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Assessment of Physicochemical Properties of Textile Wastewaters and Screening of Bacterial Strains for Dye Decolourisation

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Abstract:

Textile industries considerably contributes to water pollution of adjoining surface water bodies which in turn remarkably alters biological, chemical and physical nature of the water bodies. In the present investigation, textile wastewater samples were collected from Common Effluent Treatment Plant (CETP), Pali, Rajasthan, to monitor the quality of effluent generated by CETP. Grab Sampling was carried out in the months of October-2011 and April-2012 in accordance with standard procedures (APHA, 1998).The objectives of present study were to assess the major physicochemical parameters like temperature, pH, colour, DO, BOD, COD, alkalinity, chloride, hardness, nitrate, phosphate, TS, TDS of inlet, primary clariflocculator, aeration tank and outlet .The second objective was to isolate and characterize the indigenous bacterial strains which would decolourise textile diazo dye from the above mentioned sites. A total of 10 bacterial strains were isolated and were screened for their dye decolourising efficacy. The preliminary screening was carried out by plate assay which exhibited clear zones around bacterial colonies. The final screening was carried out in screening broth supplemented with 200mg/l diazo dye under standard conditions of temperature (37°C), pH(7.2) and incubation period(48-72 hours). Dye decolurising efficacy of bacterial strains was expressed as decrease in absorbance (λ_{max}=495.5nm) from initial 1.213 to final 0.211,0.194.0.28 for *Staphylococcus sp*.(82.54%), *Serratia sp.(84%)* and *Micrococcus sp.(76.57%*)respectively.

Keywords: CETP, Dye decolourising bacteria, Diazo dye, Physicochemical parameters.

1.0 Introduction:

Textile industry is one of the most important industries and generates enormous volumes of wastewater by virtue of its different operations like sizing, desizing, bleaching, mercerizing, dyeing and printing. (Yusuff and Sonebare,2004). The effluent from textile industry is strongly coloured and characterized by high pH, BOD, COD, chloride and suspended solids. Colour of effluent is attributed to extensive use of dyes which forms the major pollutant. Assessment of textile effluents in terms of physicochemical parameters has been both a subject of national and international repute. Nationally, it was reported that untreated or incompletely treated textile effluent from CETP, when discharged into surface water body rapidly depletes dissolved oxygen which increases BOD manifolds (Desai and Kore, 2011). Pollution indicators like COD, alkalinity, suspended solids, dissolved solids of textile mill when fail to comply with standards, affects aquatic life (Joshi and Santani,2012). Physicochemical characteristics of dried chemical sludge of CETPS were also reported. (Patel and Pandey, 2008). The physicochemical parameters of untreated textile effluent has also been reported (Rao and Prasad, 2011; Mohabansi *et al.,* 2011; Paul *et al.*, 2012). The effect of treated effluent of textile industry on biochemical constituents of fresh water fish has been reported (Noorjahan, 2010).Untreated and treated effluent of textile industry in different concentrations was studied for irrigational purposes (Garg and Kaushik, 2008). Internationally, physicochemical characterization of untreated textile effluent has also been reported (Nosheen *et al.*, 2000; Yusuff and Sonebare,2004; Jamaluddin and Nizamuddin,2012).

Colour of the effluent, being the primary pollutant is removed by various physicochemical methods which are rather costly and generates large volumes of sludge. Textile dyes are broadly categorized into azo, triphenylmethane, cationic

and anionic on the basis of chemical moiety involved. The group of atoms responsible for imparting colour to the dye are chromophores, the most important ones are azo.(-N=N-) which may be monoazo, diazo, triazo and polyazo (Chaube *et al.*, 2010). Azo dyes form the majority of dyes being discharged into effluents (Vander Zee,2005).The dye used in the present study is a diazo dye which is a sodium salt (benzidinediazo-bis-1 naphthylaminsulfonicacid) .
Formula: $C_{32}H_{22}N_6Na_2O_6S_2$) Molecular weight: 696.60g/mol (Bhattacharya *et al.,* 2011).

Treatment of textile waste water utilises different physicochemical processes like ultrafiltration , reverse osmosis, ozonation etc. These methods are very expensive and feasibility remains a matter of concern. Microbial decolourisation and degradation of textile dyes has proved to be an environmental friendly and cost effective approach (Verma and Mandawar, 2003). The key step is to isolate and characterize indigenous bacterial flora from textile effluent. Reports pertaining to isolation and characterization of autochthonous bacterial flora from textile effluent, soil contaminated with textile effluent, sludge has been well documented(Chang *et al,*2001; Mabrouk and Yousuff, 2008;Rajendran *et al.,*2011; Ponraj *et al.,2011;* Sethi *et al.*, 2012;Palani Velan *et al.*,2012;Subhatra *et al.,*2013). Previously, studies have been conducted on isolation and characterization of bacteria from CETP of textile industries. (Rajeswari *et al.,2011*). Not only prokaryotes, but fungus isolated from sludge of textile waste water has also been proved to be an efficient decolouriser of diazo dye (Shinde and Thorat, 2013).

The present study was aimed to assess the quality of untreated and treated textile effluent and isolation, characterization and screening of indigenous dye decolourising bacteria from different sites of CETP.

2.0 Materials and Methods:

2.1 Chemicals

All the chemicals used in the study were of analytical grade and procured from Hi Media and Merck. The dye used in study, diazo dye was generously provided by the Department, of Textile, The IIS University, Jaipur

2.2 Study area

Pali is one of the major textile clusters of Rajasthan and hence highly polluted with coloured effluents. In order to minimize the problem of water pollution caused primarily by textile units, a 12 MLD, Common Effluent Treatment Plant (CETP) has been set up by Pali Water Pollution Control Treatment and Research Foundation (PWPCTRF) Mandia Road. (Figure 1) which operates on biological treatment (Activated sludge)

2.3 Sampling Sites (Figure2a-2d)

1. Inlet/untreated/Raw(2a)
2. Primary clariflocculator (2b)
3. Aeration Tank/activated sludge(2c)
4. Outlet/treated(2d)

2.4 Sampling and effluent analysis

Grab effluent samples from sites 2a-2d were collected in triplicates in pre cleaned plastic cans from CETP, Mandia road, Pali in months of October-2011 and April-2012 and were transported and stored at 4°C in accordance with standard procedures.(APHA, 1998). pH, temperature and DO were recorded at the site. Other parameters BOD, COD, hardness, chloride, alkalinity, nitrate, phosphate, TS, TDS were analyzed according to their minimum retention time. For bacteriological analysis, samples were collected in pre sterilized screw capped BOD bottles (Figure 3).The bottles were washed with dilute acid, doubled distilled water and rinsed with effluent prior to sampling.

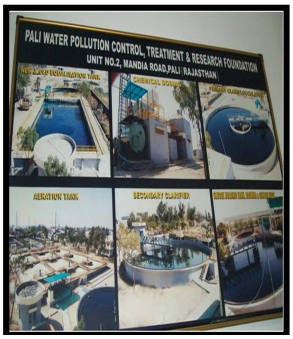


Figure1: Study area

Figure2a :Inlet

Figure 2b :Primary clariflocculator

Figure 2c: Aeration tank

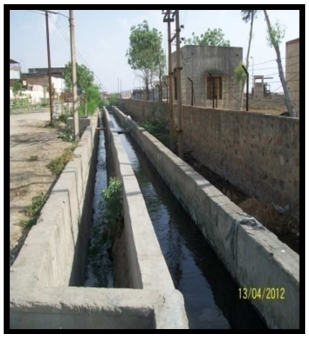


Figure 2d: Outlet

Figure 3:_Samples for Bacteriological analysis

2.5 Bacteriological analysis

Textile effluent samples were collected from 4 sites; (figure 2a-2d) in pre sterile screw capped bottles; transported and stored in accordance with standard procedures. Preliminary isolation of bacterial strains was carried out by serially diluting the samples (upto 10^{-10}) and plating onto nutrient agar with the following composition (g/l): peptone-5, meat extract-1, yeast extract-2, Nacl-5, agar-15, pH-7) and incubated at 37°C for 48 hours (Rao and Prasad, 2011). Following incubation, a mixed culture was developed in all the samples reflecting a diversity of isolates. It was then, from a mixed culture, single colonies were picked up and quadrant streaked onto nutrient agar plates amended with dye. Nutrient agar slants were prepared for preservation at 4°C, and incubated similarly. Discrete bacterial colonies that developed on nutrient agar plates were designated as master cultures and repeated subculturing was carried out in a similar manner (quadrant streaking) to obtain pure cultures. The isolates were initially grouped according to their morphological characteristics like pigmentation, growth pattern, texture, size followed by simple and differential staining (Gram staining).

2.5.1 Isolation and identification of bacterial strains

Different biochemical tests like enzymatic tests, hydrolytic reactions, fermentation of different carbohydrates, IMViC, TSIA, motility, sulfur utilization etc (Cappucino and Sherman,2002) were carried out to identify the isolates up to generic level by Bergey's Manual of determinative Bacteriology.(Holt,1994)

2.5.2 Screening of dye decolourising bacteria

2.5.2.1 Qualitative screening (Plate Assay) (Leelakriangsak and Borisut , 2012)

The bacterial isolates obtained from all samples, were tested for their dye decolourising efficacy. Media used in screening was Bushnell's and Haas Agar (BHA) with the following composition (g/l) (Magnesium sulphate =0.2; Calcium chloride=0.02; Monopotassium phosphate=1.0; Dipotassium phosphate=1.0; Ammonium nitrate=1.0; Ferric chloride=0.05 ; pH=7.0) supplemented with diazo dye (200mg/l). Preliminary screening was carried out by plate assay (Leelakriangsak and Borisut , 2012). In this procedure, a spot of a single colony using sterilized applicator stick was applied onto BHA supplemented with dye and/or 5ml of spot from pure broth culture supplemented with respective dye was applied and incubated similarly. Clear zones around colonies were indicative of decolourisation of dye. The zones around colonies were measured from the centre of the colony and expressed in centimeters. Uninoculated plates were used as controls.

2.5.2.2 Quantitative screening (Decolourisation Assay) (Manivannan et al., 2011)

The strains which were able to decolourise dye in solid form (BHA supplemented with dye) were tested for their ability to decolourise dye in liquid form. Media used for screening was Bushnell's and Haas Broth (BHB) with the following composition (g/l) (Magnesium sulphate=0.2; Calcium chloride=0.02; Monopotassium phosphate=1.0; Dipotassium phosphate=1.0; Ammonium nitrate=1.0; Ferric chloride=0.05; pH=7.0) supplemented with diazo dye (200mg/l). The strains were grown in Nutrient broth under shaking conditions.(150rpm, 37°C) until the $O.D_{660}$=0.6 was attained (Suizhou *et al.*,2006). 1ml culture (2%v/v) of respective strains were then inoculated in 100ml flasks containing 50ml BHM supplemented with dye. Initial absorbance (λ_{max}= 495.5 nm) was recorded utilizing BHM as blank and uninoculated medium as control. The experiment consisted of following set up:

1. Test/Experimental= BHB+Dye+Strain
2. Control=BHB+Dye
3. Blank=BHB.

Both test and control samples were incubated at 37°C.Cells were harvested from broth culture in 1.5 ml eppendorf tubes at regular intervals and centrifuged (1-15 PK Sartorius, SIGMA) at 10,000 rpm for 15 minutes. Pellet containing the bacterial cell mass was discarded and supernatant fraction was analysed spectrophotometerically for dye decolourising efficacy expressed in terms of percent decolourisation (Manivannan *et al.*, 2011). Percent decolourisation of different strains is graphically represented.

$$\% \text{ decolourisation} = \frac{\text{Initial O.D} - \text{Final O.D.}}{\text{Initial O.D}} X\ 100$$

3.0 Results and Discussion:

3.1 Physicochemical analysis of effluent

Table 1: Physicochemical characteristics of textile effluents

		Inlet		Primary Clariflocculator		Aeration tank		Outlet	
		Oct-2011	Apr-2012	Oct-2011	Apr-2012	Oct-2011	Apr-2012	Oct-2011	Apr-2012
Colour	-	Blackish Green	Blackish Green	Dark Green	Dark Green	Dark Green	Dark Green	Light Green	Light Green
pH	-	11.3±1.6	12±0.0	10±0.58	9±0.57	7.6±0.12	7.2±0.16	7.1±0.1	7.8±0.3
Temp	°C	21.3±0.2	28.1±0.2	20.5±0.1	28.5±0.1	20.8±0.1	29.2±0.26	20.4±0.26	28±0.1
DO	mg/l	Nil	Nil	0.3±0.01	0.2±0.01	1.6±0.02	1.8±0.06	2.1±0.12	2.4±0.18
BOD	mg/l	403.3±8.3	337.3±1.1	324.6±2.3	332.7±0.6	306.6±3.0	302.0±2.4	275.3±0.4	289.8±1.7
COD	mg/l	856.6±3.9	810.0±0.2	778.5±3.8	711.3±4.1	516.5±0.4	491.9±1.6	434.6±1.8	407.8±2.3
Alkalinity	mg/l	319.2±3.4	380.0±2.8	351.2±0.4	312±5.5	318.3±2.0	282±2.64	262.0±2.6	322.4±0.1
Hardness	mg/l	322.0±2.6	297.6±2.0	290.3±6.6	280.6±3.5	244.0±7.2	271.3±2.5	197.6±7.6	235.3±0.5
Chloride	mg/l	896.9±1.0	845.1±1.7	465.4±2.2	457.7±0.6	409.0±7.5	405.3±1.1	391.0±5.0	365.5±1.9
Nitrate	mg/l	22.1±2.7	26.3±4.2	18.0±2.6	21.8±0.7	16.1±2.3	18.4±0.3	14.2±1.0	13.6±0.1
Phosphate	mg/l	15.4±0.4	15.8±0.9	14.5±0.3	15.2±0.4	13.1±0.4	13.4±0.2	10.5±0.3	11.7±0.5
TDS	mg/l	4276.3±0.5	3270.6±1	2652±0.1	2734.5±.9	927.7±0.2	915.4±0.4	597.5±0.3	606.7±0.5
TSS	mg/l	920.6±0.1	925.3±0.5	850.0±0.1	861.5±0.3	515.4±0.1	531.7±0.9	234.8±0.3	239.7±1.1

Expressed as mean±S.D

***3.1.1 Colour**:* Colour is the major pollutant of textile sector and owes its origin by extensive use of different types of dyes. The samples from site 2a were blackish green in colour in both October and April. Brownish black raw effluent from textile industries has been reported (Desai and Kore, 2011).The colour of site 2b was slightly light as compared to site 2a , this slight lightning may be attributed to the use of coagulants and flocculants(Wong *et al.,* 2007). Sample from site 2c was grayish green in colour utilizes the phenomenon of activated sludge. Effluent generated form site 2d was though greenish but was free of turbidity and particulate matter.

3.1.2 pH: The pH of site 2a was very high(11.3±1.6-12±0) as the incoming wastewater is highly alkaline in nature. The bleaching agents and chemicals NaOCl, NaOH, surfactants and sodium phosphate used in the processes are reasons for high alkaline wastewater (Paul *et al.*, 2012). A similar trend in pH of raw textile waste water has also been reported (Ramamurthy *et al.,* 2011). pH higher than the observed range has also been reported(Desai and Kore, 2011). Effluent from site 2b had pH in the alkaline range (9±0.57-10±0.58). Waters with pH value of about 10 are exceptional and may reflect contamination by strong base such as NaOH and $Ca(OH)_2$ which are extensively used in textile sector. pH of site 2c was in the neutral to slightly alkaline range (7.2-±0.1.6-7.8±0.12).Site 2d witnessed pH in neutral range (7.1±0.1-7.8±0.3) . A similar trend in pH of outlet has also been reported (Desai and Kore, 2011).

3.1.3 Temperature: Temperature is one of the most important physiological parameters which govern the aquatic life system. Temperature of site 2a was found to be in the range (29.7±0.61°C - 31.8±0.23°C). A similar trend in temperature has also been reported for physicochemical characterization of industrial effluents. (Lokhande *et al.,* 2011). Site 2b had temperature in the range (27.4 ±0.27°C -28.5 ±0.24°C). A slight increase in

temperature of site 2c is attributed to biological activity.(28.4 ±0.34°C -29.2 ±0.27°C). Completely treated effluent from site 2d had temperature in the range (27.7 ±0.31°C -28 ±0.1°C).

3.1.4 Dissolved Oxygen (D.O.): Dissolved oxygen of site 2a was nil which reflected the highly polluted nature of effluent contaminated with organic matter. Similar findings with nil DO have been reported (Paul *et al.*, 2012). A higher value (0.46mg/l) for raw effluent has been reported (Mohabansi *et al.*, 2011). DO of site 2b was in the range (0.2±0.01 mg/l -0.3±0.01 mg/l), site 2c (1.6 ±0.02 mg/l -1.8±0.06 mg/l).This slight increase may be attributed to biodegradation of organic matter by indigenous microflora. DO of site 2d saw an increase from (2.1 ±0.12 mg/l -2.4 ±0.18 mg/l). Treated textile effluent with nil DO has been reported (Garg and Kaushik, 2008).

3.1.5 Biochemical Oxygen Demand (BOD): BOD is the most important parameter which in true sense determines the pollution load of an effluent and is expressed as a measure of the quantity of oxygen used by microorganisms in the degradation of organic matter. BOD of site 2a was (337.3±1.12 mg/l -404.3±8.3 mg/l). In previous studies conducted, BOD in this range has been reported (Nosheen *et al.*, 2000; Joshi and Santani, 2012;). Values of BOD less then (275mg/l) our findings have been reported (Rao and Prasad, 2011.). BOD on a higher side (1626mg/l) has been well documented (Garg and Kaushik, 2008).Site 2b, 2c had BOD values in the range(324.6l±2.31 mg/-332.7±0.68 mg/), (302.0±3.0 mg/-306.6±2.4 mg/). The values for site 2d were (275.3±0.42 mg/l -389.8±1.7 mg/l).A higher value of BOD (496mg/l) of textile effluent then the present investigation has been reported (Garg and Kaushik, 2008). BOD on the lower side (32mg/l-80mg/l) has been cited (Desai and Kore, 2011).

3.1.6 Chemical Oxygen Demand (COD): COD determines the oxygen required for .the chemical oxidation of organic matter and non-biodegradable matter present in it. COD is also an important pollution indicator which reflects the chemical quality of effluent. In the present investigation, high COD values are reported for all samples. Site 2a (810mg/l±0.2-856.6mg/l±3.99);site 2b (711.3mg/l±4.16-778.5mg/l±3.82);site 2c (491.9mg/l±1.67-516.5mg/l±0.47); site 2d (407.8mg/l±2.31-434.6mg/l±1.8).Higher values for both inlet (2190mg/l) and outlet (960mg/l) have been reported (Garg and Kaushik,2008). Although very high values for inlet(1088mg/l-1472mg/l) and comparatively low values for outlet then the present investigation (180mg/l-236mg/l) have been reported (Desai and Kore, 2011). Contrastingly, a very low value (45.12mg/l) of COD for inlet has also been reported (Mohabansi *et al.*, 2011).

3.1.7 Hardness: Total hardness is sum of the calcium and magnesium concentrations, both expressed as calcium carbonate, in mg/L Water is conventionally classified as hard or soft from the following classification 50 – 100 mg/l(Soft), 100 – 250 mg/l (Moderately hard) 250 – 350 mg/l(Hard),> 350 mg/ l(Excessive hard)(Mohabansi *et al.*, 2011).In the present investigation, hardness of site 2a was (297.6±2.0-322±2.6 mg/l), site 2b(280.6±3.5 mg/l -290.3±6.65 mg/l); site 2c (244±7.21 mg/l -271.3±2.51 mg/l);site 2d(197.6±7.63 mg/l -235.3±0.57 mg/l). Effluent from site 2d was moderately hard. A lower value (243mg/l) of raw effluent has been reported (Mohabansi *et al.*, 2011).

3.1.8 Alkalinity: Alkalinity is a measure of buffering capacity of water. It is an important parameter which indicates the ability of water to neutralize acids from wastewater. The higher values of alkalinity are associated with increase in the presence of bicarbonates and carbonates from effluents. In the present investigation, the values for different samples were site 2a (319.2±3.42 mg/l -380±2.82 mg/l); site 2b (312 ±5.52 mg/l -351.2 ±0.44 mg/l) ;site 2c (282 ±2.64 mg/-318.3±2.08 mg/);site 2d (262 ±2.64 mg /l -322.4±0.16 mg /l). Values in the range (280mg/l-1050mg/l) for raw effluent have been reported (Paul *et al.,* 2012).

3.1.9 Chloride: Chloride is one of the major inorganic anions in waste water. Its presence in textile in textile effluents is mainly attributed to the presence of bleaching agents. High chloride contents are harmful for metallic pipes as well as for agricultural crops if such wastes containing high chlorides are used for irrigation purposes. In the present investigation, values of chloride for different samples were site 2a (845.1±1.79 mg/l -896.9±1.0 mg/l); site 2b (457.7±0.63 mg/l -465.4 ±2.2 mg/l); site 2c (405.3±1.1 mg/l -409.0 ±7.51 mg/l); site 2d (365.5±1.99 mg/l -391.0±5.0 mg /l). Similar values then the present investigation for inlet (860mg/l) and higher values for outlet (692mg/l) have been reported (Garg and Kaushik, 2008).

3.1.10 Nitrate: Nitrogen both in the form of nitrate, nitrite, or ammonia can be health hazard

and its presence in textile effluent is credited to extensive use of different types of dyes. In the present investigation, values of nitrate for different samples were site 2a (22.1±2.70 mg/l -26.3±4.28 mg/l); site 2b (18.0±2.64 mg/l -21.8 ±0.76 mg/l); site 2c (16.1±2.37 mg/l -18.4±0.3 mg/l); site 2d (13.6±0.15 mg /l -14.2±1.05 mg /l). Nitrate content of raw textile effluent in lesser range (13.35±3.13 mg/l) has been reported (Ajao *et al.*, 2011). This reduction in nitrate content of treated effluent may be attributed to the heterotrophic nitrifying bacteria which forms a part of indigenous microflora.

3.1.11 Phosphate: Textile effluent samples are characterized by high phosphate content presumably because of inorganic and organic matter present both in dissolved and particulate forms. In the present investigation, values of phosphate for different samples were site 2a(22.1±2.70 mg/l -26.3±4.28 mg/l); site 2b (15.4±0.43 mg/l -15.8±0.93 mg/l); site 2c (13.1±0.45 mg/l -13.4±0.23 mg/l); site 2d (10.5mg /l±0.36-11.7mg /l±0.51).Values similar (10.45mg/l) have been reported for raw textile effluent(Paul *et al.*,2012). This lowering of phosphate level of effluent may be attributed to presence of micro-algae which are efficient phosphate solubilisers and scavenge phosphates.

3.1.12 Total Dissolved Solids (TDS): TDS content in water is a measure for salinity. A large number of salts are found dissolved in natural waters, the common ones are carbonates, bicarbonates, chlorides, sulphates, phosphates, and nitrates of calcium, magnesium, sodium, potassium, iron, and manganese, etc (Lokhande *et al.*,2011). In the present investigation, values of TDS were site 2a (3270.6±1.0 mg/l -4276.3±.50 mg/l); site 2b (2652±0.15 mg/l -2734.5±0.93 mg/l); site 2c (915.4±0.45 mg/l -927.7±0.23 mg/l); site 2d (597.5±0.3 mg/l -606.7±0.51 mg/l).Lower values(1700mg/l-2100mg/l) then the present investigation for TDS from inlet and contrastingly higher values(1600mg/l-2000mg/l)from outlet has been reported(Desai and Kore, 2011). Higher values (2264mg/l-7072mg/l) for TDS from inlet have also been reported (Paul *et al.*, 2012).

3.1.13 Total Suspended Solids (TSS): Solids present in dissolved form in an effluent comprise TSS. In the present investigation, values of TSS for different samples were site 2a (920.6±0.1 mg/l -925.3±0.50 mg/l); site 2b (850±0.15 mg/l -861.5±0.33 mg/l); site 2c (515.4±0.1 mg/l -531.7±0.90 mg/l); site 2d (234.8±0.3 mg /l -239.7±1.1 mg /l). Very low values of TSS from both inlet (124mg/l) and outlet (40mg/l) have been reported (Rohit and Ponmurugan, 2013). Contrastingly, very high content (3200mg/l) of TSS in raw textile has been reported (Ajao *et al.*, 2011). High values of TSS may be attributed to coloured effluents which eventually use different dye stuffs (Mohabansi *et al.*, 2011).

3.2 Isolation and Identification of bacterial strains

A total of 10 bacterial strains were isolated from all the samples. All these strains were screened for their ability to decolourise diazo dye. Different biochemical tests were conducted to identify the bacterial strains and here we report 3 genera belonging to *Staphylococcus sp and Micrococcus sp.* (primary clariflocculator).,*Serratia sp.*(aeration tank/activated sludge) grown in the presence of dye (Figure 3a). The confirmatory tests for different isolates are present in Table 2 and pure culture of isolates is represented in (Figure 3b-d)

Table 2: Biochemical characteristics of Bacterial strains(+ve= positive;-ve =negative)

Properties	**Characteristics**		
Gram Staining	+ve	+ve	-ve
Shape	Coccus	Coccus	Bacillus
Cellular arrangement	Cluster	Pairs	Single/pairs
Colony morphology	Abundant,opaque	Soft, smooth,round	Entire margin
Pigmentation	Golden –yellow	Dull yellow	Dark red
Temperature range	30-37°C	25-37°C	30-37°C
Spore	-ve	-ve	-ve

Motility	-ve	-ve	+ve
Oxygen requirement	Facultative anaerobe	Obligate aerobe	Facultative anaerobe
Nitrate reduction	+ve	+ve	+ve
Catalase	+ve	+ve	+
Indole	-ve	-ve	-ve
Methyl Red	+ve/-ve	-ve	+ve
Voges-Proskauer	+ve/-ve	-ve	+
Citrate	-ve	-ve	+ve
Hydrolysis(Gelatin)	-ve	+ve	+ve
Hydrolysis(Esculin)	-ve	+ve	+ve
Hyrolysis(Starch)	-ve	-ve	-ve
Hyrolysis(Urea)	-ve	+ve	-ve
Fermentation(Glucose)	+ve	-ve	+ve
Fermentation(Lactose)	+ve	-ve	-ve
Fermentation(Sucrose)	-ve	-ve	+ve
Fermentation (Mannitol	+ve	-ve	+ve
H_2S Production	-ve	-ve	+ve
Strains	*Staphylococcus sp.*	*Micrococcus sp.*	*Serratia sp.*

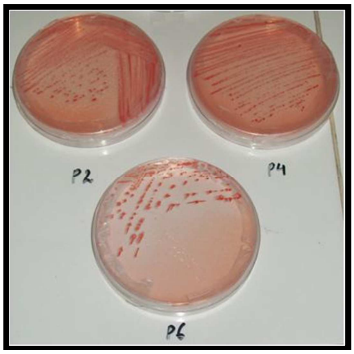


Figure 3 a: Strains in presence of dye

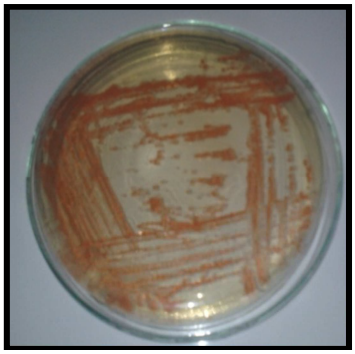
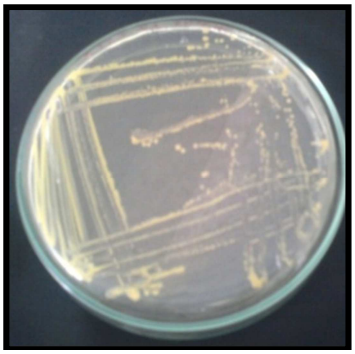


Figure 3b: *Serratia sp.*



.Figure 3 c: *Staphylococcus sp.*

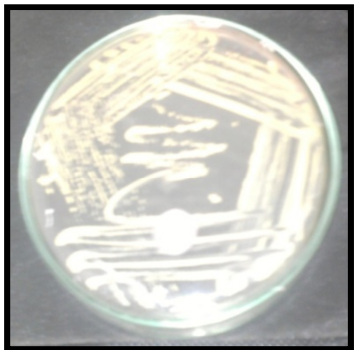


Figure 3 d: *Micrococcus sp.*

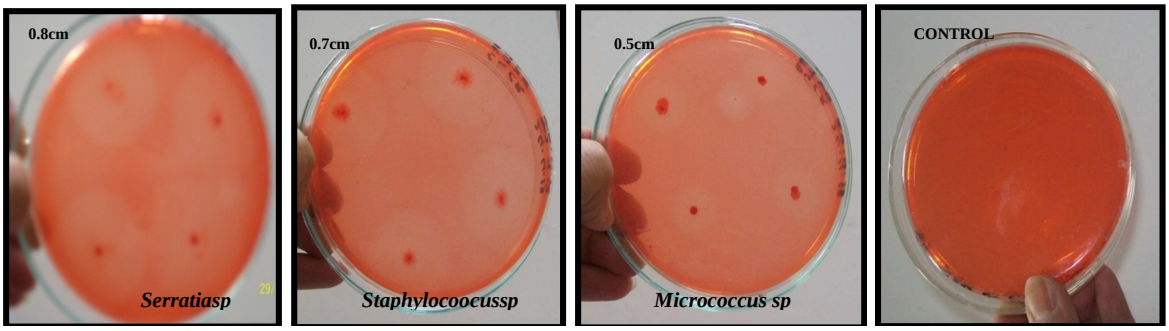


Figure 4: Decolurisation of diazo dye by bacterial strains (clear zones)

3.3 Qualitative screening by Plate Assay

Plate assay as described earlier was used to screen the bacterial strains by visual observation of clear zones around bacterial colonies. These clear zones were measured in centimeters from the centre of colony. Clear zones around colonies indicative of decolourisation of azo dyes have been reported. (Khadijah *et al.*, 2009; Chaube *et al*,2010; Leelakriangsak and Borisut ,2012; Palani Velan *et al.,*2012). The radial distance of *Staphylococcus sp*. was 0.7 cm, *Micrococcus sp.* was 0.5 cm and for *Serratia sp.*it was 0.8cm; being highest among the three stains. (Figure 4) .Uninocolated plates with BHA supplemented with dye were used as controls. (Figure 4)

3.3 Quantitative screening by Decolourisation Assay

Final or quantitative screening was carried out on three isolates using (BHM+dye) (**Figure 5).** Percent decolourisation calculated at regular intervals was expressed as decrease in initial absorbance(λ_{max}=495.5nm) from 1.213 to **0.211,0.194.0.28 for *Staphylococcus sp*.(82.54%), *Serratia sp.(84%)* and *Micrococcus sp.(76.57%*)respectively (Figure 6)** under standard conditions(pH-7.2; temperature-37°C; incubation conditions-static).Similar findings on azo dye decolourisation with this isolate have been reported (Shukor *et al.*,2009;Sethi *et al.*,2012; Vivekanandnan *et al.*,2013). The fact that *Staphylococcus sp.* and *Micrococcus sp.* are potential decolurisers of textile azo dyes has been well established.(Palani Velan *et al.*,2012; Soundararajan *et al.*,2012).Several studies in support of microbial decolourisation of diazo dye have been reported (Kumar and Sawhney,2011;Jalandoni-Buan *et al.*,2010; Bhattacharya *et a.l*,2011). Another important fact on diazo dye has been established that decolourisation takes by bioadsorption in which the colony retains or adsorbs the dye and hence the colony appears of same colour as that of dye (Sun-Young *et al.,* 2002; Bhattacharya *et al.,* 2011).

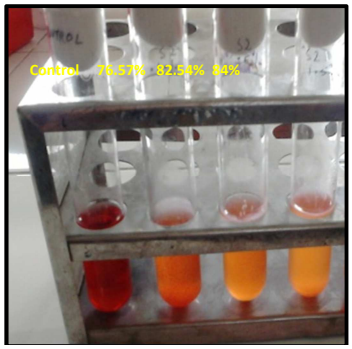


Figure 5: Decolourisation in Broth culture

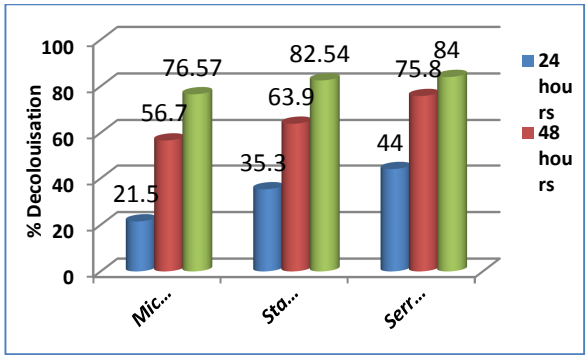


Figure 6: Percent decolourisation of diazo dye by bacterial strains

4.0 Conclusion

The fact cannot be denied that that textile industry is the major reservoir of water pollution and adversely effects the environment. To minimise the problem of water pollution caused by textile industries, Common Effluent Treatment Plant (CETP) has been set up by Pali Water Pollution Control Treatment and Research Foundation (PWPCTRF) at Mandia Road, Pali. The data on physicochemical investigations suggests that no considerable difference in pollution load of untreated and treated effluent was reported.

Moreover, in order to check for seasonal variations, sampling was conducted twice and to check for performance evaluation of CETP, samples were also collected from primary clarifier and aeration tank to study the reduction in pollution load, but alarmingly, unnoticeable differences were observed during treatment strategy, though the reduction in pollution indicators from inlet to outlet was observed. Thus, CETP needs to be monitored on regular basis to check for pollution load not only for inlet and outlet but also during intermediate stages of the treatment. Textile effluents are strongly coloured with excessive amounts of dye stuffs. Removal of colour is of utmost concern and methods of coagulation and flocculation have been used but they are not cost effective. Biotreatment offers easy, cheaper and effective alternative for colour removal of dyes. The study also discovered effluent adapted bacterial strains; *Staphylococcus sp., Micrococcus sp., Serratia sp.* which proved to be efficient decolourisers of diazo dye. Bioprospecting ,molecular characterization could offer very promising results in terms of bacterial diversity and further consortium of potential dye degraders could be established based on the criteria that a variety of dyes should be degraded by atleast 60 % in a lesser time(72 hours) for bioaugmentative approaches . The potential of these isolates should be exploited further to degrade other dyes by their enzymatic mechanisms. Further, the research work is in progress in our laboratory to isolate new bacterial strains to study their enzymatic mechanisms and immobilization techniques to retain the stability of enzymes.

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6.0 References:

1) Ajao, A.T., Adebayo, G.B. and Yakubu, S.E. (2011): Bioremediation of Textile Industrial Effluent using mixed culture of *Pseudomonas aeruginosa* and *Bacillus subtilis* immobilized on agar agar in a Bioreactor. *J. Microbiol. Biotech. Res.,* 1 (3):50-56.
2) APHA, AWWA, WPCF, (1998): Standard Methods for the Examination of Water and Wastewater. 20th Edition. American Public Health Association, Washington, DC, New York, USA.
3) Bhattacharya, S. D., Arijit, G.M. and Vignesh, S. K. (2011): Mycoremediation of congo red dye by filamentous fungi. *Brazilian Journal of Microbiology*. , 42: 1526-1536.
4) Cappucino, J. and Sherman, N. (2009): Microbiology: A laboratory Manual. 7th edition. Pearson Education Inc. New Delhi.
5) Chang, J.S., Chou, C., lin, Y., Ho, J. and Hu, T.L. (2001): Kinetic characteristics of bacterial azo dye decolourisation by *Pseudomonas luteola Water Research*.,35 :2041-2850.
6) Chaube,P., Indurkar, H. and Moghe, S.(2010): Biodegradation and decolorisation of dye by mix consortia of bacteria and study of toxicity on *Phaseolus mungo* and *Triticum aestivum. Asiatic J. Biotech. Res*., 1: 45-56.
7) Desai, P. A. and Kore, V. S. (2011): Performance Evaluation of Effluent Treatment Plant for Textile Industry in Kolhapur of Maharashtra. *Universal Journal of Environmental Research and Technology.,* 1(4): 560-565.
8) Garg, V.K. and Kaushik, P. (2008): Influence of textile mill wastewater irrigation on the growth of sorghum cultivars. *Applied Ecology and Environmental Research. ,* 6(2): 1-12.
9) Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. & Williams, S. T. (1994). *Bergey's Manual of Determinative Bacteriology*, 9th edn. Baltimore: Williams & Wilkins.
10) Jalandoni – Buan, A., Anna, L. C., Decena-Soliven, A., Ernelea, P. C., Barraquio, V. L. and Barraquio, W.L.(2010):Characterization and Identification of Congo Red Decolorizing Bacteria from Monocultures and Consortia.*Philippine Journal of Science.,* 139 (1): 71-78.
11) Jamaluddin, A. M. and Nizamuddin, M. (2012): Physicochemical Assessment of Textile Effluents in Chittagong Region of Bangladesh and Their Possible Effects on Environment. *International Journal of Research in Chemistry and Environment*., 2 (3): 220-230.
12) Joshi, V. J. and Santani, D. D. (2012): Physicochemical Characterization and Heavy Metal Concentration in Effluent of Textile Industry. *Universal Journal of Environmental Research and Technology.,* 2(2): 93-96.
13) Khadijah, O., Lee, K. K. and MohdFaiz, F.A. (2009): Isolation, screening and development of local bacterial consortia with azo dyes decolourising capability. *Malaysian Journal of Microbiology.,* 5(1): 25-32.
14) Kumar, A. and Sawhney, R. (2011): Identification of *Bacillus subtilis* subsp

subtilis"RA-29", a Congo Red Decolourizer using 16S rDNA Sequencing. *Researcher*., 3(12) : 18-22.

15) Leelakriangsak, M. and Borisut, S. (2012): Characterisation of the decolorizing activity of azo dyes by *Bacillus subtilis* azoreductase AzoR1. *Songlanakarin J. Sci. Technol*., 34(5):509-516.
16) Lokhande, P., Singare, U and Pimple, D. S. (2011): Study on Physico-Chemical Parameters of Waste Water Effluents from Taloja Industrial Area of Mumbai, India. *International Journal of Ecosystem* ., 1(1):1-9.
17) Mabrouk, M.E.M. and Yusuff, H.H. (2008): Decolourisation of Fast Red by *Lysinibacillus subtilis*. *H.M .Journal of Applied Sciences Research*.,4 : 262-269.
18) Manivannan, M., Reetha, D. and Ghosh, P. (2011): Decolourisation of textile azo Dyes by using Bacteria Isolated from Textile Dye Effluent.*Journal of Ecobiotechnology.*, 3(8):29-32.
19) Mohabansi, N.P., Tekade, P.V. and Bawankar, S.V. (2011): Physico-chemical Parameters of Textile Mill Effluent, Hinganghat, Dist. Wardha (M.S.)*Current World Environment*., 6(1): 165-168.
20) Noorjahan, C.N. (2010): Physicochemical characterization of treated textile effluent and its effects on biochemical constituents of fresh water fish, *Tillapia mossambica*. *Pollution Research.*, 29(4): 651-654.
21) Nosheen ,S., Haq, N and Rehman, K-Ur. (2000): Physico-Chemical Characterization of Effluents of Local Textile Industries of Faisalabad–Pakistan. *Int. J. Agri. Biol.,* 2(3):232-233.
22) PalaniVelan, R., Rajkumar, S. and Ayyasamy, P.M.(2012): Exploring the promising dye decolourising strains obtained from Erode and Tirrupur textile wastes. *International Journal Of Environmental Sciences.,* 2(4): 2470-2481.
23) Patel, H. and Pandey, S.(2008): Physico-chemical characterisation of textile chemical sludge generated from various CETPS in India. *Journal of Environmental Research and Development.* , 2 (3): 329-339.
24) Paul, S. A., Chavan, S. K. and Khambe, S. D. (2012): Studies on characterization of textile industrial waste water in Solapur city. *Int.J. Chem. Sci*., 10 (2): 635-642.
25) Ponraj, M., Gokila, K. and Zambare, V. (2011): Bacterial decolourisation of textile dye-Orange 3R. *International Journal of Advanced Biotechnology and Research.*, 2(1): 168-177.
26) Rao, K. V. B. and Prasad, A.(2011): Physico chemical analysis of textile effluent and decolorization of textile azo dye by Bacillus endophyticus strain VITABR13. *The IIOAB Journal, RESEARCH: BIOREMEDIATION.* ,2(2): 55-62.
27) Rajendran, R., Sundaram K., S. and Maheshwari U.K. (2011): Aerobic decolourisation of mixture of azo dye containing textile effluent using adapted microbial strains. *J. Environ.Science. Technol.,* DOI: 10/3923/jest.2011 :1-11.
28) Rajeswari, K., Subashkumar, R. and Vijayaraman, K. (2011): Biodegradation of Mixed Textile Dyes by Bacterial Strains Isolated from Dyewaste Effluent. *Research Journal of Environmental Toxicology.,* 5: 97-107.
29) Ramamurthy, N., Balasaraswathy, S. and Sivasakthivelan, P.(2011)*:*Biodegradation and physico-chemical changes of textile effluent by various fungal species*. Romanian J. Biophys.,* 21 (2):113–123.
30) Rohit,K. C. and Ponmurugan, P. (2013): Physico-chemical analysis of textile, automobile and pharmaceutical industrial effluents*. International Journal of Latest Research in Science and Technology.,* 2(2) : 115-117.
31) Sethi, S., Shubhum, Malviya, M. M., Sharma, N. and Gupta, S. (2012): Biodecolorization of Azo Dye by Microbial Isolates from Textile Effluent and Sludge. *Universal Journal of Environmental Research and Technology.,* 2(6): 582-590.
32) Shinde, K.P., and Thorat, P. R. (2013): Biodecolorization of diazo direct dye congo red by *Fusarium sp.* TSF-01. *Review of Research.,* 2(8):1-10.
33) Soundararajan, N., Gopi, V., Upgade, A. and Begam, N. (2012): Bioremediation ability of individual and consortium of non-immobilized and immobilized bacterial strains on industrial azo textile effluent. *Annals of Biological Research.,* 3 (4):1773-1778.
34) Subhathra, M., Prabakaran, V., Kuberan, T. and Balamurugan, I. (2013): Biodegradation of Azo dye from textile effluent by *Lysini bacillus sphaericus. Sky Journal of Soil Science and Environmental Management.,* 2(1):1-11.
35) Suizhou, R., Jun, G. ,Guoqu, Z. and Guoping, S. (2006): Decolourization of triphenymethane, azo and anthraquinone dyes by a newly isolated *Aeromonas hydrophila* strain. *Appl Microbiol Biotechnol.,* 72: 1316-1321.
36) Sun-Young, A., Sang-Ki, M., In-Ho, C., Young-Lark, C., Young-Su, C., Cherol-Ho, K. and Young-Choon, L. (2002): Decolourisation of

triphenymethane and azo dyes by *Citrobacter sp.Biotechnology Letters*., 24: 1037-1040.
37) Shukor, M.Y. , Syed, M.A., Sim, H.K and Khalid A. (2009):A simple method to screen for azo-dye-degrading bacteria. *J. Environ. Biol.,* 30(1): 89-92.
38) VanderZee, F.P. and Villaverde, S.(2005):Combined anaerobic-aerobic treatment of azo dyes. A short review of bioreactor studies. *Water Research.* , 39:1425-1440.
39) Verma, P. and Madamwar, D.(2003): Decolorization of synthetic dyes by a newly isolated strain of *Serratia marcescens. World Journal of Microbiology and Biotechnology. ,* 19: 615-618.
40) Vivekanandan, N., Vishwanathan, M., Shanmugam, V. and Thangavel B. (2013): Degradation and detoxification of reactive azo dyes by native bacterial communities. *African Journal of Microbiology Research. ,* 7 (20):2274- 2282.
41) Wong P. W., Teng, T. N., Rahman, T. and Abdul N. N. (2007): Efficiency of the Coagulation-Flocculation Method for the Treatment of Dye Mixtures Containing Disperse and Reactive Dye. *Water Qual. Res. J. Canada.,* 42 (1): 54-62.
42) Yusuff, R.O. and Sonibare, J.A. (2004): Characterization of Textile Industries Effluents in Kaduna, Nigeria and Pollution Implications. *Global Nest: The Int. J.,* 6 (3):212-221.