Bioresource Technology 171 (2014) 217–226

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# Novel fungal consortium for bioremediation of metals and dyes from mixed waste stream

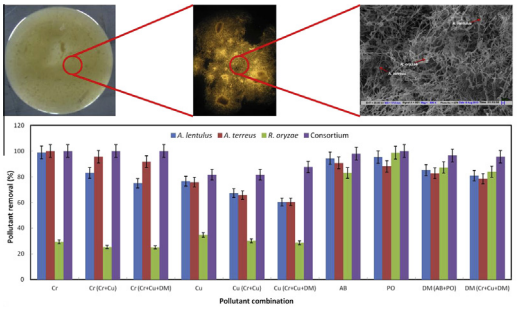
Abhishek Mishra, Anushree Malik *

*Applied Microbiology Lab, Centre for Rural Development and Technology, Indian Institute of Technology Delhi, New Delhi 110 016, India*

## HIGHLIGHTS

- *A. lentulus*, *A. terreus* and *R. oryzae* are compatible and form a stable consortium.
- First report utilizing fungal consortium for metals and dyes removal from mixtures.
- Pollutant removal efficiency improved using consortium as compared to individual.
- Faster and higher multiple pollutant uptake from metal–dye mixture using consortium.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 4 June 2014
Received in revised form 5 August 2014
Accepted 9 August 2014
Available online 25 August 2014

*Keywords:*
Consortium
Bioremediation
Heavy metals
Dyes
Mixed waste stream

## ABSTRACT

The present study is targeted towards development of a three member fungal consortium for effective removal of metals [$Cr^{6+}$ and $Cu^{2+}$] and dyes [AB and PO] from mixed waste streams. Initial studies using individual fungal strain showed that *Aspergillus lentulus* was best for $Cu^{2+}$ and AB removal, *Aspergillus terreus* for $Cr^{6+}$ removal whereas, *Rhizopus oryzae* was best for PO removal. Based on the complementary pollutant affinities and positive interactions, a consortium comprising all three strains was developed. Consortium removed 100% $Cr^{6+}$ and 81.60% $Cu^{2+}$ from metal mixture which was significantly higher than that achieved individually by *A. lentulus* ($Cr^{6+}$: 83.11%; $Cu^{2+}$: 67.32%), *A. terreus* ($Cr^{6+}$: 95.57%; $Cu^{2+}$: 65.77%) or *R. oryzae* ($Cr^{6+}$: 25.34%; $Cu^{2+}$: 30.20%). Further, 98.0% AB and 100.0% PO was removed after 48 h by the consortia. Unlike individual strains, consortium's performance was unaltered irrespective of the complexity of metal–dye mixtures, thereby establishing its superiority.



## 1. Introduction

In the recent years extensive research and development has occurred on biological methods as an eco-friendly alternative for remediation for heavy metal (Mishra and Malik, 2013; Van Nostrand et al., 2007) and dyes pollutants (Kaushik and Malik, 2009). In this context, fungi offer an efficient system due to large surface area and easy solid–liquid separation (Mishra and Malik, 2013). Fungi also possess multiple mechanisms for degradation of organic and inorganic contaminants (Awasthi et al., 2014). However, pollutant removal has been largely studied under single pollutant exposures and often using pure culture (Singh and Singh, 2014; Mishra and Malik, 2012). These studies demonstrate the utility of a certain fungal strains for removing a particular pollutant. However, fungi capable of removing a given metal effectively may not be equally effective in removing other metal or dye. Moreover, there are wide variations in the metal or dye uptake capacity among various strains. Industrial effluents are cocktail of various metals and organic contaminants (Ruta et al., 2010; Yadav et al.,

* Corresponding author. Address: Centre for Rural Development and Technology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110 016, India. Tel.: +91 11 26591158; fax: +91 11 26591121.
*E-mail address:* anushree_malik@yahoo.com (A. Malik).

http://dx.doi.org/10.1016/j.biortech.2014.08.047
0960-8524/

2010). For example, textile industry effluents contain both residual dyes (from dyeing operations) and metals (used as mordant). Also, pulp and paper industries, tannery industries and dyeing industries are some other industries generating effluents rich in metals as well as dyes (Mishra and Malik, 2014). Therefore, in order to develop a biological system capable of remediating such wastewater, diverse types of microbial strains need to be used in the form of consortia (Mahapatra et al., 2014). The use of consortium has a clear advantage for bioremediation applications as a richer metabolic network can be preserved and exploited for the bioremediation of co-contaminated matrices. Therefore, in a mixed waste stream, each of the consortium partners can specialize for uptake of a particular contaminant. As a result, simultaneous removal of several contaminants can be successfully accomplished (Mishra and Malik, 2014).

Use of multiple species consortia has proved advantageous for higher metal scavenging and more stability against environmental fluctuations, however, the investigations in this direction are very scanty. Sprocati et al. (2006) isolated seven microbial consortia from an abandoned mine through a selection for resistance to zinc. The best accumulator consortium, named Ing5, was investigated and it was observed that resistance thresholds were higher in consortium than in pure culture. Pan et al. (2009) compared the growth and metal removal individually by two fungi (*Penicillium* sp. A1 and *Fusarium* sp. A19) with that obtained through their consortium. The authors hypothesized that the different resistances to metals between A1 and A19 could be attributed to different detoxification mechanisms of the two fungi. Therefore, the co-inoculation of A1 + A19 resulted in higher resistance as well as bioaccumulation of multi-metals than single A1 or A19.

In addition, the consortium approach has also proved to be useful in biological treatment of other organic wastes via protection rendered by the metal accumulating strains to the organic degrading bacteria. Metal accumulating strains in conjunction with *Ralstonia eutropha* JMP134 have been employed (Roane et al., 2001) for a dual-bioaugmentation strategy to enhance remediation of contaminated soil containing $Cd^{2+}$ and 2,4-dichlorophenoxyacetic acid (2,4-D). A fungal consortium-SR consisting of *Trametes* sp. SQ01 and *Chaetomium* sp. R01 was developed for decolorizing of triphenylmethane dyes, which were decolorized by individual fungi with low efficiencies. Consortium had a decolorization rate of 63–96%, much higher than that of the monoculture of strain SQ01 (38–72%) (Yang et al., 2011). However, no study on the use of consortium in metal–dye matrix has been reported. Thus, this research was aimed at studying the development of fungal consortium and its efficacy over individual strains in terms of heavy metal and dye removal from mixed waste stream.

## 2. Methods

### 2.1. Chemicals

Two metals [$Cu^{2+}$ and $Cr^{6+}$] and two dyes [Acid Blue 161 (AB) and Pigment Orange 34 (PO)] were used in the present study. The properties and structures of these dyes are depicted in Table A.1. Both the dyes are water soluble and frequently used in textile industries. The stock solutions of 10 g $L^{-1}$ were prepared for each metal and dye in double distilled water. All the other chemicals used were of analytical grade and were obtained from Merck and Qualigens.

### 2.2. Isolation of efficient microbial strains for metal and dye removal

Soil samples (in duplicate) collected from dumping site of Indian Institute of Technology Delhi, India were used for isolation of microbes through serial dilution method. Sterilized nutrient agar (NA) and potato dextrose agar (PDA) amended with 50 mg $L^{-1}$ of the metal mixture [25 mg $L^{-1}$ each of $Cr^{6+}$ and $Cu^{2+}$] were used for selection of metal tolerant bacterial and fungal isolates, respectively. Agar plates were incubated at 30 °C and monitored after 24 h for bacterial and 96 h for fungal colonies. The microbial colonies that appeared on the plates were isolated, purified and subjected to further screening and selections.

### 2.3. Selection of efficient isolates

The purified isolates were screened and selected on the basis of metal tolerance and pollutant removal efficiency. Tolerance to heavy metals was determined in terms of the minimum inhibitory concentration (MIC) of metals for five bacterial and five fungal isolates. Metal solutions of required concentration were prepared by dissolving their respective salts, potassium dichromate [$K_2Cr_2O_7$] and copper sulfate [$CuSO_4{\cdot}5H_2O$] in double distilled water. In order to estimate the MIC, nutrient broth (NB) and composite broth medium (CBM) (glucose 10.0 g $L^{-1}$, yeast extract 2.5 g $L^{-1}$, $NH_4NO_3$ 0.5 g $L^{-1}$, $MgSO_4{\cdot}7H_2O$ 0.1 g $L^{-1}$, $K_2HPO_4$ 0.5 g $L^{-1}$, NaCl 1.0 g $L^{-1}$, pH 6.5 ± 0.2) were amended with respective metals to achieve the desired concentration ranging from 0.05 to 15.0 g $L^{-1}$. These flasks were inoculated with 1% (v/v) bacterial and fungal inoculum ($10^6$ cfu $ml^{-1}$) and incubated at 30 °C and 150 rpm on an orbital shaker. Bacterial and fungal growth was monitored daily for the next 2 d and 5 d, respectively. MIC was defined as the minimum concentration of the heavy metal that inhibited the growth of isolates.

### 2.4. Characterization and identification of microbial isolates

Based on the MIC results and pollutant (metal as well as dye) removal efficiency, two fungal strains (F2 and F3) were selected for molecular characterization. DNA was isolated from the agar plate using the Fungal Genomic DNA isolation kit (Qiagen). Using consensus primers, the ~1300 bp; 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA gene fragment was amplified using high-fidelity PCR Polymerase (White et al., 1990). PCR amplification was performed in 50 µl reaction mixtures containing approximately 20 ng of genomic DNA template, 1× PCR buffer with 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 µM of each primer. PCR cycling conditions were 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min, followed by an extension step of 72 °C for 10 min. The amplified DNA was purified using Qiaquick PCR Purification Kit (Qiagen) and sent for sequencing. Gene sequence of fungal strains was compared with the sequence of already reported strains, and a phylogenetic tree was constructed by Molecular Evolutionary Genetics Analysis (MEGA) software version 5.0 (Tamura et al., 2011).

### 2.5. Kinetic study in presence of metal and dye

Detailed investigations on growth and pollutant removal kinetics were conducted using identified fungal strains (*Aspergillus terreus* and *Rhizopus oryzae*). A previously isolated and identified strain *A. lentulus* FJ172995 (Sharma et al., 2011) was also used.

To evaluate the growth and pollutant removal kinetics of fungal strains (*A. lentulus*, *A. terreus* and *R. oryzae*), several sets of 250 ml flasks containing 100 ml CBM amended with individual metal ($Cr^{6+}$ or $Cu^{2+}$: initial concentration 100 mg $L^{-1}$) and dye (AB or PO: initial concentration 100 mg $L^{-1}$) were used. After sterilization, CBM was inoculated with 1% (v/v) spore suspension ($10^6$ - spores $ml^{-1}$) of the respective fungal strains and incubated at 150 rpm and 30 °C. Flasks were removed at regular intervals and the samples were analyzed for residual metal or dye concentration,