BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, April 2015. Vol. **12**(1), 281-285

Decolorization of Textile Effluent using Mixed Culture of Bacteria

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DOI: http://dx.doi.org/10.13005/bbra/1663

(Received: 03 February 2015; accepted: 09 March 2015)

The potential of different bacteria to decolorize textile waste effluents of Hindoostan mill, Karad, Maharashtra, India was evaluated under this research. Growth, Decolorization, COD and BOD were determined under controlled condition using textile waste effluent as growth medium supplemented with mineral salts. Higher decolorization was observed when a mixed culture of *Bacillus subtilis* and *Micrococcus* species was used at the pilot scale of process. This study demonstrated the use of mixed culture of bacterial species to degrade textile reactive dyes and reinforces the potential of this group of bacteria for the decolorization of various textile effluents.

Key words: Textile waste effluent,Decolorization, Bacterial Isolates, COD, BOD.

The Hindoostan Mill of Karad, Maharashtra, India is a composite cotton textile mill having both spinning and weaving activities. Most of the times, the effluents are discharged into water bodies without performing any treatment. In general, the difficulties encountered in the waste water treatment resulting from dyeing operation lies in the wide variability of the dyes used and in the excessive color of the effluents. Many dyes and other substances present in textile effluents are not radially degraded during their permanency in traditional aerobic treatment systems.[1, 2] Although, many physicochemical techniques of Decolorization have been developed over the last 20 years butfew have been implemented by the textile industries due to their high cost, low efficiency and inapplicability to a wide variety of dyes. A definitive solution of the color problem of textile effluents would provide a marked competitive advantage for this industrial sector. Since no single process is able to decolorize all textile effects, a solution for each situation should be considered, possibly involving a combination of different methods.[3]

The success of a biological process for color removal from a given effluent depends in part on the utilization of microorganism that effectively decolorize synthetic dyes of different chemical structures. Manybacteria ,actinomycetes, yeast and mitosporic fungi are able to decolorize dyes. The color removal by these microbes being mainly attributed to adsorption of the dyes.[3, 4]Decolorization of different dyes using fungi has

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been done to get the lignolytic potential and xenobiotic degradation potential.[5, 6, 7, 8] The role of some bacterial and algal species for the decolorization and degradation of textile dyes has also been reported.[7, 9, 10, 11, 12]

The objective of the present study were 1) to evaluate the potential of native soil bacteria to degrade dye of textile industry effluent 2) Batch culture study 3) to determine the decolorization of a textile industry effluent by pure and mixed culture of efficient bacterial isolates.

MATERIALS AND METHODS

Collection and chemical analysis of textile waste effluent:

The textile waste effluents sample used for this study was obtained from Hindoostan mill textile industry located at Karad, Satara District, Maharashtra, India. The textile waste effluent was analyzed in triplicates for pH, COD, BOD, Total solids, Total suspended solids, total dissolved solids, chlorides and dye using standard methods.

Isolation and Characterization of Microorganisms:

The soil sample was collected from the textile effluent wastecontaminated area from the same textile industry. The serial dilutions was prepared and 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} was selected for further study. The selected dilutions were plated in triplicates on sterile Nutrient Agar plates using spread plate method. Plates yielding 30-300 colonies were selected and colony with different morphology was selected and sub-cultured on nutrient agar to get pure culture. The isolates were identified by gram staining, colony morphology and biochemical tests using taxonomic scheme ofBergey's Manual of determination Bacteriology.[13]

Preparation of Bacterial inoculums

Mineral salt medium {1g/L; KH_2PO_4, 2 g; K_2HPO_4, 7 g/L; $MnSO_4$, 0.1 g;$Na_3C_6H_5O_7$, 0.5 g; $(NH_4)_2SO_4$}with textile waste effluent in 4:1 ratio was used to inoculate bacterial isolates.[14] The inoculated culture medium was incubated on gyratory shaker at 120 rpm with 30^0C for 24hrs. The pH of the medium was maintained at 7 using 0.1 M HCl and 0.1 M NaOH. The 5-10% (v/v) of these grown bacterial cultures were used to inoculate fresh flasks and were cultivated at the same condition for 24 hr.

Batch Culture study

Different biodegradation runs were carried out on a 7.5L capacity New Brunswick Microferm Twin fermentor designed for mass cultivation of bacteria. The reactor was equipped with a control panel to control the agitation and temperature. An autoclaved mineral salt medium (0.8L) was added into 3L of textile waste. 200 ml of prepared inoculums was added to make up 4L of working volume. The Reactor was operated at R.T. and agitation speed of 300 rpm. The aeration was maintained at flow rate of 2.0 volume of air per volume per broth per minute. The fermentation was carried out for 14 days after which samples were taken for measurement of decolorization, COD and BOD analysis.

Determination of Color removal

Decolorization of the textile waste effluents was determined by measuring the absorbance at the 600 nm. using U.V. visible spectrophotometer. The Decolorization efficiency was expressed as per the following equation:

$$\% \text{ Decolorization} = [(A-B)/A] \times 100$$

Where, A and B are the initial and final absorbance respectively.

HPLC Analysis

The textile dye degradation was monitored by HPLC as the decolorization progressed. Ten milliliters of samples were taken at the start of the experiment and daily (24 h), centrifuged and filtered through 1.2 ìm filter paper. The filtrate was extracted three times with methylene chloride and evaporated in rotary evaporator with 45 – 50^{o}C water bath, after which the residue was dissolved in 2 ml methanol. The extracted samples were analyzed using 60% acetonitryl and 40% water (mobile phase) at a flow rate of 0.5 ml/min. The elution of the samples was done isocratically using a C-18 reversed phase column (RPC – 18 phenomenex) and the UV–VIS detector was set at 285 nm.

Decolorization with mixed culture of bacteria

The textile waste effluent with 40 mg/L dye concentration was diluted with single distilled waste to obtain waste effluents with varying dye concentration of 15, 20, 25, 30 and 35 mg/L respectively. These effluents were introduced into bioreactor with mixed culture and were operated for 14 days.

RESULTS AND DISCUSSION

The values of various chemical parameters for the untreated textile waste effluents are listed in Table 1. Further a total of eight bacterial isolates were obtained from the soil contaminated with textile waste effluents. These isolates were able to utilize the textile effluent as carbon and energy source. The bacterial isolates were identified based on gram staining, colony morphology and biochemical characteristics presented in Table 2.

From the eight bacterial isolates six (B1, B2, B3, B5, B6, B7) found to capable of decolorize textile effluent. Figure 1 shows the percent dye decolorization by different bacterial isolates, which indicates that all bacterial species shows decolorization between 35-90%. *Bacillus subtilis*and *Micrococcus* shows the highest Decolorization of 90%. The percent COD and BOD reductions of the treated textile waste effluents by different bacterial species is shown in figure 2 and figure 3. Figures reflect the efficiency of Bacterial species to reduce COD is between 70-95% and BOD between 65-95%. *Bacillus subtilis*shows maximum reduction of COD and BOD as 95% and 93% in comparison to other bacterial species.The result of HPLC analysis showed the role of degradation mechanism in dye decolorization. It has been already reported Decolorization of dye effluent is a result of dye degradation mechanism .[6, 15, 16]

The similar reductions in COD and BOD were observed in the study of aerobic bioreaction of Terasile black in textile effluent by a newly isolated *Bacillus* sp. and sulfur black by *Aspergillusterus* SA 3 respectively.[12, 17] The *Pseudomonas* sp. has alsobeen recognized for high COD reduction in the treatment of textile effluent.[11]

Since *Bacillus subtilis* and*Micrococcus* sp. relatively had a higher percent color removal, COD and BOD reductions than other bacterial species, they were further used as binary mixed culture to study the biodecolorization of dye in textile waste effluent under the same experimental

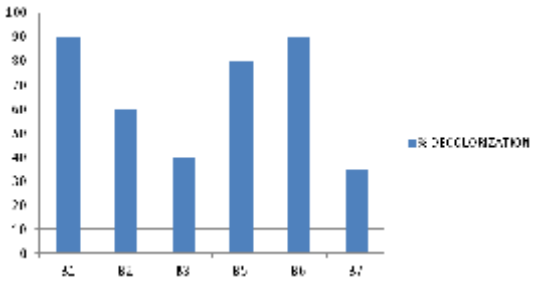

Fig. 1. Decolorization of textile waste effluents by isolated Bacterial species (B1= *Bacillus subtilis*, B2= *Pseudomonas fluorescens*, B3= *Pseudomonas nigrifacians*, B5= *Lactobacillus sp*., B6= *Micrococcus sp*., B7= *Staphylococcus sp*.)

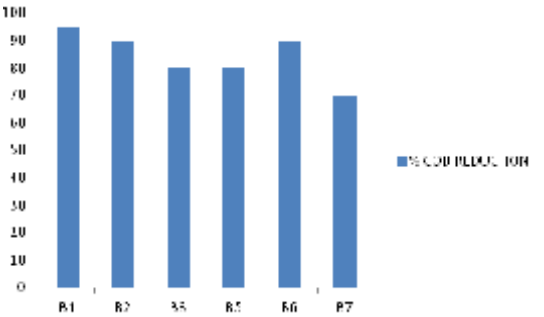

Fig. 2. COD reduction of textile waste effluents by isolated Bacterial species in a Bioreactor species (B1= *Bacillus subtilis*, B2= *Pseudomonas fluorescens*, B3= *Pseudomonas nigrifacians*, B5= *Lactobacillus sp*., B6= *Micrococcus sp*., B7= *Staphylococcus sp*.)

Table 1. Chemical analysis of the untreated textile waste effluents

Parameters	Determined value
pH	9 ± 1
BOD	4800 ± 200 mg/L
COD	6400 ±100 mg/L
Total suspended solids	2820 ±180 mg/L
Total dissolved solids	3800 ± 200 mg/L
Total solids	7200 ± 200 mg/L
Chlorides	880 ± 20 mg/L
Total dyes	28.5 ± 2.5 mg/L

Table 2. List of Isolated Bacterial Isolates

Sr. No.	Bacterial isolates
1	*Bacillus subtilis* (B1)
2	*Pseudomonas fluorescens* (B2)
3	*Pseudomonas nigrificans* (B3)
4	*Bacillus sp.* (B4)
5	*Lactobacillus sp.* (B5)
6	*Micrococcus sp.* (B6)
7	*Staphyloccus sp.* (B7)
8	*Serratia sp.* (B8)

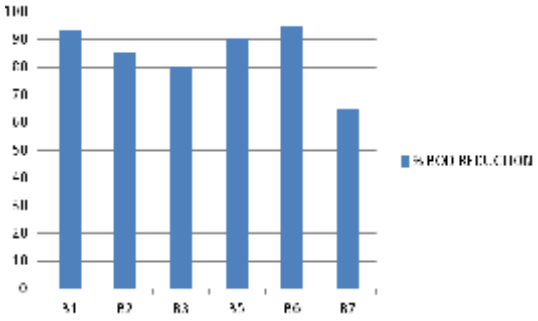

Fig. 3. BOD reduction of textile waste effluents by isolated Bacterial species in a Bioreactor species (B1= *Bacillus subtilis*, B2= *Pseudomonas fluorescens*, B3= *Pseudomonas nigrifacians*, B5= *Lactobacillus sp*., B6= *Micrococcus sp*., B7= *Staphylococcus sp*.)

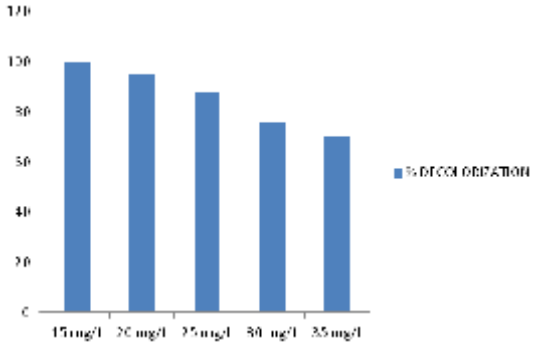

Fig. 4. Effect of Dye concentration by binary mixed culture of *Bacillus subtilis* and *Micrococcus sp.*

conditions. Maximum dye color removal of 100% was observed for 15 mg/L textile dye effluent is shown in figure 4. This indicates that percent Decolorization decreased with increased dye concentration which ultimately leads to decrease in rate of Decolorization. A similar observation has been reported for the color removal of reactive blue dye by *Polyporusrubidus*and the removal of sulfur black by *Aspergillusterreus* SA3.[17, 18]The advantages of employing mixed culture (microbial consortium) as opposed to pure culture in bioremediation have been also demonstrated.[19]

These results support the importance of studies involving mixed culture in comparison to pure culture for the decolorization of textile waste effluent.

CONCLUSION

The present results demonstrated differences between bacterial species grown in pure or mixed cultures in turns of their ability to degrade reactive textile dyes. The excellent performance of *Bacillus subtilis*and *Micrococcus* sp. in the Decolorization of dyes describe the potential of these bacteria for environmental decontamination.

ACKNOLEDGEMENT

The author is greatly acknowledgedKIBBKaradCollege to provide laboratory facility and fund.

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