

# Microbial Decolorization of Textile Dye by *Bacillus Spp.* ETL-79: An Innovative Biotechnological Aspect to Combat Textile Effluents

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**Abstract** Isolation and identification of dye decolorizing bacterial isolate from textile dye effluent was carried out. The isolates of *Bacillus spp.* ETL-79 were isolated from the textile effluent samples collected from textile industries of Ankleshwar, Gujarat, India. Different parameters were used for optimizing. Conditions for maximum decolorization depend on the bacterial isolate. The results showed that the temperature (40°C), pH (8.00), Biological Oxygen Demand (220 mg<sup>l</sup><sup>-1</sup>), Chemical Oxygen Demand (700 mg<sup>l</sup><sup>-1</sup>), Total Suspended Solids (2800 mg<sup>l</sup><sup>-1</sup>), Total Dissolved Solids (7500 mg<sup>l</sup><sup>-1</sup>) and color over the prescribed fresh water limits. A potential bacterial strain was isolated and selected from the textile effluent on the basis of rapid azo dye Crystal violet (100mg<sup>l</sup><sup>-1</sup>) decolorization and later identified as belonging to genus *Bacillus* based on Phenotypic characterization. Effects of physicochemical parameters (pH, Temperature, etc.) on the Crystal violet decolorization by the *Bacillus spp.* ETL-79 were studied. Decolorization was effective at pH 8, 35°C with starch and peptone as carbon and nitrogen sources and in static conditions. This decolorization potential increased the applicability of this microorganism for the dye removal.

**Keywords:** *Bacillus spp.*, Crystal violet, color Removal

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## 1. Introduction

The growth of the world population, the development of various industries, and the use of fertilizers and pesticides in modern agriculture have overloaded not only the water resources but also the atmosphere and the soil with pollutants [10]. In the last few decades the handling of wastewater appeared to be one of the most important. Textile industry which is one of the largest water consumers in the world produces the wastewater comprising of various recalcitrant agents such as dye, sizing agents and dyeing aid. Therefore it has to be really concerned in releasing these types of wastewater to the environment. In the disposal of textile wastewater, color is of very important due to the aesthetic deterioration as well as the obstruction of penetration of dissolved oxygen and sun light into natural water bodies [24]. The degradation of the environment due to the discharge of polluting wastewater from industrial sources is a real problem in several countries. This situation is even worse in developing countries like India where little or no treatment is carried out before the discharge [3,4,25]. In spite of the many steps taken to maintain and improve the quality of surface and groundwater, the quantities of wastewater generated by these industries continue to increase and

municipalities and industries are confronted with an urgent need to develop safe and feasible alternative practices for wastewater management. Bioremediation is a pollution-control technology that uses natural biological species to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. Xenobiotic compounds are not naturally available and hence the locally occurring microorganisms cannot readily degrade them. Hazardous materials may render harm to humans, livestock, wildlife, crops or native plants through handling, ingestion, application to land or other distributions of the contaminated materials into the environment. The textile industry leaves about 50 % of the textile azo dyes in free state to be discharged in the factory effluent and eventually to the surrounding environment. Azo compounds constitute the largest and the most diverse group of synthetic dyes and are widely used in a number of industries such as textile, food, cosmetics and paper printing [21]. The reactive azo dyes-containing effluents cause serious environmental pollution. Therefore, industrial effluents containing azo dyes must be treated before discharging into the environment to remove the dye toxicity from textile effluents [11,23,27,29]. This study aims to investigate the potential of bacterial cultures isolated from industrial dye effluent for decolorization of a textile dye, Crystal violet. Dye decolorization by bacterial cultures was optimized with respect to various nutritional

sources (carbon and nitrogen), environmental parameters (temperature, pH).

## 2. Materials & Methods

### 2.1. Sampling and Analysis of Effluent

Ankleshwar is one of the most industrialized cities in India. It is known as the chemical hub and was chosen for effluent sample collection. The Effluent sample was collected from the middle point of the area. Standard procedures (Spot and Grab) were followed during sampling. The Temperature and pH were determined at the sampling site. The pH was determined by using pH meter (Cyber scan pH meter) and temperature with laboratory thermometer. The sample was transported to laboratory at 4°C as in accordance with the standard methods [31]. The physicochemical parameters such as (Colour, Biological Oxidation Demand (BOD) Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), and Total Dissolved Solids (TDS) were determined as soon as the sample was brought to the laboratory. Sample colour was analyzed by spectrophotometer (Shimadzu UV-1700). BOD was determined by employing evaporation method by DO meter while COD was measured by COD instrument directly.

### 2.2. Chemicals

The textile dye, Crystal violet ( $\lambda_{\max}$  523 nm) was obtained from Ankleshwar Textile Industries, Ankleshwar. Nutrient broth. A stock solution of the dye (1000 mg L<sup>-1</sup>) was prepared in de-ionized water and used for all studies.

### 2.3. Isolation, Screening and Identification of Dye Decolorizing Bacteria from Effluent

The Textile Effluent was collected in sterile collection tubes from the sludge and wastewater of the ditches at industrial site located in Ankleshwar Textile Industries, Ankleshwar. The sample collected from the textile mill was screened for azo dye (Crystal violet) decolorizing bacterial strains by inoculating 10 ml of sludge solution into 250ml. Erlenmeyer flask containing 100ml nutrient broth (gL<sup>-1</sup> Peptone-5, Meat extract-1, Yeast extract-2, NaCl-5, pH-7). The flasks were incubated at 35°C under shaking conditions (140rpm). After 48h of incubation, 1.0ml. of the culture broth was appropriately diluted and plated on Nutrient Agar containing 20 mg L<sup>-1</sup> Crystal violet. The Morphologically distinct bacterial isolates showing clear zones around their colonies due to decolorization of dye were selected for further studies. The pure culture stocks of these isolates were stored at 4°C on Nutrient Agar slopes containing 1000 mg L<sup>-1</sup> of Crystal violet. These isolates were screened for their ability to decolorize Crystal violet in liquid culture. The Screening process in liquid media was carried out by inoculating a loop full of cultures exhibiting clear zones into Nutrient broth containing Crystal violet under static conditions. After 24h of incubation, 1ml. of cell suspension was transferred to fresh nutrient broth containing Crystal violet to screen the strains with color removing ability. The Screening procedure in liquid

medium was continued until complete decolorization of broth. A small amount of decolorized broth was transferred to nutrient agar plates containing Crystal violet (50 mg L<sup>-1</sup>). The bacterial isolate which tolerated higher concentration of the Azo dye was isolated by streaking plate method. The Azo dye decolorizing bacteria was identified from several aspects including morphology characters, biochemical tests as described in Bergey's manual of determinative bacteriology (Indole, Methyl Red, Voges-Proskauer test, Citrate, Catalase, Oxidase, Nitrate Reduction test, Hydrolysis of Casein, Starch, Urea and Gelatin). Assimilation of various sugars such as D-glucose, D-fructose, galactose, mannitol and D-maltose as sole carbon source were determined by inoculating the isolate into carbohydrate broth supplemented with respective carbon source. After inoculation the tubes were incubated at 37°C for 24-48h.

### 2.4. Decolorization Assay

The decolorizing activity was expressed in terms of the percentage decolorization by the modified method described previously [8]. The Decolorization process was carried out using shaking culture and static culture by inoculating 1ml. of precultured (O.D 0.85-1) *Bacillus spp.* ETL-79 into 100ml. of sterilized Nutrient broth in 250 ml. Erlenmeyer flask and incubated on rotary shaker (130 rpm) at 35°C for 24h [15]. Filter sterilized (0.22  $\mu$ m) Crystal violet (100 mgL<sup>-1</sup>) was added to the culture and incubated in shaking conditions at 140rpm and in static conditions at room temperature for decolorization to occur. At regular intervals, 4 ml. sample was withdrawn aseptically and centrifuged at 8,500 rpm for 15min. The cell free supernatant was used to determine the percentage decolorization of Crystal violet. Decolorization of dye was determined by monitoring the decrease in absorbance at the maximum wavelength of Crystal violet ( $\lambda_{\max}$  523 nm) by using a UV-Visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu, Japan). The uninoculated dye Medium supplemented with respective dye was used as blank [13]. Decolorization activity (%) was calculated by the following formula and all assays were done in triplicate:

$$\% \text{Decolorization} : \frac{\text{Initial absorbance} - \text{final absorbance} \times 100}{\text{Initial absorbance}}$$

### 2.5. Decolorization of Crystal Violet under Different Culture Conditions

The decolorization efficiency of *Bacillus spp.* ETL-79 strain was compared over a wide range of pH (5-9) by adjusting the pH with hydrochloric acid or sodium hydroxide. Decolorization at different Temperatures (RT, 35°C, 37°C, 40°C, 45°C, 50°C) was carried out by adjusting the pH to 8. Varying Carbon sources 1% each (dulcitol, starch, maltose, sucrose, dextrose, mannitol, d-xylose, lactose, mannose) and Nitrogen sources 1% each (urea, potassium nitrate, sodium nitrate, malt extract, ammonium sulphate, ammonium nitrate, ammonium chloride, peptone) were used to check the decolorizing potential of the strain. All the flasks were incubated in static conditions at pH 8 and at 35°C.

### 3. Results & Discussion

#### 3.1. Physico-chemical Characterization of Textile Effluent

The effluent sample collected from a small scale Textile Industries, Ankleshwar, Gujarat, India was dark black in color, with pungent smell and pH of slightly above neutral level and was within the permissible limits (Table 1). The temperature of the effluent was high. Total Suspended Solids (TSS) and Total Dissolved Solids (TDS) in the textile effluent were very high. The solids present in ground water, besides effecting the growth of the plants directly, also affect the soil structure, permeability and aeration, indirectly effecting the plant growth. The Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) values were within the permissible limits in the effluent sample. Different bacterial strains isolated from the textile effluent were screened for their ability to decolorize the textile Azo dye (Crystal violet) and the potential strains were characterized morphologically and biochemically.

**Table 1. Physico-chemical characterization of the textile effluent collected from Textile Industries, Ankleshwar**

Sr.No	Parameter	Unit	Effluent
1	Color	-	Dark black
2	Smell	-	pungent
3	Temperature	°C	38
4	pH	-	8.0
5	TDS	mg/l	7500
6	TSS	mg/l	2800
7	COD	mg/l	700
8	BOD	mg/l	220

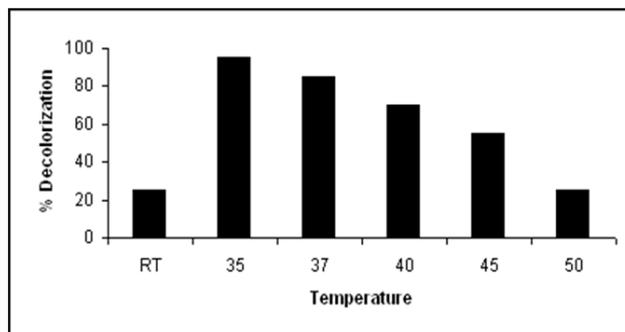
#### 3.2. Isolation and Identification

The study was started by screening for potential textile Azo dye decolorizing bacteria isolated from the textile industry effluent. Colonies surrounded by a nearly decolorized zone were isolated and then tested for dye removal capability using submerged culture. Strains isolated from the white colonies were inoculated in 100ml. of Nutrient broth in a 250ml. conical flask and incubated at 35°C under static conditions. ETL-79 exhibiting highest decolorizing activity was chosen for further studies. The gram staining test showed the isolate to be non-motile, gram positive, spore forming, and rod-shaped bacteria. The spore was terminally located and ellipsoidal in shape. Biochemical characterization of the isolate revealed it to be negative for Indole, Methyl Red test, Voges-Proskauer, Citrate, Catalase, oxidase test and Nitrate Reduction test. The isolate showed negative result for the hydrolysis of casein, gelatin, starch and urea. The strain utilized various sugars, D-Maltose, D-Glucose, D-Fructose, Mannitol and Galactose as sole carbon sources and was found to be positive.

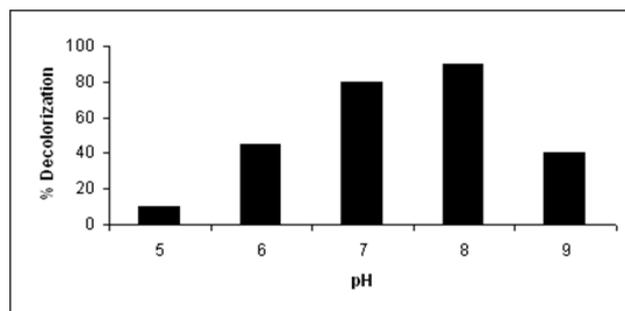
#### 3.3. Effect of pH and Temperature on Decolorization

The decolorization efficiency of *Bacillus spp.* ETL-79 was compared across a wide range of pH (5-9). The maximum decolorization (90%) was recorded at pH 8. At

neutral pH the strain exhibited percentage decolorization value of 80%. Whereas it was 45% and 40% at pH 6 and 9. The percentage decolorization decreased markedly at pH 5 (10%) due to acidic conditions (Figure 2). The optimum pH for growth and decolorization was found to be 8. The dye decolorization activity of the strain was found to decrease with increasing incubation temperature. Highest decolorization was achieved at 35°C (95%) and least percentage decolorization was at Room Temperature (RT) (25%). At 37°C there was 85% decolorization noted followed by 70%, 55% and 25% at 40°C, 45°C and 50°C respectively at the end of 24h incubation (Figure 1). No specific decolorization was observed in shaking conditions (140 rpm).

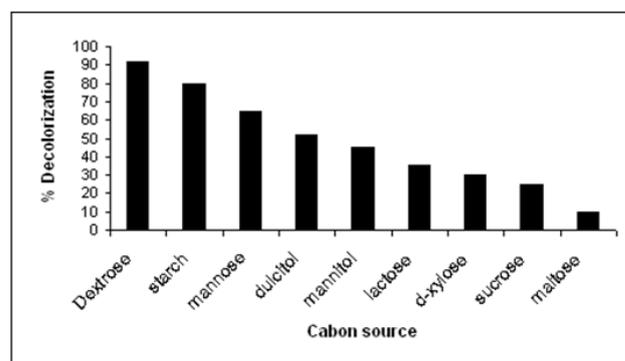


**Figure 1.** Effect of temperature on Crystal violet decolorization



**Figure 2.** Effect of pH on Crystal violet decolorization

#### 3.4. Effect of Different Carbon and Nitrogen Sources on Crystal Violet Decolorization



**Figure 3.** Effect of Carbon source on Crystal violet decolorization

Results of Crystal violet decolorization by ETL-79 with different Carbon (Figure 3) and Nitrogen sources (Figure 4) are depicted. Dextrose resulted in higher decolorization efficiency to 92% followed by starch (80%) and mannose (65%) at the end of 24h incubation period. The decolorization efficiency decreased with dulcitol (52%),

mannitol (45%), lactose (35%), d-xylose (30%) and sucrose (25%). Least decolorization was observed with maltose (10%). Maximum decolorization with nitrogen sources was achieved with Peptone (90%) and least was with Malt extract (15%). Urea and Ammonium sulphate exhibited good decolorization with 75% and 65%. The decolorization efficiency decreased markedly with Ammonium nitrate (50%), Sodium nitrate (25%), Potassium nitrate (22%) and Ammonium chloride (15%).

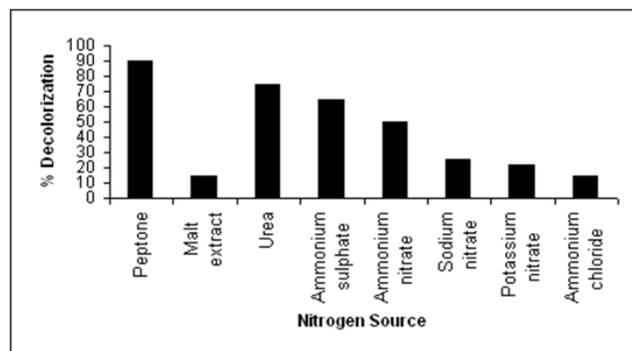


Figure 4. Effect of Nitrogen source on Crystal violet decolorization

### 3.5. Discussion

Throughout India, there is a grave concern and constant attention given to the treatment of industrial effluent from textile and dye manufacturing units. Several researchers have demonstrated the possibility of utilizing microorganisms for biotreatment of textile waste water. In India, most textiles units are scattered and/or operated from private homes. Therefore, it is necessary to collect and treat the waste in common effluent treatment plants. Microbiological methods are quite simple to use and cost of operation is low. Industrial effluent is not stable and it varies often in a wide range depending upon the process practiced. South Asian countries are experiencing severe environmental problems due to rapid industrialization. This phenomenon is very common where the polluting industries like textile dyeing, leather tanning, paper and pulp processing, sugar manufacturing, etc. thrive as clusters. Among these the Textile industries are large industrial consumers of waters as well as producers of wastewater. The effluent discharged by this industry leads to serious pollution of groundwater and soils and ultimately affects the livelihood of the poor [14]. The physico-chemical characterization of the collected textile effluent sample from Textile Industries, Ankleshwar showed a high load of pollution indicators. Colour is contributed to a water body by the dissolved compounds (dyes and pigments). The effluent color was dark black due to mixture of various dyes and chemicals used in the dyeing process. The pH of the study sample was slightly alkaline when compared to the acidic pH of the dyeing effluent in a previous study [26]. The pH of the effluent alters the physico-chemical properties of water which in turn adversely affects aquatic life, plant and humans. The soil permeability gets affected resulting in polluting underground resources of water [28]. The temperature of the effluent was high in comparison with the temperature of another effluent in one study [19]. High temperature decreases the solubility of gases in water which is ultimately expressed as high BOD/COD. The values of

BOD and COD were within the permissible limits in the present sample in comparison to the very high values of BOD and COD. TDS and TSS values of effluent sample were higher than the permissible limits. Sediments rate is drastically increased because of High value of Total Dissolved Solids which reduces the light penetration into water and ultimately decrease the photosynthesis. The decrease in photosynthetic rate reduces the DO level of wastewater which results in decreased purification of wastewater by microorganisms [9]. The current sample exhibited high values of heavy metals which were of the same order of magnitude reported in another effluent sample [18]. The nutrients of the surrounding soils are depleted as a result of high value of heavy metals thereby affecting soil fertility. High chloride contents are harmful for agricultural crops if such wastes containing high chlorides are used for irrigation purposes [2]. Majority of the textile effluent samples have permissible limits of sulphate ions. The effluent showed phenolic contents greater than 0.1 ppm which is though permissible limit of the phenolic compounds that are very toxic to fish even at very low concentrations [5]. The bleaching and dyeing process are the main causes of pollutants which include caustic soda, hypochlorite and peroxides. The isolation of different microorganisms from the effluent sample collected from the Textile Industries, Ankleshwar indicates natural adaptation of microorganisms to survive in the presence of toxic chemicals and dyes. Interest in the bioremediation of pollutants using bacteria has intensified in recent years, as many researches demonstrated the efficacy of bacterial bioremediation over fungal and Actinomycetes. Many bacteria capable of reducing Azo dyes reported were isolated from Textile effluent contaminated sites [7]. A strain of bacterium *Bacillus spp.* ETL-79 with strong decolorizing ability was isolated from Textile effluent to decolorize the textile Azo dye Crystal violet ( $100 \text{ mgL}^{-1}$ ) within 24h in aerobic and static conditions. The reason for the decreased decolorization under shaking conditions could be competition of oxygen and dye compounds for the reduced electron carriers under aerobic conditions. The percentage decolorization of Crystal violet by *Bacillus spp.* ETL-79 strain under static conditions was 90% within 24h of incubation which was equal to a similar study but with 35h of incubation period [17]. In another study conducted with *Pseudomonas putida*, *P. fluorescence*, *Bacillus cereus* and *Stentrophomonas acidaminiphila* to decolorize Acid Red 88 showed their efficiencies at 35%, 31%, 40% and 50% respectively [12]. Under aerobic conditions azo dyes are generally resistant to attack by bacteria [6]. The optimal pH for complete decolorization of Crystal violet was 8 which is slightly lower in accordance with *Cosmarium spp.* Decolorizing malachite green at pH 9 [30] and *Klebsiella pneumonia* RS-13 which completely degraded Methyl Red in pH range of 6 to 8 [20]. Optimal growth temperature was found to be  $35^\circ\text{C}$  which is consistent with the highest decolorization temperature in our study. Maximum potential of *Pseudomonas sp.* to decolorize Malachite green, fast green was noticed at  $37^\circ\text{C}$  [1]. *Vibrio loeigi* and *Pseudomonas nitroreducens* showed the highest Methyl Red degradation activity at  $30\text{-}35^\circ\text{C}$  [16]. Dextrose and Peptone were found to be the most effective carbon and nitrogen sources for decolorization of Crystal violet in the present study compared to Lactose and Yeast

extract in another similar study for decolorization of Everzol Red RBN [22].

## 4. Conclusion

The study concluded that pH, temperature, various carbon & nitrogen source have a significant influence on dye removal efficiency by *Bacillus spp.* ETL-79. This shows that the isolated bacterium have enormous potential to degrade the textile dyes and resolve the problem of unnecessary dyes present in the effluents of textile industries. Further pilot scale studies are required with these strains for actual industrial applications, and detailed study is needed to explore the mechanism involved. Although decolorization is a challenging process to both the textile industry and the waste water treatment, the result of this findings and literature suggest a great potential for bacteria to be used to remove color from dye wastewaters. The bacterial strain *Bacillus spp.* HMM-87 showed decolorizing activity through a degradation mechanism rather than adsorption. This observation has established that the bacteria are adaptive in nature and can degrade contaminants. The ability of the strain to tolerate, decolorize azo dyes at high concentration gives it an advantage for treatment of textile industry waste waters. However, potential of the strain needs to be demonstrated for its application in treatment of real dye bearing waste waters using appropriate bioreactors. Application of traditional waste water treatment requires enormous cost and continuous input of chemicals which becomes uneconomical and causes further environmental damage. Hence, economical and eco-friendly techniques using bacteria can be applied for fine tuning of waste water treatment. Biotreatment offers easy, cheaper and effective alternative for colour removal of textile dyes.

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## References

- [1] Adedayo, O.; Javadpour, S.; Taylor, C.; Anderson, WA.; Moo-Young, M.; (2004). Decolorization and Detoxification of Methyl Red by aerobic bacteria from a wastewater treatment plant. *World J Microbiol Biotechnol*, 20, 545-550.
- [2] Agarwal, SK. (1996). Industrial Environment: Assessment and strategy .APH Publishing Corporation, New Delhi, India.
- [3] Appasamy, P.Paul. et. al., (2000). Madras School of Economics, 14, 76.
- [4] Bhagirath, Behera.; Reddy, V.; Rathna, (2002). Economic and Political Weekly, Jan.19.
- [5] Coughlin, MF.; Kinkle, BK.; Tepper, A.; Bishop, PL.; (1997). Characterization of aerobic azo dye degrading bacteria and their activity in biofilms. *Water Sci Technol* 36: 215-220.
- [6] Daneshvar, N.; Ayazloo, M.; Khataee, AR.; Pourhassan, M.; (2007). Biological Decolorization of dye solution containing Malachite Green by *Microalgae Cosmarium sp.* *Bioresour. Techno* 98: 1176.
- [7] Dawkar, V.; Jadhav, U.; Jadhav, S.; Govindwar S. (2008). Biodegradation of disperse textile dye Brown 3REL by newly isolated *Bacillus sp.* *VUS J Appl Microbiol* 105: 14-24.
- [8] Deepak, KS.; Harvinder, SS.; Manjinder, S.; Swapandee, SC.; Bhupinder, SC.; (2004). Isolation and Characterization of microorganisms capable of decolorizing various triphenylmethane dyes. *J. Basic Microbiol* 44(1): 59-65.
- [9] Delee, W.; Niel, CO.; Hawkes, FR.; pinheiro, HM.; (1998). Anaerobic treatment of textile effluents: a review. *Journal of Chemical Technology and Biotechnology* 73: 323-325.
- [10] Getoff, N.; (2002). *Rad. Phys. Chem.*, 65,437-446.
- [11] Hao, O. J.; Kim, H.; and Chaing, P. C.; (2000) Decolorization of wastewater. *Critical Reviews. Environmental Science and Technology.*, pp. 30: 449-505.
- [12] Hu ,TL.; (1998). Degradation of azo dye RP2B by *Pseudomonas luteola*. *Water Sci Technol* 38: 299-306.
- [13] Jacob, Thomson.; (1998). Impact of Industries on the Ground Water Quality of Tiruppur and its Ethical implications, Ph.D. Thesis, Dept.of Zoology, University of Madras, Chennai.
- [14] Jiunkins, R.; (1982). Pretreatment of textile waste water. Proc. 37<sup>th</sup> Industrial waste Conference Purdue Uni. Lafayette, Ind p.37-139
- [15] Kalyani, DC.; Telke, AA.; Dhanve, RS. ; Jadhav, JP.; (2009). Eco-friendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated *Pseudomonas sp.* SUK1. *J Hazard Mater* 163: 735-742.
- [16] Kapdan, K.I.; F, Kargi.; G, McMullan.; and R, Marchant.; (2000). Effect of environmental conditions on biological decolorization of textile dyestuff by *C. versicolor*. *Enzyme and Microbial Technol*, 26: 381-387.
- [17] Khehra, MS.; Saini, HS.; Sharma, DK.; Chadha, BS.; Chimni, SS.; (2005). Decolorization of various azo dyes by bacterial consortium. *Dyes Pigments* 67: 55-61.
- [18] Kim, HT.; (1994). Soil reaction. In: Environmental soil science Marcel Dekker Inc., U.S.A, p. 149.
- [19] Kumar, A.; (1989) Environmental Chemistry. Wiley Eastern Limited, New Delhi, India.
- [20] Mali, PL.; Mahajan, MM.; Patil, DP.; Kulkarni, MV.; (2000). Biodecolorization of members of triphenylmethanes and azo groups of dyes. *J Sci Ind Res India* 59: 221-224.
- [21] Pandey, A.; Singh, P.; Iyengar, L.; (2007) Bacterial decolorization and degradation of azo dyes. *Int Biodeter Biodegr.*, pp.59:73-84.
- [22] Panswed, J.; Wongehaisuwan, S.; (1986). Mechanism of dye waste water color removal by magnesium carbonate-hydrate basic. *Water Sci Technol* 18: 139-144.
- [23] Rajaguru, P.; Vidya, L.; Baskarasethupathi, B.; Kumar P. A.; Palanivel, M.; and Kalaiselvi, K.; (2002). Genotoxicity evaluation of polluted ground water in human peripheral blood lymphocytes using the comet assay. *Mutation Research*. pp. 517: 29-37.
- [24] Spadaro, J.T.; Isabelle, L.; and Renganathan, Y.; (1994). *Env. Sci. Technol.*, 28,1389-1393.
- [25] Sunitha, Hooda.; and Sumanjee, Kaur.; S, Chand.; & Company Limited, (1999). New Delhi.
- [26] Tyagi, OD.; Mehra, M.; (1990). A textbook of environmental chemistry. Anmol Publications, New Delhi, India.
- [27] Umbuzeiro, G. A.; Freeman, H.; Warren, S. H.; Oliveira, D. P.; Terao, Y.; Watanabe, T.; and Claxton, L.D.; (2005). The contribution of azo dyes to the mutagenic activity of the Cristais River. *Chemosphere*. pp. 60: 55-64.
- [28] Vandevivre, PC.; Bianchi, R.; Verstraete, W.; (1998). Treatment and reuse of wastewater from the textile wet-processing industry: review of emerging technologies. *J Chem Technol Biotechnol* 72:289-302.
- [29] Wafaa, M.; Abd, El-Rahim. Moawad. H.;. and Khalafallah, M.; (2003). Microflora involved in textile dye waste removal. *Journal of Basic Microbiology.*, pp.43: 167-174.
- [30] Wong, P.; Yuen, P.; (1998). Decolorization and Biodegradation of N,N-Dimethyl-p-phenylenediamine by *Klebsiella pneumoniae* RS-13 and *Acinetobacter liquifaciens*-1. *J Appl. Microbiol* 85: 79.
- [31] Yatome, C.; Ogawa, T.; Koga, D.; and Idaka, E.; (1981). Biodegradation of Azo and triphenylmethane dyes by *Pseudomonas pseudomallei*. 13na. *J. Society. dyers colorist.*; 97: 166-169.