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Degradation and Decolourization of Textile Grade Dyes by Prominent Isolates Obtained from Textile Effluent Sample

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Abstract
Textile industries are responsible for one of the major environmental pollution problems in the world, because they release undesirable dye effluents. Textile wastewater contains dyes mixed with various contaminants at a variety of ranges. Therefore, environmental legislation commonly obligates textile factories to treat these effluents before discharge into the receiving watercourses. The present study was an attempt to examine the Decolorization ability of Prominent isolates obtained from the Industrial Effluent Sample (*Kocuria rhizophila* and *Bacillus cereus*) for two different commercial Textile Grade Dyes that are 'Perssian blue' and 'Reactive orange 13'. The primary parametric study was done and results were recorded. The isolates showed significant decolorization of both the dyes was identified by bacterial morphology, biochemical tests and also with of Automated Microbial Identification System (VITEK 2) instrument. Various physico-chemical parameters with respect to incubation condition, pH, different carbon, nitrogen and mineral sources were studied for both the dyes for dye decolorization. The effect of different carbon sources, nitrogen sources, pH and temperature on decolorization was studied to find out optimum conditions required for the decolorization of respective dyes. The test was also carried out using Textile Effluent Water Sample to check the efficiency of both the isolates on Degradation and Decolorization of dyes treatment. These Prominent isolates can be use in treatment of Textile Effluent.

Keywords
VITEK 2, Reactive orange 13, Perssian blue, Decolourization, Industrial effluent, Dyes.

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INTRODUCTION
Textile industries positively affect the economic development worldwide. China is the most important exporter of all types of textiles, followed by the European Union, India and then the USA. However, one of the problems associated with textile factories is the unacceptable effluent, especially dyes, which are difficult to degrade. Synthetic dyes are extensively used in textile dyeing, paper printing, colour photography, pharmaceutical, food, cosmetic, and other industries. Major classes of synthetic dyes include azo, anthraquinone, and triarylmethane dyes. Among these, azo dyes represent the largest and most versatile class of synthetic dyes. Color present in effluent affect photosynthetic activity in aquatic life due to reduced light penetration and may also be toxic to some aquatic organisms due to the presence of metals, chlorides (U. Aftab, et al. 2011).
Particularly reactive dyes usually have a synthetic origin and complex aromatic molecular structures,

which make them stable and difficult to biodegrade. Reactive dyes differ from all other dye classes in that they bind to textile fibers, such as cellulose and cotton, through covalent bonds.

Reactive dyes are typically azo-based chromophores combined with various types of reactive groups, which show different reactivity. The recalcitrance of azo dyes has been attributed to the presence of sulfonate groups and azo bonds, two features generally considered as xenobiotic (M. P. Shah, et al. 2013). Several physicochemical methods are used to treat textile effluents to achieve degradation and decolorization. These include filtration, coagulation, carbon activated and chemical flocculation. These methods are effective but they are expensive and involve the formation of a concentrated sludge that creates a secondary disposal problem.

Microbial degradation and decolorization is an environmentally friendly and a cost competitive alternative to chemical decomposition process. The success of a biological process for color removal from textile effluent mainly depends on the utilization of microorganisms that effectively decolorize synthetic dyes of different chemical structures. Many bacteria, actinomycetes, yeast and molds are able to remove dyes by adsorption from the effluent (K. Shinde, et al 2015).

At present, a number of studies have focused on microorganisms, which are able to decolorize and biodegrade these dyes. Several combined anaerobic and aerobic microbial treatments have been suggested to enhance the degradation of azo dyes. The present study deals with the isolation of azo dyes degrading bacterium from a dye contaminated environment, its ability to degrade reactive dyes into non-toxic product and optimization of temperature, pH, carbon and nitrogen sources for maximum dyes degradation and decolorization. So, this microbial degradation and decolorization is necessary to degrade and decolorize the harmful textile grade dyes from environment to reduce the pollution and as well as help to reduce the formation of sludge during the degradation of dyes.

MATERIAL AND METHODS

Sampling

The textile effluent sample were collected from sewage water, Ulhasnagar. Standard procedures were followed during sampling. The physical characteristics of the effluent sample like pH, temperature, colour, and odour were checked then accordingly the results were recorded. The pH was determined by using pH strip and temperature with laboratory thermometer. The sample were transported to laboratory at 4°C as in accordance with the standard methods.

Dyes and Culture Media

Reactive Orange 13, Perssian Blue, peptone, sodium chloride, meat extract, yeast extract and agar powder were obtained from Hi-Media laboratory, India. All chemicals were of highest purity and analytical grade.

Isolation of Bacteria from Industrial Effluent

Isolation were carried out by inoculating 1 ml of industrial effluent in sterile Luria Bertani (LB) broth and then incubated at Room temperature on shaker condition for 24-48 hours. Luria Bertani agar medium was used for the isolation of bacteria from the dye effluent. The loop full of sample were taken from enrichment medium after incubation and then inoculated on freshly prepared sterile LB medium plates. Then the plates were incubated at Room Temperature for 24 hrs. After completing incubation period isolated colonies were observed on plates and then these colonies were selected, brought to pure culture and preserved on slants for further studies.

Screening of Dye Decolorizing Isolates

Morphologically distinct bacterial isolates were tested for their ability to degrade the textile dyes. The isolated bacterial strains were screened out by incubating them on Nutrient Broth medium containing 100 mcg total stock concentration of Reactive Orange 13 and Perssian Blue dyes. This study was performed by using Tube Method. The different concentration of both dyes was prepared and isolates were inoculated in medium containing dyes separately. The nutrient broth medium incubated at Room Temperature for 24 hrs. After the incubation, tubes were observed for bacterial growth. The last tube in which growth observed were considered as Minimum Inhibitory Concentration (MIC) for the particular isolates. The range of MIC concentration is 10-100 mcg/ml with interval of 10mcg/ml. The screened culture was transfer to agar slant and store 4°C for further study.

Identification of selected isolates

The selected isolates were examined for their morphological properties, such as size, shape, cell arrangement and staining properties. The isolates were identified up to species using Automated Microbial Identification System (VITEK 2) instrument.

Optimization of Physico-Chemical parameters

Parameter optimization was performed using different parameters like pH, Temperature, Carbon and Nitrogen source. Minimal Salt Medium (MSM) were used for this study. The medium contains all different salts which enhance the enzymatic reaction that takes place during decolorization. For study, the media were prepared with different pH values 4.0, 5.4, 7.4, 9.0, different temperature values such as 24^{0}C, 37^{0}C, 55^{0}C, different organic Carbon sources like Glucose, Galactose, Lactose, Sucrose, Starch, Glycogen, different organic and inorganic Nitrogen Sources Peptone, Tryptone, Yeast extract, Sodium nitrate, Sodium nitrite, Potassium nitrate, Potassium nitrite. Respective flasks were inoculated with specific volume of dyes and culture at shaker and as well as static conditions for 24, 48, and 72 hrs. The aliquot was removed at specific time interval and centrifuged at 7000 rpm at 4^{0}C for 10 min. Then the Optical Density was measured on colorimeter at 530 nm.

RESULT AND DISCUSSION

The present study was aimed to isolate the bacterial strains from industrial dye effluents and find out their dye degrading capacity. Industrial effluent is not stable and it varies often in a wide range depending upon the process practiced. Asian countries are experiencing severe environmental problems due to rapid industrialization. This phenomenon is very common where the polluting industries like textile dyeing, leather tanning, paper and pulp processing, sugar manufacturing etc. thrive as clusters. Among these the Textile industries are large industrial consumers of waters as well as producers of wastewater. The effluent discharged by this industry leads to serious pollution of groundwater and soils and ultimately affects the livelihood of the poor (Jiunkins.,1982). Traditional waste water treatments require enormous cost and continuous supply of chemicals because of which the process become more tedious and uneconomical and causes further environmental damages. Microbiological treatments were easier, cheaper and an effective alternative source for hazardous textile dyes removal from aquatic ecosystem. Effluent sample is a rich source of microbial populations which are able to grow in presence of dyes. Two potential isolates were obtained which are *Kocuria rhizophila* and *Bacillus cereus* against both the dyes Reactive orange 13 & Perssian blue. Bacterial strains were isolated by Luria Bertani medium and purified by sub culturing on nutrient broth medium. The bacterial isolates were screened for the decolorization and MIC determination of dye by Tube method (Growth appearance) and results are tabulated in Table 1 and 2. The morphological and cultural characteristics of screened bacterial isolates are shown in Table 3.

Out of two morphologically distinct bacterial isolates the *Kocuria rhizophila* were effectively decolorized the Reactive orange 13 dye at alkaline pH 9 (Figure 1) and Perssian blue dye with starch as a carbon and peptone as a nitrogen source (Figure 2 and 3). Whereas the other bacterial isolate *Bacillus cereus* were effectively decolorized the Reactive orange 13 dye using yeast extract as a nitrogen source (Figure 4) and Perssian blue dye at 37^{0}c temperature and also using sucrose as a carbon source (Figure 5 and 6). The screened culture was transfer to agar slant and store 4°C for further study.

Table 1: MIC of *Kocuria rhizophila* and *Bacillus cereus* against Perssian blue dye were found to be 20mcg/ml.

Sr. No.	Concentration of dye (mcg/ml)	Growth
1	10	+ve
2	20	-ve
3	30	-ve
4	40	-ve
5	50	-ve
6	60	-ve
7	70	-ve
8	80	-ve
9	90	-ve
10	100	-ve
Positive (+ve) control	-	+ve
Negative (-ve) control	-	-ve

Table 2: MIC of *Kocuria rhizophila* and *Bacillus cereus* against Reactive orange 13 dye were found to be 100mcg/ml and 30 mcg/ml respectively.

Sr. No.	Concentration of dye (mcg/ml)	Growth *Kocuria rhizophila*	Growth *Bacillus cereus*
1	10	+ve	+ve
2	20	+ve	+ve
3	30	+ve	-ve
4	40	+ve	-ve
5	50	+ve	-ve
6	60	+ve	-ve
7	70	+ve	-ve
8	80	+ve	-ve
9	90	+ve	-ve
10	100	-ve	-ve
Positive (+ve) control	-	+ve	+ve
Negative (-ve) control	-	-ve	-ve

Table 3: Morphological and cultural characteristics of screened bacterial isolates.

Characteristics	*Kocuria rhizophila*	*Bacillus cereus*
Size	Pinpoint	2 mm
Shape	Circular	Circular
Color	Colourless	Colourless
Margin	Entire	Entire
Elevation	Low convex	Convex
Opacity	Transparent	Opaque
Consistency	Smooth	Smooth
Gram's nature	Gram positive	Gram positive
Morphology	Cocci single	Bacilli (single/ chains)

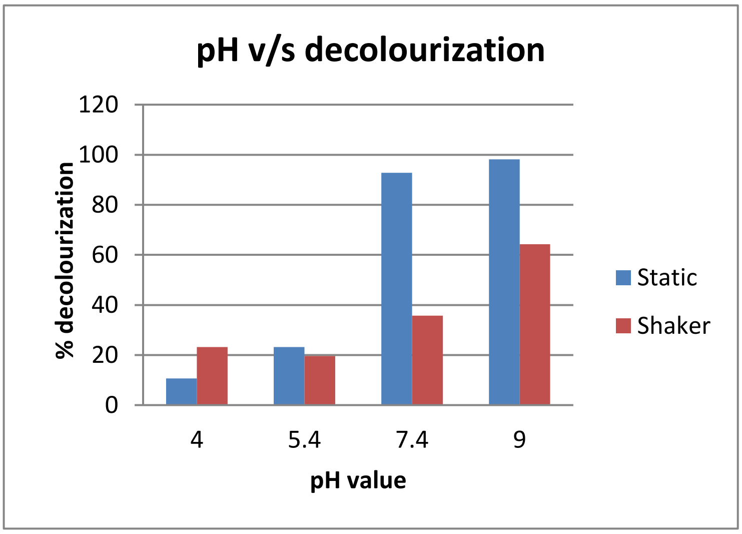

Figure No 1: Effect of pH on decolorization of Reactive orange 13 dye by *Kocuria rhizophila*

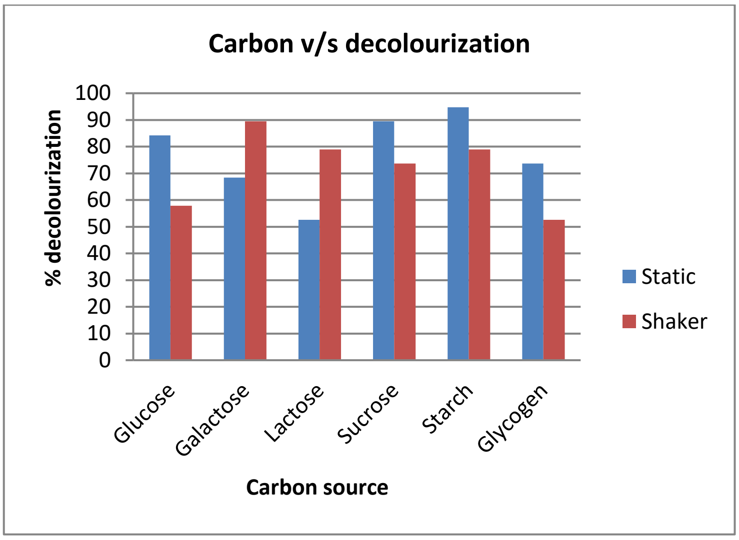

Figure No 2: Effect of Carbon source on decolorization of Perssian blue dye by *Kocuria rhizophila*

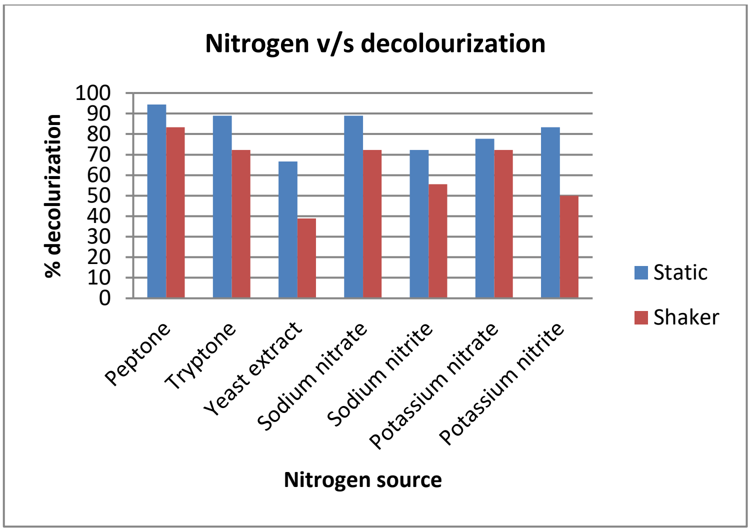

Figure No 3: Effect of Nitrogen source on decolorization of Perssian blue dye by *Kocuria rhizophila*

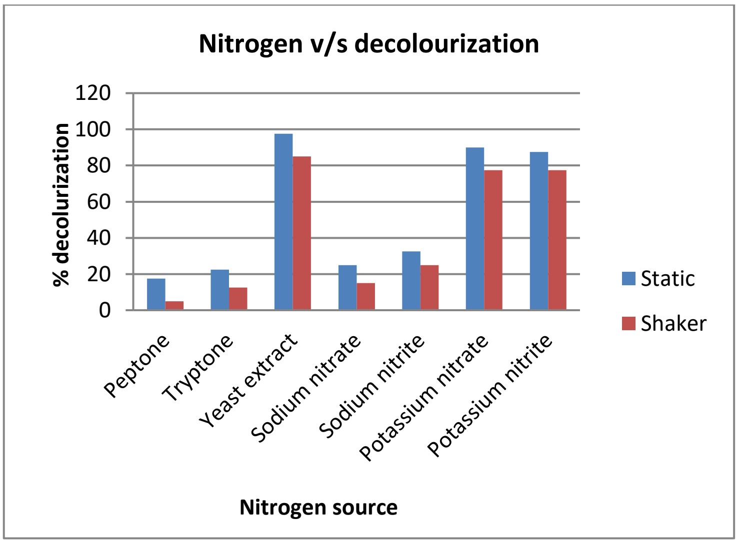

Figure No 4: Effect of Nitrogen source on decolorization of Reactive orange 13 dye by *Bacillus cereus*

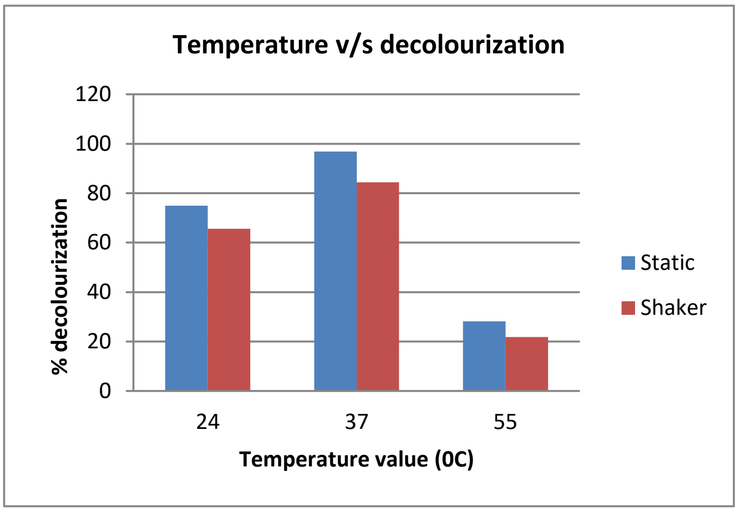

Figure No 5: Effect of Temperature on decolorization of Perssian blue dye by *Bacillus cereus*

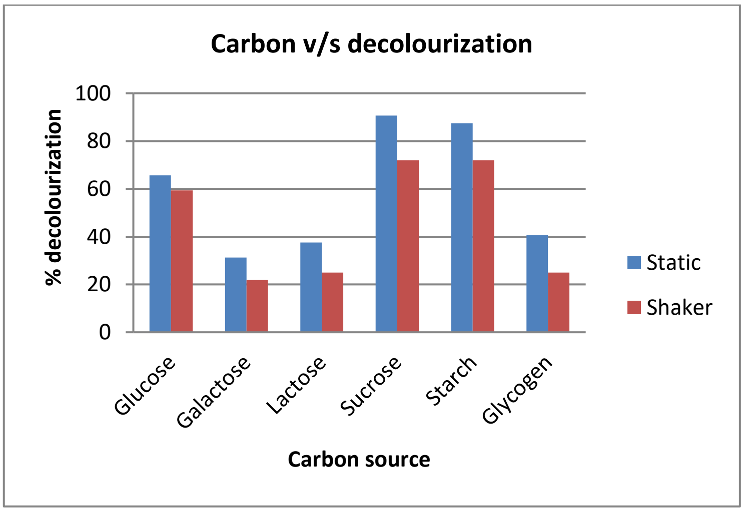

Figure No 6: Effect of Carbon source on decolorization of Perssian blue dye by B*acillus cereus*

CONCLUSION

Due to industrial effluent from textile and other dye stuff using industries to neighboring water bodies and waste water systems facing health concerns. Most of the dyes are carcinogenic in nature. The ability of the microbes to carry out dye decolorization has received much attention. Traditional waste water treatments require enormous cost and continuous supply of chemicals because of which the process become more tedious and uneconomical and causes further environmental damages.

Microbiological treatments were easier, cheaper and an effective alternative source for hazardous textile dyes removal from aquatic ecosystem. Effluent sample is a rich source of microbial populations which are able to grow in presence of dyes. This research work mainly represents the isolation and identification of bacteria able to degrade and decolurize widely used industrial dyes. In this study an attempt was made to isolate and identify the dye degrading and decolourising abilities of organisms.

The data obtained from such analysis can be useful in treating textile effluent and that treated effluent can be reused for industrial work or can be discarded in natural aquatic ecosystem without causing any pollution or damage. The two potential isolates were obtained for both the dyes (Reactive orange 13 & Perssian blue) that are *Kocuria rhizophila* and *Bacillus cereus*. The present study reveals that the both isolated bacterial strains have decolorizing capacity which can destroy the recalcitrant nature of the dye and made them easily biodegradable.

REFERENCES

1. Jiunkins R (1982) Pretreatment of textile waste water, Proceedings of 37th Industrial waste Conference. Purdue University, Lafayette, USA.
2. Keshav Shinde, Rajeshri Ghorpade, Deepak Thakare "Bacterial Decolourization of Textile Grade Coravat Red Dye by *Rhizobium Mayense*" International Journal

of Research Studies in Biosciences (IJRSB) Volume 3, Issue 5, May 2015

3. A. Tripathi, S.K. Srivastava "Ecofriendly Treatment of Azo Dyes: Biodecolorization using Bacterial Strains" International Journal of Bioscience, Biochemistry and Bioinformatics, Vol. 1, No. 1, May 2011
4. Aamr Alalewi, Cuiling Jiang "Bacterial Influence on Textile Wastewater Decolorization"
5. Anamika Pokharia, Sarabjeet Singh Ahluwalia "Decolorization of Xenobiotic Azo Dye- Black WNN by Immobilized *Paenibacillus alvei* MTCC 10625"
6. Babita Rani, Vivek Kumar, Jagvijay Singh, Sandeep Bisht, Priyanku Teotia, Shivesh Sharma, Ritu Kela "Bioremediation of dyes by fungi isolated from contaminated dye effluent sites for bio-usability" Brazilian Journal of Microbiology:2014
7. Blanca E. Barraga, Carlos Costa, M. Carmen Ma´rquez, "Biodegradation of azo dyes by bacteria inoculated on solid media" Environmental Systems Engineering Department, National School of Biological Sciences, National Politechnic Institute: Ayala,11340
8. Baljinder Kaur, Balvir Kumar, Neena Garg, and Navneet Kaur "Statistical Optimization of Conditions for Decolorization of Synthetic Dyes by *Cordyceps militaris* MTCC 3936
9. Elias Abadulla, "Decolorization and Detoxification of Textile Dyes with a Laccase from *Trametes hirsuta"* Applied and Environmental Microbiology, Aug. 2000