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Isolation and Development of Efficient Bacterial Consortia for Bioremediation of Textile Dye Effluent

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Abstract

In this study, the ability of bacterial strains isolated from the textile mill site was analyzed for de-colorization of dye effluent. The isolated strains were finally identified by 16S rDNA sequence analysis as *Bacillus species* and were used for the process of bioremediation. The physicochemical characterization of the effluent generated was also carried out. The effluent was analyzed for its physicochemical properties before and after treatment. Samples were analyzed in UV spectrophotometer and showed the absorption maxima at wavelength of 668 nm. The bacterial strain was found to have sorptive capacity, immobilized on nutrient agar medium. The effluent was treated in a flask containing minimum salt medium, 20% dye effluent and 5% (w/v) of bacterial pellet. The flask was placed in an incubator shaker at 37°C and 200 rpm. After inoculation the sample was analyzed in visible spectrophotometer after every six hours and it was found that 90% de-colorization and 70% reduction in COD was achieved after 24 hours. Heavy metals were also biosorptioned. It was concluded that the isolated bacteria represented a promising application in bioremediation process of textile industrial effluent and possible reusability of the cells for its commercial application can be achieved.

Keywords: De-colorization; *Bacillus species*; Textile dye effluent; Bioremediation

Introduction

Textile industries are the largest consumers of water and chemicals for wet processes of textile like dyeing, hence must generate high doses of effluent rich in the chemicals, which are highly toxic to the environmental components. Most synthetic dyes like azo-dyes, including reactive, acid, direct dyes and vat dyes, commonly used in the textile industry, paper printing, color photography, pharmaceutical, food, cosmetic and leather industries, are toxic, carcinogenic and highly resistant to degradation due to their complex chemical structures [1]. Effluents from the textile industries are highly colored containing dyes that vary from 2% for basic dyes to as high as 50% for reactive dyes, leading to severe contamination of surface and ground waters in the vicinity of dyeing industries [2]. In general, the wastewater from a typical cotton textile industry is characterized by high values of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), color, and pH [3]. Because of high BOD and color, the untreated dye effluents reduce dissolved oxygen content and light penetration respectively, in receiving water bodies and without suitable treatment, such wastewater would destroy the natural water environment [4]. The dye effluents also contain heavy metals that can cause several health problems. Lead for instance, can interfere with enzyme activities and function of red blood cells. It can affect nerves and brain at low concentration [5]. Heavy metals such as mercury, cadmium and chromium can bio accumulate and through the food chain to toxic levels in man [6]. Different methods are available for the remediation of dye wastewaters. Most of the physical and chemical methods like adsorption, chemical oxidation, precipitation, coagulation, filtration, electrolysis, photo degradation employed, which in spite of high cost, are low efficient and do not always ensure that the contaminants are completely removed [7]. On the other hand, biological methods involving the use of microorganisms like fungi, bacteria, yeasts, and algae for the removal of synthetic dyes from industrial effluents either by degradation or by sorption are generally considered environmentally friendly, relatively inexpensive, the running costs are low, and the end products of complete mineralization are not toxic. Bioremediation is one such process and may be considered as the most effective and the environmental friendly technology for treating industrial dye wastewaters. It is a pollution control technology that involves the use of microorganisms to catalyze the degradation or transformation or bio-sorption of various toxic chemicals to less harmful forms [8]. Bio-sorption involves the entrapment of dyes in the matrix of the adsorbent (microbial biomass) without destruction of the pollutant. Several researchers have described the use of microorganisms as bio-sorption agents in the removal of pollutants from wastewater [9-11]. Most studies on the metabolism of organic contaminants have been performed with bacteria especially in the context of bioremediation [12]. Identification of efficient indigenous microorganisms with biodegradation potential is the key step for developing bioremediation systems for effluent treatment [13]. A number of microorganisms display a remarkable ability to degrade a range of industrial contaminants (bioremediation) and to restore natural environmental conditions. Bioremediation of textile industrial effluents through bio-sorption has been studied using *Pseudomonas* and *Bacillus species* [14]. In general, potential microorganism's especially bacterial species can remove color and reduce the physicochemical parameters of solutions by bio-sorption or bioaccumulation or both [15]. Therefore, the aim of the present work is to isolate and characterize indigenous predominant adapted bacterial strains, from the textile effluent which possess the ability to cause color and COD reductions of the textile dye effluent. These isolates were used to develop an efficient bacterial consortium that can decolorize the dye effluent through bio sorption and biodegrade the organic load in the

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effluent at a faster time and can be used further to develop a continuous process of the treatment of textile effluents at large scale.

Material and Methods

Sample collection

Dye effluent sample was collected in plastic containers from the outlet of a textile dye industry in Sanganer at Jaipur district in Rajasthan, west India (GPS): 26°49'00" N, 75°47'00" E and were stored at 4C for further use to avoid changes in its characteristics. The effluent was characterized and analyzed for pH, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), total solids (TS), Alkalinity, Salinity & heavy metals based on the Standard Methods for Examination of Water and Waste-water [16].

Isolation and identification of isolated bacterial strains: For isolation of bacteria capable of decolorizing the dye effluent, 1 ml of the effluent was taken and serially diluted in 5 test tubes each containing 9 ml of sterile distilled water, in the order of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. 1 ml from each diluted test tube was spread over nutrient agar plates (g/L: Peptone 5, Beaf extract 3, NaCl 5, agar 15). The inoculated plates were incubated at 37°C for 24 hrs. The bacterial colonies appearing on nutrient agar plates were morphologically characterized and purified by repeated culturing [17]. After the incubation period the plates were observed for the growth of microorganisms. Microscopic and biochemical tests were applied to these isolates according to Bergey's manual of systematic bacteriology.

Characterization of bacterial strains and amplification of 16S rDNA: The isolated bacterial strain was selected for 16S rDNA sequencing. ARDRA and DGGE techniques were performed on the amplified fragments of genomic DNA for characterization of bacterial strains. The genomic DNA of the bacterial strains was extracted using a Genome DNA Kit (Qiagen, USA) from log phase of growth [18]. Qiaquick PCR Purification Kit (Qiagen), adjusted to 200 ng ml^{-1} was used for purifying the amplified DNA and sent for sequencing. The phylogeny relationship of the isolate was determined by comparing the sequencing data with the sequences of some members of the genus *Bacillus* available through the GenBank database of the National Centre for Biotechnology. The gene sequences of the isolate obtained in this study was compared with known 16s rDNA gene sequences in the GenBank database. The 16S rDNA was selectively amplified from genomic DNA of the isolated strains by using PCR with oligonucleotide universal primers Eubac 27 (Pf 50-AGA GTT TTG ATC CTG GCT CAG-30) and Eubac 1492 (Pr 50-CTA CGG CTA CCT TGT TAC GA-30) as described earlier [19]. Pairwise alignment giving closest match was chosen and the sequences after matching were identified as *Bacillus* species.

*ARDRA= Amplified Ribosomal DNA Restriction Analysis

*DGGE= Denaturing Gradient Gel Electrophoresis

Growth of bacterial cells, culture condition and decolorization of the dye effluent through surface adsorption: The *Bacillus spp*. was incubated at 37°c in a rotary shaker at 150 rpms in Nutrient broth. At the end of incubation, the growth media was centrifuged at 5000 rpm and the bacterial pellet was separated and preserved. The bacterial biomass separated was screened for de-colorization in 1 L flask containing Minimal Salt Medium (MSM) containing (g/L): $Ca(NO_3)_2$, $4H_2O$, 0.05, $Fe(CH_3COO)_3$, NH_4, 0.05; KH_2PO_4, 6.8; $MgSO_4$, 0.2; Na_2HPO_4 $2H_2O$, 7.8; $NaNO_3$, 0.085; 100 ml dye effluent, 5 g bacterial pellet, at pH 7 [17] in a rotary shaker (200 rpm) and incubated at 37°c for one day. The dye effluent was initially analyzed for color intensity and other physicochemical parameters. Samples were withdrawn from culture media after 6, 12, 18, and 24 h, centrifuged at 10,000 rpm for 15 min to remove all the suspended matter. The settled bacterial biomass was analyzed for change in its color and the supernatant was analyzed for determination of color intensity and other physicochemical parameters [16,20]. The peak absorbances of different colors were measured using a UV spectrophotometer and the heavy metals were analyzed using atomic absorption spectroscopy.

Screening experiments for de-colorization of dye effluent: Screening experiments were performed to select most suitable bacterial strain and pH.400 ml Minimum Salt Medium (MSM), 100 ml dye effluent without inoculum was taken as control. Invitro study was conducted in Erlenmeyer flasks containing 400 ml MSM along with 100 ml dye effluent and 5% (w/v) inoculum. Reduction in color, COD, BOD, Alkalinity, TSS, Salinity and heavy metals was analyzed after every 6 h, 12 h, 18 h and 24 h.

Results and Discussion

Isolation and characterization of bacterial strains from textile dye effluent

Mostly single type of bacterial strains appeared on the plates based on morphological differentiation of individual colonies. They were observed under a microscope, Olympus and Magnus MLX-TR, at 40 and 1009 attached with a camera. The bacterial isolates were identified as *Bacillus spp*. according to Bergey's manual of Systematic Bacteriology (Figure 1a and 1b) and the biochemical characterization indicated the

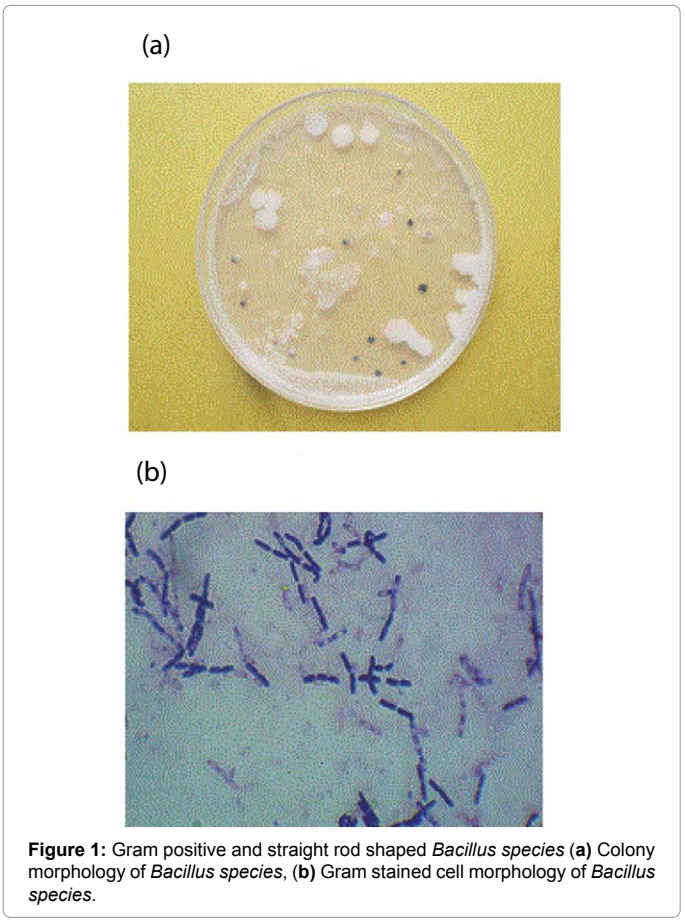

Figure 1: Gram positive and straight rod shaped *Bacillus species* (**a)** Colony morphology of *Bacillus species*, (**b)** Gram stained cell morphology of *Bacillus species*.

bacterial strains were Gram-positive (Figure 2). They were further purified using Nutrient Agar Media by repeated culturing, streaking and screened on the basis of their de-colorization potential.

Physicochemical characterization of dye effluent

The textile dye effluent collected from Sanganer, Jaipur, India indicates high level of pollution compared with standards of NEQS, (2000). The sample collected was dark blue in color. The physicochemical properties of dye effluent were analyzed before and after treatment and the evaluated parameters are shown in Table 1.

Screening of bacterial strains for de-colorization and reduction in other chemical parameters of dye effluent

Single type of bacterial strains appearing on nutrient agar plates from the diluted effluent were purified by repeated culture and then mass culture was prepared. Bacterial strain (*Bacillus spp*.) was tested for de-colorization of dye effluent and the peak absorptions of the dyes before and after treatment are shown in Figure 3 [21]. Results of the study indicated the maximum reduction in color (90%), COD (70%) and heavy metals after 24 h of treatment (Figures 4 and 5) respectively. The bacterial mass was changed to intense blue color leaving the supernatant al little light blue in color. On the basis of these results, the bacterium was selected as a potential remover of color and heavy metals through bio-sorption. Each experiment was performed in three replicates to reduce the experimental errors. The result obtained from this present investigation showed that textile effluent are highly polluted and are quiet above the acceptable limits. The removal efficiency of the color, COD and bioaccumulation of heavy metals suggested the adoption of *Bacillus spp*. for bioremediation of dye effluent and is a step towards sustainable development as both the bacteria and the color removed can be reused to reduce burden on the environment.

Conclusion

The results indicated that the isolate *Bacillus spp*. is an efficient degrader of the textile dye effluent under in-vitro laboratory conditions. The study presents a new approach for enhancing the de-colorization of textile dye effluent by bacteria under various conditions and the development of efficient bacterial consortium for bioremediation of the dye effluents is a boon for textile industries in getting rid of their effluent problem. Hence, the findings of the present study are also expected to be useful for the development of bioremediation processes for the treatment of not only the textile effluent but industrial effluent

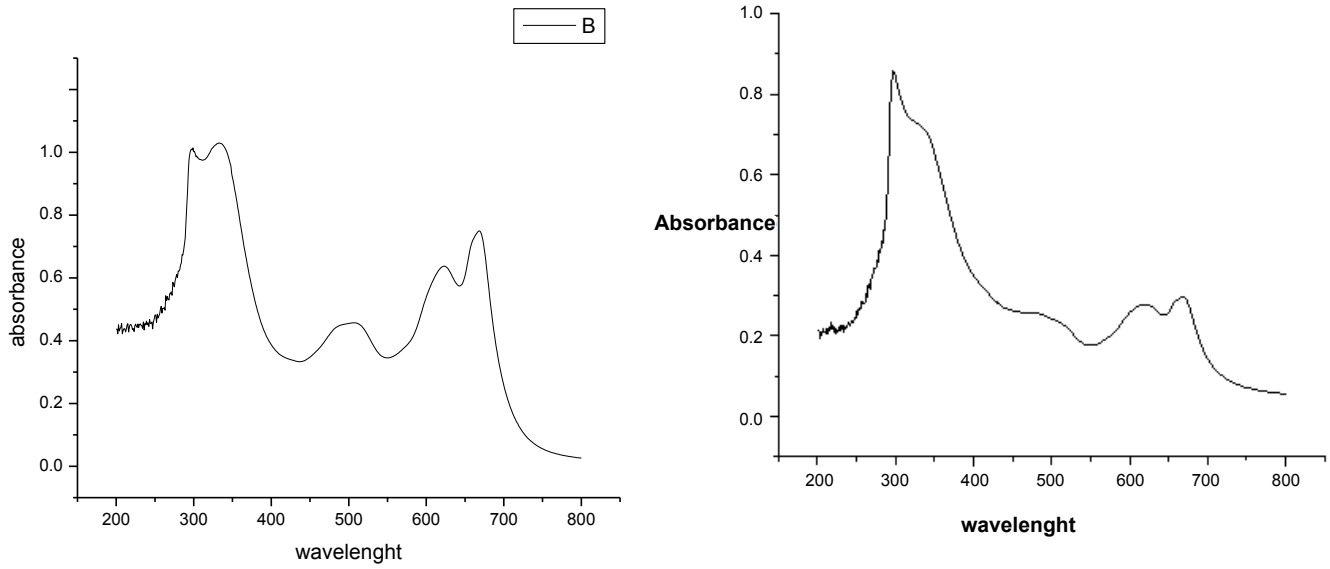

Figure 2: Peak absorbances of untreated and treated dye effluents.

Parameters	Raw	6 h	12 h	18 h	24 h	% Reduction after 24 hrs.
pH	9.3	9.1	8.7	8.3	8.00	
Color	Dark blue	blue	Light blue	colorless	Colorless	
COD	6680	5010	4008	3006	2004	70%
BOD	1120.02	896.016	728.013	593.610	392.007	65%
Alkalinity	920.50	736.5	598.5	414.5	285.25	69%
Salinity	355	301.75	230.75	159.75	106.5	70%
Hardness	120	96	84	54	61.6	68%
TSS	320	262.4	192	150.4	112	65%
Cr	0.070	0.055	0.031	0.027	0.021	-
Cu	1.051	0.873	0.0.52	0.0351	0.019	-
Fe	5.91	3.50	0.551	0.092	0.0791	-
Pb	0.12	0.095	0.061	0.032	0.011	

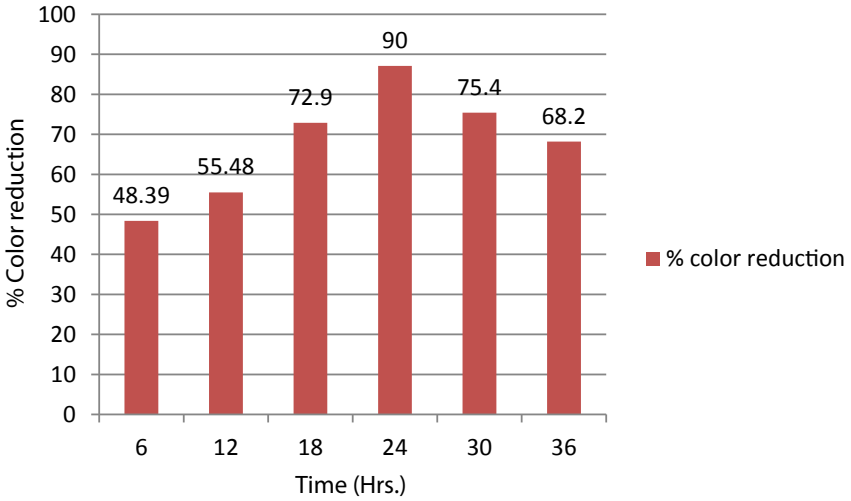

Figure 3: Percent de-colorization of textile dye effluent by *Bacillus species* at different time intervals.

Figure 4: Reduction in color: Before (1) During (2) and after (3) treatment.

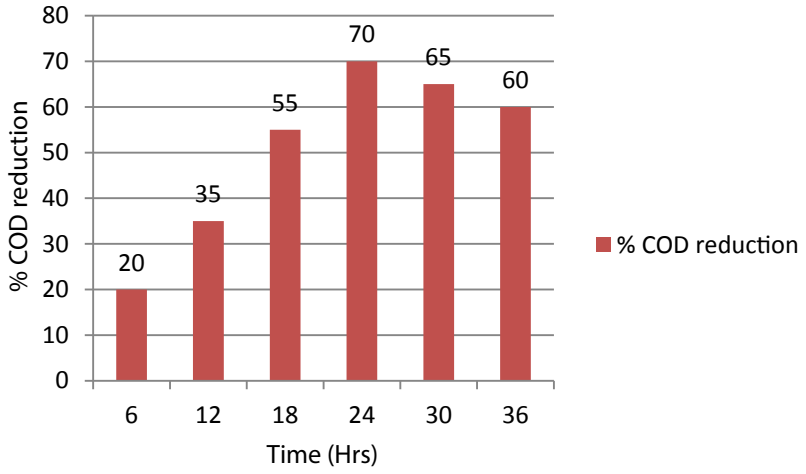

Figure 5: Percent COD reduction of textile dye effluent by *Bacillus species* at different time intervals in batch culture.

of any kind. However, further research is needed to establish the process with specific attention.

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