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Simultaneous sequestration of ternary metal ions (Cr^{6+}, Cu^{2+} and Zn^{2+}) from aqueous solution by an indigenous bacterial consortium

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ABSTRACT

Sequestration of single heavy metal contaminant from industrial wastewater using pure bacterial strains has received much attention. However, application of a bacterial consortium in multiple metals sequestration is scarce. The present study was aimed to develop a consortium from three bacterial strains for the simultaneous sequestration of ternary metal ions (Cr^{6+}, Cu^{2+} and Zn^{2+}) from aqueous solution. Kinetic studies showed that the individual strain, *Pseudomonas taiwanensis*; *Acinetobacter guillouiae* and *Klebsiella pneumoniae* were able to remove Cr^{6+}, Cu^{2+} and Zn^{2+} respectively. These bacterial strains were utilized to develop an indigenous consortium based on metal removal affinities and positive interferences between them. Consortium showed improved performance over individual strains for the removal of single as well as simultaneous removal of three metal ions. Fourier transform infrared (FTIR) spectral analysis showed the possible involvement of carboxyl, amino, hydroxyl, methyl, phosphate and sulphonate groups in metal ions sequestration. Consortium exhibits greater adaptability in ternary metal ions mixture which indicates its robust growth mechanism over individual bacterial strains.

1. Introduction

Industrialization is one of the major steps towards civilization. However, the adverse effects of industrial emanations have been destroying the environment and natural resources. Effluents of various industries such as textile, paper and pulp contain mixture of various toxic metals such as chromium (Cr), copper (Cu), zinc (Zn) etc. (Ansari and Malik, 2007; Raghuvanshi and Babu, 2009, 2010; Singh et al., 2011; Yadav et al., 2010). For example, effluents of textile industry contain metal ions (Cr^{6+}, Cu^{2+} and Zn^{2+}). The effluents generating from pulp and paper industries and tannery industries also contain cocktail of different metal ions (Cr^{6+} and Cu^{2+}) and organic pollutants (dyes) (Mishra and Malik, 2012, 2014). These toxic metal ions mix with river or sea water and create hazards to human beings.

For the last few years, various microorganisms have been successfully utilized to remove these toxic metal ions

Abbreviations: A, absorbance; M_o, initial metal ion concentration at time $t=0$, $mg\,L^{-1}$; M_t, final metal ion concentration at time t h, $mg\,L^{-1}$; q, uptake capacity of metals (mg metal g^{-1} biomass); R, percentage removal (%); t, time, h; T, temperature, °C; X, biomass concentration (dry weight), $g\,L^{-1}$; μ, specific growth rate, h^{-1}; o, initial condition; AG, *Acinetobacter guillouiae*; APHA, American Public Health Association; FTIR, Fourier transformed infrared spectroscopy; KP, *Klebsiella pneumoniae*; MEGA, molecular evolutionary genetics analysis; MSM, minimal salt medium; NCBI, National Centre for Biotechnology Information; PT, *Pseudomonas taiwanensis*.

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(Bejestani et al., 2013; Cerino-Córdova et al., 2012; Das et al., 2014; Kumar and Thatheyus, 2013; Majumder et al., 2013, 2014). However, different microorganisms have different levels of resistance to metals. One specific microorganism may not be capable of removing all the metal ions present in the industrial effluents. It imposes a great challenge to tackle water pollution through biological route (bioremediation) in case of industrial effluents contaminated with multiple metal ions. Therefore, researches have been initiated to remove mixture of different metal ions and organic wastes using consortia of diverse microbial strains. A consortium culture comprising of six Gram-negative bacteria (*Flavobacterium* sp., *Serratia* sp., *Pseudomonas* sp., *Chryseomonas* sp., *Xanthomonas* sp., and *Agrobacterium* sp.) and three Gram-positive bacteria (*Arthrobacter* sp., *Bacillus* sp. and *Micrococcus* sp.) was used to treat an aqueous solution containing different heavy metal ions (Cd^{2+}, Cr^{6+}, Cu^{2+}, Ni^{2+} and Pb^{2+}) (Sannasi et al., 2008). A fungal consortium from three different fungal strains (*Aspergillus lentulus*, *Aspergillus terreus*, *Rhizopus oryzae*) was developed for the removal of metal ions (Cr^{6+} and Cu^{2+}) and dyes (Acid Blue 161 and Pigment Orange 34) (Mishra and Malik, 2014). Consortium was able to remove 100% Cr^{6+} and 81.60% Cu^{2+} as compared to removal achieved by three individual fungal strains. A mixed consortium containing three sulfate-reducing bacteria (*Desulfovibrio desulfuricans*, *Desulfomonas pigra* and *Desulfobulbus* sp.) was utilized to remove Ni^{2+}, Mn^{2+} and Cu^{2+} from aqueous solution (Barbosa et al., 2014). Results demonstrated more than 90% removal of the metals

Microorganisms possess ingenious mechanisms for metal resistance and remediation (Roane and Pepper, 2000). For example, the Gram-negative bacteria remediate various metal ions (cationic species) from aqueous solution due to the presence of surface functional groups in their peptidoglycan layers (Warren and Haack, 2001). Gram-positive bacteria possess higher carboxylic site densities as they have thick peptidoglycan layers as compared to Gram-negative bacteria. Warren and Haack (2001) reported least removal of metal ions using Gram-neagative bacteria *Escheritia coli*. In a seprate study, it was demonstrated that the various metal ions were adsorbed prefentially when the Gram-negative *Citrobacter* strain MCM B-181 was exposed to a multiple metal ions (Co^{2+}, Ni^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+}) solution (Puranik and Paknikar, 1999). The intracellular metal resistance mechanisms of bacteria involve metal binding or the metal sequestration by proteins (metallothioneins) (Lee et al., 2008). During the bioremediation of multiple metal ions, each of the partner microorganism in the consortium exhibits better uptake capacity for a particular metal. Due to the robust metal uptake mechanism and better growth rate of consortium partners, consortium shows significantly higher removal capacity of several metal ions present in the mixed stream (Romo et al., 2013; Sprocati et al., 2006).

Consortia can also display better and stable sequestration of metal ions against fluctuating input conditions in the environment which may be useful during in situ application. In spite of several advantages mentioned above, limited studies have been carried out for the removal of multiple metal ions using bacterial consortia. Till date, no investigation on the application of bacterial consortium for the simultaneous sequestration of ternary toxic metal ions Cr^{6+}, Cu^{2+} and Zn^{2+} has been reported. The present study was aimed to develop an indigenous consortium using three different isolated bacterial strains. For the first time an attempt was made to investigate the efficacy of the developed bacterial consortium over three isolated individual bacterial strains for the sequestration of single metal ion as well as simultaneous sequestration of three metal ions (Cr^{6+}, Cu^{2+} and Zn^{2+}).

2. Materials and methods

2.1. *Preparation of reagents and media*

Analytical grade (Merck, India) reagents and media were used in the experiments. One litre of nutrient, minimal salt media (MSM) solution was prepared by dissolving specific amount of mineral salts (K_2HPO_4, KH_2PO_4, $CaSO_4 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, $(NH_4)_2SO_4$ and $FeSO_4$) in distilled water. The composition of the mineral salts was taken from earlier study (Majumder et al., 2014). A 10 g of D-glucose was dissolved in 100 mL distilled water to prepare 10,000 mg L^{-1} of stock glucose solution. The nutrient agar (NA) media for microbial growth was prepared by dissolving 5 g of peptone, 1.5 g of beef extract, 1.5 g of yeast extract, 15 g of agar and 5 g of sodium chloride in 1 L of distilled water (APHA, 1985). The pH of MSM and NA media were adjusted to 7.0 ± 0.2 by using 1 M HCl or 1 M NaOH. All the media were sterilized in a Vertical Autoclave (MSW-101, Macro Scientific Works, India) at 15 kPa at 120 °C for 30 min. The MSM and NA media were used for cultivating the bacterial species. Three different aqueous stock solutions (1000 mg L^{-1}) of Cr^{6+}, Cu^{2+} and Zn^{2+} were prepared by dissolving 2.828 g of anhydrous $K_2Cr_2O_7$ salt, 3.92 g of $CuSO_4 \cdot 5H_2O$ salt and 4.4 g of $ZnSO_4 \cdot 7H_2O$ salt respectively in distilled water, and the solution volumes were made up to 1000 mL. These solutions were used in the batch studies and also used for the preparation of simulated industrial effluents.

2.2. *Preparation of simulated industrial effluents*

Five sets of simulated industrial effluents of tannery industries were prepared by varying the concentrations of Cr^{6+}, Cu^{2+} and Zn^{2+} (Lokhande et al., 2011). These sets were used in the batch studies as mentioned in Section 2.10.3. The compositions of five sets of simulated industrial effluent are given in Table 1.

2.3. *Treatment of activated sludge*

An activated sludge consisting of aerobic mixed microbial culture was collected from the Municipal Sewage Treatment Plant of Birla Institute of Technology & Science (BITS-Pilani), Pilani Campus, India. The pre-treatment and enrichment of the culture was carried out in the similar way as reported in earlier study (Majumder et al., 2014). The pre-treated and enriched culture was used for the isolation of Cr^{6+}, Cu^{2+} and Zn^{2+} tolerant microbial strains.

Table 1 – Actual volume of stock solutions used and composition of heavy metal ions in simulated industrial effluent.

Set no	Composition in simulated industrial effluent (mg L^{-1})		
	Cr^{6+}	Cu^{2+}	Zn^{2+}
1	30.40	47.70	30.50
2	30.40	21.50	15.80
3	19.20	47.70	30.50
4	19.20	21.50	15.80
5	25.50	35.50	21.50