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PHYSICO CHEMICAL ANALYSIS OF TEXTILE EFFLUENT AND DECOLORIZATION OF TEXTILE AZO DYE BY *BACILLUS ENDOPHYTICUS STRAIN* VITABR13

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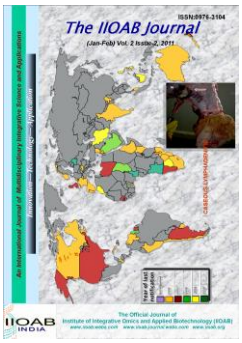
ABSTRACT



The physicochemical characterization of the textile industry effluent collected from Coimbatore in Tamil Nadu,India was been carried out and the results showed high rates of temperature (40°C), pH (7.51) and Electrical Conductivity (9565 µmhos/cm), Biological Oxygen Demand (275 mgl^{-1}), Chemical Oxygen Demand (789 mgl^{-1}), Total Suspended Solids (1750 mgl^{-1}), Total Dissolved Solids (5875mg l^{-1}), heavy metal ions, Total hardness (Ca^{2+}, Mg^{2+}, Cl^{-} & SO_4^{2-}) and colour over the prescribed fresh water limits. A potential bacterial strain(VITABR13) was isolated and selected from the textile effluent on the basis of rapid azo dye Acid Red 128(100mgl^{-1})decolorization and later identified as belonging to genus Bacillus based on Phenotypic characterization and phylogenetic analysis of the 16s rRNA gene sequence. Effects of physicochemical parameters (pH, Temperature, Carbon and Nitrogen sources) on the Acid Red 128 decolorization by the selected bacterium 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.

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Keywords: *textile Industry effluent; Bacillus endophyticus VITABR13; acid red 128; decolorization*

[I] INTRODUCTION

Water pollution control is at present one of the major areas of scientific activity. Textile industries are large industrial consumers of waters as well as producers of wastewaters. With the increased demand for textile products, the textile industry and its wastewaters have been increasing proportionally, making it one of the main sources of severe pollution problems worldwide [1,2]. The diversity in composition of chemical reagents used in textile industries contributes to much of the water pollution. The reagents range from inorganic compounds to polymers and organic products [3]. Waste water generated by different production steps of a textile mill have high pH, temperature, detergents, oil, suspended and dissolved solids, dispersants, leveling agents, toxic and non-biodegradable matter, color and alkalinity. [4]. Important pollutants in textile effluent are mainly recalcitrant organics, color, toxicants and surfactants, chlorinated compounds (AOX). The textile wastewaters are characterized by extreme fluctuations in many parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH, colour and salinity [5].The process of adding colour to the fibres is known as dyeing which normally requires large volumes of water not only in the dye bath, but also during the rinsing step? The process of dyeing involves the use of different chemicals like salts, metals, surfactants, organic processing assistants, sulphide and formaldehyde. There are more than 8,000 chemical products associated with the dyeing process and over 100,000 commercially available dyes exist with over 7×10^5 metric tons of dyestuff produced annually [6].

Approximately a half of all known dyes are azo dyes, making them the largest group of synthetic colorants. Azo dyes consist of a diazotized amine coupled to an amine or phenol. Atleast 3000 different varieties of azo dyes are extensively used in the textile, paper, food, cosmetics and pharmaceutical industries [7]. Azo dyes are characterized by the presence of one or more azo groups (R_1-N=N-R_2) which are responsible for their colorations and when such a bond is broken (degraded) the compound loses its color [8].A related topic having received considerable interest is the presence of color in effluents associated with production of dyes. Color in the effluent is one of the most obvious indicators of water pollution and the discharge of highly colored synthetic dye effluents is aesthetically displeasing and can damage the receiving water

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body by impeding penetration of light [9].Dyes are recalcitrant molecules which are difficult to degrade biologically. Some of azo dyes are either toxic or mutagenic and carcinogenic [10].Azo dyes are designed to resist chemical and microbial attacks and to be stable in light and during washing [11].Treatment of dye waste water involves chemical/physical methods, among which are coagulation, precipitation, adsorption by activated carbon, oxidation by ozone, ionizing radiation and ultrafiltration.These physico- chemical methods are generally costly, produce wastes which are difficult to dispose of, are less efficient and of limited applicability [12].As a viable alternative, biological processes have received increasing interest owing to their cost effectiveness, ability to produce less sludge, and environmental friendliness [13].Microorganisms capable of degrading azo dyes include *proteus spp* [14],*Enterococcus spp* [15], *Streptococcus spp*[16],*Bacillus cereus* [17], *Streptomyces spp* [18], and the white rot fungus *Chanerochaetes chrysosporium* [19].Decolorization of azo dyes normally begins with initial reduction or cleavage of azo bond anearobically, which results into colorless compounds. This is followed by complete degradation of aromatic amines strictly under aerobic conditions [20].The effectiveness of microbial decolorization depends on the survival, adaptability, and activity of the microorganism [21].Azo dyes generally resist aerobic microbial degradation, only organisms with specialized azo dye reducing enzymes were found to degrade azo dyes under fully aerobic conditions [22]. Although there are many studies on microbial decolorization of azo dyes by various microbial species, it is necessary that new experiments be conducted for finding new resources and microorganisms with suitable biological properties for decolorization.These new microorganisms which have capability to grow in polluted environment, minimal nutritional requirements and rapid growth can be used to achieve good results in decolorization experiments [23]. Hence, the Objective of our present study was: 1) to characterize the textile effluent for its physico-chemical parameters 2) To assess the potential of *Bacillus endophyticus* VITABR13 strain isolated from textile effluent to decolorize an azo dye (Acid Red 128) and to optimize the various physico chemical parameters such as pH, Temperature, Carbon and Nitrogen sources for efficient dye decolorization.

[II] MATERIALS AND METHODS

2.1. Sampling and analysis of effluent

Coimbatore is one of the most industrialized cities in Tamil Nadu, India. It is known as the textile capital of South India or the Manchester of the South India (Lat.11º 00'N, Long. 77º 00' E) 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 (Hanna digital pH meter, model-671-p) and temperature with laboratory thermometer. The sample was transported to laboratory at 4ºC as in accordance with the standard methods [24].

The physicochemical parameters such as (Colour, Electrical Conductivity (EC), Biological Oxidation Demand (BOD) Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), heavy metal ions) were determined as soon as the sample was brought to the laboratory. Sample colour was analysed by U-3010 spectrophotometer (Hitachi, Japan) while Electrical Conductivity(EC) was determined by conductivity meter (Jenvway EC meter, model-4070).BOD was determined by employing evaporation method by DO meter while COD was measured by COD instrument directly. Chloride and Sulphate contents were assessed by titrimetric and turbidity method, respectively [25]. The phenolic compounds were determined by photometric method. Different metal ions present in the effluent sample were determined by Atomic Absorption Spectrophotometer (AAS) as per the standard methods.

2.2. Chemicals

The textile dye Acid Red 128(λmax. 523nm) was obtained from a small Dyeing Industry in Coimbatore, Tamil Nadu. Nutrient broth (gL^{-1}Peptone-5, Meat extract-1, Yeast extract-2, Nacl-5, pH-7) A stock solution of the dye (1000mg L^{-1}) 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 Coimbatore. The sample collected from the textile mill was screened for azo dye (Acid Red 128) decolorizing bacterial strains by inoculating 10 ml. of sludge solution into 250ml.Erlenmeyer flask containing 100ml. Nutrient broth (gL^{-1}Peptone-5, Meat extract-1, Yeast extract-2, Nacl-5,pH-7). The flasks were incubated at 35°C under shaking conditions (130rpm).After 48h of incubation, 1.0ml. of the culture broth was appropriately diluted and plated on Nutrient Agar (gL^{-1} Peptone-5, Meat extract-1, Yeast extract-2, Nacl-5, Agar-15, pH – 7.0) containing 20mg L^{-1} Acid Red 128.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 1000mg L^{-1} of Acid Red 128.These isolates were screened for their ability to decolorize Acid Red 128 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 Acid Red 128 under static conditions. After 24h of incubation, 1ml. of cell suspension was transferred to fresh nutrient broth containing Acid Red 128 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 Acid Red 128 (50 mg L^{-1}).The bacterial isolate which tolerated higher concentration of the Azo dye was isolated by streak plate method. The Azo dye decolorizing bacteria was identified from several aspects including morphology characters, biochemical tests, and sequence analysis of nearly complete 16srRNA gene.

The identification and characterization of the potential isolate was done by gram staining, motility, presence of spores, spore position and spore

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morphology and by following 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 was determined by inoculating the isolate into carbohydrate broth supplemented with respective carbon source. After inoculation the tubes were incubated at 37ºC for 24 - 48h.The Molecular identification of the potential strain was carried out by amplifying the 16srRNA gene as described previously [26].The PCR product was purified using the QIA quick PCR purification kit (Qiagen).Sequencing was performed by using an ABI PRISM model 3700 automatic DNA sequencer and the big dye terminator cycle sequencing kit (both from Applied Biosystems).The almost complete 16srRNA gene sequence (1565 bp) was aligned with closely related sequences retrieved from EMBL by using CLUSTAL W [27]. Pairwise evolutionary distances were computed by using MEGA-4 software. Phylogenetic tree was constructed using NEIGHBOR, UPGMA, KITSCH, FITCH and DNAPARS of the PHYLIP package [28].The stability among the clades of a phylogenetic tree was assessed by taking 1000 replicates of the dataset with a cut off value of 50.

2.4. Decolorization assay

The decolorizing activity was expressed in terms of the percentage decolorization by the modified method described previously [29]. The Decolorization process was carried out using shaking culture and static culture by inoculating 1ml. of precultured (O.D 0.8-1) *B.endophyticus* VITABR13 into 100ml. of sterilized Nutrient broth in 250ml.Erlenmeyer flask and incubated on rotary shaker (130rpm) at 35ºC for 24h [30]. Filter sterilized (0.22 µm) Acid Red 128 (100 mgL^{-1}) was added to the culture and incubated in shaking conditions at 130rpm and in static conditions at room temperature for decolorization to occur. At regular intervals, 4ml.sample was withdrawn aseptically and centrifuged at 10,000 rpm for 15min.The cell free supernatant was used to determine the percentage decolorization of Acid Red 128.Decolorization of dye was determined by monitoring the decrease in absorbance at the maximum wavelength of Acid Red 128(λmax. 523nm) by using a UV-Visible spectrophotometer (UV-1700 pharmaspec, shimadzu). The uninoculated dye Medium supplemented with respective dye was used as blank [31]. Decolorization activity (%) was calculated by the following formula and all assays were done in triplicate:

$$\% \text{ decolorization} = \left(\frac{\text{Initial Absorbance} - \text{Final Absorbance}}{\text{Initial Absorbance}} \right) \times 100$$

2.5. Decolorization of acid red 128 under different culture conditions

The decolorization efficiency of *Bacillus endophyticus* VITABR13 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.

2.6. Statistical analysis

Data was statistically defined by one-way ANOVA using Microsoft excel. Results in each experiment were interpreted depending upon probabilities. Probability (p-value) was less than 0.05 which was found to be significant.

[III] RESULTS

3.1. Physico chemical characterization of textile effluent

The effluent sample collected from a small scale Textile Dyeing Industry in Coimbatore, Tamil Nadu, India, was black in colour, with pungent smell and pH of slightly above neutral level and was within the permissible limits. The temperature of the effluent was high. Electrical Conductivity (EC) of the effluent was low. 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. The results of heavy metals analyzed in the textile effluent such as $Cu2^{+}$, Cd^{2+}, Zn^{2+}, Fe^{2+}, Cr^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+} had showed their amounts to be considerably high. Among the metals the level of Iron was the highest. The levels of Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) metal cations in the effluent whose combined effect leads to the total hardness of water was also found to be very high. The sulphate ion (SO_4^{2-}) content was found to be within permissible limits but the chloride (Cl^{-}) content was found to be remarkably high which is an index of surface pollution level. The high salt concentration in ground water leads to formation of a saline soil and is a serious hazard to agriculture. The phenolic content was found to be greater than 0.1ppm which is though permissible but still toxic **[Table–1]**. Different bacterial strains isolated from the textile effluent were screened for their ability to decolorize the textile Azo dye (Acid Red 128) and the potential strains were characterized morphologically, biochemically and at molecular level for identification. The bacterial count (CFU/ml) was significantly high.

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. One strain

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exhibiting highest decolorizing activity was chosen for further studies. The gram staining test showed the isolate to be non-motile, gram positive, spore forming, 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 **[Table–2]**. The potential strain was phylogenetically identified as *Bacillus endophyticus* VITABR13 on the basis of 16S rRNA gene sequence (1556bp).The sequence was compared with other sequences from GenBank database using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).The strain VITABR13 was closely related to *B.endophyticus* (2DT) AF295302 (98%) **[Figure–1]**. The sequence of the 16S rRNA gene of the strain VITABR13 is available under the GenBank accession number GU014293.

Table: 1. Physicochemical characterization of the textile effluent

S.No	Parameter	Units	Effluent
1.	Colour	-	Black
2.	Smell	-	Pungent
3.	Temperature	° C	40
4.	pH	-	7.51
5.	EC	µ mhos/cm	9565
6.	TSS	mg l^{-1}	1750
7.	TDS	mg l^{-1}	5875
8.	COD	mg l^{-1}	789
9.	BOD	mg l^{-1}	275
10.	Cu^{2+}	mg l^{-1}	3.62
11.	Cd^{2+}	mg l^{-1}	0.5
12.	Zn^{2+}	mg l^{-1}	1.0
13.	Fe^{2+}	mg l^{-1}	6.42
14.	Cr^{3+}	mg l^{-1}	1.5
15.	Mn^{2+}	mg l^{-1}	5.4
16.	Ni^{2+}	mg l^{-1}	0.4
17.	Pb^{2+}	mg l^{-1}	0.37
18.	Ca	mg l^{-1}	1500
19.	Mg	mg l^{-1}	889
20.	Cl^{-}	mg l^{-1}	2013
21.	SO_4^{2-}	mg l^{-1}	240
22.	Phenol	mg l^{-1}	0.143
23.	Bacterial Count	CFU/ml	11.6×10^5

3.3. Effect of pH and temperature on decolorization

The decolorization efficiency of *B.endophyticus* VITABR13 was compared across a range of pH (5-9).The maximum decolorization (90%) was recorded at pH 8. At neutral pH the strain exhibited percentage decolorization value of 77%.Where as it was 47% and 44% at pH 6 and 9.The percentage decolorization decreased markedly at pH 5 (8%) 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

Table: 2. Morphological and biochemical characteristics of VITABR13.

Character	VITABR13
Gram staining	+
Morphology	Rods
Motility	-
Spore	+
Spore position	Terminal
Spore shape	ellipsoidal
Indole	-
Methyl Red	-
Voges-Proskauer	-
Citrate test	-
Catalase test	-
Nitrate Reduction test	-
Oxidase test	-
Hydrolysis of:	
Casein	-
Gelatin	-
Starch	-
Urea	-
Acid from:	
D-Maltose	+
Mannitol	+
D-Glucose	+
D-Fructose	+
Galactose	+

35°C (90%) and least percentage decolorization was at Room Temperature (RT) (27%).At 37°C there was 82% decolorization noted followed by 67%, 50% and 29% at 40°C, 45°C and 50°C respectively at the end of 24h incubation **[Figure–3]**. No specific decolorization was observed in shaking conditions (130rpm). (Data not included)

3.4. Effect of different carbon and nitrogen sources on acid red 128 decolorization

Results of Acid Red 128 decolorization by VITABR13 with different Carbon **[Figure–4]** and Nitrogen sources **[Figure–5]** are depicted. Dextrose resulted in better decolorization efficiency with 91% followed by starch (78%) and mannose (62%) at the end of 24h incubation period. The decolorization efficiency decreased with dulcitol (56%), mannitol (42%), lactose (37%), d-xylose (34%) and sucrose (28%).Least decolorization was observed with maltose (11%). Maximum decolorization with nitrogen sources was achieved with Peptone (87%) and least was with Malt extract (16%).Urea and Ammonium sulphate exhibited good decolorization with 77% and 61%.The decolorization efficiency decreased markedly with Ammonium nitrate (57%), Sodium nitrate (26%), Potassium nitrate (22%) and Ammonium chloride (21%).

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[IV] DISCUSSION

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 [32]. The physico-chemical characterization of the collected textile effluent sample from Coimbatore 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 black due to mixture of various dyes and chemicals used in the dyeing process. [33]. The pH of the study sample was slightly alkaline when compared to the acidic pH of the dyeing effluent in a previous study [34]. 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 [35]. The temperature of the effluent was high in comparison with the temperature of another effluent in one study [36]. 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 in one effluent study.

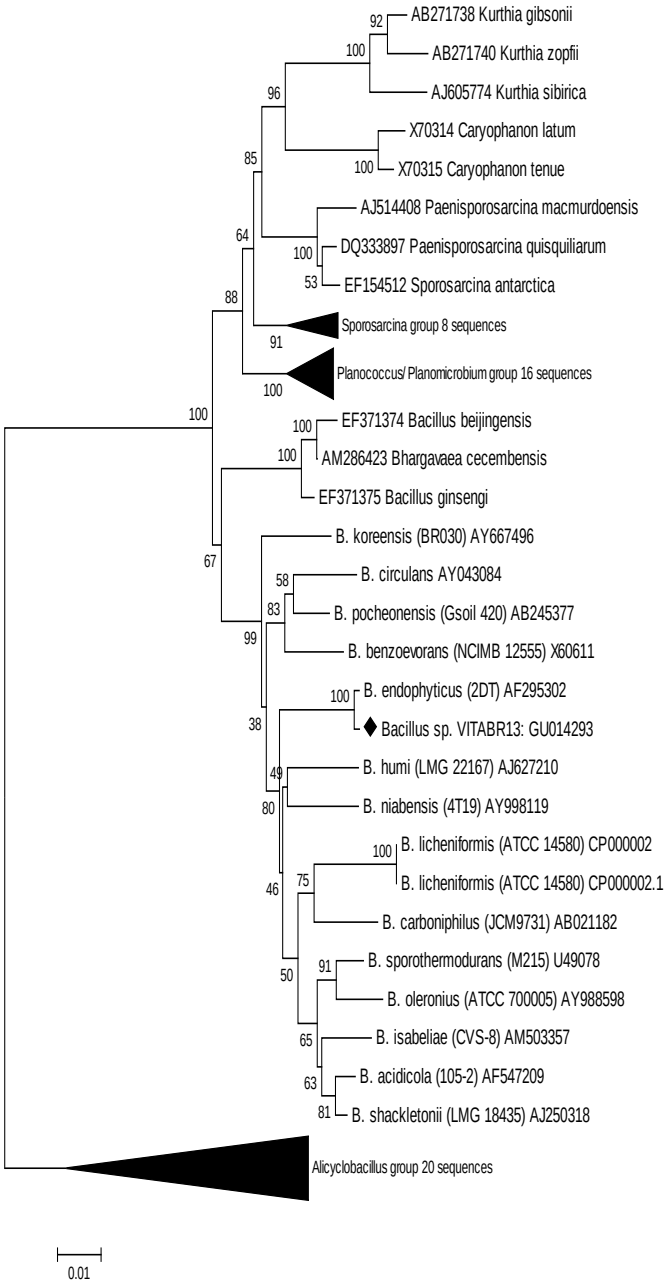

Fig: 1. Phylogenetic analysis of 16s rRNA sequence of Bacillus sp.VITABR13. The per cent numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analyses of 1,000 replicates.The scale bar (0.01) indicates the genetic distance.

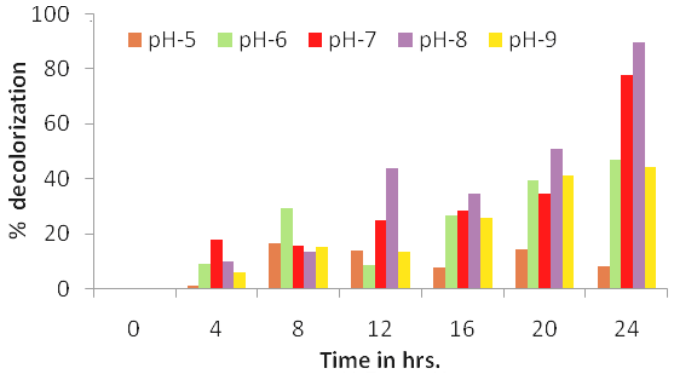

Fig.2 Acid Red 128 decolorization at different pH

The Electrical Conductivity of the sample was low. TDS and TSS values of effluent sample was high than the permissible limits but when compared to a textile effluent collected from a mill near Hisar (Haryana) was found to be low [37].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 [38].The current sample exhibited high values of heavy metals which was of the same order of magnitude reported in another effluent sample [39].The nutrients of the surrounding soils are depleted as a result of high value of heavy metals thereby affecting soil fertility.The Textile effluent had low values of sulphate and very high values of chloride content which are nearly equal to the value of one of the dyeing effluent sample from Faisalabad, Pakistan [40] .High chloride contents are harmful for agricultural crops if such wastes containing high chlorides are used for irrigation purposes. [41]. Majority of the

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textile effluent samples have permissible limits of sulphate ions. The effluent showed phenolic contents greater than 0.1ppm which is though permissible limit of the phenolic compounds still these compounds are very toxic to fish even at very low concentrations [42]. The bleaching and dyeing process are the main causes of pollutants which include caustic soda, hypochlorite and peroxides.

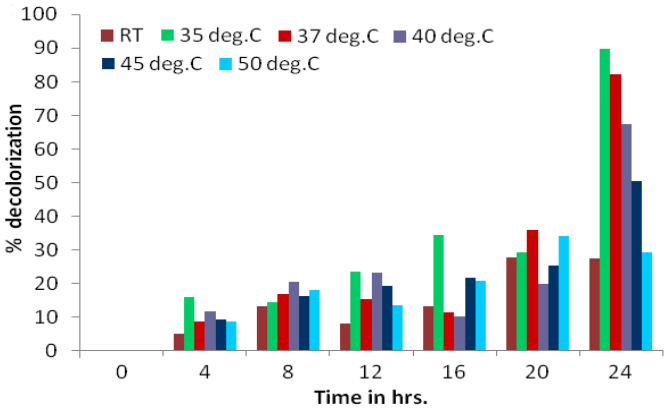

Fig: 3. Acid Red 128 decolorization at different Temperatures

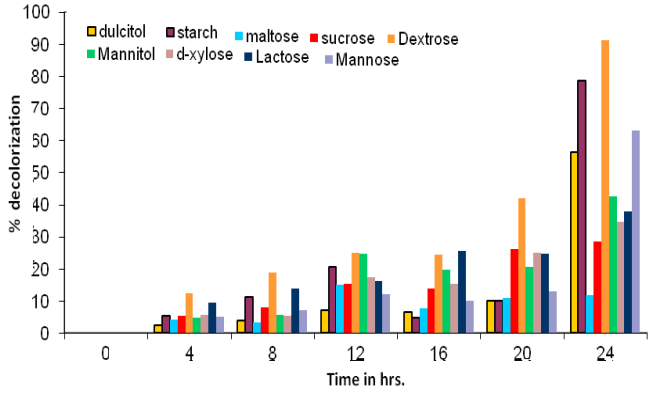

Fig: 4. Acid Red 128 decolorization in different C-sources

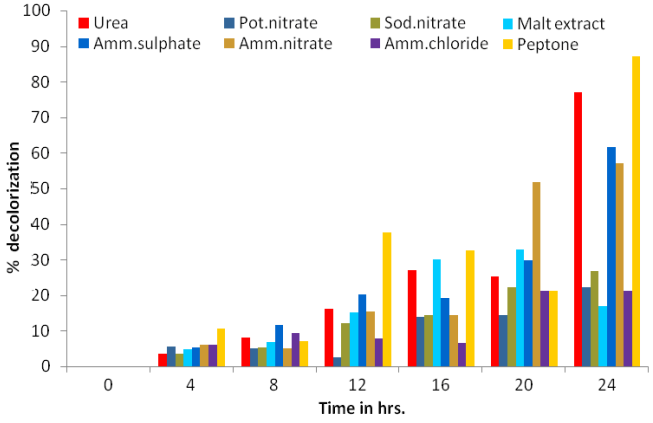

Fig: 5. Acid Red 128 decolorization in different N-sources

The isolation of different microorganisms from the effluent sample collected from the Textile Industry in Coimbatore indicates to 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 [43]. A strain of bacterium *Bacillus endophyticus* VITABR13 with strong decolorizing ability was isolated from Textile effluent to decolorize the textile Azo dye Acid Red 128(100 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 [44]. The percentage decolorization of Acid Red 128 by Bacillus *endophyticus* VITABR13 strain under static conditions was 90% within 24h of incubation which was equal to a similar study but with 35h of incubation period[45]. 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 [46]. Under aerobic conditions azo dyes are generally resistant to attack by bacteria [47]. The optimal pH for complete decolorization of Acid Red 128 was at 8 which is slightly in accordance with *Cosmarium sp.* Decolorizing malachite green at pH 9 [48] and *Klebsiella pneumonia* RS-13 which completely degraded Methyl Red in pH range of 6 to 8[49]. Optimal growth temperature of VITABR13 was found to be 35°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°C [50].*Vibrio logei* and *Pseudomonas nitroreducens* showed the highest Methyl Red degradation activity at 30-35°C [51].Starch and Peptone were found to be most effective carbon and nitrogen sources for decolorization of Acid Red 128 by VITABR13 in the present study compared to Lactose and Yeast extract in another similar study for decolorization of Everzol Red RBN [52].

[V] CONCLUSION

Although decolorization is a challenging process to both the textile industry and the wastewater treatment, the result of this findings and literature suggest a great potential for bacteria to be used to remove color from dye wastewaters. Interestingly, the bacterial species used in carrying out the decolorization of Azo dye Acid Red 128 in this study was isolated from the textile dye industry waste effluent. The bacterial strain *Bacillus endophyticus* VITABR13 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 wastewaters. However, potential of the strain needs to

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be demonstrated for its application in treatment of real dye bearing wastewaters using appropriate bioreactors.

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REFERENCES

[1] Asia IO, Oladoja NA, Bamuza-pemu EE. [2006] Treatment of Textile Sludge using anaerobic technology. *African Journal of Biotechnology* 5 (18): 1678–1683.

[2] Andre dos BS, Francisco JC, Jules van BL. [2007] Review paper on current Technologies for decolourisation of textile wastewaters: Perspectives for anaerobic Biotechnology. *Bioresource Technology* 98: 2369–2385.

[3] Mishra G, Tripathy M. [1993] A critical review of the treatments for decolourisation of textile effluent.*Colourage* 40: 35–38.

[4] Kumar P, Prasad B, Mishra IM, Chand S.[2007] *Haz Mater* 149: 26–34.

[5] Talarposhti AM, Donnelly T, Anderson GK. [2001] Colour removal from a simulated dye wastewater using a two-phase anerobic packed bed reactor. *Water Res* 35: 425–432.

[6] Zollinger H. [1987] Colour Chemistry-Synthesis, Properties and Application of Organic dyes and pigments.*VCH publishers*, New York, p. 92–100.

[7] Brown MA, De Uito SC. [1993] Predicting azo dye toxicity.*Crit Rev Environ Sci Technol* 23: 249–324.

[8] Chagas EP, Durrant LR. [2001] Decolorization of azo dyes by *Phanerochaete chrysosporium* and *Pleurotus sajorcaju*. *Enzyme Microb Technol* 29: 473–477.

[9] Khehra MS, Saini HS, Sharma DK, Chadha BS, Chimni SS. [2006] Biodegradation of azo dye C.I.Acid Red 88 by an anoxic-aerobic sequential bioreactor. *Dyes and Pigments* 70: 1–7.

[10] Hildenbrand S, Schmahl FW, Wodarz R, Kimmel R, Dartsch PC. [1999] Azo dyes are carcinogenic aromatic amines in cell cultures. International Archieves of Occupational and Environmental Health 72 (suppl.) M52vM56.

[11] Rajaguru P, Kalaiselvi K, Palanivel M, Subburam V. [2000] Biodegradation of azo dyes in a sequential anaerobic-aerobic system. *Applied Microbiology and Biotechnology* 54: 268–273.

[12] Quezada M, Linares I, Buitron G. [2000] Use of sequencing batch biofilter for degradation of azo dyes (acids and bases).Water Sci Technol 42: 329–336.

[13] Verma P, Madamwar D. [2003] Decolorization of synthetic dyes by a newly isolated strain of *Serratia marcescens*.World *Journal of Microbiology and Biotechnology* 19: 615-618.

[14] Roxon JJ, Ryan AJ, Wright SE. [1966] Reduction of tartrazine by a *Proteus* species isolated from rats. Food Cosmet Toxicol, 4: 419-426.

[15] Scheline RR, Longberg B. [1965] The absorption, metabolism, w 55-58.

[16] Wuhrmann K, Mechsner K, kappeler T. [1980] Investigation of rate- determining factor in the microbial reduction of azo dyes. *Eur J Appl Microbiol Biotechnol* 9: 325–338.

[17] Paszcynski A, Pasti MB, Goszczynski S, Crawford DL,Crawford RL. [1991] New approach to improve degradation of recalcitrant azo dyes by *Sptreptomyces spp* and *Phanerochaete chrysosporium*. *Enzyme Microbiol Technol* 13: 378–383.

[18] Glenn GK, Gold MH. [1983] Decolorization of several polymeric dyes by the lignin-degrading basidomycete *Phanerochaete chrysosporium. Appl Environ Microbiol* 45: 1741–1747.

[19] Sponza DT, Isik M. [2002] Decolorization and azo dye degradation by anaerobic/aerobic sequential process.*Enzyme and Microbial Technology* 31: 102–110.

[20] Kim SJ, Ishikawa K, Hirai M, Shoda M. [1995] Characterization of newly isolated fungus Geotrichum candidum Dec 1 which decolorizes various dyes.*J Ferment Bioeng* 79: 601–607.

[21] Ganesh R, Boardman GD, Michelsen D. [1994] Fate of azo dyes in sludges. *Water Res* 28(6): 1367–1376.

[22] Churchley JH. [1994] Removal of dye wastewater colour from sewage effluent-the use of a full scale ozone plant. *Water Sci Tech* 30: 275–284.

[23] Greenberg AE, Clesceri AE, Eaton AD. [1992] Standard Methods for the examination of water and wastewater, 18th ed. American Public Health Association, Washington, DC, p.212.

[24] Yatome C, Ogawa T, Koga D, Idaka E. [1981] Biodegradability of azo and triphenylmethanes dyes by *Pseudomonas pseudomallei* 13NA. *Journal of Society of Dyers and Colorists* 97: 166–169.

[25] Shivaji S, Vijaya Bhanu N, Aggarwal RK. [2000] Identification of *Yersinia pestis* as the causative organism of plague in india as determined by 16S rDNA sequencing and RAPD- based genomic fingerprinting. *FEMS Microbiol Lett* 189: 247–252.

[26] Thompson JD, Higgins DG, Gibson TJ. [1994] CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position- specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673–4680.

[27] Felsenstein J. [1993] PHYLIP (Phylogeny inference package), version 3.5c.Department of Genetics, University of Washington, Seattle, USA.

[28] Rump HH, Krist K. [1992] Laboratory manual for the examination of water, wastewater and soil. 2nd ed. VCH Publishers, New York, p.321.

[29] Deepak KS, Harvinder SS, Manjinder S, Swapandeep SC, Bhupinder SC. [2004].Isolation and Characterization of microorganisms capable of decolorizing various triphenylmethane dyes. *J.Basic Microbiol* 44 (1): 59–65.

[30] 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.

[31] 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.

[32] Jiunkins R. [1982] Pretreatment of textile waste water.Proc. 37th Industrial waste Conference Purdue Uni. *Lafayette, Ind* p. 37–139.

[33] Buckley CA. [1992] Membrane technology for the treatment of dyhouse effluents. *Water Sci. Techno*, 25 (10): 203–209.

[34] Tyagi OD, Mehra M. [1990] A textbook of environmental chemistry.Anmol Publications, New Delhi, India.

[35] 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.

ISSN: 0976-3104

[36] Kumar A. [1989] Environmental Chemistry. Wiley Eastern Limited, New Delhi, India.
[37] Senthilnathan S, Azeez PA. [1999] Water Quality of Effluents from Dyeing and Bleaching Industry in Tiruppur, TamilNadu India. *Journal of Industrial Pollution Contro*, 15(1): 79–88.
[38] 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.
[39] Kim HT. [1994] Soil reaction. In: Environmental soil science. Marcel Dekker Inc., U.S.A, p. 149.
[40] Pan GX, Chen JM, Gao JQ, Lui SL, Zhen JW. [2000] On the change of status of some metal elements in soil environment under economical development. *Resources and Environment in the Yangtze Basin* 9: 51–55.
[41] Agarwal SK. [1996] Industrial Environment: Assessment and strategy .APH Publishing Corporation, New Delhi, India.
[42] 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.
[43] 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.
[44] Junnarkar N, Murty JD, Bhatt NS, Madamwar D. [2006] Decolorization of diazo dye Direct Red 81 by a novel bacterial consortium. World J Microbiol Biotechnol 22:163–168.
[45] Khehra MS, Saini HS, Sharma DK, Chadha BS, Chimni SS. [2005] Decolorization of various azo dyes by bacterial consortium. *Dyes Pigments* 67: 55–61.
[46] Hu TL. [1998] Degradation of azo dye RP_2B by *Pseudomonas luteola.Water Sci Technol* 38: 299–306.
[47] 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.
[48] Wong P, Yuen P. [1998] Decolorization and Biodegradation of N,N-Dimethyl-p-phenylenediamine by *Klebsiella pneumonia RS-13* and *Acinetobacter liquifacienss*-1.*J Appl.Microbiol* 85: 79.
[49] 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.
[50] Adedayo O, Javadpour S, Taylor C, Anderson WA, Moo-Young M. [2004] Decolourization and Detoxification of Methyl Red by aerobic bacteria from a wastewater treatment plant. *World J Microbiol Biotechnol*,20: 545–550.
[51] Kapdan IK, Kargi F, McMullan G, Marchant R. [2000] Decolorization of textile dyestuffs by a mixed bacterial consortium.*Biotechnology Letters* 22: 1179–1181.
[52] Panswed J, Wongehaisuwan S. [1986] Mechanism of dye waste water color removal by magnesium carbonate-hydrate basic. *Water Sci Technol* 18: 139–144.

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