RESEARCH PAPER | Microbiology | Volume : 5 | Issue : 8 | August 2015 | ISSN - 2249-555X

Physiochemical Analysis of Textile Industry Effluent and Bioremediation of Dyes by Bacterial Species

KEYWORDS: Azo dye decolorization, bacteria,textile industry effluent, Microbial biotechnology, Technical Textiles

Tambekar, DH
Post Graduate Department of Microbiology, SGB Amravati University, Amravati 444602, India

RP Sontakke
Post Graduate Department of Microbiology, SGB Amravati University, Amravati 444602, India

ABSTRACT *In the present study an attempt was made toexamine the potential of different bacterial strains fordecolorization of Congo Red from highly polluted drain, locally known as Buddha Nala, Ludhiana. The bacterial strains used in the study wereKlebsiella sp Staphylococcus sp, Pseudomonas sp and Bacillus sp.Outof theseBacillus sp. emerged out to be most potentdecolourizer, being selected for further studies.Over the years, with the active spread and development of the industries, Heavy Metal, which are either used, or produced as by-products by numerous manufacturing, industrial, refining and mining processes have become ubiquitous, persistent environmental pollutants. To overcome the difficulties posed by conventional wastewater treatment systems bioremediation has emerged as a promising technology in the past few years for the treatment of industrial dye effluents and contaminated soil.*

INTRODUCTION:

The textile industry uses large volumes of water in their processing and this one of key inputs. To produce 1 ton of textile product is consumed 200-270 tonnes of water. The effluent carries with it a high polluting load, since about 90% of the products chemicals used in textile processing are eliminated after completing your goals. The textile industry uses vegetable fibres such as cotton, animal fibres such as wool, silk, and synthetic materials such as nylon, polyester, and acrylics (Sahin, 1996). The process of adding colour to the fibres is known as dyeing which normally requires large volumes of water not only in the dye bath, but also during the rinsing step. The process of dyeing involves the use of different chemicals like salts, metals, surfactants, sulphide and formaldehyde (Khataee and Kasiri, 2010).

Dyes contributed to overall toxicity at all process stages. Also dye baths could have high level of BOD/COD, colour, toxicity, surfactants, fibers and turbidity and may contain heavy metals (AEPA, 1998). Dyes are recalcitrant molecules which are difficult to degrade biologically. Some of azo dyes are either toxic or mutagenic and carcinogenic (Hildenbrand ***et al.,*** 1999). Azo dyes are designed to resist chemical and microbial attacks and to be stable in light and during washing (Rajaguru ***et al.,***2000). The ability of microbes to degrade a vast array of pollutants makes bioremediation a technology that can applied in different soil conditions. A new type of bioremediation (the successful transformation of toxic polluting agents to non-toxic, usable substrates for further 'macro'-degradation) has become a major research now a day. Again the guidelines for the textile processing industries by the pollution control boards create concern over the environment-friendliness of the processes.

Materials and methods

Sampling and Analysis of Effluent Sampling: Highly polluted drain, locally known as Buddha Nala, Ludhiana was chosen for effluent sample collection. Sampling site covers the effluent from 8 textile mills in the woven fabric and knit fabric finishing industry.

Physiochemical analysis of effluent: Determination of Physio-chemical parameters such as DO, pH, TDS, TSS, total alkalinity, total hardness, chloride, were analyzed according to standard methods. Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) determination was made according to the standard methods for examination of water and waste water (APHA, 2002).

Isolation and Identification of bacterial strains and Screening for dye Decolorization: Isolation and identification of bacteria were carried out by plate counting technique. One gram of soil and sludge were weighed individually and suspended in 99mL of sterile distilled water. Discrete bacterial colonies that developed on Agar plates were initially grouped on the basis of colony morphology, pigmentation followed by Gram staining and motility.

Biodecolorization and biodegradation analysis The flasks were observed for decolorization of the azo dye present in the medium. At every 12h interval 5 mL aliquot of the decolorized culture broth was collected and centrifuged at 10,000 rpm for 5 min. The supernatant was recovered and analyzed spectrophotometrically at 497 nm. The analysis was done using UV-VIS spectrometry, Decolourization extent was calculated using the following formula:

$$\text{Decolorization extent (\%)} = \frac{C_0 - C_t}{C_0} \times 100$$

Where C_0 refers to the initial absorbance, C_t refers to the absorbance after incubation; and t refers to the incubation time.

Effect of pH and temperature: Decolorization was studied at varying pH (3.0–13.0) and temperature (20°C-50°C).

RESULTS AND DISCUSSION:

Physiochemical analysis of textile effluent: The TSS values of effluent were found too significantly with Industries as well as with sampling days and found to be 788 ppm. The increased amount of TSS is due to increased chemical dosing dye fixation and partial dissolution of fibre materials. In other studies the amount of TSS in different textile wastewater samples was found to be in the range of 1020-3680 ppm which is considerably higher than the result of our findings (Abraha ***et al.,*** 2014). The total dissolved solids in sugar mill effluent, tannery waste and textile industries were also reported in the level of

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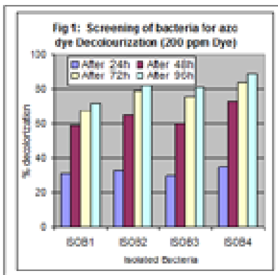
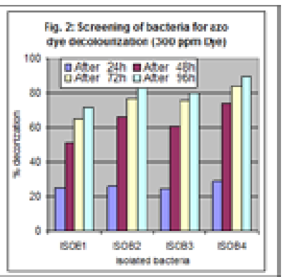
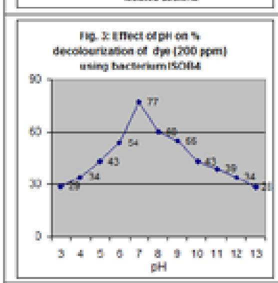
400 - 1650 ppm (Avasan and Ramkrishana, 2001), 1000 - 2850 ppm and 8500 – 15000 ppm (Rao ***et al.,*** 1993) respectively. In the present investigation COD of untreated textile effluents were 2395 ppm and this value of the COD is beyond the permissible limit (250 ppm) of CPCB (CPCB, 1995). This indicates that the effluents were unsuitable for the existence of aquatic organisms due to the reduction of DO content (Shobana, 2008).

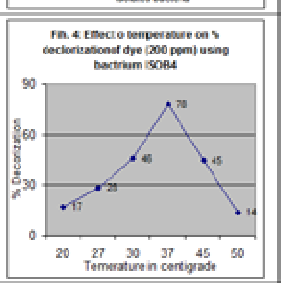
Table 1: Physicochemical quality of Effluent			
Parameters	ResultS	Parameters	ResultS
Colour	Blackish Golden brown	Total hardness as CaCo3(ppm)	715
Odour	pungent	BOD(ppm)	276
Temperature (°C)	35	COD(ppm)	2395
pH	6.5	Total Alkalinity(ppm)	1297
Total Suspended Solids (ppm)	788	Chlorides(ppm)	2752
Total Dissolved Solids(ppm)	7993	Sulphates (ppm)	1676

Isolation and identification of bacterial strains:
From 25 morphologically distinct strains isolated from the textiles effluent, only 4 isolates were found to possess the ability to decolorize the Dye Congo Red and marked them as BACT-1, BACT-2, BACT-3 and BACT-4. Dye degrading isolates were identified on the basis of morphological and biochemical tests according to Bergey's Manual of Systematic Bacteriology (Sneath ***et al.,*** 1984) Selected bacterial isolates were further purified and sub cultured for further study. The percentage decolourization found to be 67, 79, 76 and 86 respectively for isolate BACT-1, BACT-2, BACT-3 and BACT-4 after 72 h of Incubation using concentration of 100 ppm of Congo red dye. In case of 200 ppm of dye the percentage decolourization was found to be 66, 78, 76 and 83 respectively for isolate BACT-1, BACT-2, BACT-3 and BACT-4 after 72 hours of Incubation and 66, 75, 76 and 84 percentage of decolourization at 300 ppm of congo red Dye. [Fig. 1, 2] The decolourizing activity of the bacterial consortium was studied using Congo Red Dye at different initial concentrations varying from 100 to 300 ppm. The maximum decolourization was observed up to 200 ppm. The maximum decolourization observed by isolate BACT-4 i.e. 83 % followed by isolates BACT-2 (78%) BACT-3 (76%) and BACT-1 (66%).









The pure bacterial isolates BACT-1, BACT-2, BACT-3 and BACT-4 were identified as ***Klebsiella sp Staphylococcus sp, Pseudomonas sp*** and ***Bacillus sp***. by respectively biochemical characteristics [Table:2] using standard microbiological procedures based on the methods of in Bergey's Manual of Systematic Bacteriology (Rigas and Dritsa, 2006). In another study conducted with ***Pseudomonas putida. P. fluorescence, Bacillus cereus*** and ***Stentrophomonas acidaminiphila*** to decolorize Acid Red 88 showed their efficiencies at 35%, 31 %, 40% and 50% respectively. Under aerobic conditions azo dyes are generally resistant to attack by bacteria (Daneshvar ***et al.,*** 2007).

Table 2 : Morphological and biochemical characterization of Bacterial Isolates				
Test	BACT-1	BACT-2	BACT-3	BACT-4
Gram Reaction	-ve	+ve	-ve	+ve
Motility	-ve	-ve	+ve	+ve
Glucose fermentation	AG	A	AG	AG
Sucrose fermentation	AG	A	-	AG
Lactose fermentation	AG	A	-	AG
Mannitol fermentation	AG	AG	-	AG
Indole	-	-	-	-
MR	-	+	-	-
VP	+	+	-	-
Citrate Utilization	+	-	δ	-
H_2S Production	-	-	-	-
Catalase	+	+	+	-
Urease	+ (late)	δ	δ	-
Where: A- Acid, G-Gas				

Effect of pH and temperature on decolourization of dye: The pH and temperature are important factor for the optimal physiological performance of microbial cultures and decolorization of dyes. These factors affect the cell growth and various biochemical and enzymatic mechanisms. In present study the decolorization of Congo Red Dye by potential isolate ***Bacillus sp*** (BACT-4) was found in the pH range of 3.0-11.0. The maximum decolorization (78%) was observed at pH 7.0. and at 37 ^{0} C of temperature. A further increase or decrease in pH and temperature from the optimum value decreases the decolorization rate (Fig. 3 & 4). It was found out that under agitation conditions, presence of oxygen deprives the azoreductase from obtaining electrons needed for cleavage of azo dyes.

Conclusion:
This study addresses the physiochemical characteristics of the effluents and the result revealed that the most of the parameters were not within the permissible limit of PCB standard. Some of the industrialist discharged the untreated effluents to the nearby rivers that are being completely polluted by the effluents also polluting the ground water causing various health problems. Government legislation is increasingly becoming more stringent especially in the more developed countries, regarding the removal of dyes from industrial effluents. Moreover, further research on these strains could explore new tools and techniques to evolve viable and eco friendly microbial solutions for treatment of dyeing industrial effluent.

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