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Eco-friendly Microbial Decolorization and Detoxification of Reactive dyes by Isolated Three Culture of *Bacillus* sp.

Running title: - Enhanced Decolorization and Detoxification of Reactive dyes; Reactive Yellow 15, Reactive Yellow 145 and Reactive Red 195 by Three Isolated Cultures of *Bacilllus* sp., *Fictibacillus gelatini*, *Bacillus subtilis inaquosorum* and *Bacillus subtilis subtilis*.

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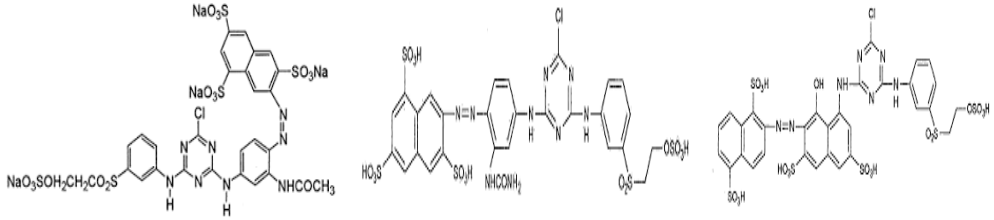
ABSTRACT
Complete decolorization of Reactive dyes i.e. Reactive yellow 15, Reactive yellow 145 and Reactive red 195 has been observed at concentration of 500 mg/l within minimum time of 4-18 hrs in static condition of growth by three different bacterial isolates namely, *Fictibacillus gelatini*, *Bacillus subtilis subsp. inaquosorum* and *Bacillus subtilis subsp. subtilis* isolated from soil and dry sludge, near textile dyeing industry. These selected isolates showed even higher decolorization in simple nutrient broth media than in optimized media having combination of maltose and yeast extract at 37°C and neutral pH. Either they showed similar results as obtained in nutrient broth or showed less decolorization with supplementation. This suggests that carbon and nitrogen sources acted as a catabolite repessor. Current study is environmental-friendly due to use of biological methods and without any hazardous end products, highly economical as microbes utilized dyes as carbon and nitrogen source for their effective growth apart from complete decolorization that is occurring very fast within hours.

Key words: - Decolorization; *Bacillus sp*; Reactive dyes; Bacterial isolates; Optimum conditions.

INTRODUCTION
A large number of small textile processing units are scattered all over India. It is one of the most complicated among manufacturing industries that discharge large quantities of contaminants in wastewater, with organic pollutants (pesticides, herbicides, phenols, polycyclic aromatic hydrocarbons (PAHs), aliphatic and heterocyclic compounds) [1]. Azo dyes are characterized by the presence of one or more azo groups (-N=N-), which are responsible for their coloration and when such bond is broken or degraded, the color is lost [2]. Out of all chromogenic dyes, the reactive groups of azo dyes are predominantly used due to superior fastness for the fabric, high photolytic stability and resistance towards microbial degradation [3]. Dyes are most difficult to treat because of their complex & synthetic origin. Due to this, total degradation of azo dyes is the only solution for the elimination of intermediate xenobiotic compounds from the environment because alternations in the chemical structures result in formation of new xenobiotic compounds which may be more or less toxic than parental. The existing physico-chemical methods were found to be less effective, since they exhibit operational complexity, are expensive and unable to completely remove azo dyes & their metabolites and also generate large amount of secondary pollutants [4].
This poses a challenge to researchers in finding alternative biological methods that are efficient, inexpensive, eco-friendly and produce less amount of sludge [5], [6]. In most of the studies, decolorization is occurring in many days with very low rate of decolorization or the microorganisms are capable of degrading at a very low concentration of dyes. Many workers have reported that rate of dye decolorization gradually decreases with increasing concentration of dyes

due to the toxic effect of dyes on degrading microorganisms or the blockage of active sites of enzymes by dye molecule with different structures [7]. Decolorization percentage of RY145 by *B. boroniphilus* was inversely proportional to the dye concentration. Therefore at conc. of 500 mg/l of dye there was nearly 0% decolorization in 24 h [8]. The maximum decolorization of 86.2% of Reactive Red HE8b by DN1 was observed at 200 mg/l of dye concentration [3]. This is not the case with our strains. The present investigation with *Fictibacillus gelatini*, *Bacillus subtilis subsp. inaquosorum* and *Bacillus subtilis subsp. subtilis* is quite significant as it reports decolorization of very large (500 mg/l) concentration of different Reactive dyes (Figure 1) in a very short time.



(A) (B) (C)

Figure 1: Different dye structures: (A) Reactive Yellow 15 (B) Reactive yellow 145 (C) Reactive Red 195

MATERIALS AND METHODS

Chemicals and Dye collection: All the chemicals of analytical grade were purchased from Hi-Media Laboratories and Textile dye Reactive Yellow15 (λ max=420nm), Reactive Yellow 145(λ max=418nm) and Reactive Red 195(λ max=542nm) were collected from Bhiwani Textile Mill, Haryana, India.

Isolation, Screening and Identification of Bacteria

Different sludge samples were collected from Nahar Textile Industry, Lalru (Punjab) and the isolation of bacterial colonies was done by serial dilution method. The pure colonies were obtained by spreading & streaking techniques and incubating at 37°C for 24 h. A preliminary identification of the cultures was based upon biochemical tests. The culture was sent for 16 S r-DNA sequencing at MTCC IMTECH, Chandigarh for confirmation of species level identification.

Strain, Media and Culture Condition

Modified Zhou and Zimmermann (ZZ) agar medium (Y.E-5 g/l, glucose-5 g/l, $(NH_4)_2SO_4$- 0.5 g/l, KH_2PO_4- 2.66 g/l, Na_2HPO_4- 4.32 g/l) was used to study the effect of different parameters on decolorization of Reactive yellow 15, Reactive yellow 145 & Reactive red 195. Decolorization experiments were carried out in nutrient broth (Peptone-3 g/l, Yeast extract-5 g/l, NaCl-3 g/l).

Decolorization of Dyes

An inoculum size of 10% of pre-cultured cells was inoculated in modified ZZ medium containing Reactive dyes. Experiments were performed in triplicates and control (without microorganisms) was included.

Decolorization activity was expressed in terms of percentage decolorization and was determined by monitoring the decrease in absorbance at absorption maxima of Reactive dyes. The uninoculated medium supplemented with respective dye was used as reference. The culture suspension was centrifuged at 10,000 rpm for 10 min for removal of the biomass as they interfere with the measurement. The degree of decolorization of dyes was measured by reading the absorbance of supernatants at λ max of specific dyes by UV-visible spectrophotometer. The decolorization was calculated according to the following formula:

$$\text{Decolorization (\%)} = \frac{\text{Ao} - \text{A}}{\text{AoA}} \times 100$$

Where, Ao is the initial dye absorbance before decolorization and A is the absorbance after decolorization [9], [10].

Optimization of pH, Temperature, Inoculum size, and Concentration of dyes

Decolorization was studied by varying different parameters such as pH (6,7,8,9 and 10), Temperature (25,30,37,40,45 and 50 °C), Inoculum size (1,3,5,7.5 and 10%) along with dye

concentrations (100, 200, 500, 1000 mg/l of the Reactive dyes). The Percentage decolorization was measured at different time intervals & determined by decolorization equation given above.

Enzyme screening assays: Quantitative assay

Quantitative results are reported numerically and compared against accompanying reference interval for interpretation. Laccase, Azo-reductase, Tyrosinase and Lignin peroxidase (LiP) activities were analyzed in cell free extracts as well as in culture supernatants.

Laccase assay (EC. 1.10.3.2) was performed by a modified protocol from Zarvazina *et al*. (2004) [11], which used 2, 2'-azino-bis-(3-ethylthiazoline-6-sulfonate) (ABTS) as a substrate at pH 6.0. The reaction was started by adding 1 ml of sample and was monitored spectrophotometrically at 436 nm. One unit of activity was regarded as the amount of enzyme capable of converting 1µM ABTS per min per ml. Laccase activity was also determined by using protocol from Bain *et al*., (2003) [12], which used Guaicol as substrate at pH 6.5 and was monitored spectrophotometrically at 465 nm. One unit was the amount of enzyme that increases absorbance by 0.001 units at 37°C. **Azoreductase** (EC. 1.7.1.6) was determined by protocol from Chen *et al*., (2005) [13], with 4.45 M of dyes, 100 µM NADH in 50 mM phosphate buffer (pH 7.4) and 1ml of enzyme solution and monitored for the decrease in color absorbance (430 nm) at room temperature. Azo reductase activity was expressed in terms of azo dye reduction which was calculated by using molar absorption coefficient of $23M^{-1}cm^{-1}$ at 430 nm. **Tyrosinase** was determined by reaction mixture of 2 ml, containing 0.01% catechol in 0.1M phosphate buffer (pH 7.4) at 475 nm (Zhang *et al*., 1997) [14]. **Lignin peroxidase activity** was determined by monitoring the formation of propanaldehyde at 300 nm in a reaction mixture of 2.5 ml containing 100 mM n-propanol, 250 mm tartaric acid, and 10mM H_2O_2 (Shanmugan *et al*., 1999) [15]. All enzyme assays were run in triplicates and average results were calculated and one unit of enzyme activity was defined as the change in absorbance unit/min/ mg of enzyme, where blank test tube contained all components except the enzyme solution. Protein estimation was carried out by Lowry *et al*. (1951) [16].

RESULTS

Isolation, Screening and Identification of Microorganisms

Pure bacterial colonies obtained were tested for their decolorization ability in nutrient broth medium supplemented with 500 mg/l of each textile dyes separately (Reactive yellow 15, Reactive yellow 145 and Reactive red 195). Three isolates were found to be capable of complete decolorization of these dyes in very less time and therefore chosen for further studies. Selected bacterial strains were identified by 16 S rRNA as *Fictibacillus gelatini*, *Bacillus subtilis inaquosorum* and *Bacillus subtilis subtilis*.

Optimization studies for maximum dye decolorization

The identified bacterial cultures were taken for decolorization assays with Reactive yellow 15, Reactive yellow 145 and Reactive red 195 dyes in simple nutrient broth and evaluated for percent decolorization at time interval of 2 hrs for 2 days and thereafter 24 hrs for rest of the days (8 days) at their optimum conditions of pH 7 and 37°C of static growth. Initial screening of cultures was found to be efficient to decolorize all the three dyes completely in 4 to 18 hrs. During first few hours the rate of decolorization of RY15, RY145, and RR195 by *Fictibacillus gelatini*, *Bacillus subtilis inaquosorum* & *Bacillus subtilis subtilis* was fast. Decolorization rate increased consistently from 4 to 12 hrs and finally complete decolorization was observed in case of the three dyes (Figure 2).

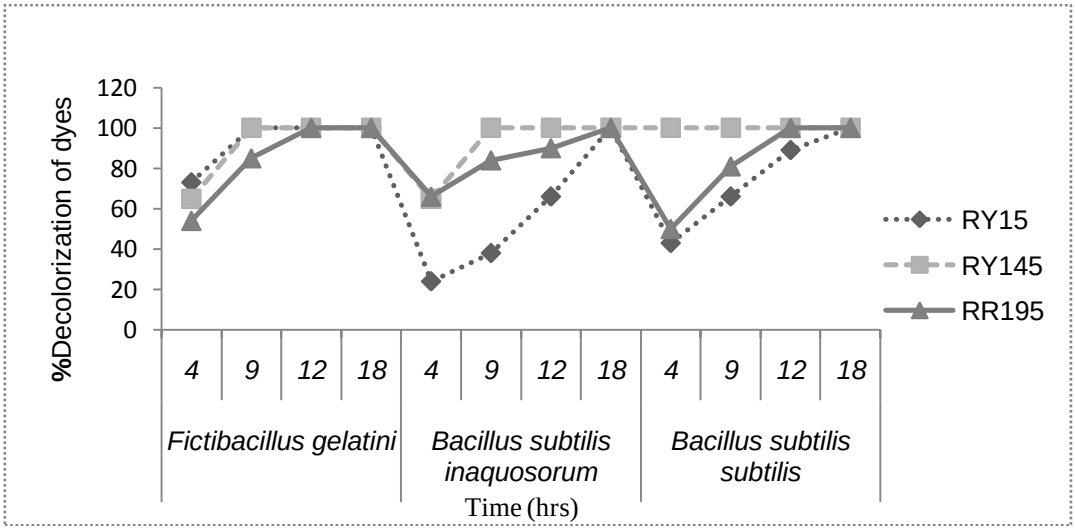


Figure 2: Decolorization of reactive dyes in simple nutrient broth medium by *Fictibacillus gelatini*, *Bacillus subtilis inaquosorum* and *Bacillus subtilis subtilis*.

All the three isolates were grown and optimized in modified ZZ medium (containing C and N sources) at same conditions as mentioned above. Different carbon sources (glucose, galactose, fructose, mannitol, maltose, sucrose & starch) and nitrogen sources (peptone, yeast extract, beef extract, malt extract, sodium nitrate & ammonium nitrate) were supplemented to all the three isolates. Out of these, medium with combination of Maltose and Yeast extract showed good results but decolorization was not as high as in case of nutrient broth (Table 1 & Figure 3). Either they showed similar results as obtained in nutrient broth or showed less decolorization with supplementation. This suggests that carbon and nitrogen sources acted as catabolite repessor as their presence did not show any good results as observed in simple nutrient broth and decreased their decolorization in modified medium. At a dye conc. of 500 mg/l and in absence of yeast extract the decolorization was found to be 81.94%, after 70 hrs suggesting that the organism could utilize organic dye as sole C source [17].

Table: 1 showing comparative study of decolorization of reactive dyes in simple nutrient broth medium and in modified ZZ medium (with maltose and yeast extract) by three different *Bacillus* sp

Dyes → / Isolates ↓	RY15		RY145		RR195	
	M1	M2	M1	M2	M1	M2
Fictibacillus gelatini	C/9h	C/12h	C/9h	C/18h	C/12h	C/18h
Bacillus subtilis inaquosorum	C/18h	33/8D	C/9h	C/12h	C/18h	58.6/1D
Bacillus subtilis subtilis	C/18h	50/8D	C/4h	C/18h	C/12h	C/12h

M1- Nutrient Broth, M2- Modified ZZ medium, C- 100%

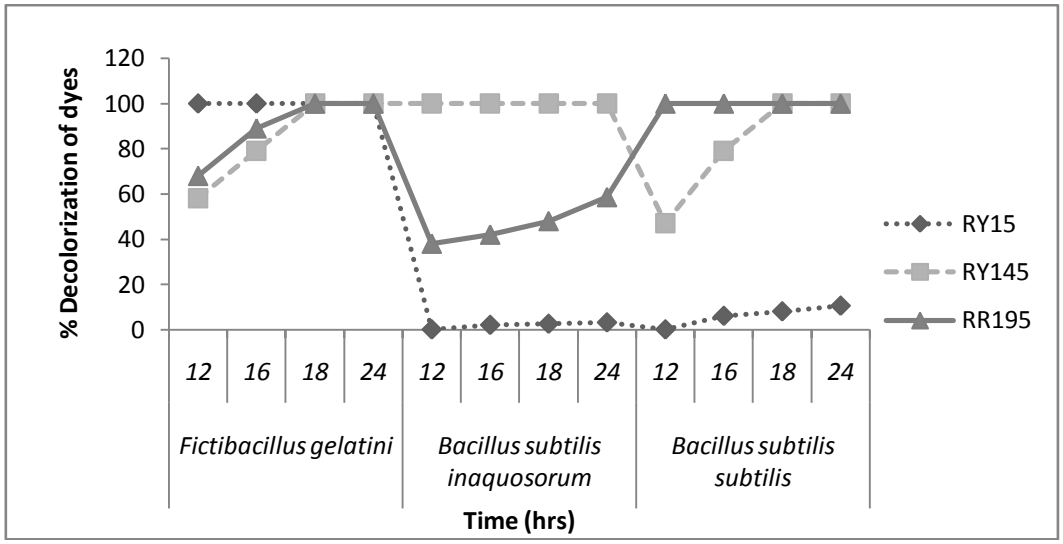


Figure 3: Decolorization of reactive dyes with co-substrate by *Fictibacillus gelatini, Bacillus subtilis inaquosorum* and *Bacillus subtilis subtilis.*

Effect of pH & Temperature on Reactive dyes decolorization

pH of the medium greatly affect the percentage of decolorization. All the three isolates showed optimum results at pH 7 and the pH range of our culture was reported to be between 6 -10. Temperature is the key factor, which affect the cells and its metabolic reactions during breakdown or utilization of complex carbon compounds in form of reactive dyes. The effect of temperature ranging from 25- 50°C was investigated and checked the percentage of decolorization. At higher temperature decolorization slowly decreased and efficient decolorization was reported at 37°C. This may be due to greater synthesis of enzymes and the decolorization at optimal temperature may be owing to higher respiration and substrate metabolism.

Effect of inoculum size on Reactive dyes decolorization

Inoculum size plays an important role because of sufficient biomass required to decolorize and degrade the substrate in form of dye to product. The effect of inoculum size on dye decolorization was studied with the addition of different inoculum sizes from 1 to 10% and observed the percentage of decolorization. Optimum inoculum size for complete decolorization of dyes was observed to be 10% (v/v).

Effect of dye concentration on Reactive dyes decolorization

The Reactive dye molecule is a complete structure. So, the concentration of dye strongly influences the decolorization ability of the organism. Complete decolorization of all three reactive dyes in different conc. ranges as 100, 200, 500 & 1000 mg/l were observed in very less time period by all the three isolates. Isolate *Fictibacillus gelatini* completely decolorized all three initial conc. of RY15 dye in 12h, RY145 dye was completely decolorized; 100 mg/l in 36h, 200 mg/l in 2days & 500 mg/l decolorized to 33% in 8days and RR195 dye was completely decolorized; 100 mg/l in 12h, 200 mg/l in 18h & 500 mg/l decolorized to 50% in 8 days and the isolate showed no decolorization of all three dyes at conc. of 1000 mg/l.

Isolate *Bacillus subtilis inaquosorum* showed complete decolorization of RY15; 100 mg/l in 12h, 200 mg/l in 18h, 500 mg/l also in 18h & no decolorization at conc. of 1000 mg/l, initial conc. of RY145 dye was completely decolorized in 12h but at conc. of 1000 mg/l upto 86.4% decolorization was observed in 2 days. In case of dye RR195 all three conc. upto 500mg/l were completely decolorized in 18h but 1000 mg/l conc. was decolorized upto 80.1% in 3 days.

Similarly *Bacillus subtilis subtilis* showed complete decolorization of 100, 200 and 500 mg/l of RY15 in 12h, 18h, 18h respectively and no decolorization was observed at 1000 mg/l of the dye, RY145 dye at conc. 100 mg/l was decolorized in 12h, 200 mg/l also in 12h but 500 mg/l was decolorized upto 98.6% in 1day & 1000 mg/l upto 66.8% in 3 days. All three initial conc. of dye RR195 were completely decolorized in 12h but at conc. of 1000 mg/l upto 67.3% decolorization was achieved in 3 days. So, decolorization percent is constantly or progressively increased with dye conc. upto 500 mg/l of dye after which decolorization percent started to decrease (Table 2).

Table 2: Decolorization of all three dyes by selected three isolates in different conc. of dyes

	Fictibacillus gelatini				*Bacillus subtilis inaquosorum*				*Bacillus subtilis subtilis*			
Dyes (in mg/l)	100	200	500	1000	100	200	500	1000	100	200	500	1000
RY15	C/12h	C/12h	C/12h	0	C/12h	C/18h	C/18h	0	C/12h	C/18h	C/18h	0
RY145	C/36h	C/2d	33/8d	0	C/12h	C/12h	C/12h	86.4/2d	C/12h	C/12h	98.6/1d	66.8/3d
RR195	C/12h	C/18h	50/8d	0	C/18h	C/18h	C/18h	80.1/3d	C/12h	C/12h	C/12h	67.3/3d

C-100%

Enzyme analysis

Cell free intracellular content of the organism has shown the presence of laccase, lignin peroxidase, azoreductase and tyrosinase. The results of different enzyme activity assessed are shown in Table 3. Presence of these enzymes in the extracellular content of cells indicates the harmless fate of primary aromatic amines produced after reductive cleavage of azo bond in dyes.

Fictibacillus gelatini & *Bacillus subtilis inaquosorum* showed higher Laccase & Azoreductase activity, as compared to other enzymes & *Bacillus subtilis subtilis* possessed higher activity of enzyme azoreductase at pH 7 and 37°C.

Table: 3 Enzyme activities of extracellular enzymes of *Bacillus* sp.

	Enzyme assessed	Substrate	Enzyme Activity of *Fictibacillus gelatini* (IU/ml)	Enzyme Activity of *Bacillus subtilis inaquosorum* (IU/ml)	Enzyme Activity of *Bacillus subtilis subtilis* (IU/ml)
1.	Laccase Assay	Guaicol & ABTS[a]	6.33±0.001 8.55±0.14	0.007±0.001 2.33±0.03	0.004 ± 0.001 0.001 ± 0.000
2.	Azoreductase Assay	RY145/RR15/RR195[b] Dye+NADH[c]	3.73±0.03	1.39±0.12	1.23 ± 0.02
3.	Tyrosinase Assay	Catechol	0.004±0.0001	0.003±0.001	0.007 ± 0.001
4.	Lignin Peroxidase	n-Propanol	0.010±0.0007	0.006±0.001	0.018 ± 0.0013

a- 2, 2'-azino-bis-(3-ethylthiazoline-6-sulfonate)**,** b- Reactive yellow 145, Reactive yellow 15, Reactive red 195**,** c- nicotinamide adenine dinucleotide.

Effect of Carbon and Nitrogen sources

Carbon and nitrogen sources seemed to be effective to promote the decolorization of textile dyes. In the present study different carbon sources (glucose, galactose, fructose, mannitol, maltose, sucrose & starch) and nitrogen sources (peptone, yeast extract, beef extract, malt extract, sodium nitrate & ammonium nitrate) were supplemented to all the three isolates for decolorization of dyes. Out of above all, combination of Maltose and Yeast extract showed good results.

As number of studies also reported, supplementation of co-substrate (C & N sources) showed no significant effect on decolorization. In present study combination of Maltose and Yeast extract showed good decolorization percent but not as high as in case of simple nutrient broth (Table 1 & Fig.3).

Decolorization of Textile Effluents by Consortium

Due to efficient decolorization capability, these isolates could be used to develop microbial consortium for the decolorization and complete mineralization of dyes from textile effluent. The textile effluents from Nahar textile industry Lalru (Punjab) were designated as L1, L2 & Bhiwani textile mill (Haryana) were designated as B1, B2. The combination of yeast extract and maltose was able to decolorize textile effluent L1, L2, B1 & B2 in concentration of 0.3% of both C & N sources in 12 h, 3 days, 12 h and 4 days with 100, 88, 100 & 100% respectively. All three microbes in their combination showed effective decolorization of the effluent (Table 4).

Table: 4 Consortium *Fictibacillus gelatini*/ *Bacillus subtilis inaquosorum*/ *Bacillus subtilis subtilis* showed % decolorization of effluents

Textile effluents	Decolorization by consortium (In %)
L-1 (Reactive dye effluent)	100 in 12h
L-2 (Reactive dye effluent)	88 in 3D
B-1 (Reactive dye effluent)	100 in 12h
B-2 (Disperse dye effluent)	100 in 4D

Textile effluent samples (L1, L2, B1 & B2) collected were purple, brown, dark green and dark brown in color with pungent smell. It was found that pH of untreated effluents depends upon the types of process being used in particular industry. Generally, processes in textile industries were carried out at alkaline pH; it was observed that different untreated raw L1, L2, B1, B2 textile effluents showed variations in their properties; pH variations of the four effluents were 14, 12, 10 & 9.8, COD: 558, 800, 500 & 520 mg/l, TDS: 3442, 3860, 2793 & 2421 mg/l and TSS: 1344, 1380, 1440 & 1540 mg/l. All four effluents have been treated at pH between 7-8 and their physio-chemical properties improved with decrease in, COD: 130, 365, 210 & 265 mg/l, TDS: 2101, 2293, 1120 & 1181 mg/l and TSS: 140, 180, 100 & 202 mg/l (Table 5), their values got reduced to half or less than half after treatment.

Table: 5 Treatment of industrial effluents by Physio- chemical Analysis

Parameters	Observation before treatment of raw textile effluent				Observation after treatment of textile effluent			
	L1	L2	B1	B2	L1	L2	B1	B2
pH	14	12	10	9.8	7-8	7-8	7-8	7-8
Color	Purple	Brown	Dark Green	Dark Brown	Light Purple	Light Brown	Light Green	Brown
COD(mg/l)	558	800	500	520	130	365	210	265
TDS(mg/l)	3442	3860	2793	2421	2101	2293	1120	1181
TSS(mg/l)	1344	1380	1440	1540	140	180	100	202

DISCUSSION

Twenty two morphologically distinct strains were isolated from the different samples collected from Nahar Textile Industry. Purified isolates were screened for dye decolorization activity by using the nutrient broth amended with dye inoculated with purified isolates. Khadijah *et.al*., (2009)[18], Mahmood *et.al.*, (2011)[19], also used liquid media amended with dye for screening of bacterial isolates for their ability to decolorize the dye. Out of twenty two isolates tested, three isolates (RS6, DS6 & S6IV) exhibited best activity of dye decolorization. So, all three isolates showing ability of dye decolorization were taken for further studies. This paper describes a novel study into the identification of diversity of microbes in MTCC Chandigarh, using 16 S rRNA analyses. Selected bacterial strains were identified as *Fictibacillus gelatini*, *Bacillus subtilis inaquosorum* and *Bacillus subtilis subtilis*, which showed complete decolorization of reactive dyes in minimum times of hours without requirement of any external supplement. Optimization of culture conditions was desirable for enhancing the dye decolorization activity, which is greatly influenced by the physical factors (pH, temperature, inoculum and incubation time) and nutritional factors (carbon and nitrogen sources). These factors play important role in the growth of bacteria and dye decolorization. No defined medium has been established for the best dye decolorization. Each organism or strain has its own special requirements for maximum dye decolorization activity.

pH has a major effect on the efficiency of dye decolorization; bacterial cultures generally exhibit maximum decolorization at neutral pH [20] and exhibit decolorization ability in the range of pH 5-8 [21]. All the three isolates showed optimum results at pH 7 and the pH range of our culture was reported to be between 6 -10. *Klebsiella pneumonia RS-13* has been reported to completely degrade methyl red in the pH range of 6-8 [22]. *E. coli* and *Pseudomonas luteola* both exhibited best decolorization at pH 7 with constant decolorization rate upto pH 9.5 [22], [23]. Maximum

decolorization of 97.92% of dye RR195 by *Georgenia* at pH-7 was reported by Sahasrabudhe *et al*., (2012) [21]. Temperature is another very important parameter for the treatment of wastewater. The growth of microorganisms can be inhibited at one temperature but it can be activated at another temperature. So, it is essential to incubate microorganisms at their optimum incubation temperature for their successful growth. In present study at higher temperature decolorization slowly decreased and efficient decolorization was reported at 37°C. *Alcaligenes sp*. APO4 exhibited higher potential at 37°C to decolorize and detoxify the dyes effluent up to 92.6% and 83.9% respectively after 24 days of incubation [24].

The amount of inoculum used for dye decolorization also affects the activity of dye decolorization. To ensure a maximum decolorization of dye in the limited volume of medium, the microorganism inoculum size should therefore be controlled. Optimum inoculum size for complete decolorization of dyes was observed to be 10% (v/v). These results are similar to Sahasrabudhe *et al*., 2012 [21]. They suggested optimum inoculum size of 10% in their study on decolorization of the Reactive Red 195 by bacterial isolate *Georgenia*. Mohan *et al*., 2013 [25], also reported high decolorization % of Coractive blue-3R with 10% inoculum sizes of *Bacillus* sp. & *Plaococcus* sp.

Selection of best microorganism was done on the basis of effective decolorization even at high dye concentrations (100, 200, 500 and 1000mg/l). In this case decolorization percent is constantly or progressively increased with dye conc. upto 500mg/l of dye after which decolorization percent starts to decrease at higher conc. (Table 2).Shilpa *et al*., (2012) [8] reported 100% decolorization of RY145 (50 mg/l) by *B. boroniphilus* within 9 h at shaking conditions but they observed percent decolorization inversely proportional to dye concentration, where at conc. of 500 mg/l there was negligible decolorization of reactive yellow 145 at 24 h. The maximum decolorization of Reactive red HE8b by DN1 was observed at 200 mg/l of dye concentration [3] and of Reactive yellow 107, Reactive red 198, Reactive black 5 and Direct blue 71 by *Bacillus* spp. ETL-1979 at 100 mg/l of dye concentration [26]. *Georgenia* Sp.CC-NMPT-T3 could decolorize Reactive red 195 at 50 mg/l of dye conc. in 5 h whereas it took 48 h to decolorize 92.08% at 250 mg/l. The culture showed decolorizing ability upto 200 mg/l at a faster rate after which the rate began decreasing [21]. It has been proposed that dye concentration can influence the efficiency of microbial decolorization through a comination of factors including the toxicity imposed by dye at higher concentration [22], [27].

The most important dye degrading enzymes are: azoreductases, laccases and peroxidases. Some bacterial strains produce different kinds of enzymes whereas others produce only one or two of them. These enzymes have a great potential in various biotechnological processes mainly due to their high non-specific oxidation capacity, the lack of requirement for cofactors, and the use of the readily available molecular oxygen as an electron acceptor [28-30]. *Fictibacillus gelatini* & *Bacillus subtilis inaquosorum* showed higher Laccase & Azoreductase activity, as compared to other enzymes & *Bacillus subtilis subtilis* possessed higher activity of enzyme azoreductase at pH 7 and 37°C with 100% decolorization of all three Reactive dyes after 4-18 hrs of incubation period. Shah et al., 2013 [31] reported azoreductase activity at 37°C, pH 7, for decolorization of 93% of Reactive black dye.

Textile industrial effluent that is deficient in carbon content and biodegradation without any extra carbon source was very difficult [32]. Out of all above mentioned C&N sources combination of Maltose and Yeast extract showed good results. In present study combination of Maltose and Yeast extract showed good decolorization percent but not as high as in case of simple nutrient broth (Table 1 & Fig.3). Either they showed similar results as obtained in nutrient broth or showed less decolorization with supplementation. This suggests that carbon and nitrogen sources acted as catabolite repessors. Accordingly to other reports study decolorization efficiency of *Bacillus* sp. CH12 was significantly enhanced with carbon (≥98%) and organic nitrogen (~100%) supplements with concentrations (50−250 mg/l) of dye Reactive red 239 [33]. Similarly with 1% glucose, 1% yeast extract and 1% starch, where % decolorization was upto 92.22%, 94.00% and 92.88% respectively [34]. At a dye conc. of 500 mg/l and in absence of yeast extract the decolorization was found to be 81.94%, after 70 hrs suggesting that the organism could utilize organic dye as sole C sources [17]. Similarly reports observed by Throat et al., 2010 [35] supports our study by decolorizing crystal violet azo dye to 91.03 and 91.90%, when CD11 culture were used without and with co- substrate (1% glucose) respectively.

The textile effluents from Nahar textile industry, Lalru (Punjab) & Bhiwani textile mill (Haryana) were used for the study of decolorization and complete mineralization of dyes from textile effluent. These isolates not only decolorized the reactive dyes effluent but also disperse dye effluent along with the reduction in cost of effluent treatment process. The significance of this study is that, isolates almost completely decolorize these textile effluents and convert them from opaque to transparent. These isolates not only degrade or remove the dyes from these effluents but also decreased their COD, TDS and TSS within permissible limit. The values of BOD and COD were less in the treated sample in comparison to the very high values of BOD and COD in effluent [36], [37].

CONCLUSIONS

Textile effluent and sludge produced by effluent treatment plant is rich source of dye decolorizing bacterial population. From the present study, it can be concluded that these three isolates have very high decolorization potential and take only upto 4-18 h at temperature of 37°C, pH-7 and at static condition of growth for completely degradation of Reactive dyes: Reactive yellow 15, Reactive yellow 145 and Reactive red 195 at very high concentration of 500 mg/l, with no requirement of any carbon and nitrogen sources, which has not been reported earlier.

The present study indicates that biodegradation of Reactive dyes by *Bacillus sp.* were mediated by different enzymes like laccase, lignin peroxidase, azoreductase and tyrosinase that were produced in simple medium of nutrient broth. This study is quite significant as the rate of dye decolorization does not decrease with increasing concentration of dyes which is reported by due to the toxic effect of dyes on degrading microorganisms or the blockage of active sites of enzymes by dye molecule with different structures. So, these isolates may serve as remarkable tool for decolorization and detoxification of reactive dyes in cheaper way by decolorizing maximum (500 mg/l) conc. of textile dyes in lesser time with no or very less additional requirement of co-substrate.

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