Decolourization, degradation and removal of heavy metals of textile effluent with the help of mixed bacterial consortium

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This paper discusses the role of mixed bacterial consortium in decolourisation, degradation and removal of heavy metals from textile effluent. The bacterial strains, used as consortium, were isolated from textile effluent and were comprised of Enterobacter asburiae and E. cloacae. This bacterial consortium (0.1 g/100 mL bacterial consortium biomass) efficiently decolorized (up to 98%) the effluent under aerobic condition within 10 min, at pH 1.67 and 32°C. Ultraviolet (UV) visible analysis, fourier transform infrared spectroscopy (FTIR) study and colourless bacterial cells indicate the capacity of bacterial consortium to decolorize the textile effluent through biodegradation instead of adsorption on the surface. Cytotoxicity and phytotoxicity studies also signify the lesser toxicity of textile effluent after the treatment with bacterial consortium. Thus the present bacterial consortium can be successfully employed for decolourisation, biodegradation and metals removal from acidic textile effluents.

Keywords: Decolourization, degradation, heavy metals removal, mixed bacterial consortium, textile effluent

Introduction

Textile industries generate huge quantity of dye effluent. Dyes contain carcinogenic amines, pentachlorophenol, halogen carriers, bleaching chlorine, biocides, formaldehyde, softeners and fire retardants along with a number of heavy metals1. Textile waste water also contains organic and inorganic chemicals, which cause biological and chemical oxygen demand in receiving water2. Dyes cause serious human health hazards like cancer, tumor formation and allergies3. The heavy metals like Cr, Ni, Zn and Cu present in the form of various complexes or free ionic metals in the textile waste water are hazardous for environment4. Therefore, degradation of dye and removal of its colour and heavy metals is imperative.

A number of physico-chemical methods have been reported for the treatment of textile effluents. These methods are not only expensive but also produce secondary sludge. The heavy metals can be removed from the textile effluent through techniques like adsorption, ion exchange, precipitation and through membrane processes5. Tarley et al6 considered such methods to be labour intensive operation and have low efficiency. However, bioremediation is an environmental friendly and cheaper technology for textile effluent treatment. In recent years, bacteria have been reported to work as a cheaper, efficient and ecofriendly tool for textile effluent treatment2-8. Microbial consortia are found to be more efficient compared to the pure culture for the degradation of textile effluent2.

In the present investigation, bacteria were isolated from the textile effluent and their consortium was used for decolourisation, degradation and removal of heavy metals from the textile effluent.

Materials and Methods

Sample Collection

Textile effluent samples were collected in sterilized plastic bottle from a textile industry of Varanasi district (Uttar Pradesh), India. The samples were mixed together to make a composite sample and transported to the laboratory. There it was kept in a refrigerator following standard procedure10 till further use.

Chemicals

All the culture media including glucose, peptone, yeast extract, nutrient broth, potassium bromide (KBr) and paradichlorobenzene were procured from HiMedia Laboratory, Delhi, India.

Physico-chemical Analysis

The physico-chemical parameters like pH, oxidation-reduction potential (ORP), conductivity,
total dissolved solid (TDS) and salinity were measured immediately at the site using water analysis kit procured from Decibel Dynamics Ltd., New Delhi (India). The heavy metals, such as, Cu, Cr, Ni, Zn, Co, Pb, Cd, Mn and Fe, of dye effluent were measured in the laboratory within 24 h of the collection of effluent following standard method (Eaton et al11) in an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 800 Make, Singapore).

Isolation, Identification and Enrichment of Textile Effluent Decolourizing Bacteria

The effluent sample collected from textile industry was screened out for isolation of highly efficient bacterial strains. The effluent sample was diluted 10^6 times in sterile distilled water and was inoculated on agar plates containing glucose 1%, peptone 0.5% and yeast extract 0.5% (GPY) nutrient. The plates were incubated for 24 h at 37°C and the bacterial colonies grown on the agar plates were picked up. The purified bacterial isolates were characterized by standard microbiological methods and one of them was identified as *Enterobacter cloacae* on the basis of 16S rDNA study from the Indian Institute of Vegetable Research (IIVR), Varanasi. Another bacterium was also isolated from the same textile effluent using similar methodology and was identified as *E. asburiae* by the Institute of Microbial Technology (IMTECH), Chandigarh, India. Mass cultivation of both the strains was carried out to treat the textile effluent.

Genomic DNA Isolation and 16S rDNA Gene Amplification and Sequencing

Genomic DNA was isolated using GeneiPure™ bacterial DNA purification kit (GeNei™, Bengaluru, India) following the manufacturer’s protocol. Universal eubacterial primers12 F-D1: 5’-ccgaattctgcacaacagagttagcttgctgctag-3’ and R-D1: 5’-ccggtatccagtgaggtgatcagcc-3’, were used to amplify 1500 bp region of 16S rRNA gene using a thermal cycler (BioRad, USA). Amplification products were resolved by electrophoresis in agarose (1%) and visualized using a gel documentation system (Alfa Imager, Alfa InfoTech Corporation, USA). The amplicons were purified using GeneiPure™ quick PCR purification kit (GeNei™, Bengaluru, India) and quantified at 260 nm using a spectrophotometer taking calf thymus DNA as a control. The purified partial 16S rDNA amplicon was sequenced in an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems®, CA, USA).

Analysis of 16S rDNA sequences

The partial gene sequences were compared with sequences from DNA databases and sequences showing >95% similarity were retrieved by Nucleotide Basic Local Alignment Search Tool (BLAST) program available at the National Center for Biotechnology Information (NCBI) BLAST server (www.ncbi.nlm.nih.gov/BLAST).

Preparation of Bacterial Consortium

Both the bacterial strains (*E. cloacae* R6-355 & *E. asburiae* AB004744) were grown separately overnight in GPY medium. A mixed bacterial consortium was prepared by mixing 1 mL inoculum of each strain in 10 mL GPY broth and incubated at 30°C for 24 h with continuous shaking at 120 rpm.

Acclimatization of Bacterial Consortium

The bacterial consortium was inoculated in 200 mL GPY growth medium at pH 7 containing 5 mL of dye effluent and incubated for 10 h at 37°C. The optical density (OD) of acclimatized bacterial consortium was allowed to reach 0.7 at 660 nm wavelength.

Decolourization Assay

The textile effluent (20 mL) was mixed with nutrient broth (10 mL) at pH 7 under sterile condition. Then it was treated with acclimatized bacterial cell pellets harvested in mid exponential phase. The bacterial biomass of 1 g/L at 35±2°C was used for different contact periods of colour removal. The bacterial treated (2 mL) textile effluent sample was withdrawn at 5 min interval and centrifuged at 10,000 rpm for 5 min. Subsequently, supernatant was measured optically at 490 nm λ max of dye effluent using Hitachi U-2900 spectrophotometer.

Metal Estimation

Textile effluent was digested with HNO₃:HClO₄ (10:1) for complete metal digestion by the method of Eaton et al11 and filtered with Whatman filter paper. The supernatant was analyzed for heavy metals like Pb, Cd, Cr, Ni, Zn, Mn and Fe in an Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer AAnalyst 800, Singapore). Mixed bacterial consortium treated textile effluent was centrifuged and supernatant was analyzed for the determination of heavy metals. The analyzed data was compared with control samples (textile effluent) and water standards of the Environment (Protection) Rules, 1986.

UV-Vis and FTIR Spectroscopic Studies

Untreated textile effluent (control) and treated effluent with 1 g/L mixed bacterial consortium biomass
were separately centrifuged at 10,000 rpm. The UV-Vis absorption spectra of the supernatant of untreated and treated textile effluents were recorded using a UV-Visible spectrophotometer at ambient temperature. Textile effluent treated with 1 g/L bacterial consortium biomass for 10 min was centrifuged at 10,000 rpm and supernatant was freeze-dried. The freeze-dried sample was used for FTIR study. The pellets prepared with KBr were observed under the FTIR spectra in the range 400-4000 cm⁻¹.

Cytotoxicity Study

For cytotoxicity study, bulbs of *Allium cepa* (diam 20-25 mm, wt 8-10 g) were obtained from local market and washed properly in running tap water. The loose outer scales were removed before use and the dry bases were scrapped. For root tip growth, such three onion bulbs were partially embedded in sterile sand and separately wetted with tap water, effluent (100%) and bacterial treated supernatant of textile effluent for 5 d. The experiment was carried out in triplicate. After sufficient growth, the root tips were excised and kept in saturated paradichlorobenzene (PDB) containing 0.1% scoline at 4°C for 4 h. Subsequently, they were washed thrice with double distilled water and fixed in acetic acid and ethanol (1:3). After 24 h, fixative was poured off and 70% alcohol was added to the vial for preservation of the root tips. Slide was prepared for microscopic observation using acetocarmine and 1 N HCl.

Phytotoxicity Assay

The toxicity of the degraded product can be defined using phytotoxicity assay. Therefore, seeds of *Triticum aestivum* (wheat) var. Balram-011, *Phaseolus mungo* (urdu bean) var. Malviya-36 and *Vigna radiata* (mung bean) var. HUM-12 were collected. These seeds were surface-sterilized with sodium hypochlorite (0.1%) for 2 min to avoid microbial contamination and then rinsed with distilled water (thrice). The sterilized seeds were soaked in sterile distilled water for 1 h. Empty and undeveloped seeds floating in sterile distilled water were discarded before transferring to the surface of the sterilized filter paper in Petri plates. Two layers of sterile filter paper moistened with 10 mL of distilled water was placed at the bottom of each sterile Petri plate (9 cm). Phytotoxicity assay of test seeds (10 seeds per plate) was performed at room temperature (32±2°C) using effluent, bacterial treated effluent and double distilled water as control. Length of plumule (shoot) and radical (root), germination index and germination percentage were recorded every day.

**Statistical Analysis**

The data represented mean of triplicate experiments and mean±SE was subjected to one way ANOVA analysis. Means were separated by Tukey’s multiple range test when ANOVA was significant (p≤0.05) (SPSS 16.0; Chicago, IL, USA).

**Results and Discussion**

**Identification of Dye Decolorizing Bacterial Strain Isolated from Dye Effluent**

The bacterial strains isolated from the textile effluent were identified on the basis of 16S rDNA gene sequence. The 1358 base pair gene sequence obtained from one isolate showed 99.26% similarity with the gene sequence of *E. asburiae* AB004744 located in the ribosomal database (Fig. 1). However, the 2457 base pair gene sequence obtained from the other isolate was found to be 100% identical to the gene sequence of *E. cloacae* R6-355 of ribosomal database (Fig. 2).
Decolourization Assay

The decolourization of textile effluent by mixed bacterial consortium was carried out for various contact time (Fig. 3). The colour removal mechanism was observed in two phases: in the first phase, the rate of removal was optimum in 10 min; in the second phase, it attained the saturation phase after a contact time of 15 min. Therefore, experiment was not extended further. It was seen that 1 g/L of the mixed bacterial consortium biomass was efficient to remove 98.89% acidic textile effluent colour within 10 min (Fig. 3).

It was also observed that the pH of reaction mixture increased from 1.63 to 1.74 in 5 min (Table 1). The rise in pH could be attributed to release of carbon dioxide (CO$_2$) and the subsequent formation of carbonic acid (H$_2$CO$_3$), thereby establishing equilibrium within the atmosphere. Moreover, during the colour removal process, surface interaction happened between the cell surface and dye molecules.

Bacterial consortium has been found more efficient in colour removal from textile effluents as compared to the individual strains of bacterium, probably because of enhanced effect of coordinated metabolic acquaintances. In the present investigation, the bacterial consortium could remove 98% of colour within 10 min; while in other studies, it took 7 to 21 d by white rot fungi and other bacteria. It has also been shown that the consortium decolourizes textile effluent through the process of biotransformation and biodegradation, and the process is controlled by the enzymatic activity.

Metal Removal with Help of Bacterial Consortium

Bacterial consortium acts as a tool for the removal of metals from the effluent and the removal is performed through active and passive pathways. The active pathway is energy dependent process and it transports metals across the cell membrane inside the cell. The bacteria accumulate heavy metals inside the cell in polyphosphate bodies for their metabolic needs and for various enzymatic activities. However, in the passive way, metals binds on bacterial surface only. In the present study, metal concentrations like Pb, Cd,
Cr, Ni, Zn, Mn and Fe were reduced under permissible limit in the mixed bacterial consortium treated textile effluent as compared to non treated textile effluent (Table 1).

UV-Vis and FTIR Spectroscopic Studies

Almost complete disappearance of the peaks at ~490 and at ~650 nm in the corresponding UV-Vis spectrum of the treated textile effluent (Fig. 4) strongly indicates the degradation of dye effluent due to the action of bacterial consortium. The decolourization of dyes may occur by adsorption\(^{20}\) or degradation\(^{21}\). In case of adsorption, dyes are only adsorbed on the surface of bacterial cells, whereas formation of new compounds is the result of dye degradation by bacterial enzymes. Absorption spectrum peaks will decrease in proportion to each other in case of adsorption. If the dye removal is through biodegradation, then major visible light absorption peak will completely disappear or new peaks will appear\(^{22}\). Dye adsorption can also be easily observed by clearly coloured cell pellet, whereas those retaining their original colours are accompanied by the occurrence of biodegradation\(^{22}\). In the present investigation, 98% decolourization was achieved in 10 min and the bacterial cell pellets were not pigmented. These results strongly support the evidence of biodegradation of textile effluent by mixed bacterial consortium.

The FTIR spectrum of textile effluent had the characteristic sharp peaks at 3445, 1636.7, 1123.63, 1096 and 599.76 cm\(^{-1}\). In the textile effluent, the amine group is supported by the peak at 3417.18 cm\(^{-1}\) for N-H stretching vibrations, 1633.94 cm\(^{-1}\) for stretching of amines, 1403.26, 1167.89, 1057.80 cm\(^{-1}\) for C-F bending for alkyl halide, 952.54 cm\(^{-1}\) for =C-H bending for alkenes, 880.03 cm\(^{-1}\) for aromatic benzene vibrations, and 672.62, 615.52, 603.36, 592.33, 451.69 cm\(^{-1}\) for chloroalkanes vibrations.

In mixed bacterial consortium treated effluent, stretching was observed in amines and it was supported by the peak at 1730.4 cm\(^{-1}\). Aromatic stretch in C=C was supported by the peak 1403.5 cm\(^{-1}\). Peak at 1172.11 cm\(^{-1}\) was an evidence of C-O bond stretching of ester. Peak at 1059.69 cm\(^{-1}\) supported the formation of primary alcohol. Aromatic C-H meta-disubstituted benzene was evidenced by the peak at 874.40 cm\(^{-1}\), however 591.60 cm\(^{-1}\) peak indicated the formation of bromo alkanes. The formation of new peaks in the treated textile effluent as compared to untreated textile effluent and complete lack of five peaks of chloroalkanes was the result of degradation of textile effluent by bacteria.

Reduction in Textile Effluent Toxicity after Treatment

Use of textile effluent water is hazardous even for irrigation of agricultural field. Cytotoxicity test on A. cepa was carried out in the presence of bacterial consortium treated and untreated textile effluents. In the present study, textile effluent had a negative effect on the cell division of A. cepa. Consequently, abnormalities were seen in the anaphase of cell division like presence of bridges and laggard’s chromosome, alteration in telophase stages, loss of chromosomes and binucleated cells (Fig. 5). Several

![Fig. 4](image-url)  
Fig. 4 — UV-Visible spectra of untreated and treated textile effluents with mixed bacterial consortium.

![Fig. 5](image-url)  
Fig. 5 (A-F) — Aberrations induced by textile effluent in A. cepa root tips: A, Normal metaphase; B, Normal anaphase; C, Normal telophase; D, Spindle abnormalities in anaphase; E, Vagrant chromosome in telophase; & F, Anaphase-telophase bridge.
earlier studies have also reported such effects on cell division\textsuperscript{24-25}. Dyes directly affect the spindle apparatus as a result of increase in polyploidy in cells. Chromosomal aberrations were observed when \textit{A. cepa} was treated with azo dyes contaminated industrial effluent\textsuperscript{26,27}. Abnormalities produced in \textit{A. cepa} under mixed bacterial consortium treated and untreated effluents are shown in Table 2. Under treated dye effluent condition, a significant reduction in the number of total aberrant mitotic cells was observed. Further, phytotoxicity studies revealed that the number of germination and length of radical and plumule of \textit{V. radiata}, \textit{T. aestivum} and \textit{P. mungo} were less in untreated effluent as compared to the mixed bacterial consortium treated effluent (Table 3). It further supports the evidence of degradation of textile effluent with the bacterial consortium.

### Conclusions

In the present investigation, mixed bacterial consortium comprising of \textit{E. asburiae} and \textit{E. cloacae} was used for the treatment of effluent. The bacteria were isolated from the textile effluent. FTIR spectroscopic study reveals the formation of new functional groups in the bacterial consortium treated textile effluent. Five peaks (672.62, 615.52, 603.36, 592.33, 451.69 cm\textsuperscript{-1}) of chloro alkanes completely disappeared due to degradation of the dye effluent. The bacterial consortium could decolorize 98.89% acidic textile effluent within 10 min through degradation. The heavy metals like Pb, Cd, Cr, Ni, Zn, Mn and Fe were reduced substantially. A significant reduction in the number of total aberrant mitotic cells was observed in the \textit{A. cepa} root tip. Phytotoxicity test reveals a decrease in the number of germination and length of radical and plumule of \textit{V. radiata}, \textit{T. aestivum} and \textit{P. mungo} in the untreated effluent as compared to the treated effluent.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Control</th>
<th>Treated</th>
<th>Effluent</th>
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<tbody>
<tr>
<td>Anaphase bridge</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Binucleated cell</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Trinucleated cell</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Quadrinucleated cell</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vagrant chromosome</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Vagrant chromosome in telophase</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Spindle disturbance in telophase</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sticky chromosome</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Penta nucleated cell</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total cell of abnormality(TCA)</td>
<td>5</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td>Total mitotic cells observed</td>
<td>94</td>
<td>75</td>
<td>80</td>
</tr>
<tr>
<td>Frequency of TCA</td>
<td>5.31±0.57\textsuperscript{c}</td>
<td>16±0.33\textsuperscript{b}</td>
<td>33.75±2.51\textsuperscript{a}</td>
</tr>
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</table>

| Table 2 — Effect of untreated and mixed bacterial consortium treated textile effluent on number and frequency of chromosome aberrations obtained for the \textit{Allium cepa} tests |

<table>
<thead>
<tr>
<th>Days</th>
<th>Plume</th>
<th>Radicle</th>
<th>Plume</th>
<th>Radicle</th>
<th>Plume</th>
<th>Radicle</th>
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<tbody>
<tr>
<td>\textit{V. radiata}</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>NF</td>
<td>1.06±0.006\textsuperscript{a}</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>0.747±0.02\textsuperscript{c}</td>
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<tr>
<td>2</td>
<td>0.053±0.003\textsuperscript{b}</td>
<td>1.90±0.058\textsuperscript{b}</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>1.16±0.08\textsuperscript{d}</td>
</tr>
<tr>
<td>3</td>
<td>0.123±0.002\textsuperscript{b}</td>
<td>2.86±0.009\textsuperscript{a}</td>
<td>NF</td>
<td>NF</td>
<td>0.197±0.009\textsuperscript{a}</td>
<td>1.7±0.058\textsuperscript{c}</td>
</tr>
<tr>
<td>\textit{P. mungo}</td>
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<td></td>
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</tr>
<tr>
<td>1</td>
<td>NF</td>
<td>0.66±0.025\textsuperscript{b}</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>0.43±0.007\textsuperscript{b}</td>
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<td>NF</td>
<td>1.09±0.009\textsuperscript{ab}</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>1.4±0.06\textsuperscript{ab}</td>
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<tr>
<td>3</td>
<td>0.45±0.05\textsuperscript{a}</td>
<td>1.62±0.019\textsuperscript{a}</td>
<td>NF</td>
<td>NF</td>
<td>0.31±0.023\textsuperscript{b}</td>
<td>0.59±0.44\textsuperscript{bc}</td>
</tr>
<tr>
<td>\textit{T. aestivum}</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>NF</td>
<td>1.516±0.012\textsuperscript{b}</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>0.95±0.12\textsuperscript{d}</td>
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<tr>
<td>2</td>
<td>1.09±0.001\textsuperscript{b}</td>
<td>1.56±0.18\textsuperscript{b}</td>
<td>NF</td>
<td>NF</td>
<td>0.863±0.15\textsuperscript{c}</td>
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</tr>
<tr>
<td>3</td>
<td>1.60±0.014\textsuperscript{a}</td>
<td>1.76±0.014\textsuperscript{a}</td>
<td>NF</td>
<td>NF</td>
<td>1.103±0.05\textsuperscript{b}</td>
<td>1.26±0.02\textsuperscript{c}</td>
</tr>
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</table>

NF= Not Found
Acknowledgement

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References