Decolourisation of reactive textile dye effluent by using Bacterial strain

T. Marimuthu[1], S. Rajendran[2],M.Manivannan[3]

[1]Research and Development Centre, Bharathiar University, Coimbatore, Tamil nadu, India.
[2]Corrosion Research Centre, PG and Research department of chemistry, GTN Arts college, Dindigul, Tamil nadu, India.
[3]Department of chemistry, chettinad college of engineering and Technology, Karur, Tamil nadu, India

ABSTRACT

The study was investigated the decolorization potentials of certan selected microbial species (bacterial) to an actual textile dyeing industry effluent. All the microbes tested namely (bacterial) Bacillus sp, pseudomonas sp, Acineto bacter, Legionella and Staphylococcus (bacterial) showed greater potential in decolorizing the dye effluent. Bacterial decolorization of textile effluents were critically analysed and discussed. Bioremediation is an option that offers the possibility to destroy harmless various pollutants using natural biological activity.

Key words: reactive textile dye effluent, bacterial strain, decolorization potential

INTRODUCTION

A dye is a synthetic chemical used to impart color to materials of which it becomes an integral part. Dyes are carbon based organic compounds while pigments are normally inorganic compounds, often involving heavy toxic metals (Balakrishnan et al.2008). Dyes are classified to their application and chemical structure. They are composed of a group of atoms responsible for the dye color, called chromophores, as well as an electron withdrawing or donating substituents that cause or intensify the color of the chromophores, called auxophores.

Dyes are of various types based on their dissociation in aqueous solutions i.e. acid dyes, direct reactive dyes (anionic), basic dyes (cationic) and disperse dyes (nonionic). They are used on several substrates in food, cosmetics, paper, plastic and textile industries. Solutions retain them by physical adsorption by making compounds with metals and salts using covalent bonds.

A very small amount of dye in water (10-50 mg L-1) affects the aesthetic value, transparency of water and gas solubility of water bodies. The presence of even very low concentrations of dyes in effluent is highly visible and degradation products

of these textile dyes are often carcinogenic (Kim et al., 2003). Further, the adsorption of light by these textile dyes creates problems for photosynthetic aquatic plants and algae (Singh and Singh, 2006). These reactive dyes are a highly water-soluble polyaromatic molecule, which means they do not adsorb to solids and are prevalent in high concentration in the effluents (Ganesh et al., 1994).

Laccases are involved in the biodegradation of lignins, which constitute the main noncarbohydrate component in wood and are among the most abundant groups of biopolymers in the biosphere (Elias Abdulla, et. al. 2000). Faryal and Hameed (2005) carried out the textile effluent analysis for presence of Mn, Zn, Mg etc. and reported subsequent decolourising bacteria. Guendy (2007) discovered a method for treatment of wide conc. range of dye waste water through ozonization. Khadijah (2009) repoted 1540 bacterial isolates and screened for their ability to degrade selected azo dyes. [1 – 11]

Sapna Kochher & Sandeep Kumar (2012) studied about screening for potential textile dye decolorizing bacteria. The result of this study suggesed a great potential for bacteria to be used remove colour from dye wastewaters. The bacterial species used in carrying out the decolorization of dyes in the study were isolated from the textile dye industry waste effluent. The batcterial strain Bacillus sp. Showed decolorizing activity through a degradation mechanism rather than adsorption. This study has established that the bacteria are adaptive in nature and can degrade contaminants. [12].

Leena.R and Selva raj. D (2008) studied about Bio-decolourization of textile effluent containing Reactive Black-B by effluent – adapted and non- adapted bacteria. The used five effluent adapted bacteria namely (1).Alcaligenes sp, (2).Eubacterium sp,(3).Arthrobacter sp. (4). Pseudomonas aeruginosa & (5).Bacillus sp. The also used four effluent non-adapted bacteria namely (1).Kluyvara ascorbata, (2).Bacillus sp, (3).Pseudomonas sp & (4).Pasteurella sp.for decolourization in ten days. Effluent-adapted bacillus sp. gave 35.68% reduction in colour wheresas the non-adapted isolate of the same species showed 30.04% colour removal. Similarly, effluent-adapted P.aeruginosa showed 44.2% decolourization which was better than non-adapted Pseudomonas sp. (41.73%).[13-20]

MATERIALS AND METHODS:

The present study has been carried out to find the suitable bioremediation measures of textile dye effluent by using Biological and chemical agents.

Sample Collection:

The dye effluent was collected from the Balammalpuran in Karur. The samples were collected by using sterile sample bottles and transported to the laboratory within 24 hrs of collection. The collected samples were stored at low temperature for further analysis.

Enumeration of total microbes in the dye effluent sample: Total microbial counts in the dye effluent sample serially diluted ($10^{-1}$to 10^{-9}). One ml from the dilution 10^{-3} was plated in Nutrient agar plates, using spread plate method and incubated at 37°C for 24 hrs for bacterial counts and PDA agar for fungal counts incubated at 28°C for 48 hrs. Colonies on the plates were counted by using colony counter.

Enumeration of dye degrading microbes:

The dye effluent sample was serially diluted. One µl of sample from dilution 10^{-3} was plated on pre dried mineral salt agar using spread plate technique and PDA agar. A filter paper saturated with sterile dye effluent sample was aseptically placed on the inside of the inverted petridishes and the culture plates were incubated for 4 days. Plates yielding 30 to 250 colonies were enumerated for bacterial isolates and 15 -105 colonies for fungal colonies.

Isolation and Identification of Bacterial sample:

The Bacterial cultures were isolated and identified by pure culture and Biochemical methods. Bacterial culture isolated from dye effluent sample, dilution 0.1ml of sample was inoculated on Nutrient Agar plates and kept for incubation at 37°C for 24 hrs.

Gram staining

A thin smear of cultures were made, air-dried and heat fixed. The slides were flooded with primary stain (Crystal violet) for one minute. It was washed with water and flooded with mordant (Grams iodine) for a minute. After washing of the mordant, the decolourizer (alcohol) was added and washed and stained with the counter stain (Safranin) for a minute. After washing, the slides were air-dried and observed under the phase contrast microscope. The bacterial strain later confirmed as *Bacillus* sp. *Pseudomonas* sp. at Easma Institute of Technology, Aravakurichy.

Motility

Motility test medium was used to check the motility of the bacterium. Bacterial motility can be shown using different types of motility medium. The composition of these preparations gives freedom of movement comparable to that of

broth culture. Motile bacteria were identified by the presence of growth away from the line of inoculation whereas non – motile organisms grow only the initial stab line.

Biochemical tests

Catalase test

Catalase test was used to detect the presence of the enzymes Catalase. A capillary tube was dipped into 3% H_2O_2 and the colony was touched. There was bubble formation, it was positive.

Indole test

Tryptone broth was inoculated and incubated at 37^o C for 48 hours and added Kovac's reagent and read immediately. The results were interpreted based on the change of colour from yellow to cherry red ring formation.

Methyl red test (MR)

Buffered glucose broth was inoculated and incubated at 37^oC for 48 hours. A few drop of methyl red solution was added to culture and the results were read immediately. The results were interpreted based on the change of colour from yellow to red.

Voges Proskauer test (VP)

The organisms were inoculated in buffered glucose broth and were incubated at 37^0 C for three days and 3 ml of alpha napthol was added followed by 1 ml of 40% KOH. It was mixed well and allowed to stand for 30 min. The results were interpreted based on the change of colour to pink.

Citrate utilization

The organisms were streaked onto Simmons Citrate agar plate and incubated at 37^oC for 48 hours. The results were interpreted based on the change of colour from initial green to deep blue if it was positive.

Assay of decolorization activity

The bacterial strains (Bacillus, Pseudomonas, Acineto bacter,Legionella,Staphylococcus) and were grown on PDA agar plates and were

streaked on plates containing dyes in media. The plates contained MM2 – Carbon, MM2 – Nitrogen, MM2 – Nitrogen – Carbon. Decolorization of the dye was visually observed for the extent of zone clearing on the plates.

The extent of dye decolorization by the microbial cultures in broth was determined by spectrophotometer at the maximum absorbance of the respective dyes in the cell free extracts. The percentage of dye decolorization by the cells was done using the modified method of Yatome *et al*., (1991).

Cultures were grown in 50 ml of broth for overnight at 37^{o} C and 80 rpm to an OD of 1.00 at 600nm. The cultures were centrifuged at 10,000 rpm for 10 min. and washed twice with sterile saline (0.85%) and resuspended in 10 ml of saline solution. 0.1ml of the inoculum was added to the broth containing dye and incubated at 37°C, 85 – 110 rpm for 24 hr. The supernatant was collected after centrifugation for absorbance measurement at respective wavelengths. The percentage decolorization was calculated as follows:

$$\% \text{ age Decolorization} = \frac{\text{Initial O.D - Final O.D}}{\text{Initial O.D}} \text{X100}$$

SAMPLE ANALYSIS:

Analysing of physico-chemical parameters of the dye effluent sample:

The dye effluent sample was used for various Physico-chemical analysis viz., colour of the sample, Odour by direct smelling of the sample, Standard thermometer was used for temperature measurements, The pH of the sample was determined by pH meter.

ADMI color was determined with a Spectrophotometer in accordance with the ADMI Tristimulus method 2120 D detailed in *Standard Methods* (1998). The spectrophotometer was calibrated before each use with standard platinum cobalt color solutions of 100, 200, 300, 400, and 500 ADMI color units. (Edwards, 2000). Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) were analyzed.

Biological Oxygen Demand (BOD):

Adjust the pH of water sample to neutrality using 1N acid or 1N alkaline solutions. Fill the water sample in 6 BOD bottles without bubbling. Add 1 ml of Allyl thiourea to each bottle. Determine dissolved oxygen content in 3 of the 6 BOD bottles by titration method. Take the mean of the 3 readings (D1). Incubate the rest of 3 BOD bottles at 27ºC in a BOD incubator for 3 days. Estimate the oxygen concentration in all the 3 incubated samples. Take the mean of 3 readings (D2). Calculate the BOD of water in mg/l by using the following formula

BOD (mg/l) = D1-D2 ,Where,

D1 = Initial dissolved oxygen in the sample (mg/l)

D2 = Dissolved oxygen in the water sample (mg/l) after 3 days of incubation.

Chemical Oxygen Demand (COD):

Take three , 100 ml conical flasks and pour 50 ml of water sample in each (i.e., in triplicate). Simultaneously run distilled water blank standards (also in triplicate). Add 5 ml of potassium dichromate solution in each of the 6 flasks. Keep the flasks in water bath at 100ºC for 1 hour. Allow the samples to cool for 10 minutes. Add 5 ml potassium iodide solution in each flask. Add 10 ml of H2SO4 in each flask. Titrate the contents of each flask with 0.1 M of sodium thiosulphate solution until the appearance of pale yellow colour. Add 1 ml of starch solution to each flask (solution turns blue colour). Titrate the again with 0.1 M sodium thiosulphate until the blue colour disappears completely. Find out the COD (mg/l) of the water sample by using the following formula

$$\text{COD of the sample (mg/l)} = \frac{8*C*(V_B-V_A)*1000}{S}$$

Where, C = Concentration of titrant (0.l M)

V_B = Volume of titrant used for sample (ml)

V_A = Volume of titrant used for blank (ml)

S = Volume of sample taken (ml)

RESULTS:

Sample collection:

The textile dye effluent sample was collected from Sengunthapuram, Karur Dt. Tamil Nadu.

Sample : Textile dye effluent

Color : Wine red color

Odour : Alkali odour.

Enumeration of total and dye utilizing microbes in the dye effluent samples:

The count of total bacterial counts in dye effluent sample ranged from 4.12 X 10^3 $CFUg^{-1}$ while in the fungal the counts ranged 1.98 X 10^3 $CFUg^{-1}$.

S. No	Sample	Microbial Counts(CFU g^{-1})
1	Bacterial sample	4.12X 10^3

Isolation and Identification of Bacterial sample:

The dye utilizing bacterial isolates were identified based on the Biochemical characterization. The species of *Bacillus* sp, and *Pseudomonas* sp, Acineto bacter, Legionella and Staphylococcus were more dominantly isolated among the bacteria respectively.

DEGRADATION ANALYZING:

For Bacteria:

The isolated Bacterial samples Bacillus sp, Pseudomonas sp, Acineto bacter, Legionella and Staphylococcus was checked for the extent of dye degradation both in solid media plates as well as in liquid media. All the isolates gave maximum degradation. Visual degradation indicated that degradation was higher in case of broth as compared to minimal media.

The overnight broth cultures (Bacillus sp, Pseudomonas sp, Acineto bacter, Legionella and Staphylococcus) with crude oil were centrifuged and resuspended in 10 ml of saline solution and 0.1ml of the inoculum was added to the broth containing crude oil and incubated at 37°C, 85 – 110 rpm for 24 hr. The supernatant was collected after centrifugation for absorbance measurement at 650 nm wavelengths. Among the five isolated bacterial strains, *Pseudomonas* showed higher degradation than *Bacillus*. The percentage degradation was calculated and given in Table-1.

Table – 1 bacterial decolorization of dye effluent

S. No	Bacterial cultures	Percentage Degradation (%)
1	*Bacillus* sp	65
2	*Pseudomonas* sp	73
3	Acineto bacter	52

4	Legionella	45
6	Staphylococcus	58

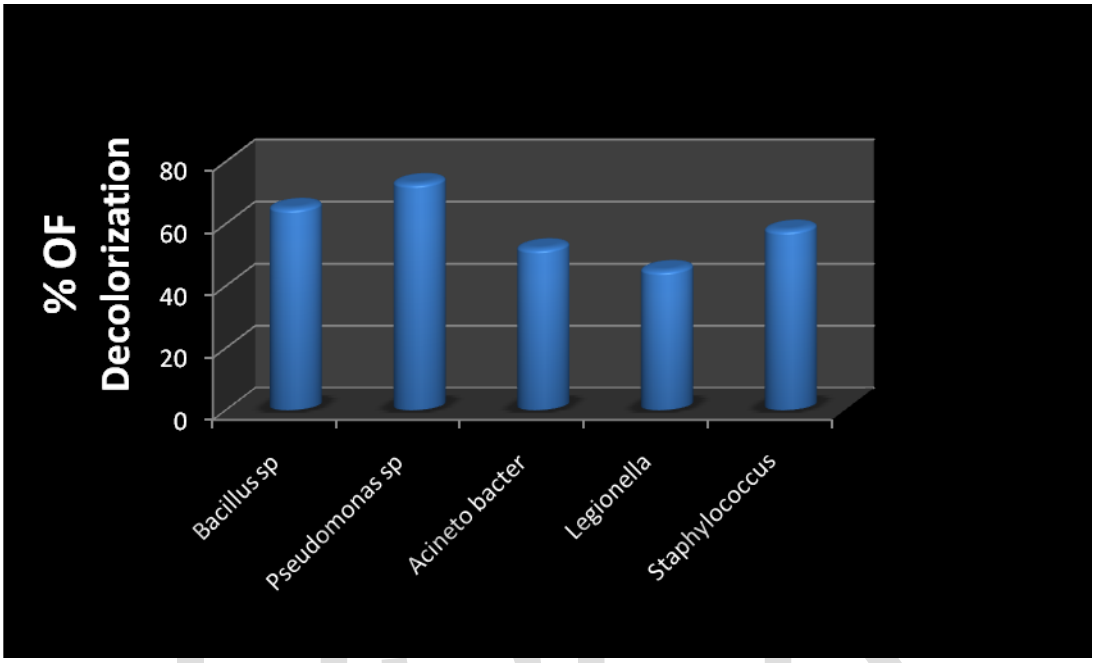

Fig -1 Bacterial Decolorization

After inoculation of isolated microorganisms, the physico-chemical parameters were analyzed for 2 weeks intervals.

Table -2 COD Removal by bacterial strain

S.NO	Bacterial strain	COD mg perlitre					
		FIRST DAY	3 DAYS	6 DAYS	9 DAYS	12 DAYS	%OF COD REMOVAL AFTER 12 DAYS
1	Bacillus sp	938	872	801	743	688	26.65
2	Pseudomonas sp	938	863	737	624	502	46.48
3	Acineto bacter	938	789	671	409	374	60.12
4	Legionella	938	857	789	737	645	31.23
5	Staphylococcus	938	865	798	714	632	53.94

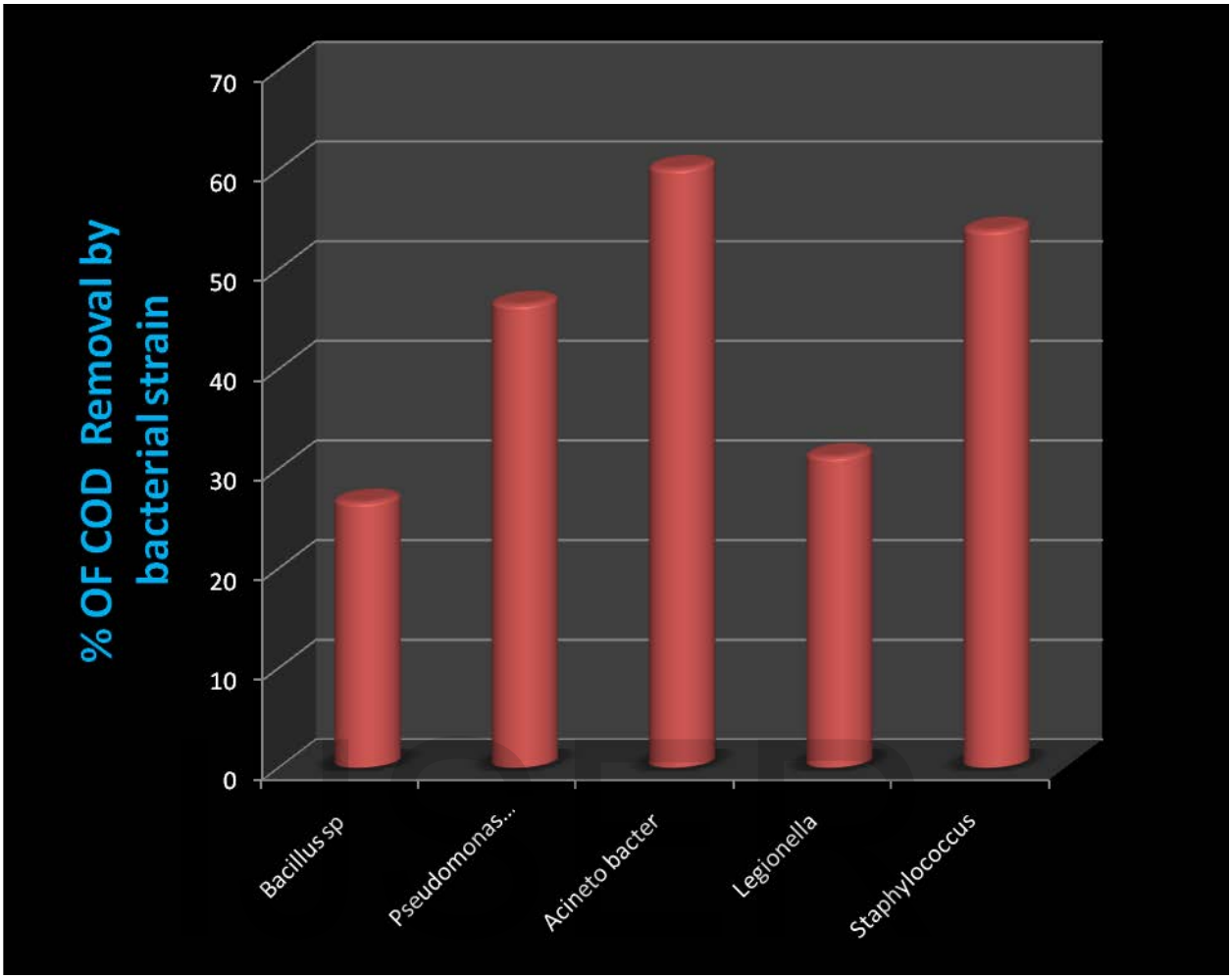

Fig -2 COD Removal by bacterial strain

Table -3 BOD Removal by Baterial strain

.S.NO	Bacterial strain	BOD mg perlitre					
		FIRST DAY	3 DAYS	6 DAYS	9 DAYS	12 DAYS	%OF COD REMOVAL AFTER 12 DAYS
1	*Bacillus* sp	45	41	35	29	26	42.22
2	*Pseudomonas* sp	45	38	34	29	24	46.66
3	Acineto bacter	45	37	32	30	27	40.0
4	Legionella	45	42	38	33	29	35.5
5	Staphylococcus	45	43	37	35	32	28.88

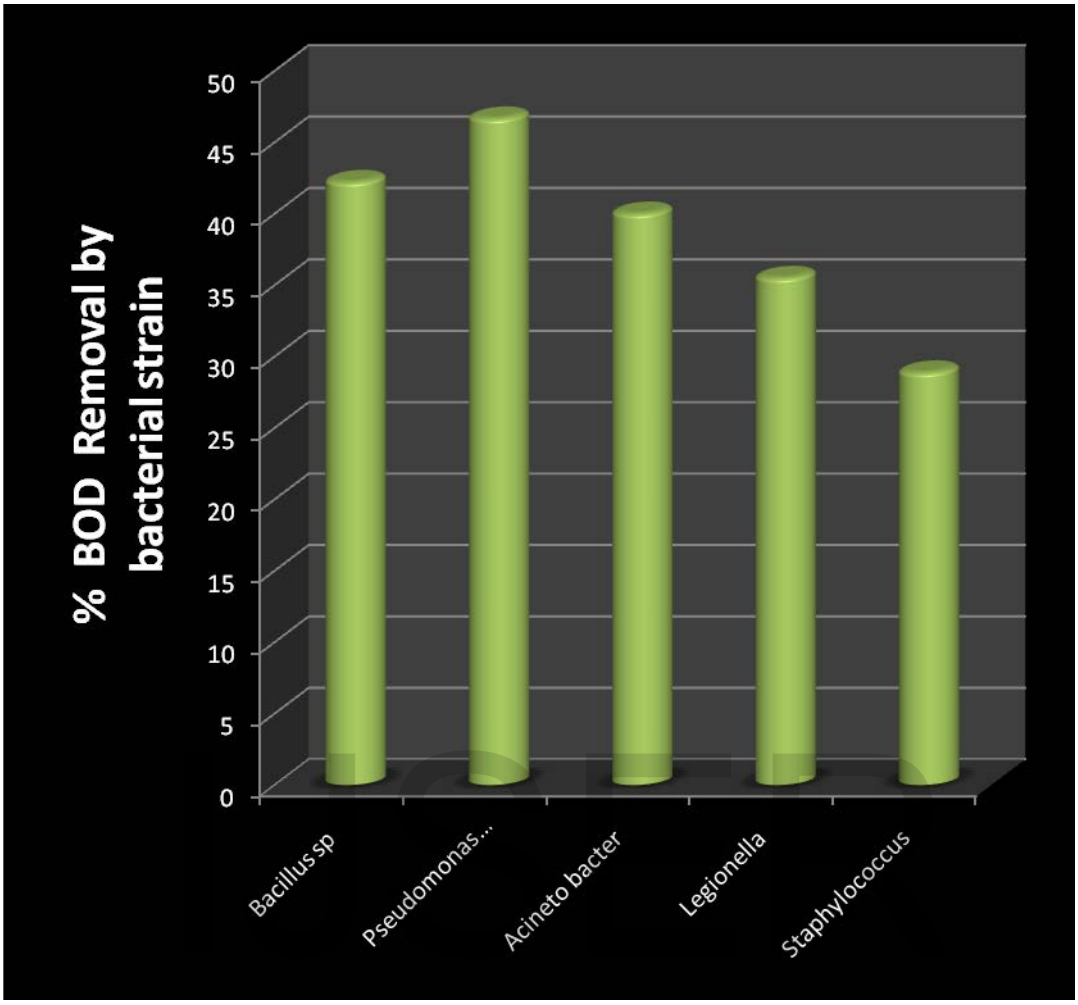

Fig -3 BOD Removal by Baterial strain

Table -4 P^H variations by bacterial strain

S.NO	Bacterial strain	P^H				
		FIRST DAY	3 DAYS	6 DAYS	9 DAYS	12 DAYS
1	*Bacillus* sp	8.1	7.7	7.5	7.3	7.2
2	*Pseudomonas* sp	8.1	8.O	7.8	7.7	7.6
3	Acineto bacter	8.1	7.9	7.7	7.6	7.3
4	Legionella	8.1	7.8	7.6	7.5	7.4
5	Staphylococcus	8.1	8.O	7.9	7.7	7.5

DISCUSSION:

In the present study, decolorization proceeded gradually even up to 12 days in effluent-adapted bacterial treatment. Effluent adapted bacillus sp gave 35.68% reduction in color [20] according to olukanni et al. (2006) , but present work gave 73%.

The microbes utilized carbon, nitrogen and sulphate found in effluent medium for their nutrition. Decolorization % will be further increased and prolonged by supplementing the effluent medium with other cheaper effective carbon or energy source such as sucrose, starch and hydrolysed starch. Ability of the microbial isolates to utilize starch as a co-substrate could be encouraging from commercial point of view (moosvi et al., 2005).[19]

Acineto bacter had high COD removal % (60.12) and pseudomonas sp had high BOD removal % (46.66)

REFERENCES :

[1.] Shenai,V.A. (1994). Chemical index international. Chemical weekly.145-149.
[2.] Guendy,H.R. 2007. Ozone treatment of textile wastewater relevant to toxic effect elimination in marine environment. Egyptian Journal of Aquatic Research.98-115.
[3.] 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 Technoloy, 26, 381-387.
[4.] Faryal,R., Hameed,A. 2005. Isolation and characterization of various fungal strains from textile effluent for their use in bioremediation. Pak.J.Bot.; 1003-1008.
[5.] Khadijah,O., Lee,K.K., Mohd Faiz F., Abdullah. 2009. Isolation, screening and development of local bacterial consortia with azo dyes decolourising capability. Malaysian Journal of Microbiology.;25-32.
[6.] Balakrishnan, M., S. Arul Antony, S. Gunasekaran, R.K. Natarajan, 2008. Impact of dyeing industrial effluents on the ground water quality in Kancheepuram (India). Indian Journal of science and Technology. Vol.1no.7, B-117-123.
[7.] Kim, S., C. Park, T.H. Kim, J. Lee, S.W. Kim(2003): COD reduction and decolorization of textile effluents using a combined process. J. Biosci. Bioeng., 95;;102-105.
[8.] Singh, V.K., Singh, J. 2006. Toxicity of industrial wastewater to the auatic plant Lemna minor. L. J. Environmental Biol., 27;; 385-390.
[9.] Ganesh, R., G.D. Boardman and W.C. Tincher(1994): Fate of azo dyes in sluges. Water Res., 28, 1367-1376
[10.] Wiegel,J., Zhang,X., Wu,Q. 1999. Anaerobic dehalogenation of hydroxylated polychlorinated biphenyls by Desulfitobacterium dehalogenans.Applied and Environmental Microbiology.,2217-2221.

[11.] 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

[12.] Sapna Kochher & Dr.Sandeep Kumar, "Screening For Potential Textile Dye Decolorizing Bacteria", "International Journal for Science and Emerging Technologies with Latest Trends" 2(1): 36-48 (2012)"

[13]Abadulla E, Robra KH, Gubitz G, Silva L, Cavaco-Paulo A (2000).Enzymatic decolourization of textile dyeing effluents. Textile Res. J.70: 409-414.

[14] Boer CG, Obici L, Sourza CG, Piralta, RM (2004). Decolourization of synthetic dyes by solid state cultures of *Lentinula (Lentinus) edodes* producing manganese peroxidase as the main lignolytic enzyme.Bioresour. Technol. 94: 107-112.

[15] Chen KC, Huang WT, Wu JY, Houng J–Y (1999). Microbial decolourization of azo dyes by *Proteus mirabilis.* J. Ind. Microbiol. Biotechnol. 23: 686-690.

[16] Hao OJ, Kim H, Chang PC (2000). Decolourization of wastewater. Crit.Rev. Environ. Sci. Technol. 30: 449-505.

[17] Maier J, Kandelbauer A, Erlacher A, Cavaco-Paulo A, Gubitz GM (2004). A new alkalithermostable azo reductase from *Bacillus sp*. Strain SF. Appl. Environ. Microbiol. 70(2): 837- 844.

[18] McMullan G, Meehan C, Conneely A, Kirby N, Robinson T, Nigam P, Banat IM, Marchant R Smyth WF (2001). Microbial decolourization and degradation of textile dyes. Appl. Microbiol. Biotechnol. 56: 81-87.

[19] Moosvi S, Keharia H, Madamwar D (2005). Decolourization of textile dye Reactive Violet 5 by a newly isolated bacterial consortium RVM 11.1. World J. Microbiol. Biotechnol 21: 667-672.

[20] Olukanni OD, Osuntoki AA, Gbenle GO (2006). Textile effluent biodegradation potentials of textile effluent-adapted and non-adapted bacteria. Afr. J. Biotechnol. 5(20): 1980-1984.