



Decolourization of Simulated Dye in Aqueous Medium using Bacterial Strains

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ABSTRACT

India is the one of the world's largest producers of textiles and garments. Textile processes requires large volume of fresh water in various cloth processing operations. The inputs of wide range of chemicals/dyes, which, if not incorporated in the final products (fabric), become waste and turn out to be part of water ecosystem. This experiment was designed to optimize the various parameters for dye removal and to study the removal of dye from treated and untreated textile industry effluent by isolated bacterial strains in optimized conditions. Observations revealed that the rate of dye decolourization efficiency by bacterial strains was significantly influenced by initial dye concentration, pH, temperature of incubation and incubation time. About 60-70% colour removal from the samples was achieved after the incubation period of four days, and a little change in decolourization rate was observed thereafter. It was also observed in the study that optimal conditions for CR dye removal by using strain 1 were found to be pH of 6 (70.4%), incubation temperature, 35°C (76.5%) and by using strain 2 it was found to be pH of 6 (77%), incubation temperature of 30°C (77.1 %) at a constant string speed of 150 rpm with 25 ppm dye concentration. While in case of PR dye optimal conditions were pH 6 (69-74%) and incubation temperature of 30°C. Dye degrading capacity of both the strains was evaluated and it was found that strain 2 namely *Lysinibacillus fusiformis* isolated from untreated effluent was found to be highly effective for the removal of both the dye from solution as compare to strain 1.

Key words: Cibacron red, procion red, textile industry, *Lysinibacillus xylanilyticus*, *Lysinibacillus fusiformis*

INTRODUCTION

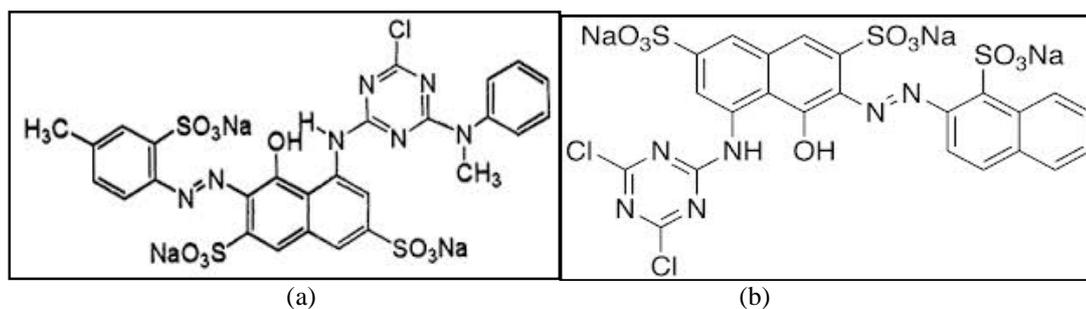
India is the one of the world's largest producers of textiles and garments. Textile processes requires large volume of fresh water in the cloth processing operations. The wastewater volume varies from industry to industry. Textile industries consume large amount of water (60– 400 litre/kg of fabric) with an average 170 litre/kg of fabric produced and chemicals for wet processing. It is the most problematic industries in terms of dye release to the environment in the form of wastewater. The chemical reagents used in textile sector are diverse in chemical composition ranging from inorganic to organic. Dyes released from the textile processing and dyestuff industries result in the increase of organic load of the natural reservoirs [1]. The uncontrolled release of these compounds (dyes) in the environment causes severe environmental problems. Since they are designed to be chemically and photolytically stable, they are highly persistent in natural environments. They are xenobiotic compounds because they do not exist as natural products and therefore contain structural elements that cannot be synthesized biochemically [2-3].

In aquatic bodies, dyes decrease the light absorption capability, which may significantly affect photosynthetic activity of aquatic life and may be toxic due to the presence of aromatics or heavy metals [4-6]. A very small quantity of dye in water (10 to 50 mg/l) affects the aesthetic value, transparency, and gas solubility of water bodies [4]. During evolution of catabolic enzymes and pathways microorganisms were not exposed to these structures and have not developed the capability to use those compounds as sole sources of carbon and energy. In this study, attempt has been made for the degradation of two dyes using potential dye decolourizer bacterial strains, isolated

from treated and untreated effluent collected from a textile industry. Effect of various parameters, such as pH, temperature, dye concentration, incubation time etc. have been studied in this experiment.

MATERIAL AND METHODS

For decolourization experiment, two dyes were selected i.e Cibacron red and Procion red. The dyes were procured from a textile industry located at Ambala, Haryana (India). In order to study the effect of initial concentration of Cibacron red (CR) dye and Procion red (PR), the experiments were carried out at a fixed bacterial concentration (1 ml inoculum v/v) at different dye concentrations (25, 50, 75 and 100 ppm) for different time intervals (0, 2, 4, 6 and 8 days) at 35°C under shaking condition on 7 pH at 150 rpm. Maximum absorption wavelength was recorded for CR and PR was 510nm and 515nm respectively.



Molecular structure of Cibacron Red (CR) dye (a) and Procion Red (PR) dye (b)

Physico-chemical Characteristics of Untreated and Treated Textile Effluent

The physico-chemical characteristics of untreated and treated textile effluent are shown in Table 1. Textile untreated effluent was brownish black with alkaline pH 8.7 and treated effluent in muddy grey in colour with alkaline pH (8.2). High pH value was because of bases, like NaOH and several other alkaline chemicals that were used during processing and finishing of cloths and fabrics. Similarly, Yusuff and Sonibare [7] observed the pH of five different textile industries and interestingly, the pH of all samples was in alkaline range (more than 10). Dissolved oxygen of the effluent was found almost nil because of very high temperature during the discharge of effluent, because during processing of fabrics a large number of organic matter and solids are mixed with wastewater. Electrical conductivity (EC) of the effluent was higher (3.9 mS cm^{-1}) before treatment and after treatment it declined (2.3 mS cm^{-1}). EC is sensitive to variation in dissolved ions and mineral salts. Permissible limit of COD for textile effluent is only 100 mg/l [8]. While considerably, high COD value of 3467 mg/l was out of permissible limits for textile effluents discharge standards. Higher COD value may be due to the presence of high organic load, solids and several other pollutants in the textile wastewater, because there are several processes which results into elevated chemical oxygen demand. Suspended solids value in untreated effluent is 180 mg l^{-1} while after treatment value was decreased to 95 mg l^{-1} indicated mildly polluted nature of textile effluent. But in case of total solids value was too high 2900 mg l^{-1} (untreated) and after treatment was 1780 mg l^{-1} . Sodium, Potassium and Calcium values were found to be 150 mg l^{-1} , 60 mg l^{-1} and 350 mg l^{-1} before treatment, while after treatment, the values were 132 mg l^{-1} , 24 mg l^{-1} and 265 mg l^{-1} respectively, indicated considerable hardness nature of the effluent. The concentrations of chloride, sulphate and phosphate were 796 mg l^{-1} , 342 mg l^{-1} and 19 mg l^{-1} before treatment and 584 mg l^{-1} , 315 mg l^{-1} and 13 mg l^{-1} respectively after treatment. High level of above discussed pollutants and toxicants signify the study of decolourization of effluent.

Table - 1 Physico-chemical Properties of Treated and Untreated Textile Industry Effluent

Parameters	Untreated effluent	Treated effluent
Colour	Brownish black	Muddy grey
pH	8.7 ± 0.32	8.2 ± 0.20
EC (mS cm^{-1})	3.9 ± 0.03	2.3 ± 0.17
Suspended solids (mgL^{-1})	180 ± 11	95 ± 4.9
Total Solids (mgL^{-1})	2900 ± 128	178 ± 23
Total D S (mgL^{-1})	2720 ± 90	1685 ± 110
COD (mgL^{-1})	2400 ± 143	820 ± 27
Potassium (mgL^{-1})	60 ± 2.5	24 ± 1.7
Calcium (mgL^{-1})	350 ± 23.3	265 ± 10
Chloride (mgL^{-1})	796 ± 45	584 ± 23
Sulphate (mgL^{-1})	342 ± 87	315 ± 17
Phosphate (mgL^{-1})	19 ± 1.35	13 ± 1.1
Sodium (mgL^{-1})	150 ± 9.5	132 ± 9.7

Isolation and Screening of Bacterial Strains

Fifteen bacterial isolates were obtained from effluent (7 from treated and 8 from untreated) samples collected from different sites of textile industry premises by repeated streaking of pure colonies of bacteria on solid agar surface. Out of these 15 isolates 2 were selected (1 each from treated; *Lysinibacillus xylanilyticus*) and untreated effluent; *Lysinibacillus fusiformis*) on the basis for their dye removal capacity of their respective effluent. These two selected strains were maintained for further experiments. Use of different type of effluent as a substitute of distilled water for making media had an advantage, since only dye degrading strains appeared on this media. Finally stock cultures were maintained, through fortnightly sub culturing at 40°C on agar media. These microbes were further explored for further dye decolourization experiment.

Decolourization of Simulated Dyes Solution

Colour degradation study was performed with simulated dyes solution. For this purpose, 100 ml of sterilized dye effluent in 250 ml Erlenmeyer's flasks were inoculated with microbial isolates. Various essential nutrients for finest growth of microbial cultures were added in the form of Nutrient broth (1.4% w/v) bacteria. Process parameters like pH, temperature, Carbon source and nitrogen source were varied to achieve the optimal conditions for maximum decolorization. pH varied in the range of 4.0 to 9.0, pH of the experimental solution was adjusted with 0.1N NaOH and 0.1N HCl. Temperature variations in range of 25°C - 40°C were maintained under aerobic shaking at 150 rpm in an orbital shaking incubator. Decolourization experiments were performed for the incubation period of 8 days, 1 ml of liquid aliquots were repeatedly withdrawn from flasks after every 24 h intervals in a laminar flow system under extremely hygienic conditions, filtered, centrifuged in a centrifuge machine at 10000 rpm for bacteria for the time period of 15 min, clear supernatant was scanned spectrophotometrically (Systronic-106 UV-Visible Spectrophotometer) at lambda max for percent colour degradation up to the incubation period of 8 days. The following formula was used to calculate the percent decolorization:

$$\text{Decolourization (\%)} = \frac{(C_0 - C_e)}{C_0} \times 100$$

Where, C_0 is the initial absorbance (OD) and C_e is the final absorbance (OD) at different time intervals. Chemical oxygen demand (COD) was measured according to the standard dichromate reflux method and percent reduction in chemical oxygen demand was measured daily up to the incubation period of 8 days.

RESULTS AND DISCUSSION

The potential of a number of bacterial strains for decolourization and treatment of textile wastewater was studied in recent years. Bioaugmentation with pure bacterial and mixed cultures has shown enormous potential for decolorization of textile dyes in real wastewater and stimulated dye solutions [9]. Sometimes pure cultures of bacteria are capable of decolorizing textile wastewater, sometimes mixed cultures perform better than pure cultures due to synergistic metabolic activities [10]. A number of textile dyes degrading microbial strains belonging to different genera of bacteria and fungi have been isolated. These include microbial strains of *Pseudomonas sp.* [11], *Armillaria sp.* [12], *Bacillus sp.* [13], *Lysinibacillus sp.* [14] and *Staphylococcus sp.* [15]. In this study, two *Lysinibacillus* species (*Lysinibacillus xylanilyticus* and *Lysinibacillus fusiformis*) were selected on the basis for their dye removal potential.

Effect and Optimization of CR Dye Concentration by Strain 1 (*Lysinibacillus Xylanilyticus*)

Four dye concentrations (25, 50, 75 and 100 ppm) were taken in aqueous solution, incubated for 8 days at 35°C and pH 7. It was found that there was a linear decrease in decolourization percentage of dye solution with increasing dye concentration. The decolourization at the lowest (25 ppm) and highest dye concentration (100 ppm) was 72.6% and 40.8%, respectively. It was clear from Fig. 1 that maximum decolourization (72.6%) was recorded at 25 ppm concentration of dye at 35°C by strain *Lysinibacillus xylanilyticus* after incubation time up to 4th days. There was slide decrease in decolourization by strain *Lysinibacillus xylanilyticus* after 4 days of incubation period. Other researchers also reported 75.85-100% and 46.4-99.3% decolourization of textile dye Acid Sulphone Blue and Fast Red A, respectively [16]. The higher concentration of dye may inhibit nucleic acid biosynthesis and cell growth, so the effect of dye concentration on growth of organisms is an important consideration for its field application [17].

Effect and Optimization of CR dye Concentration by *Lysinibacillus Fusiformis* (Strain 2)

In this experiment with different concentrations of dye maximum removal was achieved at 25 ppm (79.8%) after 4 days of incubation there was no further significant increase in dye removal was observed at higher concentration (Fig 2). At 50 ppm concentration dye removal was 43.9%, at 75 ppm it was 37.5% at 100 ppm it was 37.5. Lesser dye removal at higher concentration may be attributed to the inhibition of microbial growth. Similarly Shen and Wang, [18] evaluated the optimum concentration for reduction by *Lysinibacillus fusiformis* within a concentration range of (25-100 ppm) reported that the optimum concentration for better CR dye reduction was 67.8 mg/l. Das and Mishra, [19] studied the degradation of red dye within a concentration range of (25-100 ppm) and optimum

result reported at 50 ppm using *Brevibacterium casei*. Khehra et al. [20] suggested that the decrease in decolourization efficiency with increasing dye concentration may be due to the toxic effect of dyes.

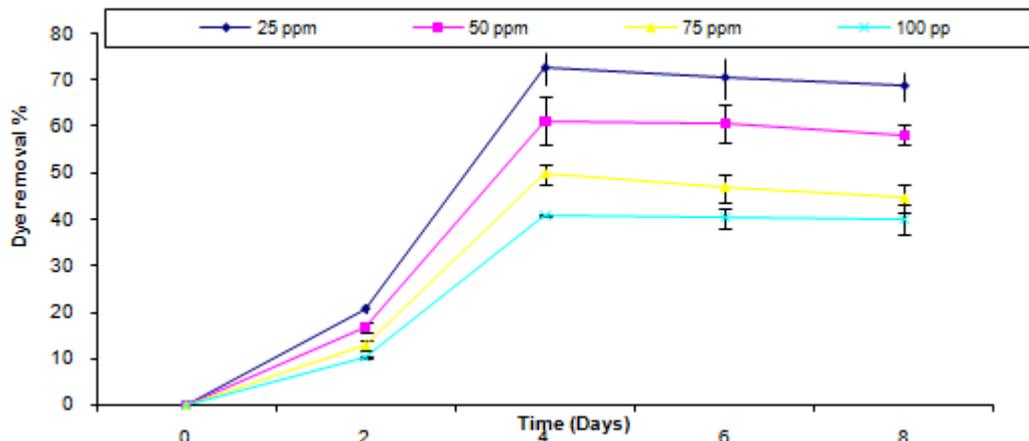


Fig. 1 Effect of dye concentration with incubation time on removal of dye (Cibacron red) by *Lysinibacillus xylanilyticus* (strain-1) at pH = 7.0, stirring speed = 150 rpm, Temp. = 35 °C

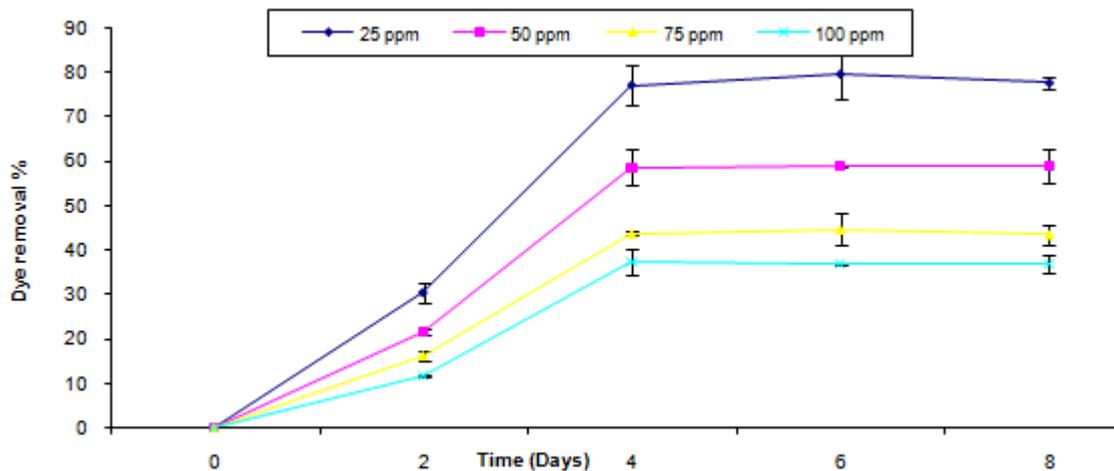


Fig. 2 Effect of dye concentration with incubation time on removal of dye (Cibacron red) by *Lysinibacillus fusiformis* (strain-2) at pH = 7.0, stirring speed = 150 rpm, Temp. = 35 °C

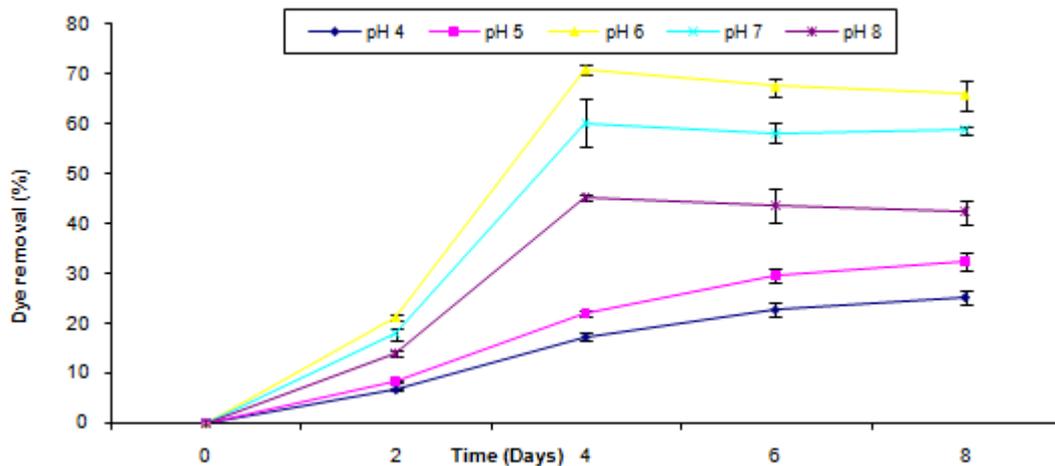


Fig. 3 Effect of pH with incubation time on removal of dye (Cibacron red) by *Lysinibacillus xylanilyticus* (strain-1) at 25 ppm dye concentration, stirring speed = 150 rpm, Temp. = 35 °C

Effect and Optimization of pH on Decolourization (%) of CR by *Lysinibacillus Xylanilyticus* (strain 1)

It was noticed in study that initial pH had a great influence on the decolourization of textile dyes. The dye removal was in the range of (70-80%) by strain *Lysinibacillus xylanilyticus* when studied at different pH values ranging from 4-9 at constant concentration of dye i.e. 25 ppm and temperature was 35°C. Removal of dye was maximum at pH 6 i.e.70.4% after four days of incubation where as it was 60% at pH 7, 49% at pH 8, 45.2% at pH 4 and 32.5%

at pH 5 (Fig 3). Dye removal was significantly lower at pH 4, 5 and 8 as compare to pH 6 and 7. Dye removal increase initially up to 4 days of incubation, but after 4 days there was no further dye removal up to 8th day i.e. experiment termination day. Among the various pH values, pH 7 was found to produce more amount of cell mass and resulted in better degradation of dye. In a study Bandyopadhyay et al, [21] studied the dye degradation by *Pseudomonas putida* MTCC 1194 and found that at pH 7 the degradation was optimum.

Effect and Optimization of pH on Decolourization of CR by *Lysinibacillus Fusiformis* (strain – 2)

In this experiment with varying range of pH i.e. 4-9 were taken and maximum removal was observed at pH 6 i.e. 77.6% with dye concentration 25 ppm and temperature was 35°C by strain *Lysinibacillus fusiformis* (Fig 4). After six days of experiment no further increase in dye removal was observed below pH 6 and above this, so pH 6 was supposed to be the best pH for dye removal and growth of microbial broth (Fig 4). The fact also be noticed that when we increase the pH with incubation time in respect of concentration of broth also increasing trend that was shown a decrease in decolourization. In a similar study Zeng et al., [22] reported two new isolate of high-degrading strains, micrococcus sp. and *Alcaligenes faecalis*, gave highest degradation and growth at pH 7.0. Similarly, Veenagayathri and Vasudevan, [23] studied the degradation of 100 mg/L dye concentration under the effect of different pH ranging from (5.5 to 8.5) by the bacterial consortium. They reported maximum dye degradation with 99% efficiency at pH 7 followed by 5, 9, 24, 42 and 74% at different pH 5.5, 6, 6.5, 8.5 and 8, respectively. Decolourization of Orange 3R dye by using bacterial isolates (*Pseudomonas sp.* and *Bacillus sp.*) showed maximum dye decolorization of 89% at the end of 144h under optimum pH 6 was also reported by other researchers [24].

Effect and Optimization of Temperature on Decolourization (CR) by *Lysinibacillus Xylanilyticus* (strain - 1)

The effect of temperature on dye removal by *Lysinibacillus xylanilyticus* was studied with time (Fig. 5). Temperature had a profound effect on decolourization rate as shown in experiment. Maximum colour removal was obtained at 35°C after four days of incubation at dye concentration of 25 ppm and pH was 6.0 (Fig. 5). There was no significant increase in decolourization rate after four days of incubation. There was a linear relationship between increasing time and dye removal up to 35°C, beyond which the effect was detrimental. The dye degradation by *Lysinibacillus xylanilyticus* is optimum only at 30°C beyond that temperature it did not show better degradation. So it can only be possible to maintain the temperature condition at 30°C. Murty et al. [25] isolated a strain which showed complete decolorization of the selected dye (RB 250-100 mg/l) within 8 hr in static condition. During the study the optimum pH, temperature, inoculum size and carbon and nitrogen sources for the decolorization was studied at 7.0, 37°C, glucose (0.2 %) and nitrogen (0.5 %) respectively.

Effect and Optimization of Temperature on Decolourization (CR) by *Lysinibacillus Fusiformis* (Strain - 2)

It was found that there was a linear increase in dye removal with increase in temperature but up to 30°C after six days of incubation i.e. 77.1% when the concentration of dye was 25ppm and pH was 6 by strain *Lysinibacillus fusiformis* (Fig 6). As at 25°C it was about 55% but beyond 30°C a sharp decline was observed the decrease in colour removal activity is due to poor growth of microbial culture in broth at higher temperatures. So 30°C temperature was considered an ideal for maximum removal of CR dye under optimized conditions. Achour et al. [26] showed the existence of good scope for the bacterial consortium composed of *Sphingomonas paucimobilis*, *Bacillus sp.* and *Staphylococcus epidermidis* in the decolourization (98.67 %) of textile wastewater at optimum temperature of 28°C.

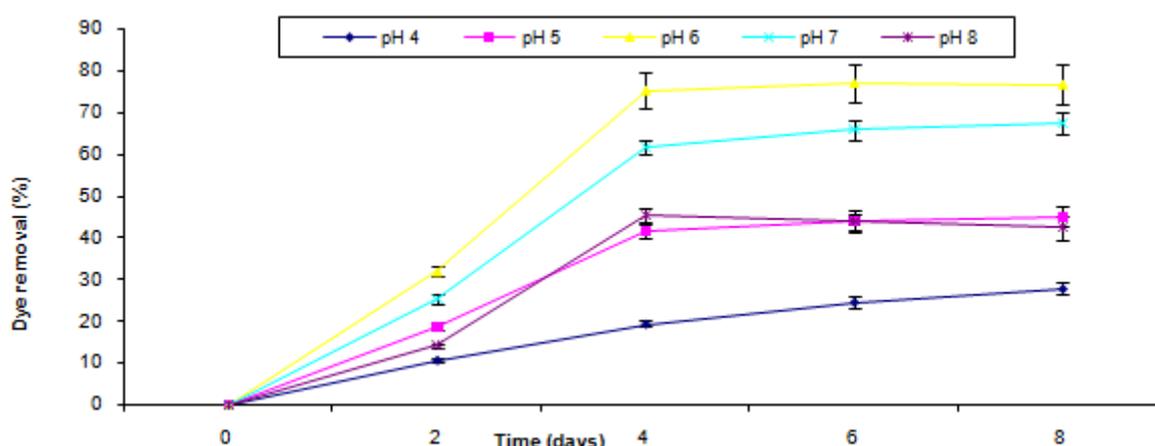


Fig. 4 Effect of pH with incubation time on removal of dye (Cibacron red) by *Lysinibacillus fusiformis* (strain-2) at 25 ppm dye concentration, stirring speed = 150 rpm, temp. = 35 °C

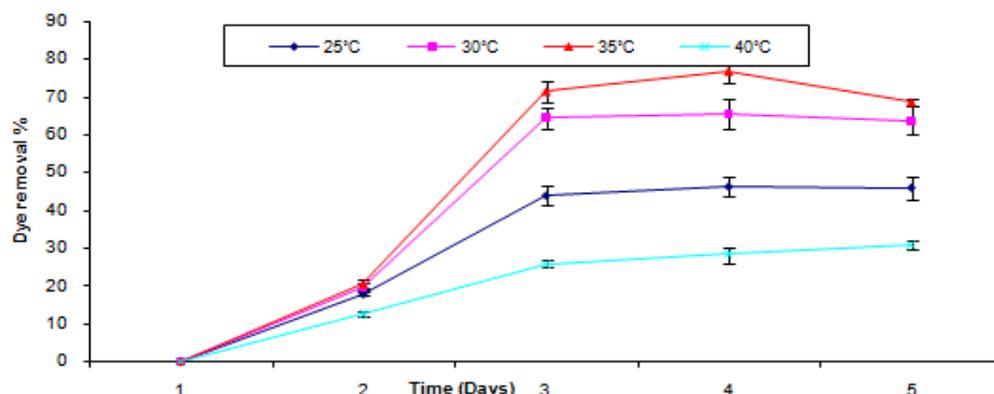


Fig. 5 Effect of temperature with incubation time on removal of dye (Cibacron red) by *Lysinibacillus xylanilyticus* (strain-1) at 25 ppm dye concentration, stirring speed = 150 rpm, pH = 6.0

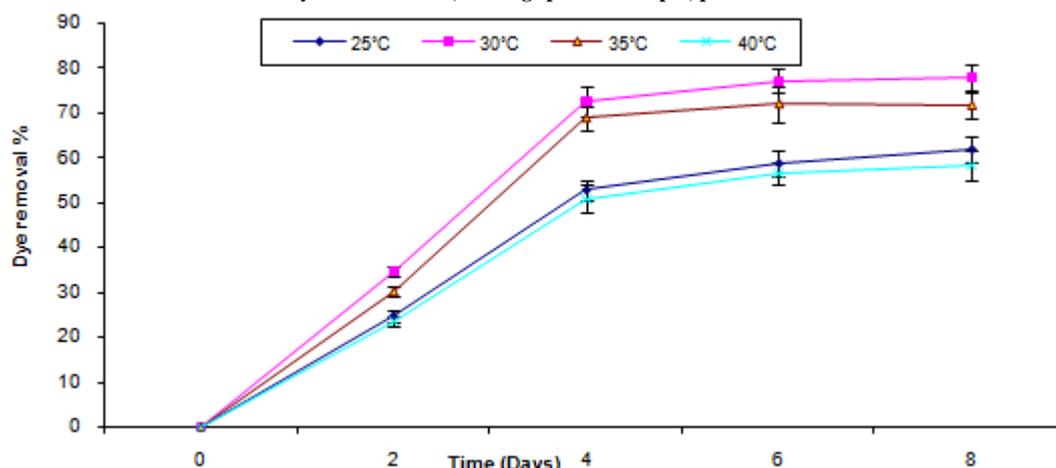


Fig. 6 Effect of temperature with incubation time on removal of dye (Cibacron red) by *Lysinibacillus fusiformis* (strain-2) at 25 ppm dye concentration, stirring speed = 150 rpm, pH = 6.0

Effect of Concentration and Optimization of Maximum Removal of Dye (PR) by *Lysinibacillus Xylanilyticus* (Strain -1)

The effect of concentration on colour degradation potential of *Lysinibacillus xylanilyticus* was shown in Fig. 7. It was found that there was a linear increase in % decolourization of dyeing effluent with increasing concentration of broth such as 25, 50, 75 and 100 ppm with respect of incubation time also increased as range of 0, 2, 4, 6 and 8 days (Fig 7). Therefore, the concentration of broth was shown maximum peak of dye (PR λ 510nm) removal in percentage 68.7 mg/l at 25 ppm and day 6, beyond that which shown a sharp decrease in % decolourization was observed, which may be attributed to poor growth of bacterial culture at advanced incubation time. There was slight decrease in decolourization by strain *Lysinibacillus xylanilyticus* was observed with incubation time. Similarly Das and Mishra, [19] studied the degradation of red dye within a concentration range of (25-100 ppm) and optimum result reported at 50 ppm using *Brevibacterium casei*.

Effect of Concentration and Optimization of Maximum Removal of dye (PR) (%) by *Lysinibacillus Fusiformis* (Strain -2)

The effect of concentration on colour degradation potential of *Lysinibacillus fusiformis* was shown in Fig. 8. It was found that there was a linear increase in % decolourization of dyeing effluent with increasing concentration of broth (ppm) such as 25, 50, 75 and 100 ppm with respect of incubation time also increased as range of 0, 2, 4, 6 and 8 days. Therefore, the concentration of broth was shown maximum peak of dye (PR) removal in percentage 71.0% at 25 ppm and day 8, beyond that which shown a sharp decrease in % decolourization was observed, which may be attributed to poor growth of bacterial culture at advanced incubation time. Das and Mishra, [19] studied the degradation of red dye within a concentration range of (25-125 ppm). Similarly Bae et al. [27] evaluated the optimum concentration for reduction by *Lysinibacillus fusiformis* within a concentration range of (25-100) reported that the optimum concentration for better PR dye reduction was 67.8 mg/l.

Effect and Optimization of pH on Decolourization (PR) by *Lysinibacillus Xylanilyticus* (Strain – 1)

The effect of pH on decolourization of untreated textile effluent by *Lysinibacillus fusiformis* was shown in Fig. 9. The results revealed that pH 6.0 was optimal for decolourization (64.6 mg/l) with respect of broth concentration 25ppm by strain -1 after 8 day's incubation. It was also shown a maximum decolourization. One more fact have

been noted that when we increased pH with incubation time in respect of concentration of broth also in increasing trend, that was shown a decreasing in decolourization. Shah et al. [28] optimised various condition for *Bacillus cereus* it was found to be 1% sucrose, 0.25% peptone, pH 7, temperature 37°C and 8% inoculum and that for *Bacillus megaterium* was found to be glucose 1%, 0.25% yeast extract, pH 6, temperature 37°C and 10% inoculum. Decolourization of textile dye recorded by *Bacillus cereus* under ideal conditions was 95% and that by *Bacillus megaterium* was 98%.

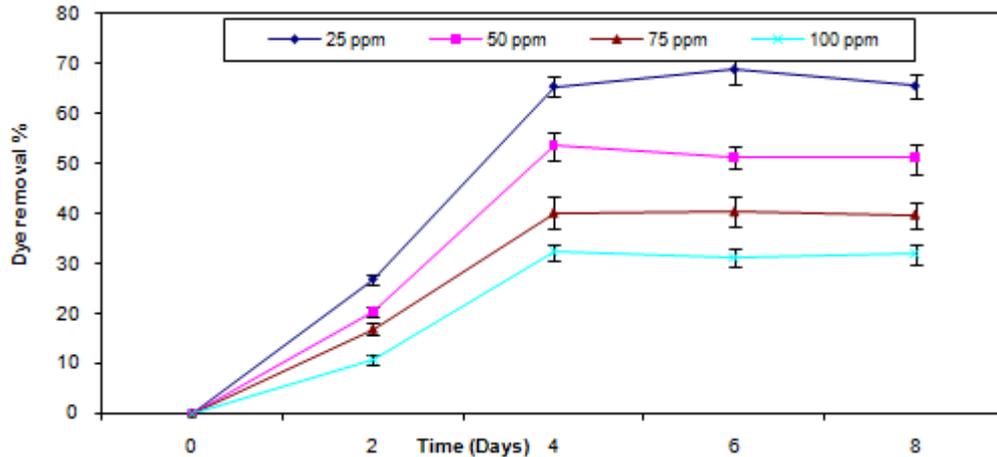


Fig. 7 Effect of dye concentration with incubation time on removal of dye (Procion red) by *Lysinibacillus xylanilyticus* (strain-1) at pH = 7.0, stirring speed = 150 rpm, temp. = 35 °C

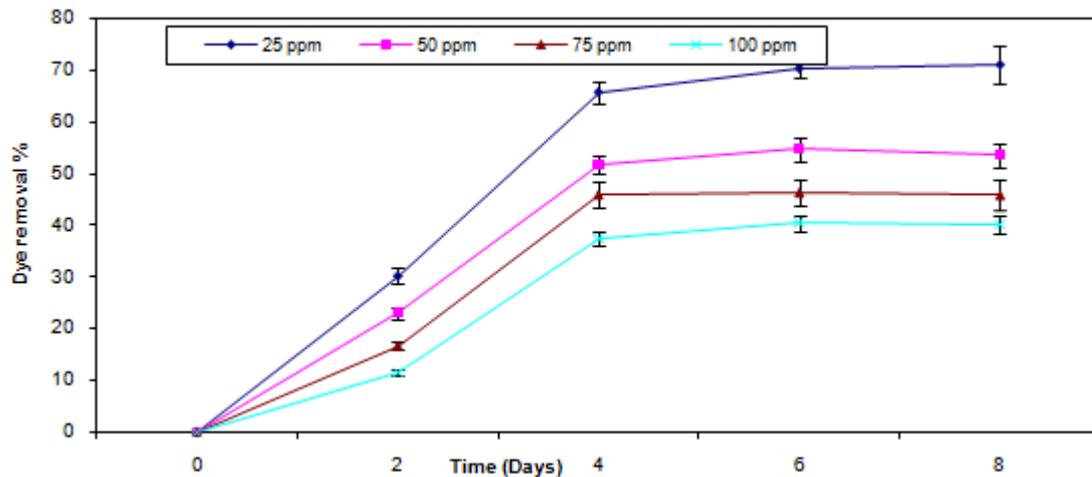


Fig. 8 Effect of dye concentration with incubation time on removal of dye (Procion red) by *Lysinibacillus fusiformis* (strain-2) at pH = 7.0, stirring speed = 150 rpm, Temp. = 35 °C

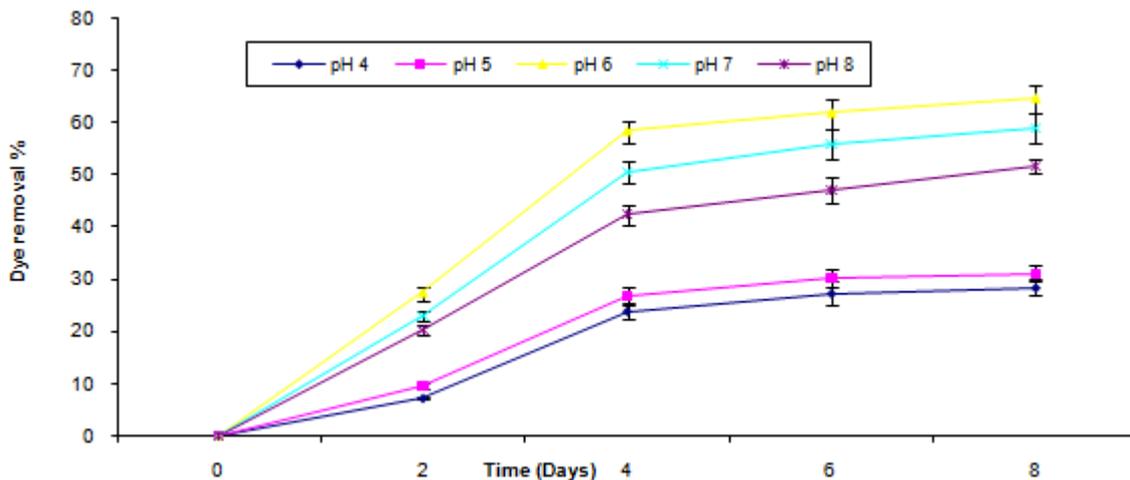


Fig. 9 Effect of pH with incubation time on removal of dye (Procion Red) by *Lysinibacillus xylanilyticus* (strain-1) at stirring speed = 150 rpm, Temp. = 35 °C, Dye concentration 25 ppm

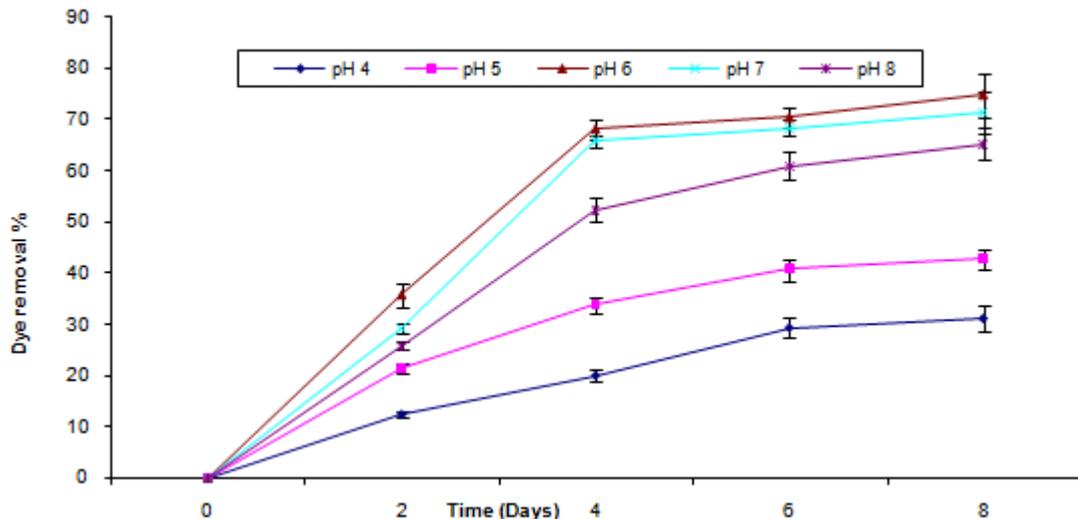


Fig. 10 Effect of pH with incubation time on removal of dye (*Procion Red*) by *Lysinibacillus fusiformis* (strain-2) at stirring speed = 150 rpm, Temp. = 35 °C, Dye concentration 25 ppm

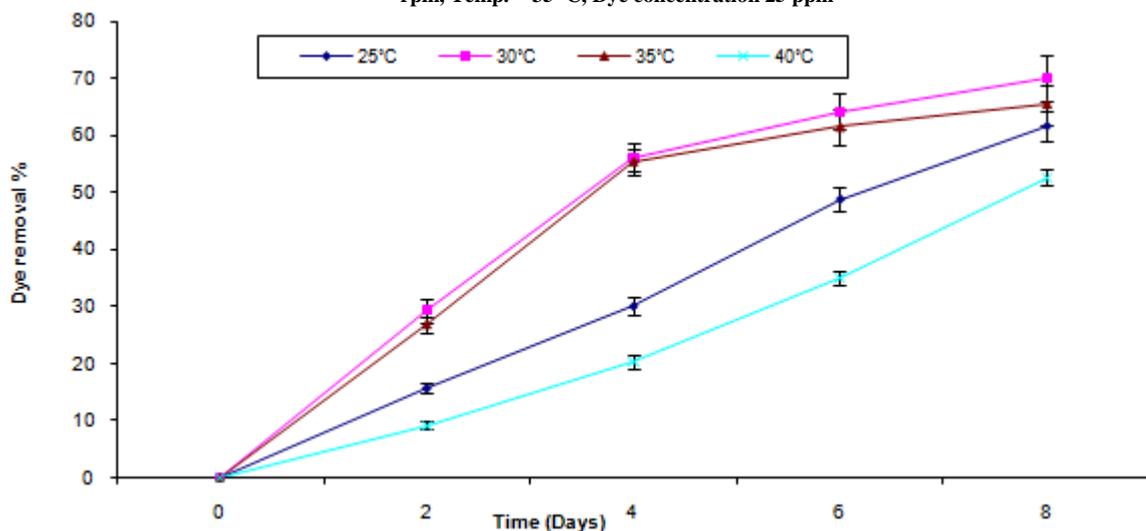


Fig. 11 Effect of temperature with incubation time on removal of dye (*Procion Red*) by *Lysinibacillus xylanilyticus* (strain-1) at stirring speed = 150 rpm, pH = 6.0, Dye concentration 25 ppm

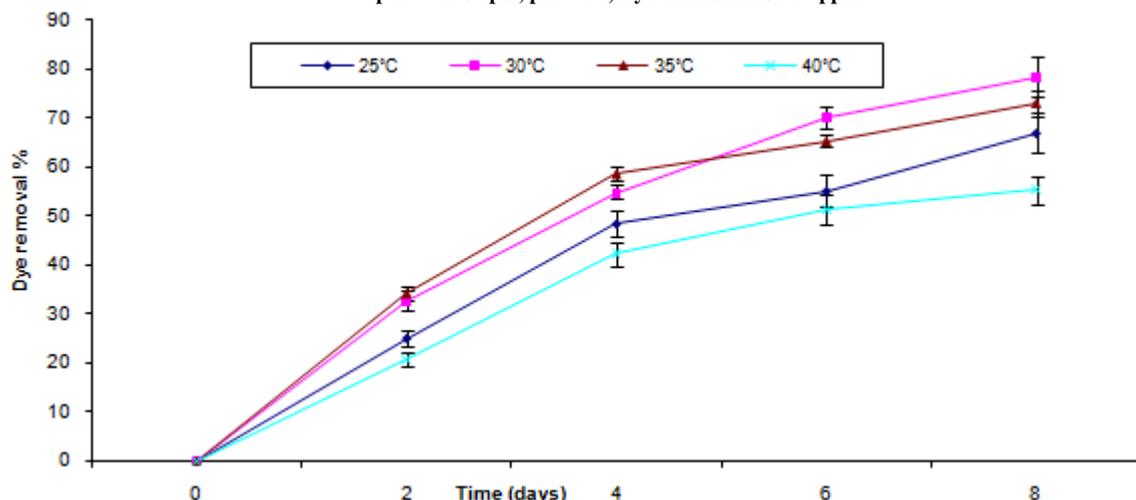


Fig. 12 Effect of temperature with incubation time on removal of dye (*Procion Red*) by *Lysinibacillus fusiformis* (strain-2) at stirring speed = 150 rpm, pH = 6.0, Dye concentration 25 ppm

Effect and Optimization of pH on Decolourization (PR) by *Lysinibacillus Fusiformis* (Strain - 2)

The effect of pH on decolourization of untreated textile effluent by *Lysinibacillus fusiformis* was shown in Fig. 10. The results revealed that pH 6.0 was optimal for decolourization (74.7 mg/l) with respect of broth concentration 25 ppm by strain-2 after 8 day’s incubation. It was also shown a maximum decolourization. One more fact also be

noted that when we increased pH with incubation time in respect of concentration of broth also in increasing trend, that was shown a decreasing in decolourization. In a similar study Zeng et al. [22] reported two new isolate of high-degrading strains, *Micrococcus sp.* and *Alcaligenes faecalis*, gave highest degradation and growth at pH 7.0.

Effect and Optimization of Temperature on Decolourization (PR) by *Lysinibacillus Xylanilyticus* (Strain - 1)

The effect of temperature of untreated textile effluent by *Lysinibacillus xylanilyticus* (strain - 1) was studied in Fig. 11. Temperature had an intense effect on decolourization rate as shown. The maximum colour removal 70.1 mg/l was attained at temperature of 30 °C after 8 days of incubation and was considered as optimal period for decolourization by strain-1. No significant increase in decolourization rate was observed after 8 days of incubation time at 30 °C. There was a linear relationship between increasing temperature and colour removal up to 30 °C, beyond which the effect was detrimental. The decline in colour removal activity by the strain -1 at high temperature can be attributed to the loss of cell viability or denaturation of enzymes. Therefore to ensure the stability for long term operation, decolourization with microbial cells should not be undertaken at high temperature. The dye removal was in range of 65 to 70% when studied at different temperature conditions where the pH was 6 and dye concentration was 25 ppm it was 70.1 % after a days of incubation at temperature 30°C a linear increase in dye removal was shown when temperature increase from 25-30°C as it was 61.7% at 25°C and 70.1% at 30°C but beyond this it seemed to be decreased i.e 65.4% on 35°C and 52.7% on 40°C. Therefore to ensure the stability for long term operation, decolourization with microbial cells should not be carried at higher temp. So it can only be possible to maintain the temperature condition at 30°C.

Effect and Optimization of Temperature on Decolourization (PR) by *Lysinibacillus Fusiformis* (Strain - 2)

It was found in the studies that there was a linear increase in decolourization % of dye at all temperature conditions with respect to time up to 8th day of incubation when dye concentration was 25 ppm and pH was 6. Fig. 12 depicted that maximum dye removal was achieved at 30°C, as it was 78.4% followed by 67%, 73.1% and 55.3% dye removal at 25°C, 35°C and 40°C, respectively. So at higher temperature dye removal seemed to decrease which may be due to inhabitation of microbial growth.

CONCLUSION

It was found that concentration, temperature and pH have significant effect on dye removal efficiency. During experiment, it was observed that more than 60-70% colour removal from the samples was achieved after the incubation period of four days, and a little change in decolourization rate was observed thereafter. Out of the 15 bacterial isolates, *Lysinibacillus xylanilyticus* and *Lysinibacillus fusiformis* were selected from treated and untreated effluent respectively, on the basis for their dye removal capacity of their respective effluent. The study concluded that pH, initial dye concentration and temperature have a significant influence on dye removal efficiency by bacterial strains. The optimal conditions for CR dye removal by using strain 1 were found to be pH of 6 (70.4%), incubation temperature, 35°C (76.5%) and by using strain 2 it was found to be pH of 6 (77%), incubation temperature of 30°C (77.1 %) at a constant string speed of 150 rpm with 25 ppm dye concentration. While in case of PR dye optimal conditions were pH 6 (69-74%) and incubation temperature of 30°C (70.1-78.4%) for both the strains at a constant string speed of 150 rpm with 25 ppm dye concentration. This shows that these bacteria have enormous potential to degrade the textile dyes and resolve the problem of unnecessary dyes present in the effluents of textile industries. If dye degrading capacity of both the strains was evaluated and it was found that strain 2 namely *Lysinibacillus fusiformis* was found to be highly effective for the removal of both the dye from solution as compare to strain 1. The major importance of the study is the use of local bacterial strains isolated from the untreated effluent itself having potential of decolourization.

REFERENCES

- [1] RS Dhanve, DC Kalyani, SS Phugare and JP Jadhav, Coordinate Action of Exiguobacterial Oxidoreductive Enzymes in Biodegradation of Reactive Yellow 84A Dye, *Biodegradation*, **2009**, 20, 245-255.
- [2] A Stolz, Basic and Applied Aspects in the Microbial Degradation of Azo Dyes, *Applied Microbiology Biotechnology*, **2001**, 56, 69-80.
- [3] PG Rieger, HM Meier, M Gerle, U Vogt, T Groth and HJ Knackmuss, Xenobiotics in the Environment: Present and Future Strategies to Obviate the Problem of Biological Persistence, *Journal of Biotechnology*, **2002**, 94, 101-123.
- [4] IM Banat, P Nigam, S Datel and R Marchant, Microbial Decolourization of Textile Dyes Containing Effluents: A Review, *Bioresource Technology*, **1996**, 58, 217-227.
- [5] YM Slokar and AML Marechal, Methods of Decoloration of Textile Wastewaters, *Dyes Pigments*, **1998**, 37, 335-356.
- [6] PA Carneiro, ME Osugi, CS Fugivara, N Boralle, M Furlan and MVB Zanoni, Evaluation of Different Electrochemical Methods on the Oxidation and Degradation of Reactive Blue 4 in Aqueous Solution, *Chemosphere*, **2004**, 59, 431-439.

- [7] RO Yusuff and JA Sonibare, Characterization of Textile Industries Effluents in Kaduna, Nigeria and Pollution Implications, *Global Nest the International Journal*, **2004**, 6, 212–221.
- [8] CPCB, Annual Report of Central Pollution Control Board, New Delhi, India **2010**.
- [9] M Imran, E David, Crowley, A Khalid, S Hussain, MW Mumtaz and M Arshad, Microbial Biotechnology for Decolorization of Textile Wastewaters, *Reviews in Environmental Science and Biotechnology*, **2015**, 14,73-92.
- [10] BD Tony, D Goyal and S Khanna, Decolorization of Textile Azo Dyes by Aerobic Bacterial Consortium, *International Biodeterioration and Biodegradation*, **2009**, 63, 462–469.
- [11] S Hussain, Z Maqbool, S Ali, T Yasmeen, M Imran, F Mahmood and F Abbas, Biodecolorization of Reactive Black-5 by a Metal and Salt Tolerant Bacterial Strain *Pseudomonas* sp. RA20 Isolated from Paharang Drain Effluents in Pakistan, *Ecotoxicology and Environmental Safety*, **2013**, 98, 331-338.
- [12] T Hadibarata, ARM Yusoff, A Aris, T Hidayat and RA Kristanti, Decolorization of Azo, Triphenylmethane and Anthraquinone Dyes by Laccase of a Newly Isolated *Armillaria* sp. F022, *Water Air and Soil Pollution*, **2012**, 223, 1045–105.
- [13] VV Dawkar, UU Jadhav, DP Tamboli and SP Govindwar, Efficient Industrial Dye Decolorization by *Bacillus* sp. VUS With its Enzyme System, *Ecotoxicology and Environmental Safety*, **2010**, 73, 1696– 1703.
- [14] RG Saratale, GD Saratale, DC Kalyani, JS Chang and SP Govindwar, Enhanced Decolorization and Biodegradation of Textile Azo Dye Scarlet R by Using Developed Microbial Consortium-GR, *Bioresour Technol*, **2009**, 100, 2493–2500.
- [15] A Khalid, F Kausar, M Arshad, T Mahmood and I Ahmed, Accelerated Decolorization of Reactive Azo Dyes Under Saline Conditions by Bacteria Isolated from Arabian Seawater Sediment. *Microbiology Biotechnology*, **2012**, 96, 1599–1606.
- [16] P Kaushik and A Malik, Microbial Decolourization of Textile Dyes Through Isolates Obtained from Contaminated Sites, *Journal of Scientific & Industrial Research*, **2009**, 68, 325-331.
- [17] KC Chen, JY Wu, DJ Liou and SCJ Hwang, Decolorization of the Textile Dyes by Newly Isolated Bacterial Strains, *Journal of Biotechnology*, **2003**, 101, 57-68.
- [18] YS Shen and DK Wang, Development of Photoreactor Design Equation for the Treatment of Dye Wastewater by UV/H(2)O(2) Process, *Journal of Hazardous Material*, **1994**, 89(2-3), 267-277.
- [19] AP Das and S Mishra, Bioreduction Based Bioremediation of Hexavalent Chromium Cr (Vi) Through Potential Indigenous Microbes, M.Tech Thesis, National Institute of Technology, Rourkela, India, 2009.
- [20] MS Khehra, SS Harvinder and DK Sharma, Biodegradation of Azo Dye CI Acid Red 88 by an Anoxic-aerobic Sequential Bioreactor, *Dyes Pigments*, **2005**, 70, 1-6.
- [21] K Bandyopadhyay, D Das and B R Maiti, Kinetics of Phenol Degradation Using *Pseudomonas putida* MTCC 1194, *Bioprocess Engineering*, **1998**, 18, 373 – 377.
- [22] HY Zeng, H Jiang, K Xia, YJ Wang and Y Huang, Characterization of Phenol Degradation by High-Efficiency Binary Mixed Culture, *Environment Science Pollution Research*, **2010**, 17, 1035–1044.
- [23] K Veenagayathri and N Vasudevan, Effect of pH, Nitrogen Sources and Salts on the Degradation of Phenol by the Bacterial Consortium Under Saline Conditions, *International Journal of Biotechnology and Biochemistry*, **2010**, 6, 783–791.
- [24] M Ponraj, K Gokila and V Zambare, Bacterial Decolorization of Textile Dye- orange 3R, *International Journal of Advanced Biotechnology and Research*, **2011**, 2 (1), 168-177.
- [25] SD Murty, SD Patel, R Soni and N Bhatt, Isolation and Identification of Bacterial Culture for Azo Dye Degrading Capability, *International Journal of Research in Chemistry and Environment*, **2012**, 2, 69-79.
- [26] S Achour, E Khelifi, L Ayed, AN Helal and A Bakhrouf, Response Surface Methodology for Textile Wastewater Decolourization and Biodegradation by a Novel Mixed Bacterial Consortium Developed via Mixture Design, *Desalination and Water Treatment*, **2013**, 52, 1539-1549.
- [27] WC Bae, KT Gu, IK Kang, YJ Won and BC Jeong, Reduction of Hexavalent Chromium by *Escherichia coli* ATCC 33456 in Batch and Continuous Cultures, *Journal of Microbiology*, **2000**, 38, 36-39.
- [28] MP Shah, KA Patel, SS Nair and AM Darji, Potential Effect of Two *Bacillus* spp on Decolorization of Azo dye. *Journal of Bioremediation and Biodegradation*, **2013**, 4,199.