Bioremediation of multi-metal contaminated soil using biosurfactant – a novel approach

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Abstract An unconventional nutrient medium, distillery spent wash ((1:3) diluted) was used to produce di-rhamnolipid biosurfactant by *Pseudomonas aeruginosa* strain BS2. This research further assessed the potential of the biosurfactant as a washing agent for metal removal from multi-metal contaminated soil (Cr-940 ppm; Pb-900 ppm; Cd-430 ppm; Ni- 880 ppm; Cu-480 ppm). Out of the treatments of contaminated soil with tap water and rhamnolipid biosurfactant, the latter was found to be potent in mobilization of metal and decontamination of contaminated soil. Within 36 hours of leaching study, di-rhamnolipid as compared to tap water facilitated 13 folds higher removal of Cr from the heavy metal spiked soil whereas removal of Pb and Cu was 9-10 and 14 folds higher respectively. Leaching of Cd and Ni was 25 folds higher from the spiked soil. This shows that leaching behavior of biosurfactant was different for different metals. The use of wastewater for production of biosurfactant and its efficient use in metal removal make it a strong applicant for bioremediation.

Keywords *Pseudomonas aeruginosa* · Rhamnolipid Biosurfactant · Distillery waste · Metals · Column studies

Introduction

Soil is being contaminated with metals due to direct contact with industrial discharge, improper disposal of wastes, incidence of spills, and failure of land disposal facilities. Metals such as lead, chromium, cadmium, arsenic, copper, and zinc have been detected in most of the sites, with mercury and nickel present in some of the sites. Metals, unlike many hazardous organic constituents, cannot be degraded or readily detoxified. The existence and fate of the metals in soil is a matter of concern not only because of their potential impact on microbial communities, presents a potential health hazard for humans but also because of the potential for groundwater contamination and hence toxicological impact on human health [1].

Metal contaminants in the environment are usually tightly bound to colloidal particles and organic matter. This represents a major constraint to their removal using currently available *in situ* remediation technologies. The promising new remedial strategy for treating the soil bound metal contamination in soil is the use of biosurfactants that are non-toxic to the soil structure [2]. Metal removal from multi-metal contaminated soil using biosurfactant offer the advantage of being nontoxic and easily biodegradable than some synthetic surfactants which are being used to mobilize [3, 4] and increase the availability of recalcitrant or sorbed contaminants from soil. Moreover, biosurfactant have diversity of chemical forms so that they can be selected on the basis of their high specificity for different metals and thus metal removal efficiency.

The main objective of the present research is to provide a novel strategy for use of di-rhamnolipid produced from distillery wastewater for mobilization of metals from

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multi-metal contaminated soil. Results so obtained from column studies for metal removal and demonstration of selectivity of heavy metals for their mobilization from multi-metal contaminated soil will be discussed.

Materials and Methods

Production of biosurfactant: Distillery wastewater was processed before sterilization by earlier described method [5]. Characterization of the distillery waste has been described earlier [5]. Batch fermentation was carried out in fermenter (New Brunswick BIO FLO II C) using (1:3) diluted distillery (post methanated) spent wash as nutrient medium for proliferation of *Pseudomonas aeruginosa* BS2 [5] (isolated from oily sludge, Mathura Refinery, U.P, India, MTCC 5266) and biosurfactant production under following conditions i.e. temperature 37°C, agitation 400 rpm, incubation period 96 hours and aeration at 2 SLPM, 1 and ½ VVP (volume per air per volume liquid) [5]. After 96 hours of batch fermentation, foam was completely removed by foam fractionation method [6]. The yield of biosurfactant using distillery waste was found to be 0.91 g/l. Di-rhamnolipid biosurfactant present in the collapsed foam was recovered by adsorption-desorption processes using wood activated carbon as an adsorbent at the rate of 4g/litre of collapsed foam and acetone as an biosurfactant eluant [7].

Preparation of metal contaminated soil: The garden soil collected from NEERI premises was contaminated artificially in the laboratory with the solution of poly metal salts ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (500 ppm), $3\text{Cd SO}_4 \cdot 8\text{H}_2\text{O}$ (500 ppm), $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (1000 ppm), $(\text{CH}_3\text{COO})_2\text{Pb} \cdot 3\text{H}_2\text{O}$ (1000 ppm), and K_2CrO_4 (1000 ppm)). The salts were dissolved in distilled water to achieve concentrations mentioned above and were added together into the soil sample (without adjusting the pH value). The soil was shaken for 3 d on a rotary shaker at room temperature ($30.0 \pm 0.2^\circ\text{C}$), and subsequently centrifuged (Remi, C24) at 5000 rpm for 10 min to remove the unadsorbed metals if present in solution. The supernatant was discarded and the contaminated soil was air dried and sieved through 200 mesh (0.074 mm).

Physico-chemical properties of soil: Physical properties of soil were studied-Bulk density, maximum water holding capacity, porosity (K-R box method), textural class (International pipette method). Soil to distilled water ratio of 1:2 was used for measuring pH (Eutech, pH Tutor). Electrical conductivity (Systronic Conductivity Cell) was measured using conductivity method. For measuring total metal concentration soil, samples were digested with acid and

then analyzed using an inductively coupled plasma atomic emission spectrophotometer (ICP-AES-JY-24, Jobin Yvon, France). The characteristics of the both garden soil and heavy metal contaminated soil were measured and they are listed in Table 1.

Column studies for metal removal using biosurfactant: Heavy metal spiked soil (50 g) was filled in 1st and 2nd glass columns ($2.0 \text{ cm} \times 15 \text{ cm}$), while into the 3rd and 4th columns of same dimensions, 50 g of garden soil was filled. Di-rhamnolipid biosurfactant solution (0.1 %) was prepared in distilled water previously adjusted to pH 8.0 with NaOH for mobilization and leaching of heavy metals from spiked soil (column 2 and 4) at a rate of 0.2 ml/minute using multi-channel peristaltic pump and total volume of each washing solution was kept constant i.e. 200 ml. Removal of metals from garden soil and metal contaminated soil using tap water served as controls (column 1 and 3 respectively). Sampled regularly after 12 h of time interval. Leachates (1 ml) were acidified with 5 ml of concentrated HNO_3 and digested in a sand bath and the volume was reduced to 1/4th. These digested samples were diluted to 100 ml and the concentrations of heavy metals were analyzed using ICP-AES. Biosurfactant solution and tap water were also similarly digested to determine the presence of heavy metal contaminants if any.

Metal mass balances: Metals remaining in the soil after soil washing was estimated using a mass-balance approach based on the initial soil metal and the aqueous phase metal measured in the extractant solution during the soil-washing experiment.

Results and Discussion

Till now, pure substrates such as glucose and hydrocarbons like hexadecane, palm oil, olive oil etc have been used for biosurfactant production [8, 9]. Di-rhamnolipid biosurfactant produced from distillery wastewater as no-cost complete nutrient medium, by *Pseudomonas aeruginosa* BS2 is highly efficient in regard to its surface-active properties. Biosurfactant produced is a di-rhamnolipid and has two types of surfactant molecules, viz. rhamnolipid-A and rhamnolipid-B with molecular weight as 504 and 605, respectively and low Critical Micellar Concentration (CMC) of 28 mg/L as compared to high CMC of 40–50 mg/L reported for mono rhamnolipid and mixture of mono and di-rhamnolipids [5]. The use of di-rhamnolipid alone produced from unconventional substrate such as distillery waste water, has not been reported so far for its ability to decontaminate metals from multi-metal spiked soil.

The use of multi-metal solutions for preparation of artificially contaminated soil leads to competitive metal sorption in soil. The sorption of metals to soil ranged from 86–96%, which may be attributed to competitive sorption onto the soil as well as difference in initial metal concentrations used for contamination. Results of comparative analysis on removal of five different heavy metals from the multi-metal spiked soil by using di-rhamnolipid and tap water has shown high potential of di-rhamnolipid in mobilization and decontamination of contaminated soil.

Effect of time on removal of metals from soils using tap-water and biosurfactant is depicted in Fig. 1(a–d). A general increase in elution of metal with time was observed. Among the five model heavy metals, marked specificity in case of Cr and Cd as overall removal of 865 and 396 ppm from 940 and 430 respectively was observed. In the case of Pb and Cu

whereas, 792 and 423 ppm from loaded concentration of 900 and 480 ppm of the respective metal was seen followed by Ni, 687 ppm removal off 880 ppm, after 36 hours (Table 2). Di-rhamnolipid as compared to tap water facilitated 13 folds higher removal of Cr from the heavy metal spiked soil whereas removal of Pb and Cu was 9–10 and 14 folds higher respectively. Leaching of Cd and Ni was 25 folds higher from the spiked soil. This shows that leaching behavior of biosurfactant was different for different metals. This difference is not only due to specificity of biosurfactant for different metals but also due to the co-existence of metals in the soil [10]. Biosurfactant specificity observed towards specific metal will help preferential elution of specific contaminant using di-rhamnolipid. The low removal of metals from garden soil using biosurfactant was determined by low metal concentrations in the soil and the fact that garden soil acts

Table 1 Physico-chemical Characteristics of Garden Soil and Heavy Metal Spiked Soil

Parameters	Garden Soil	Heavy Metal Spiked Soil
Physical		
Bulk density, g/cc	1.18	1.34
Maximum water holding capacity, %	62.16	38.60
Porosity, %	53.92	44.40
Sand, %	26	No change
Silt, %	26	No change
Clay, %	48	No change
Textural Class	Clay	Clay
Chemical		
pH	7.80	6.40
EC, mS/cm	0.20	0.30
Cation Exchange Capacity (CEC), mg/100g	75.57	68.70
Total Heavy Metals, mg/kg		
Chromium,	47.8	940.5
Lead	120.6	900.4
Cadmium	18.5	430.4
Nickel	52.2	880.6
Copper	52.6	480.6

Table 2 Metal mass balances in soil after washing with rhamnolipid biosurfactant

Metals	Concentration of metal (ppm)			
	Before treatment	Removal	Remaining in soil	Total Recovery(%)
Chromium	940.5	865.3	80.0	100.5
Lead	900.4	792.4	120.5	101.4
Cadmium	430.4	396.0	40.5	101.4
Nickel	880.6	686.9	220.5	103.0
Copper	480.6	423.0	60.5	100.6

Fig. 1 Effect of time on removal of metals from soils (a) Garden soil treated with tap water (b) Heavy metal spiked soil treated with tap water (c) Garden soil treated with 0.1% di-rhamnolipid biosurfactant (d) Heavy metal spiked soil treated with 0.1% di-rhamnolipid biosurfactant

as aged contaminated soil and there the binding of metals to the soil is very strong [11].

The results above indicates that di-rhamnolipid selectively favours mobilization of metals in the order of $\text{Cd}=\text{Cr}>\text{Pb}=\text{Cu}>\text{Ni}$. Apart from differential sorption of metals to soil the extraction and mobilization of metals depends on various factor like stability constant of the metals of concern with the di-rhamnolipid [12].

Few column studies have been reported for the metal removal using rhamnolipid. Though our results are comparable with that reported, the present study showed higher removal of few metals as compared to the others. Wang [13] conducted column experiments to evaluate the feasibility of using rhamnolipid foam to remove Cd and Ni from a sandy soil contaminated with Cd (1706 ppm) and Ni (2010 ppm). Rhamnolipid solution (0.5%) with an initial pH value of 10.0 after flushing with 20-pore-volume of solution removed 73.2% of the Cd 68.1% of the Ni.

Mass balances were found to be significant during the experiments. Remaining Cr, Pb, Cd, Ni, Cu in the soil was found

to be 80, 120, 40, 220 and 60 ppm respectively. Unaccounted metal concentrations ranged between 1–3%. This may be due to the analytical and/or operational errors, which are unavoidable but expected during the experiments (Table 2).

This experimentation of removal of metals from multi-metal contaminated soil by the virtue of biosurfactant revealed the positive indication for its use in bioremediation of these metal contaminated soils.

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