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Original Research Paper

# Biological Decolourisation of Two Synthetic Textile Dyes and an Actual Textile Dyeing Industry Effluent by Selected Bacterial Isolates

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**Key Words:**
Biological decolourisation
Synthetic textile dyes
Dyeing industry effluent
Bacterial isolates
Bioremediation

## ABSTRACT

The study was undertaken to assess the decolourisation potential of selected bacterial species on two synthetic dyes and an actual textile dyeing industry effluent. All the four bacterial species tested namely *Kluyvera ascorbata*, *Bacillus* sp., *Pseudomonas* sp. and *Pasteurella* sp. showed greater potential in decolourising the synthetic dyes Orange G and Direct Blue 71. The bacterial isolates were comparatively less efficient in degrading the complex effluent medium that contained the dye Reactive Black 5. Factors affecting the efficacy of bacterial degradation of textile effluents were critically analysed and discussed. The outcome of the study contributes in taking bacterial dye remediation from laboratory to field conditions.

## INTRODUCTION

Synthetic dyes are used extensively in textile dyeing industries. Wastewater generated by textile dyeing process contains dyes at concentrations ranging from 10-200 mg/L. About 10-20% of the dyes are lost in the effluent along with organic and inorganic accessory chemicals (Murugesan & Kalaichelvan 2003) mainly due to the inefficiencies in the industrial dyeing process. The untreated or partially treated effluents released from the dyeing units pose a major threat to the environment.

Release of coloured compounds into the environment is undesirable not only because of their aesthetic appearance and colour, but also because of their breakdown products, which may be toxic or mutagenic to life (Weisburger 2002). Textile factories daily discharge millions of litres of untreated effluents in the form of wastewater into public drains that eventually empty into rivers (Olayinka & Alo 2004). This alters pH, and increases biochemical oxygen demand (BOD) and chemical oxygen demand (COD) giving the rivers intense colourations (Ajayi & Osibanjo 1980), thus, limiting the use of these water resources.

Textile wastewater is rated as one of the most polluting among the industrial sectors considering both volume and composition of effluent (Vanndevivera et al. 1998). Textile industries are multi-chemical utilizing concerns. In a typical dyeing plant, the major chemical usage include dyes, pigments, finishing agents, acids, alkalis, surfactants, dispersants, leveling agents, carriers and auxiliaries (Cooper 1995). Though, the textile effluent is a complex and highly variable mixture of many polluting substances ranging from inorganic compounds and elements to polymers and organic products (Banat et al. 1996), it is difficult to remove dyes from effluents since they are highly stable to light, heat and oxidizing agents and are usually nonbiodegradable.

Commonly used waste treatment methods like coagulation, flocculation, adsorption, chemical

transformation, incineration and photocatalysis do not adequately eliminate many azo dyes from effluent waters (Kimura 1980). Most current physical and chemical technologies do not achieve total decolourisation of coloured effluents or they have operational difficulties or are too expensive, and traditional biological wastewater treatments have low removal efficiencies (Robinson et al. 2001). Bioremediation constitutes an alternative to such conventional physico-chemical methods. Biological treatment of textile effluents can be aerobic, anaerobic or a combination of both depending on the type of microbes being employed (Keharia & Madamwar 2004). Azo aromatic dyes are reductively cleaved into colourless amines by several bacterial species (Chung & Stevens 1993, Stolz 2001).

In the present investigation, assessment of the dye decolourising potential of four bacterial species namely *Kluyvera ascorbata*, *Bacillus* sp., *Pseudomonas* sp. and *Pasteurella* sp.; examination of the capacity of these organisms to continuously degrade or decolourise two commonly used textile azo dyes namely Orange G and Direct Blue 71 under aerobic conditions; and ability of the isolated bacterial species to decolourise an actual textile dyeing industry effluent containing the azo dye Reactive Black 5 were studied. The results obtained from the study were expected to provide a breakthrough in discovering efficient wild bacterial isolates with the potential for use in *in-situ* biological treatment of textile effluents.

## MATERIALS AND METHODS

**Chemicals and dyes:** All chemicals used were of analytical grade. The dyes, Orange G (OG) and Direct Blue 71 (DB 71), were procured from Jegatham Dyes and Chemicals, Nagercoil. Stock solution of the dyes was prepared by dissolving 2.0g of each dye in 100 mL distilled water (20 mg/mL).

**Effluent source:** Highly coloured textile effluent containing the diazo dye Reactive Black 5 (RB 5) was collected from a textile dyeing unit located in Arulpuram area of Tamil Nadu. The concentrated solution from the rinsing step was collected in a 10 L airtight plastic can. The effluent was filtered through ordinary filter paper to remove large suspended particles and stored at $4 \pm 1$°C until further analysis.

**Sterilization technique:** All glasswares were initially washed with detergent and rinsed thoroughly with distilled water followed by sterilization in hot air oven at 120°C for 1.5 hours. Inoculations were performed with flame sterilized loops and the entire experiment was carried out under strict sterile conditions.

**Source and isolation of microorganisms:** Isolation of bacterial species was carried out from soil samples taken from a municipal landfil site located at Vadasery, Nagercoil. Soil samples were subjected to serial dilution and inoculated onto sterile nutrient agar plates with the nutrient medium composed of beef extract (3 g/L), peptone (5 g/L), sodium chloride (5 g/L) and agar (15 g/L) at a neutral pH and $30 \pm 1$°C for 3 days. Bacterial colonies were identified and characterized based on Bergey's Manual of Determinative Bacteriology (Holt et al. 1994). Counting and identification procedures were carried out under a stereo binocular microscope. Stock cultures of the isolated bacterial species were preserved on agar slants at 4°C.

**Preparation of bacterial inocula:** Standard inoculum was prepared by subculturing a single colony of the respective bacterium from the stock culture in 125 mL conical flasks containing 10mL modified Minimal Medium (MM) (sodium chloride 4.0, magnesium sulphate 0.42, potassium chloride 0.29, D-glucose 0.1, ammonium sulphate 0.1, potassium phosphate dibasic 1.27, calcium chloride 0.02, ammonium nitrate 1.0, sodium carbonate 0.1, yeast extract 0.6, EDTA 0.5 g/L) (Oranusi & Ogugbue 2005) at 30°C in a rotary incubation shaker (200 rev/min) for 3 days.

**Inoculation of bacteria into dye medium**: 1 mL aliquot of each of the four isolated bacteria was inoculated into 500mL conical flask containing 100 mL MM with 100 mg/L of the dye and the pH was adjusted to 7 to 8.5 and incubated at 30°C for 10 days. Control flasks contained only dyestuff and nutrients but received no bacterial inoculum.

**Introduction of bacteria into effluent medium:** 100 mL of the filtered effluent was taken in 500mL conical flasks. 1 mL aliquot of each of the bacterial species was inoculated into the effluent medium. Raw effluent without bacterial inoculum was treated as control. Incubation was at 30 ± 2°C for 10 days in static condition. 1 hour shaking was given at 2 days interval on a mechanical shaker. In both the sets of experiments, incubations were performed in triplicate and results were expressed as mean ± SD.

**Decolourisation studies:** Samples were withdrawn at zero day and at 24 hours interval for determination of optical density (OD). 2 mL sample was aseptically withdrawn from each flask and centrifuged at 6000 rpm for 3 minutes in a bench centrifuge. Absorption spectrum of the clear supernatant was recorded using a spectrophotometer at λmax for each dye. Orange G, Direct Blue 71 and Reactive Black 5 had λmax values of 490, 330 and 598 nm respectively. Percentage dye decolourisation was calculated using the following formula.

$$\% \text{ Dye decolourisation } = \frac{OD_{\text{Zero day}} - OD_{\text{Sample}}}{OD_{\text{Zero day}}} \times 100$$

## RESULTS AND DISCUSSION

**Identity of bacterial isolates:** The soil samples collected were screened for bacteria subjecting to serial dilution and plating methods. The species were identified by studying the structural arrangements using stereomicroscope, and biochemical characteristics based on standard manual. Four bacterial species (Table 1) were isolated and used for the decolourisation studies of which *Bacillus* sp. and *Pseudomonas* sp. (Zimmermann et al. 1982, Chang et al. 2001, Paar et al. 2001, Olukanni et al. 2006) are well studied and documented for their decolourisation and degradation potentials. However, *Kluyvera ascorbata* and *Pasteurella* sp. are somewhat lesser explored species in textile dye effluent remediation.

**Biodecolourisation of synthetic azo dyes:** It was found that there was a decrease in the absorption spectrum of the samples treated with the 4 species of bacteria in both the synthetic dye media when the incubation period increased. Decolourization percentage of Orange G and Direct Blue 71 was proportional to the incubation period (Figs. 1 and 2).

Orange G was effectively decolourised by most of the selected bacterial species, and a maximum decolourisation of 94.92 ± 0.37 % was observed in the sample treated with *K. ascorbata* on 10$^{th}$ day of incubation followed by *Bacillus* sp. (84.09 ± 1.72). With *Pseudomonas* sp. and *Pasteurella* sp., 61.89 ± 0.91 and 45.86 ± 1.58 % colour removal was obtained. Combined treatments of *K. ascorbata* with *Bacillus* sp. gave 91.71 ± 1.70% decolourization and *Pseudomonas* sp. combined with *Pasteurella* sp. showed 53.74 ± 0.68%. Individual bacterial treatments were better than combined treatments in the Orange G dye medium. Thus, the phenomenon of additive effect or synergism of bacteria on decolourization was ruled out in the study. The result contradicted the observation of Knapp & Newby (1994) who suggested a synergistic role of bacterial species in the decolourisation of a diazo dye.

Decolourization rate was slow during the initial period of incubation (0-24 hours) and the peak

Table 1: Biochemical characteristics of the bacterial isolates.

| S. No. | Name of the test | Colony morphology and biochemical characteristics | | | |
|---|---|---|---|---|---|
| | | Round, white colony | Rhizoidal colony | Cream colony | White colony |
| 1 | Gram staining | Gram negative rod | Gram negative rod | Gram negative rod | Gram negative rod |
| 2 | Indole test | +ve | - ve | - ve | +ve |
| 3 | Methyl red test | +ve | - ve | - ve | +ve |
| 4 | Voges-Proskauer test | -ve | -ve | -ve | -ve |
| 5 | Citrate test | -ve | + ve | + ve | + ve |
| 6 | Triple sugar iron agar test | Acid slant/Acid butt | Acid slant/Acid butt | Acid slant/Acid butt | Acid slant/Acid butt |
| 7 | Mannitol motility test | + / + | - / + | - / + | + / - |
| 8 | Urea hydrolysis test | -ve | +ve | - ve | -ve |
| 9 | Nitrate utilization test | +ve | +ve | +ve | +ve |
| 10 | Catalase test | +ve | +ve | - | - |
| 11 | Coagulase test | +ve | -ve | - | - |
| 12 | $H_2S$ production test | -ve | -ve | -ve | -ve |
| 13 | Oxidase test | -ve | -ve | +ve | +ve |
| 14 | Lysine decarboxylate test | +ve | +ve | +ve | +ve |
| 16 | Blood agar plate | αHC | αHC | - | - |
| | Identified organisms | *Kluyvera ascorbata* | *Bacillus* sp. | *Pseudomonas* sp. | *Pasteurella* sp. |

Note: αHC Alpha Haemolytic Colony

activity was observed during 2nd and 3rd day in almost all the cultures. Rate of decolourisation proceeded gradually form the 3rd to 10th day. *K. ascorbata* showed better Orange G decolourising efficiency of all the four bacterial species studied. It is suggested through this study that this species can be commercially exploited for bioremediation as it is proved by earlier reports (Farmer et al. 1981) that it is widely spread and can be easily isolated from the environment, has a potential to grow fast in artificial culture conditions and is clinically insignificant.

*Bacillus* sp. also had a relatively higher percentage of dye decolourization. This species is worthy to be considered for bioremediation of textile mill effluents as reported earlier (Maier et al. 2004). Though, in the present study, the species showed lower rates of decolourization, their potential as an azo dye degrader is already established and needs further in-depth studies at the molecular level. Several previous works (Oranusi & Ogugbue 2001, Jones & Falkinham 2003, Nachiyar & Rajkumar 2003) suggested *Pseudomonas* as an organism with dye decolourization potential. The outcome of this study also indicated the same and the enzyme system of the microbes responsible for the degradation needs further analysis.

A maximum of 84.5 ± 0.6 % colour removal was achieved by *Pseudomonas* sp. in Direct Blue 71, but the combined treatment of *K. ascorbata* and *Bacillus* sp. resulted in 78.02 ± 2.1 % decolourization. With *Pseudomonas* sp., decolourization may be due to azoreductase of the bacterium that utilize NADPH and NADH as co-factors and reductively cleaved several sulphonated azo dyes (Zimmermann et al. 1984). Almost all the cultures showed maximum activity between 4th and 8th day of incubation in decolourising Direct Blue 71, and decolourization proceeded gradually up to the 10th day irrespective of the bacterial strain.

**Biodecolourisation of textile effluent:** The complex effluent contained the dye Reactive Black 5. By appearance the effluent was bluish black in colour and moderately alkaline with pH 9.17. It was totally devoid of dissolved oxygen with a heavy load of BOD, COD, TDS and TSS (values not

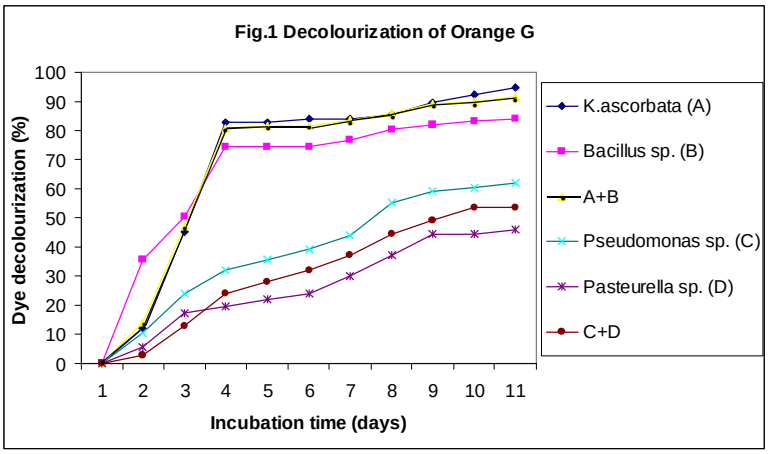


Fig.1 Decolourization of Orange G
Dye decolourization (%)
Incubation time (days)
K.ascorbata (A)
Bacillus sp. (B)
A+B
Pseudomonas sp. (C)
Pasteurella sp. (D)
C+D

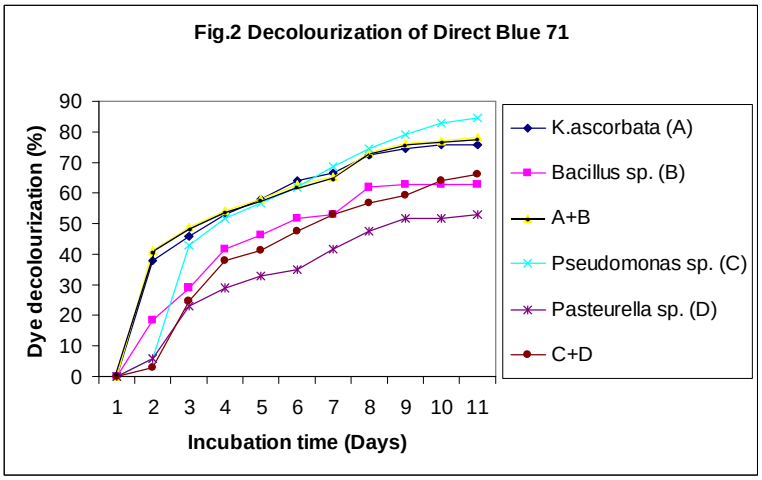


Fig.2 Decolourization of Direct Blue 71
Dye decolourization (%)
Incubation time (Days)
K.ascorbata (A)
Bacillus sp. (B)
A+B
Pseudomonas sp. (C)
Pasteurella sp. (D)
C+D

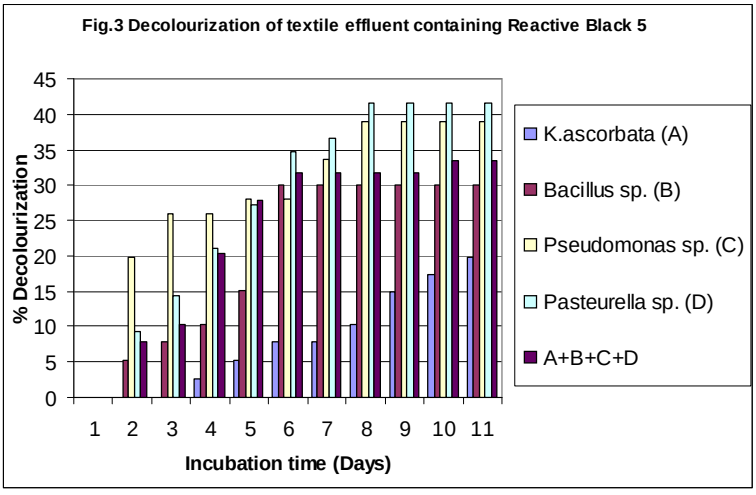
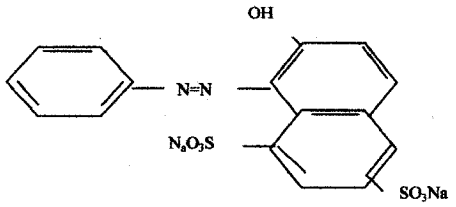
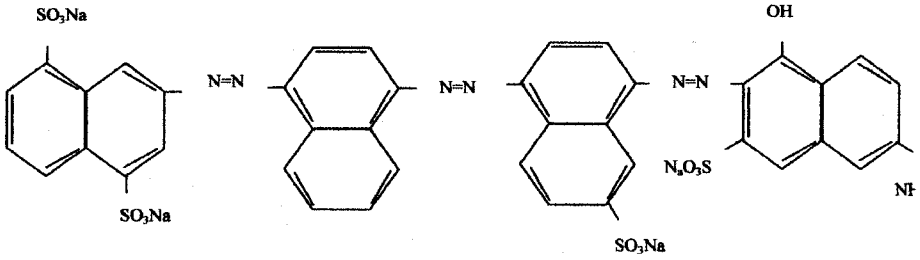


Fig.3 Decolourization of textile effluent containing Reactive Black 5
% Decolourization
Incubation time (Days)
K.ascorbata (A)
Bacillus sp. (B)
Pseudomonas sp. (C)
Pasteurella sp. (D)
A+B+C+D

(a) Orange G (OG)




(b)Direct Blue 71 (DB71)




(c) Reactive Black 5 (RB5)

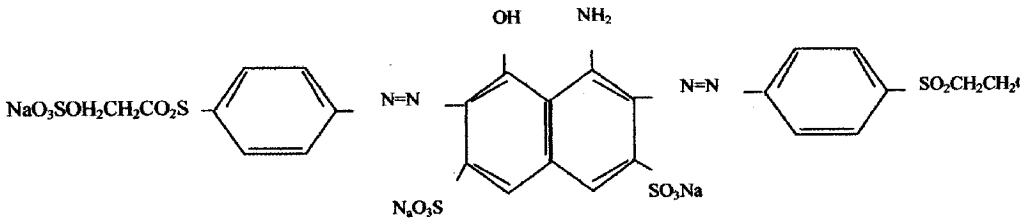



Fig. 4: Structural formulae of dyes used in the work.

shown) than the permissible levels as per Tamil Nadu Pollution Control Board (Eswaramoorthi et al. 2004) norms for textile dye effluent discharge into natural water systems. Maximum decolourization of 41.73 ± 1.07 was attained in the effluent by *Pasteurella* sp. on the 7th day followed by *Pseudomonas* sp. (39.03 ± 0.16) on the same day. *Bacillus* sp. proceeded decolourization only up to the 5th day and attained 30.94 ± 0.11% colour removal (Fig. 3). From 2nd day onwards, all the cultures were subjected to 30 minutes agitation in a mechanical shaker. On the subsequent days, the decolourization rate proceeded significantly. Settling of certain particles at the bottom of culture flasks was noticed in the treated samples after agitation. The reason for this setting and the role of flocculating agents present in the effluent needs to be analysed further.

The decolourization patterns showed by the 4 bacterial cultures were different in the 3 different dye media. Decolourization generally occurs by adsorption of dyestuff on bacteria (Yuxin & Jian 1998) and some bacteria can degrade dyestuff by azoreductase activity (Maier et al. 2004) or flavin reductase (Roxon et al. 1976, Walker 1970). Effectiveness of decolourization also depends on the structure and complexity of the dyes as reported by Kim et al. (1995). Results of the current study revealed that the selected species of bacteria were more capable in degrading Orange G than Direct Blue 71 as evidenced form the maximum percentage of dye removal achieved by individual bacterial

species. Previous reports attributed such results to the number of azo bonds (Oranusi & Ogugbue 2005). It is supposed that Orange G, an anionic monoazo component with a single azo bond (-N=N), was decolourised easier than Direct Blue 71 with trisazo bonds and Reactive Black 5 in the effluent is a diazo component (Fig. 4).

Though, the bacterial isolates were capable of decolourizing the dye to some extent, it was not able to achieve 100% decolourization in any of the crude treatments. Effectiveness of microbial treatment depends on survival, adaptability and activity of the selected organisms (Cripps et al. 1990, Pasti-Grigsby et al. 1992). Biodegradation of pollutants in natural ecosystems is influenced by innumerable environmental factors including pH, temperature, salinity, cations, anions, BOD and COD (Ganesh et al. 1994).

## CONCLUSION

Although, several bacteria are capable of azo dye degradation, very few strains can withstand the conditions of dyeing effluents in terms of the above mentioned extremes of parameters. The study contributes to the efforts of bringing out the phenomena of bacterial remediation of azo dyes merely from laboratory conditions to commercially applied field conditions.

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