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Evaluation of *Pseudomonas aeruginosa* an innovative bioremediation tool in multi metals ions from simulated system using multi response methodology

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HIGHLIGHTS

- The negative values −0.181 and −0.175 for ORP (mV) and Fe confirming the closeness in the responses.
- Addition of the Mn(II) did not significantly reduced the Fe(II) removal in the effluents.
- To optimize Cd, Cr, Fe and Mn removal process.
- Grouping of the metallic ions on the basis of their removal potential.

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ABSTRACT

Under certain conditions bacteria can act as a good biosorbent towards heavy metals in simultaneous removal from effluents. The present study explores overlay plots of multi response surface methodology for simulated wastewater treatment potential. *Pseudomonas aeruginosa* was used for bioremediation of metallic ions, where removal of Cd (80–90%), Mn (85–90%), Fe (50–55%), Cr (70–75%) can be achieved by fixing the pH, oxidation reduction potential (mV) and one of the metallic constituent in the simulated effluent. The metal ions Cd and Cr (T), Fe and ORP (mV) are relatively closely located to each other in the loading plot indicating co-variance between these components. However Cr(VI) transformation and Mn removal are distantly placed in the bi-plot indicating the existed significant difference. Elevated reductase enzyme activity (31.75 μg/min mg) observed in the isolate showing the ability to effectively reduce metals ions.

1. Introduction

Worldwide environmental problem has been invited over the past few decades due to tremendous increase in the metallic contents in the environment, a growing concern of health risks. Heavy metals, such as chromium (Cr) and cadmium (Cd) are released into the environment with industrial and domestic wastewater discharge. Cr(VI) is a priority toxic metal present in wastewater discharged from the various industries such as electroplating, pigment, and lumber and wood products processes. In natural environment, chromium can exist in oxidation states ranging from Cr(0), Cr(II) to Cr(VI), Cr(III) and Cr(VI) are the most dominant oxidation states in natural systems (Dogan et al., 2011; Srivastava and Thakur, 2012). Due to highly solubility and mobility, Cr(VI) enters the environment as a results of anthropogenic activities. As Cr(VI) is released into the environment, it can exist in a number of anionic forms, such as $HCrO_4^-$, CrO_4^{2-} and $HCr_2O_7^-$, in the pH range generally observed in the environment. Many microbes are able to reduce Cr(VI) to Cr(III) through direct microbial reduction, either enzymatically or non-enzymatically and can accumulate chromium by active transport of chromate (Cr(VI)) using the sulfate pathway (Nies and Silver, 1995). Bacteria can reduce Cr(VI) under both aerobic and anaerobic conditions through electron-transport systems containing cytochromes. Aerobic chromate reduction is associated with soluble proteins which use NADH as an electron donor, whereas under anaerobic conditions chromate acts as a terminal electron acceptor through a membrane-bound reductive activity (Levinaa et al., 2007).

The case of Cd is also of great interest due to its non essentiality and high toxicity to human being. The possibility of Cd^{2+} metal ion having anionic radius of 0.92 Å close to that of calcium (Ca^{2+}, 0.94 Å) enters through calcium channels and is one of the potential

Abbreviations: ORP, oxidation reduction potential; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; RSM, response surface methodology; ANOVA, analysis of variance; CV, coefficient of variance; Cr(VI), hexavalent chromium; Cr(T), total chromium.

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routes (Schaller et al., 2011). The biosorbents available are of particular interest since they have high metal binding capacities due to their surface polysaccharides, proteins or lipids, thereby providing numerous metal binding sites (such as carboxyl, hydroxyl, carbonyl, sulfhydryl, thioether, sulfonate, amine, imine, amide, imidazole, phosphonate and phosphodiester groups) (Yu et al., 1999).

There are several modification methods to enhance metal binding efficiency of the biomass. Iron has been used for the modification of biomass or other materials, since it possesses a natural affinity towards chromium. The following general reaction of ferrous sulfate to reduce Cr(VI) from the metal industry process effluents has suggested.

$$Cr(VI)(aq) + 3Fe(II)(aq) \rightarrow Cr(III)(aq) + 3Fe(III)(aq)$$

As the pH of the solution comes near neutral, then the $Cr(OH)_3$ precipitates could be formed rapidly.

$$Cr(III) + 3OH^- \rightarrow Cr(OH)_3 \downarrow$$

Hence it is essential to develop a technology having high metal removal efficiency with minimal environmental impact that purifies the wastewater generated. Biological remediation is an innovative technology available for treatment of heavy metal polluted effluents. The microbes have the ability to bind the metals ions from the external environment at the surface or to transport intracellularly for various functions. The mechanisms by which heavy metal are removed include electrostatic interactions, van der Waals forces, covalent bonding, redox interactions and extracellular precipitate formation. Microbial exudates are ubiquitous in the subsurface environment and as such may play an important role in microbial Cr(VI) reduction and subsequent mobility or immobility in systems contaminated with chromium (Dogan et al., 2011). The biological removal of iron and manganese by Fe–Mn oxidizing bacteria is gradually placing the conventional physicochemical treatments because it does not generate secondary pollution, no potentially hazardous chemically derived products and no extra costs of chemistry (Gallard and Gunten, 2002). The biological manganese oxidation was proven by the facts of good manganese removal in wetlands at chemical unfavorable conditions (such as ORP and pH) and the isolation of manganese oxidizing strains from the wetlands (Xu et al., 2009). Though single toxic metallic species rarely exist in natural and wastewater, much research has been focused on the removal of single species, whereas very little attention has been drawn to remove the many metals simultaneously (Sag and Kustal, 1997). The environmental factors such as pH, ORP, multi metal ions and microbial strains are more crucial and present unique influence on metals removal potential from the effluents. With variation of these parameters, the removal of metals would be producing some significant variations. The present isolate has been successfully applied in the removal of the Cd (50 mg L^{-1}), Cr(VI) (50 mg L^{-1}) and COD removal kinetic at the loading rate of (≈9520 mg L^{-1}) in the simulated effluent contaminated heavy metals (Singh et al., 2013).

As a collection of statistical and mathematical techniques for developing, improving, and optimizing processes, while applying the Response Surface Methodology the two commonly models used are Central Composite Design (CCD) and Box–Behnken Design (BBD) in optimization studies. The BBD optimization technique is more suitable for estimating quadratic response chiefly in cases when predicting the response at the extreme level is not objective of the model. The metallic ions removal, Cr(VI) reduction, chromate reductase activity, effect on pH and oxidation reduction potential (ORP mV) can be predicted by developing unique approach for evaluating the process efficiency in the simulated effluents. In this study multi metals removal system as synergistic role of Fe(II), Mn(II) and Cr(VI) was investigated on the Cd removal in the system augment with *Pseudomonas aeruginosa*.

2. Methods

2.1. Analytical methods and quantification of heavy metals

Screw caped flasks (250 ml) were used for entire study. The culture was harvested as whole flask at predetermined intervals for various parameters estimation. The pH, ORP (mV), Cr(VI), chromate reductase activity and protein contents were measured immediately whereas the supernatant was preserved as per standard methods for further analysis. The oxidation reduction potential (mV) and pH were measured before centrifuging the culture using Eutech meter, calibrated prior to use by standard calibration solutions. Prior to analysis of metallic contents, protein contents and chromate reductase activity, the samples were centrifuged and the supernatant fraction was analyzed for various parameters. Cr(VI) concentration was analyzed by 1,5-diphenylcarbazide methods at 540_{nm} using UV/VIS spectrophotometer. The soluble/residual heavy metals contents in the effluent were determined using Atomic Absorption Spectrophotometer (Shimadzu – 6300 Japan) after digesting the supernatant according to APHA (2005). All chemical analysis was carried out in duplicate to ensure the validity of the results. The removal (%) of heavy metal was determined using the following equation.

$$\text{Removal } (\%) = ((C_i - C_f) \times 100)/C_i$$

where, C_i is the initial heavy metal concentration; C_f is the final heavy metal concentration.

2.2. Preparation of simulated contaminated wastewater samples

Metal ion solutions of Cr(VI), Cd(II), Fe(II), and Mn(II) were prepared by dissolving their respective salts, namely potassium dichromate ($K_2Cr_2O_7$), cadmium sulfate ($3CdSO_4{\cdot}8H_2O$), ferric chloride ($FeCl_3$), and manganese sulfate ($MnSO_4$), in double distilled water for entire study. The chemicals used for the preparation of samples were of analytical grade. The required heavy metals stock solutions were prepared by dissolving potassium chromate ($K_2Cr_2O_7$) and cadmium sulfate $3CdSO_4{\cdot}8H_2O$ salts respectively in 100 ml double distilled water to prepare 10,000.0 mg L^{-1} stock solutions. The ferric chloride ($FeCl_3$) and manganese sulfate ($MnSO_4$) were prepared to 10 mM stock solutions. Simulated effluent was further prepared by adding the appropriate amount of the stock solutions.

2.3. Growth medium for isolate and its inoculum preparation

The isolate *P. aerugnosa* (Opportunistic pathogen) employed for heavy-metal removal was isolated using standard isolation techniques (Singh et al., 2013). Tris minimal modified medium (Michel et al., 1986) (g L^{-1}): Tris base 6.0, KH_2PO_4 0.67, $(NH_4)_2SO_4$ 4.0, KCl 0.62, $MgSO_4$ 0.063, $FeSO_4$ 0.003 and glucose 6.0 were used to maintain the culture and further metals removal study. The inoculum was maintained by transferring 1 ml fully grown culture into 250 ml conical flask containing 100 ml sterilized nutrient broth. The inoculated flasks were incubated in BOD shaker cum incubator at 120 rpm for 48 h at 37 °C. Optical density (OD) was measured using UV–VIS spectrophotometer at 600_{nm}. The culture was almost fully grown within 48 h and fresh fully grown culture was used as inoculum.