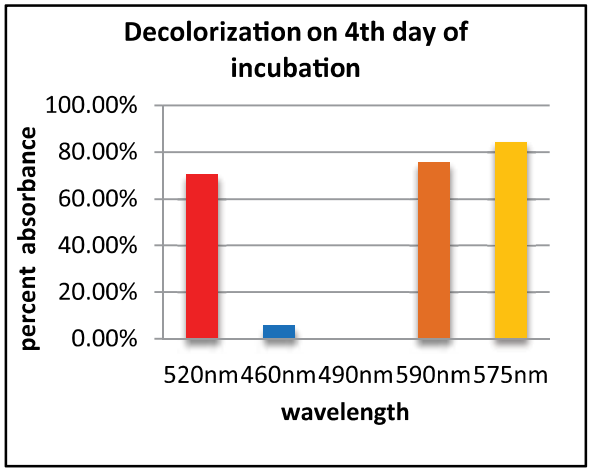

Figure 2(f): Bar Diagram Show's Degradation by *Bacillus spp. (S6)*

Key: Red (3BS), Orange (R.R), Yellow (3RS), Blue(R), and Turquoise (V.S.C)

fold dilutions and streak on nutrient agar plate for primary screening, colonies were picked up with different morphological characteristics. Six bacteria were isolate by (n=5) soil and effluent sample and represent as (S1, S2, S3, S4, S5 and S6) were isolate and shown on (table I-II). The identified microorganisms are: *Staphylococcus aureus*, *Bacillus spp*, *Pseudomonas aeruginosa*, *Streptococcus spp*, *Bacillus spp*, and *Pseudomonas aeruginosa*. These isolate were tested for the ability to decolorize study dyes. Secondary screening were performed by checking the ability of these organisms by incorporated into Nutrient Broth (NB) containing 300ppm of study dye's .These dye's include, Red (3BS), Orange (R.R), Yellow (3RS), Blue (R), and Turquoise (V.S.C), as shown in fig: 1.

After 4 day's of decolorization the decolorizing percentages can be calculated by their absorbance using UV-visible spectrophotometer.

In the undertaken study, bacterial isolates were screened for their ability to degrade different textile dyes. Adequate dye decolorization was observed by the selected bacteria after 4 days of incubation. The highest decolorization (87%) was found for the dye Orange (R.R) by *Pseudomonas aeruginosa*. Almost all dyes were found to be decolorized by the tested isolates. Only Turquoise (V.S.C) could not be decolorize by isolates S2, S3, S4, S5, and S6 after 4 days of incubation. Decolorizing percentages of the experimental dyes by tested bacteria were demonstrated in (Table III). In present study, the *Staphylococcus aureus* can degrade Red (3BS), 54%, Blue(R), 75%, Turquoise (V.S.C), 50%, Yellow (3RS), 80% and Orange (R.R), 77%. Prior research studies have displayed that *Staphylococcus sp.*, which were isolated from soil in a textile effluent treatment plant, have the ability to decolourize the sulfonate azo dye, congo red and four azo dyes (RY107, RR198, RB5 and DB71) (Elisangela *et al.*, 2009).The degradation percent shows *Staphylococcus spps* can decolorize up to 52% degradation at day 3, 54.08% degradation at day 6, 59.07% degradation at day 9 and 60% degradation at day 12 (Abioye *et al.*, 2015). *Pseudomonas aeruginosa* (97.33%) was identified as the best decolourizer of Congo Red. The best decolourizer of Direct Green was *Bacillus subtilis* (99.05%) (Manivannan *et al,* 2011). The best dye decolourizer of Red RR was found to be *Bacillus subtilis* (91%). Moreover, *Bacillus* subtillis best decolorizes the dye Yello RR (65%). *Pseudomonas aeruginosa* was very fine decolorizer of navy blue (70.58%) and *Psuedomonas putida* (95%) was identified as

the excellent decolourizer of Blue RR (Shah, 2014). Decolorization capacity of *Pseudomonas aeruginosa* (97.44%), *Bacillus subtilis* (86.66%) and *Pseudomonas fluorescens* (84.28%) was also studied and it was observed that the isolated bacterial strain have the capability to degrade these complex dyes. It was noticed that the bacterial isolate namely *Bacillus subtilis* (97.97%) showed maximum decolorization ability followed by *Pseudomonas fluorescens* (90.07%) and *Pseudomonas aeruginosa* (64.54%) (Rajaganesh and Basha, 2014). Present study showed similar results that *Bacillus* spp. (S2) can degrade Red (3BS), 67.80%, Blue(R), 80.5%, Yellow (3RS), 84.80%, Orange (R.R), 61% except Turquoise (V.S.C). .*Bacillus* spp. (S6) can degrade Red (3BS), 70.30% , Blue(R), 5.79% , Yellow (3RS), 81.70% , Orange (R.R), 84% and but could not degrade turquoise ,*Pseudomonas aeruginosa* (S3) can degrade Red (3BS), 77.30%, Blue(R), 74%, Yellow (3RS), 75.85%, Orange (R.R), 87% and ,*Pseudomonas aeruginosa* (S5) can degrade Red (3BS), 71%, Blue(R), 86%, Yellow (3RS), 81%, Orange (R.R), 77.5% except Turquoise (V.S.C). It was observed that Streptococcus spps were able to degrade the dye Concentration present in the Effluent. A maximum of 87.03% of dye was successfully removed from the Dye effluent (Lawrence and Kannan, 2013). *Streptococcus faecalis* showed the highest volumetric decolorization activity the removal in color was found to be 55% after 42 hrs of incubation period (Sivaraj *et al.*, 2011). Similar result found in present study that Streptococcus spp. degrade Red (3BS), 73.20%, Blue(R), 70.30%, Yellow (3RS), 85%, and Orange (R.R), 70.9%. In this study almost all bacteria have the ability to degrade all dyes except Turquoise (V.S.C) in literature only *Bacillus megaterium* demonstrated utmost decolourization of turquoise blue dye within 48 hours (Joshi *et al.*, 2013). *Bacillus megaterium* PMS82 able of degrading acid orange as a sole source of carbon under minimal nutritional condition under static condition (Shah, 2014).

In our study *Staphylococcus aureus* showed potential to degrade Turquoise (V.S.C), up to 50% after 4 days of incubation. The other bacteria still not identify to degrade turquoise in text and more exploration is desirable to understand their decolorizing potential. This study ensures the screening of microorganism which has the capacity to remove reactive dyes from textile industry waste water. The isolates showed ability to degrade the complex structure of study dyes by producing enzymes which break the complex structure of these dyes therefore it become the promising approach for detoxification of textile effluent.

REFERENCES

Abd El–Rahim WM, Moawad H and Khalafallah M. 2003. Microflora involved in textile dye waste removal. *J. Basic Microbial.*, 43(3):167-174.

Abioye, OP, Iroegu, VT and Aransiola SA. 2015. Biodegradation of Methyl Red by Staphylococcus aureus Isolated from Waste Dump Site. *J. Environ. Sci. Technol.*, 8(3): 131.

Balakrishnan M, Antony SA, Gunasekaran S and Natarajan RK. 2008. Impact of dyeing industrial effluents on the groundwater quality in Kancheepuram (India). Indian *J. Sci. Technol.*, 1(7):1-8.

Bayoumi MN, Al-Wasify RS and Hamed SR. 2014. Bioremediation of Textile Wastewater Dyes using Local Bacterial Isolates. Int. *J. Curr. Microbiol. Appl. Sci.*, 3(12): 962-970.

Blánquez P, Sarrà M and Vicent MT. 2006. Study of the cellular retention time and the partial biomass renovation in a fungal decolourisation continuous process. *Water Res.*, 40(8):1650-1656.

Elisangela F, Andrea Z, Fabio DG, de Menezes Cristiano R, Regina DL and Artur, CP. 2009. Biodegradation of textile azo dyes by

a facultative Staphylococcus arlettae strain VN-11 using a sequential microaerophilic / aerobic process. *Int Biodeterior. Biodegradation*, 63(3): 280-288.

Field, JA, de Jong E, Feijoo-Costa G and de Bont JA. 1993. Screening for ligninolytic fungi applicable to the biodegradation of xenobiotics. *Trends Biotechnol.* , 11(2): 44-49.

Joshi B, Kabariya K, Nakrani S, Khan A, Parabia FM, Doshi HV and Thakur MC. 2013. Biodegradation of turquoise blue dye by *Bacillus megaterium* isolated from industrial effluent. *AJEP*, 1(2): 41-46.

Lawrence S and Kannan R. 2013.Textile Dye Removal from Wastewater Effluents by Using Potential Microbes. *IJSR*, 2319-7064.

Lokesh KN and Sivakiran RR. 2014. Biological methods of dye removal from textile effluents-A review. *J. Biochemical Technol.*, 3(5): 177-180.

Mahbub KR, Mohammad A, Ahmed MM and Begum S. 2012. Decolorization of synthetic dyes using bacteria isolated from textile industry effluent. *Asian J. Biotechnol*, 4(3): 129-136.

Manivannan M, Reetha D and Ganesh P, 2011. Decolourization of textile azo dyes by using bacteria isolated from textile dye effluent. *J. Ecobiotechnol.*, 3(8): 29-32.

Pierce J. 1994. Colour in textile effluents-the origins of the problem. *J. Soc. Dyers Colour*, 110: 131-134.

Rajaganesh K and Basha SA. 2014. Decolorization of selected Reactive azo dyes by bacterial isolates from textile dye effluents .*International Journal of Environmental Biol.*, 4(3): 215-220.

Shah MP. 2014. Azo dye reduction by Methanogenic Granular Sludge Exposed to Oxygen. *Int. J. Environmen. Bioremed. & Biodegradation*, 2(1): 18-24.

Shah MP. 2014. Biodegradation of azo dyes by three isolated bacterial strains: An environmental bioremedial approach. *J. Microbial Biochemical Technol.*, S3: 007. doi:10.4172/1948-5948.S3-007.

Shah MP, Patel KA, Nair SS and Darji AM. 2013. Microbial degradation of textile dye (Remazol Black B) by *Bacillus spp.* ETL-2012. *J. Bioremed. Biodegradation*, 4: 180. doi:10.4172/2155-6199.1000180.

Sivaraj R, Dorthy CAM and Venckatesh R. 2011. Isolation, characterization and growth kinetics of bacteria metabolizing textile effluent. *J. Biosci. Technol.*, 2: 324-330.

Sudha M, Saranya A, Selvakumar G and Sivakumar N. 2014. Microbial degradation of azo dyes: a review. *Int. J. Curr. Microbial Appl. Sci.*, 3: 670-690.