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ORIGINAL RESEARCH ARTICLE

Decolourization of Acid Red 131 by using *Shigella* sp. Isolated from Tannery Effluent

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ABSTRACT

Dyeing of leather in leather industries result in dye-containing wastewaters. Removal of dyes from these wastewaters is desired, not only because of their colour but also because of their toxicity, mutagenicity and carcinogenicity. Different physical, chemical and biological techniques can be used to remove dyes from wastewater. Each technique has technical and economic limitations. Most physicochemical dye removal methods have drawbacks because they are too expensive, have limited versatility, are greatly interfered by other wastewater compounds and/or generate waste products that must be handled. Alternatively, biological treatment holds promise as a relatively inexpensive way to remove dyes from wastewater. At least dyes belonging to the largest class of dyes, the azo dyes are prone to bacterial biodegradation. The present study confirms the ability of bacteria to degrade the leather dye Acid Red 131 with degradation efficiency of 99%, thus suggesting its application for degradation of dye bearing of industrial wastewaters. Presence of a Co-substrate (Dextrose & Ammonium formate) is the essential conditions for attaining maximum degradation efficiency. The bacteria isolated from tannery waste water are a facultative anaerobe that shows positive or negative result for some biochemical tests.

Key words: Tannery effluent, Acid Red 131, *Shigella* sp. and Decolourization.

1. INTRODUCTION

Industrial pollution has been and continues to be a major factor causing the degradation of the environment around us, affecting the water we use, the air we breathe and the soil we live on. Water is polluted not only by industries but also by households. Both industries and household wastewater contain chemicals and biological matter that impose high demands on the oxygen present in water. Polluted water thus contains low levels of dissolved oxygen as a result of the heavy biological oxygen demand (BOD) and chemical oxygen demand (COD) placed by industrial and household waste materials discharged into water bodies and water systems, both above and below the earth's surface. In addition to low levels of dissolved oxygen in water, industrial wastes also contain chemicals and metals that are directly harmful to human health and the ecosystem. The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years, because they are indestructible and most of them have toxic effects on organisms [1]. Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems [2].

Dyes are recalcitrant by design and not readily amendable to common treatment methods, imposing a challenge for closed water systems. Extensive research in the field of biological azo dye decolourization has shown promising results, but much of this work has been done with single model compounds [3]. However, industrial textile wastewater presents the additional complexity of

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dealing with unknown quantities and varieties of many kinds of dyes [4], as well as low BOD/COD ratios, which may affect the efficiency of the biological decolourization. India's dye industry produces every type of dyes and pigments.

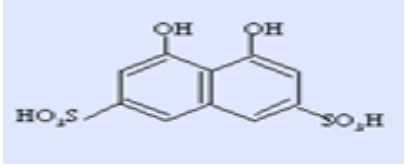
Production of dye stuff and pigments in India is close to 80,000 tones. India is the second largest exporter of dye stuffs and intermediates after China. The textile industry accounts for the largest consumption of dyestuffs, at nearly 80%. Industrialization is vital to a nation's economy because it serves as a vehicle for development. However, there are associated problems resulting from the introduction of industrial waste products into the environment.

Dyes are stable against breakdown by many microorganisms and most dyes do not biodegrade under the aerobic biological treatments in a municipal sewage plant. Many dyes, including the azo dyes, degrade under anaerobic conditions and the aromatic amines thus formed have been found to degrade further aerobically. Out of several methods that are used in the treatment of textile effluents to achieve decolourization, including physiochemical methods like filtration, specific coagulation, use of activated carbon and chemical flocculation some of the methods are effective but quite expensive. Biotreatment offers a cheaper and environmentally friendlier alternative for colour removal in textile effluent [5]. Bioremediation is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. This natural process, bioremediation, includes bioengineering the capabilities of intrinsic microorganisms, to clean up the environment is an effective alternative to conventional remediation methods [6].

2. MATERIALS AND METHODS

Dye

Acid red 131 used in this study were obtained from the Tannery Division of CLRJ, Chennai. Acid Red 131 is an azo dye which commercial name is COLODERM RED 3BL. It is having the solubility at 30g/L. It doesn't form precipitation with either acid or with hard water. It having the naphthalene intermediate namely Benzoyl H acid, its commercial name is 4:5 Dihydroxy 2:7 napthalene Disulfonic Acid, its Mol. Wt 320, Emprical formula is $C_{10}H_8O_8S_2$ and its structure is



Sample collection

Tannery effluent was collected from CLRI, Chennai, Tamilnadu, India. The collected Tannery effluent was stored in the plastic container at room temperature for further use.

Isolation and identification of microorganism from Tannery effluent

Nutrient agar medium were prepared, sterilized and dispensed into sterile petriplates. Samples from primary culture (the unknown microorganisms were already isolated from the tannery waste water by serial dilution in CLRI, CHORD lab) was inoculated into Nutrient agar plates. Inoculated plates from then incubated at 37°C for 24 hours. Identification of the bacterial isolates was carried out by the routine bacteriological methods *i.e.*, By the colony morphology, preliminary tests like Gram staining, capsule staining, endospore staining, motility, catalase and oxidase, plating on selective medias and performing biochemical tests.

Measurement of dye decolourization

Measurement of dye concentration was measured with a UV/VIS spectrophotometer (Cary 100-spectrophotometer) at regular intervals during the decolourization process. The concentration of azo dye was detected spectrophotometrically by reading the culture supernatant at its specific max (550 nm) after centrifugation at 10,000 rpm for 20 minutes. The dye **d**egradation % was calculated as follows

$$\text{Degradation \%} = \frac{\text{Initial Conc - Final Conc}}{\text{Initial absorbance}} \times 100$$

Degradation using parameters

Dye (Acid red 131) degradation was studied using various parameters. First, by changing various pH ranging from 4.5 - 8. Second, by changing various carbohydrate sources like dextrose ($C_6H_{12}O_6$), lactose ($C_{12}H_{22}O_{11}$), mannitol (C_6H_8 (OH) $_6$), starch (($C_6H_{10}O_5)_n$) & sucrose ($C_{12}H_{22}O_{11}$). Third, by changing various nitrogen sources like ammonium acetate (CH_3COONH_4), ammonium formate (NH_4HCO_2), ammonium chloride (NH_4CI), Glycine (NH_2CH_2COOH), L - glutamine ($C_5H_{10}N_2O_3$) & L - methionine ($HO_2CCH(NH_2)CH_2CH_2SCH_3$). For the above studies, the dye concentration in the range of 100 mg/l was used constantly. Finally by changing different dye concentration (100 - 300ppm) the degradation were studied.

Degradation of Acid red 131 at various Carbon sources

The effect of various carbon sources *viz.*, Dextrose, Lactose, Mannitol, Starch and Sucrose

was analyzed in this present study. The Carbon sources (1:100) were added in the Nutrient Broth containing Acid red 131 (500 mg/l) and incubated at 30°C for 4 days in a rotary shaker running at 180 rpm. Decolourization assay was measured in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated.

Degradation of Acid red 131 at various Nitrogen sources

The effect of various nitrogen sources *viz*., Ammonium acetate, Ammonium chloride, Ammonium formate, Glycine, L - Glutamine and L - Methionine was analyzed in this present study. The Nitrogen sources (1:100) were added in the Nutrient Broth containing Acid red 131 (500mg/l) and incubated at 30°C for 4 days in a rotary shaker running at 180 rpm. Decolourization assay was measured in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated.

Degradation of Acid red 131 at various pH

Colonies of an overnight growth were suspended in normal saline to obtain an optical density of 0.6 at 610 nm wavelength. One milliliter of the cell suspension was inoculated in 250 ml Erlenmeyer flasks containing Nutrient Broth and Acid red 131 (500 mg/l). The pH of the medium was adjusted to 4.5, 5, 5.5, 6, 6.5, **7**, 7.5 and 8 with hydrochloric acid and sodium hydroxide. The cultures were incubated at 30°C for 4 days in a rotary shaker running at 180 rpm. Decolourization assay was measured in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated.

Degradation of Acid red 131 at various dye concentration

Degradation activity of the bacteria was studied using Acid red l31 at different initial dye concentrations varying from 100 – 300 mg/l. The cultures were incubated at 30°C for 4 days in a rotary shaker running at 180 rpm. Decolourization assay was measured in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated.

3. RESULTS AND DISCUSSION

In the present study, the bacteria was isolated from the tannery effluent and identified. From the biochemical test results, the unknown microorganism used for this study was facultative anaerobic bacteria and it is an Enterobacter family. The unknown organism may be *Shigella* sp. Saranraj [a] *et al*. [7] isolated a bacterial strain from tannery effluent and identified as *Enterococcus casseliflavus.* Sadeeshkumar *et al*. [8] isolated a bacterial strain from tannery effluent and identified as *Enterococcus casseliflavus.*

The preliminary studies revealed that the dye could not be used as the sole carbon and nitrogen source by the organism required additional carbon and nitrogen source sources to co-metabolize the dye. Hence, different carbon sources were evaluated for dye decolourization at 100 mg/1 dye concentration. Among various carbon sources, Dextrose was the best carbon source and decolourization was maximal at 0.5g/l of Dextrose concentration is shown in the (**Fig 2 & 3**).

The result on the effect of various organic and inorganic nitrogen sources (1g/l) on dye degradation showed that among the organic nitrogen sources L-glutamine shows moderate level of degradation. Other inorganic nitrogen source Ammonium formate shows efficient degradation so it is the best nitrogen source for that strain A is shown in the (**Fig 4 & 5**).

Bacterial culture generally exhibited maximum degradation at pH values at 7. Decolourization of CI Acid red 131 at various pH values by strain A. It shows that an increase in pH from 5.0 to 7 (**Fig 1**) while the degradation rate value decreased as pH was increased range from 7 to 8 with marked reduction in decolourization activity at pH 5.0. Both *E.coli* and *Pseudomonas luteola* exhibited best degradation rate at. pH 7 with constant decolourization rates up to pH 9.5 [9]. *Klebsiella pneumoniae* RS. 13 completely degraded methyl red in pH range of 6.0 to 8 [10]. They found that a pH value between 6 and 9 was optimum for decolourization of triphenylmethanes and azo dyes by *Pseudomonas* spp [11]. Moreover, it has been reported that generally azo dye reduction to more cultures to more basic aromatic amines leads to a rise in pH of the medium by a 0.8 - 1.0 [12, 13].

Degradation activity of the bacteria was studied using Acid red l31 at different initial dye concentrations varying from 100 – 300 mg/l is shown in figure 6. Rate of degradation increased with increase in initial dye concentration up to 200 mg/l . Further increase in dye concentration resulted in reduction in degradation rates. Lower decolourization efficiency is due to higher inhibition at high dye concentration [14]. The dye concentration in the reactive dye bath effluent was observed within narrow range of 0.1 - 0.2 g/1 [15]. This strain A shows maximum degradation at 100mg/l is shown in (**Fig 7**). The direct red 81 degradation rate was increased with increase in

initial dye concentration up to 200ppm by using bacterial consortium NBNJ6. Further, the 16srRNA from this microorganism was isolated and it was used as a primer to amplify that particular DNA responsible for dye degradation, by PCR and it is used for degradation directly.

Saranraj [b] *et al.* [16] investigated the decolourization and degradation of Direct azo dyes and biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. They isolated five different bacterial species from the textile dye effluent sample and the isolates were identified as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Escherichia coli*. The bacterial inoculums were inoculated into flasks containing Direct azo dyes (500 mg/l) with trace amounts of yeast extract, glucose and sucrose and then sterilized and incubated for 4 days. In their research, *Pseudomonas aeruginosa* (97.33%) was identified as the best decolourizer of Congo Red. Similar results we also obtained in our present study. The best decolourizer of Direct Green-PLS was *Bacillus subtilis* (99.05%). *Klebsiella pneumoniae* (87.27%) highly decolourized the Direct Violet-BL. *Escherichia coli* (61.56%) was the best decolourizer of Direct Sky Blue-FF. The best decolourizer of Direct Black-E was *Klebsiella pneumoniae* (92.03%).

Fig 1: Effect of pH on dye Degradation

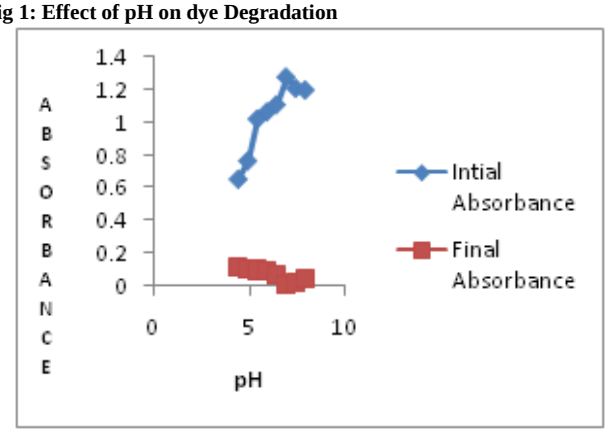


Fig 2: Effect of Carbon source on dye degradation at initial level

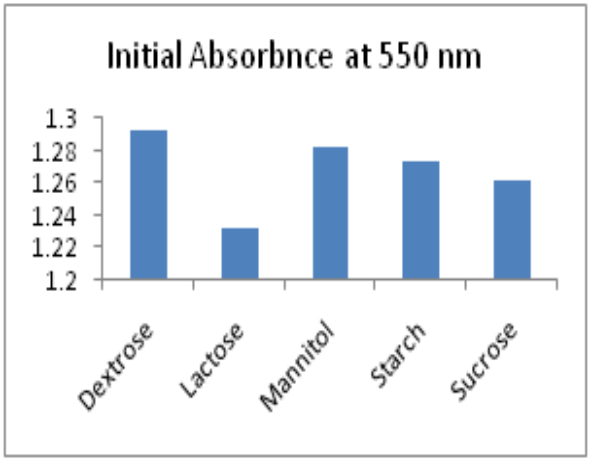


Fig 3: Effect of Carbon source on dye degradation at final level

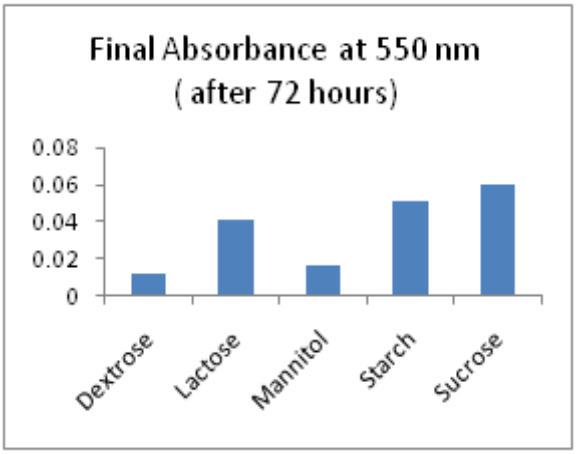


Fig 4: Effect of nitrogen source on Dye degradation at initial level

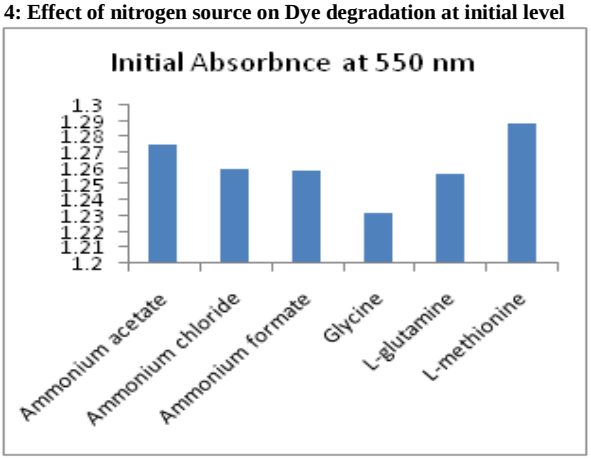


Fig 5: Effect of nitrogen source on Dye degradation at final level

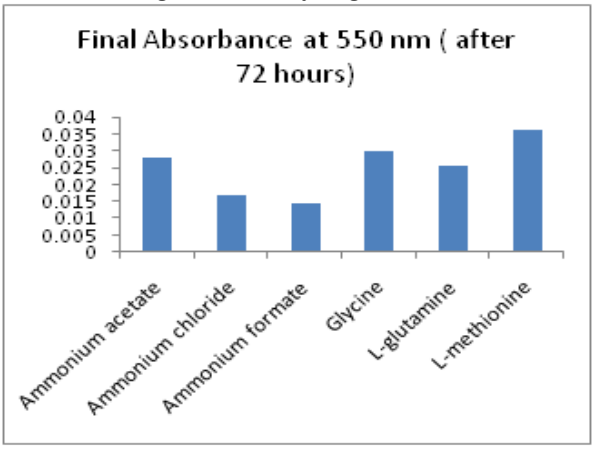
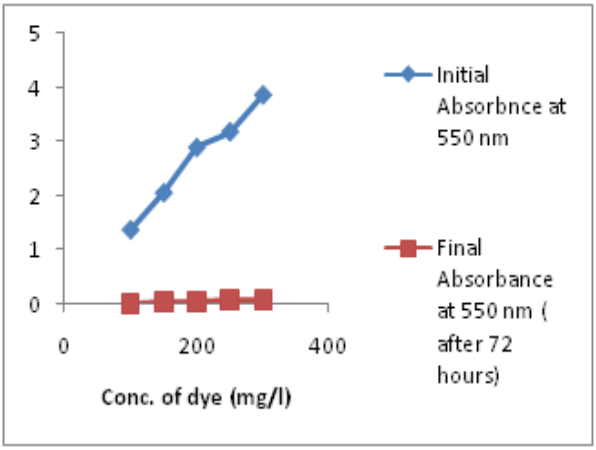


Fig 6: Effect of different dye concentration on degradation



4. CONCLUSION

This present study strongly recommends the usage of dyes should be handled carefully and it should

be discharged in highly proper way without affecting the environment. Dyes especially the non-degradable ones, even in minute levels are causing stress to environment so, and an effort has to taken to handle the dyes properly and save the environment.

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