DEVELOPMENT OF BIOLOGICAL SYSTEM EMPLOYING MICROBIAL CONSORTIUM FOR POLLUTANT REMOVAL FROM MIXED WASTE STREAM

by

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Dedicated to

Scientific Community

CERTIFICATE

This is to certify that the thesis entitled **"Development of biological system employing microbial consortium for pollutant removal from mixed waste stream"** being submitted by **Mr. Abhishek Mishra** to the Indian Institute of Technology Delhi for the award of the degree of 'Doctor of Philosophy' is a record of bonafide research work carried out by him. He has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis. To the best of my knowledge the results contained in this thesis have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

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Akhanndda-Mannddala-Akaaram, Vyaaptam Yena Cara-Acaram ||

Tat-Padam Darshitam Yena, Tasmai Shrii Gurave Namah ||

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ABSTRACT

The present study is targeted towards development of a compatible consortium for simultaneous removal of metals and dyes from mixed waste streams. Hence, systematic studies were conducted in relatively complex situations viz single and binary pollutant uptake in terms of metal-metal and dye-metal combinations as well as the performance of individual strain versus the consortium. Initially a previously isolated robust strain (*Aspergillus lentulus*) was investigated for its performance under multiple metal stresses. Results established that *A. lentulus* had remarkable tolerance against Ni(II), Cu(II), Pb(II), and Cr (III) and it also showed significant growth coupled metal uptake. Under the binary metal stress (Cu(II) and Ni(II) at 75 mg L^{-1} each), 32.3 % and 65.1 % removal was observed for Ni(II) and Cu(II), respectively, as compared to 42.1 % and 78.4 % removal, respectively, under single metal stress. Further, simultaneous removal of various hazardous metals such as Cr (71.2 %), Cu (56.7 %) and Pb (100.0 %) was accomplished from nutrient supplemented electroplating effluent, which is remarkable. Hence, *A. lentulus* proved to be a competent strain that could be employed in conjunction with other partners for multi-pollutant removal.

Next, the potential of other fungal strains (identified as *A. terreus* and *Rhizopus oryzae* along with *A. lentulus*) for selected heavy metal [Cr(VI) and Cu(II)] and industrial dye (Acid Blue 161 or AB and Pigment Orange 34 or PO) removal at 100 mg/l initial concentration was evaluated. In view of the pollutant removal rates and uptake capacity, *A. lentulus* was best for Cu(II) removal (76.6 % in 120 h) and AB removal (96.7 % in 42 h), *A. terreus* was best for Cr(VI) removal (100.0 % in 96 h) whereas, *R. oryzae* was best for PO removal (98.4 % in 24 h). The mechanisms of pollutant removal in different fungal strains were investigated employing desorption studies as well as SEM, TEM, EDX, spectroscopic and FTIR analysis. Metal removal was found to be mediated simultaneously by biosorption and bioaccumulation phenomenon. Desorption studies revealed that *A. terreus* binds chromium principally on its surface (59.7 %), whereas *R. oryzae* efficiently binds copper on its surface (48.4 %). *A. lentulus* shows maximum bioaccumulation of chromium within the cell (44.2 %) and cell membrane (11.2 %), it also stores a significant fraction of copper (27.3 % and 32.8 %, respectively) on these locations. SEM-EDX proved the highest deposition of chromium on *A. terreus* and that of copper on *R. oryzae*. TEM-EDX mapping more precisely confirmed localized copper deposition on cell wall/membrane in *R. oryzae* and heavy chromium depositions on cell surface in *A. terreus*. Multiple mechanisms such as biosorption, bioaccumulation and biodegradation were implicated in dye removal process. *A. lentulus* and *A. terreus* showed good binding efficiency for AB and PO dye. In the case of *R. oryzae*, the internalized dye gets further degraded in due course of time as demonstrated by HPLC analysis. The FTIR analyses confirmed the role of amino, carboxyl, hydroxyl and phosphate groups in metal adsorption and that of amine, amide and carboxyl groups in the dye adsorption on the fungal biomass. These studies established that the principle mechanism for

removal of a particular pollutant varies among different fungal strains. Moreover, the same strain responds to and sequesters different pollutant via different routes.

Based on the complementary affinities and mechanisms of pollutant removal as well as positive interactions; development of stable consortium comprising *A. lentulus*, *A. terreus* and *R. oryzae* was attempted for efficient metal and dye removal. Much faster and higher removal percentages were obtained as compared to the individual strains. Consortia could bring about complete removal of Cr(VI) in 96 h and 81.6 % removal of Cu(II) within 120 h. Whereas, 98.0 % Acid Blue 161 and 100.0 % Pigment orange 34 dye was removed after 48 h employing fungal consortium. None of the individual strains could perform with such efficiency. Further, a 50% reduction in the nutrient cost incurred for multiple pollutant removal from mixtures using consortium, was obtained by response surface methodology based optimization. To further evaluate the efficacy of the fungal consortium, pollutant removal from three industrial effluents (dye, textile and electroplating) was carried out where substantial pollutant [Cr(VI) and Cu(II) and dye] removal was obtained. The results suggest the utility of fungal consortium in simultaneous removal of dyes and metals from complex synthetic solution as well as industrial effluents.

To facilitate the industrial application, easily storable product (myco-tablet and myco-capsule) of the member fungal strains was developed. The myco-tablets thus produced had the vital parameters within the appreciable range (viability < 30 x10 ^{8} cfu g^{-1}, > 75 % Cr(VI) removal, > 65 % Cu(II) removal, > 90 % dye removal, hardness < 1.5 kg/cm^2, friability < 1.3 %, disintegration time < 4 min), thus making them suitable for storage, packaging, transportation and on-site use. Still lower dispersion time was obtained with myco-capsules (1.15 min). Also, the performance of myco-capsules for all the three fungal isolates was considerably better than myco-tablets in terms of biomass production and pollutant removal. Further, the combined use of all three myco-capsules improved the pollutant removal rates. The present innovation in terms of low cost myco-capsules with multiple metal and dye removal efficacy shall prove to be instrumental in simplifying the bioremediation of complex waste streams. The application of multi-pollutant targeted products invented in this study were demonstrated and disseminated to ensure translation of research into actual application.

In short, academic importance of the present study can be highlighted by the development of a novel consortium targeted towards multi-pollutant removal. The present study also delivers an easy-to-handle and low cost product which is inimitable in its application in handling waste streams containing cocktails of metals and dyes with proven field applicability.

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