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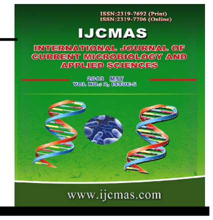


**Original Research Article**

# Biosorption of textile mill effluent by using indigenous microorganisms

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**A B S T R A C T**

Nowadays most of the textile industries consumes enormous amount of water and chemical substances for wet processing of textile industries. Textile effluents have higher density of organic and inorganic compounds and strong colour, caused by several residual dyes. Colour is imparted to textile effluents because of various dyes and pigments are generally used. Most of the dyes are visible in water concentrations as low as 1mg/litre. Most of the sediments, suspended and dissolved solids are also important repositories for toxic heavy metals. The presence of dyes and metals causes very serious damage to the aquatic and other ecosystem. Still, there is an urgency to need of valued research to unravel potential of various microorganisms for the remediation of natural sources. Sampling of effluent was carried out during dying and washing at three different stations in Erode region, Tamil Nadu state. pH and temperature of the effluents were determined at the sampling stations. The physico- chemical Characteristics of effluents were studied. Some effective microorganisms were screened out especially the potential strains for the degradation of textile dye effluents. In this context, the overall advantage in textile dye effluent colour was reduced by the degradation method. In the same time the toxicity of the textile effluents was reduced. The results are discussed with the available literature.

**Keywords**

Residual dye;
Biosorption;
Effluent;
Indigenous
microorganism.

## Introduction

Textile industries consumes large amount of water and chemicals for wet processing (AEPA, 1998). The chemical reagents used in textile sector are diverse in chemical composition ranging from inorganic to organic. The inputs of wide range of chemicals, which, if not incorporated in the final products, become waste and turn out to be part of water biology. Generally, textile effluent is contain high pH, temperature, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) reported by Ghoreishi and Haghighi (2003).

With the increased demand for textile products, the textile industries and its wastewaters have been increasing proportionately, making it one of the main sources of severe pollution problems worldwide (Wesenberg, 2002). Effluent treatments from dyeing and finishing processes in the textile industries is one of the most important environmental problems (Sen and Demirer, 2003). Since most of the synthetic dyes have complex aromatic molecular structures which make them inert and biodegradable difficult when discharged into the environment (Shenai, 1996). Through the textile processing employs a variety of chemicals depending on the nature of the raw material and product. Some of these chemicals are enzymes, detergents, dyes, acids, sodas and salts (Aslam *et al*., 2004). The high consumption of reactive dyes, mainly in the industries, increases the environmental problems, due to their low degree of exhaustion (60 - 90%) (Lopez-Grimau and Gutierrez, 2006).

Though some physicochemical processes have been shown to be effective in dye removal, their application is limited due to the excess usage of chemicals, sludge generation with subsequent disposal problems and high installation as well as operating costs and sensitivity to a variable wastewater input (Robinson *et al*., 2001).

The presence of very low concentrations of dyes in effluent is highly visible and undesirable (Nigam *et al*., 2000). There are more than 1,00,000 commercially available dyes with over $7\times10^5$ of dye-stuff produced annually (Zollinger, 1987). Due to their chemical structure, dyes are resistant to fading on exposure to light, water and many chemicals (Poots and McKay, 1976; McKay, 1979). Many dyes are difficult to decolourise due to their complex structure and synthetic origin. There are many structural varieties, such as, acidic, basic, disperse, azo, diazo, anthroquinone based and metal complex dyes. Discoloration of textile dye effluent does not occur when treated aerobically by municipal sewerage systems (Willmott *et al*., 1998).

Despite the existence of a variety of chemical and physical treatment processes, public acceptance of biotechnological treatment is better than any other chemical or physical treatment. Hence many investigators tried to solve the problem by biological methods by using bacterial strains (Georgiou and Aivasidis, 2006). The efficiency of actual biotechnological application, however, depends on its design, process optimization and cost minimization. As environmental regulation becomes stringent, novel processes are needed for efficient treatment of various kinds of dye effluent at relatively low operating costs. The present study is focused on the bacteriological evaluation of a local textile effluent. Self purification ability of the waste water is inspected besides analyzing the potential of different dyes resistant bacterial isolates at laboratory scale.

## Materials and Methods

### Collection of Sample

The sample was collected from the textile during the discharge of effluent near Pallipalayam region, Namakkal district, India by using water bag. The sample were immediately covered tightly and preserved at room temperature for further analysis.

### Sampling and analysis of effluent

Sampling of effluent was carried out during dyeing and washing at three sites. Standard procedures were followed during sampling. pH, temperature and other physicochemical parameters were determined instantly after bringing the samples in the laboratory.

**Isolation and identification of bacterial strains from the effluent**

Effluent samples collected from the textile industry were screened out for the isolation of potential bacterial strains. Samples after being serially diluted in sterile distilled water were plated onto nutrient agar plates and incubated for 48 h at 30˚C. Discrete bacterial colonies that grew on agar plates were initially grouped on the basis of gram staining and different morphological characteristics, such as pigmentation, motility and colony forms. Bacterial isolates were then picked, sub-cultured and subjected to further biochemical tests for identification according to Bergey's Manual of Determinative Bacteriology.

**Composition of mineral salt (ms) medium (Naeem *et al*, 2009)**

Mineral salt medium was made by adding per liter of distilled water; acetic acid (99.9%) 0.15 ml, (NH2)2CO 100.0 mg, KH2PO4 67.0 mg, NaHCO3 840.0 mg, MgSO4·7H2O 38.0 mg, CaCl2 21.0 mg, FeCl3·6H2O 7.0mg and Glucose 6.0 gm. Agar (1.5%) was added as solidifying agent.

**Screening of bacterial isolates against effluent** (Pasti-Grigsby *et al.*, 1996; Lee *et al.*, 2001)

The bacterial isolates were sequentially adapted to higher concentrations (10–10,000mgl$^{-1}$) of effluent after repeated sub-culturing on solid culture media of mineral salt and agar in petri plates. Salts used for Mn2+, Zn2+, Fe3+, Cd2+, Ni2+, Cr3+, Cr6+, Cu2+ and Pb2+ metal ions included MnSO4·H2O, ZnSO4·7H2O, K2Fe(CN)6, (CH3 COO)2Cd·2H2O, Ni(NO3)2·6H2O, Cr(NO3)3, K2Cr2O7, CuCl2·2H2O and Pb(NO3)2, respectively. The maximum resistance limits (MRL) beyond which the bacterial growth was completely inhibited at different concentration of dyes and metal ions were determined. In addition, apparent decolorization abilities of the bacterial strains for different dyes were also monitored at 37 ˚C for 8 days

Decolorization ability of the bacterial isolates was analyzed for effluent (at 20mgl$^{-1}$) in liquid culture of mineral salt (100 ml in 250 ml Erlenmeyer flask) under static condition for eight days. Freshly prepared inocula (10%) of selected bacteria in nutrient broth were taken and used in the experiments. Samples of treated dye containing medium were drawn into plastic vials (size 2.5 ml). These samples were initially filtered through 0.2m syringe filter. The residual amounts of each dye in medium was monitored though Shimadzu UV/VIS spectrophotometer at its respective max. Absorbance units were converted into concentrations by using standard curves and % decolorizations were determined by using the following formula:

$$\%\text{Decolorization} = \frac{\text{Initial concentration of dye} - \text{Final concentration of dye}}{\text{Initial concentration of dye}} \times 100$$

## Results and Discussion

**Physicochemical and microbiological status of textile effluent**

Apparently, the effluent samples was collected from the Pallipalyam region near by Erode were of true color, with disagreeable smell and temperature of about 24.6°C. The pH of the effluent was alkaline (7.48). Electric conductivity (EC) (µs/m) of the effluent was 7890. TSS in the effluent was 97mg/Lit and TDS were 5166mg/Lit. Overall, there was observed a significant high load of COD of about 1667mg/Lit than BOD of about 537mg/Lit though both followed by decreasing trend towards sink. The physicochemical parameters of the effluent sample was collected from the outlet of dyeing industry is compared with an environmental standards and shown in Table 1.

Physicochemical status of coloured effluent sample of Pallipalayam region revealed a reasonably high load of pollution indicators compared to the prescribed environmental standards (Table 1).

Initially the temperature of the effluent was generated from the Pallipalayam region was considerably high. However, declined to mesophilic, which could have favored biologically mediated remediation of effluent. High temperature reduces solubility of gases in water that ultimately express as high BOD/COD. BOD and COD levels recorded in effluent samples declined down the stream, specifically BOD which almost touched the permissible limits of standards. Nevertheless, high values of BOD/COD as observed in present case demands significant amount of dissolved oxygen for enhanced intrinsic remediation of wastewater.

Generally alkaline pH of textile effluents was associated with the process of bleaching and it is extremely undesirable in water ecology. Both chemically and biologically mediated adsorption and reduction of dyes were initiated with decreasing pH level under redox-mediating compounds. Decrease in pH of the effluent down the stream significantly improved the bacterial count and thereby associated remediation.

Conductivity was measured to establish a pollution zone around an effluent discharge. It is sensitive to variation in dissolved ions and mineral salts. Electric conductivity in effluent was considerably low and it gradually decreased and paralleled with decreasing metal ions concentrations thereby suggesting their biotic and abiotic removal. TSS and TDS in effluents corresponds to filterable and no filterable residues, respectively. There was observed increase in TSS in effluent from the source to sink compared to desired limits of standards. Though, decrease in TDS with an increase in bacterial count and declining pH and EC suggested process of flocculation (as TSS). Microbial community (both aerobic and anaerobic) establishes itself in granulated floc as activated sludge plays a vital role in biodecolorization /bioremediation of wastewater. The genus level of unknown bacteria isolated from effluent for decolourization were identified and confirmed by using various biochemical tests. The positive and negative result of different tests is shown in Table 2

The positive result in citrate test, nitrate test, starch hydrolysis, catalase test and the negative result in indole test and glucose fermentation tests confirmed that the unknown organism 1 was *Bacillus* spp. The negative result in nitrate, starch, catalase and positive result in glucose fermentation broth confirmed that the unknown organism 2 is of *Lactobacillus* spp. In the same way the positive and negative results in the biochemical tests

**Table.1** Comparison of physicochemical parameters of effluent with environmental standards

| S.No | Parameters | Sample | Domestic | Industrial |
|---|---|---|---|---|
| 1 | pH | 7.48 | 6.5-8 | 6 |
| 2 | Conductivity (µS/cm) | 7890 | 5000 | 5550 |
| 3 | Temperature (°C) | 24.6 | Source+5 | Source+5 |
| 4 | Total dissolved solids (mg/Lit) | 5166 | 500 | 500 |
| 5 | Total suspended solids (mg/Lit) | 97 | 50 | 100 |
| 6 | Biological oxygen demand (mg/Lit) | 537 | 150 | 300 |
| 7 | Chemical oxygen demand (mg/Lit) | 1667 | 250 | 750 |

**Table. 2** Result of biochemical tests

| Biochemical tests | Unknown Bacterial *Spp* | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** |
| Urease | – | + | + | – | – |
| Gelatin | – | – | – | + | – |
| TSI | + | – | + | – | + |
| Indole | – | – | – | – | + |
| Citrate | + | – | – | + | – |
| Nitrate | + | – | + | + | + |
| Starch | + | – | – | – | – |
| Catalase | + | – | + | + | + |
| Oxidase | – | – | – | + | + |
| Glucose fermentation test | – | + | + | – | + |
| Shape | Rod | Rod | Cocci | Rod | Rod |
| Gram positive/negative | + | – | + | – | + |

Where + indicates positive result and – indicates negative result.

**Table.3** Decolourization (%) of effluent in liquid culture medium by different bacterial strains

| **Wavelength** | **480nm** | **512nm** | **620nm** |
|---|---|---|---|
| **Bacteria** | **Average** | **Average** | **Average** |
| *Bacillus* spp. | 96.72 ± 1.41 | 85.27 ± 2.82 | 62.31 ± 1.11 |
| *Lactobacillus* spp. | 65.34 ± 0.11 | 53.01 ± 2.91 | 71.34 ± 2.69 |
| *Staphylococcus* spp. | 76.71 ± 0.57 | 61.38 ± 3.92 | 87.38 ± 1.57 |
| *Pseudomonas* spp. | 50.73 ± 0.81 | 78.13 ± 2.11 | 93.10 ± 2.60 |
| *E.coli* | 89.90 ± 0.64 | 90.08 ± 3.92 | 77.35 ± 7.85 |

**AVE ± SE**

**Table.4** Decolourization (%) at varying pH

| **Parameter** | **pH** | | | | |
|---|---|---|---|---|---|
| **Bacteria** | **1** | **2** | **3** | **4** | **5** |
| *Bacillus* spp. | 98 | 89 | 62 | 83 | 77 |
| *Lactobacillus* spp. | 71 | 64 | 76 | 77 | 61 |
| *Staphylococcus* spp. | 84 | 62 | 88 | 99 | 85 |
| *Pseudomonas* spp. | 60 | 90 | 73 | 68 | 90 |
| *E.coli* | 59 | 71 | 90 | 87 | 72 |

**Table.5** Decolourization (%) at varying temperature

| **Parameter** | **Temperature(°C)** | | | | |
|---|---|---|---|---|---|
| **Bacteria** | **30°C** | **40°C** | **50°C** | **60°C** | **70°C** |
| *Bacillus* spp. | 93 | 96 | 92 | 81 | 66 |
| *Lactobacillus* spp. | 95 | 98 | 91 | 83 | 70 |
| *Staphylococcus* spp. | 94 | 98 | 89 | 73 | 74 |
| *Pseudomonas* spp. | 80 | 83 | 77 | 69 | 64 |
| *E.coli* | 72 | 75 | 71 | 82 | 76 |

was confirmed that the unknown organism 3 was *Staphylococcus* spp., 4 were *Pseudomonas* spp. and 5 were *E.coli* respectively. The bacterial isolates was adapted to effluent after repeated sub-culturing on MS-agar plates shows that different percent of decolourization in liquid medium at a varying absorbance, pH and temperature and are shown from Table 3 to 5.

Most of the bacterial isolates from effluent were previously reported to be used in the degradation of dyes and related products. Bacterial isolates sequentially adapted against higher concentration of effluent on solid medium exhibited varying MRLs. At OD 480nm *Bacillus* spp. shows higher decolourization and *Pseudomonas* spp. shows lower percent of decolourization. At 512 nm *E.coli* shows higher percent of decolourization were as *Lactobacillus* spp. shows lower value. At 620 nm *Pseudomonas* spp. shows higher percent of decolourization.

Bacterial isolates sequentially adapted against higher concentration of effluent on solid medium exhibited varying MRLs. Besides, diffused decolorization halos around the bacterial colonies were evident in at least 8 days. Similarly, Pasti-Grigsby *et al.,* (1996) reported that slow biodecolorization of dyes on solid culture medium compared to liquid culture. Overall, biodecolorization abilities of the bacterial isolates confirmed through experiments in liquid broth under anoxic (static) conditions remained below 80%. It clearly indicated need of improvements in culture conditions (aeration and agitation) to further augment the decolorization processes.

The textile mill effluent was collected from Pallipalayam region near by Erode clearly imparting a high load of chemicals indicated in the form of different pollution indicators. But, the phenomenon of natural remediation seemed to be occurring on-site and it was furthermore confirmed through laboratory studies where the bacterial isolates indicated high resistant and transformation abilities for dyes. A detailed physiological understanding of such microbes is much needed for standardization of bioremediation technologies in future.

The experimental result shows that the integrated biological method with electrochemical oxidation and photo catalytic oxidation is a viable alternate to reduce colour of the dye effluent containing the bio-recalcitrant reactive dye. The overall colour reductions obtained at end of the sequential study for the different effluent streams respectively to the effluent streams containing bacterial strains. Further, the reductions in colour obtained respectively to the effluent streams containing bacteria. Biosorption method is useful not only to reduce the pollution load but also to remove microbes. Hence the treated effluent can be reused in the rinsing section of dye house after the removal total dissolved solids.

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