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Research Article

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Removal of Heavy Metals Contamination By Bio-Surfactants (Rhamnolipids)

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ABSTRACT

The problem of heavy metals contamination is a global issue and the challenge is to develop methods to remove heavy metals from soil and water. Recently, technologies using microorganisms and microbial products to remove metals have been successfully applied to waste water streams such as sewage sludge, and industrial effluents. Anionic bio-surfactants (Rhamnolipids) produced by the bacterium of Pseudomonas species has been effectively used as heavy-metal cleanup. This study investigates the feasibility of using bio-surfactants (Rhamnolipids) to remove or reduce heavy metals (Cd^{2+}, Pb, Ni^{2+}, Ba, Zn and Sr). Different concentrations (20, 40, 80 ppm) of Rhamnolipids were evaluated and compared for the efficiency of heavy metals removal. Results showed that, 80 ppm of bio-surfactants were required to reduce up to (53%, 62%, 56%, 28%, 20% and 7%) of Cd^{2+}, Pb, Ni^{2+}, Ba, Zn and Sr respectively. Rhamnolipid at concentration of 80 ppm could be successfully used as bio-surfactants to remove heavy metals from contaminated water.

Key words: Bio-surfactant, Rhamnolipid, contaminated water, heavy metals.

INTRODUCTION

Water pollution has been suggested as the leading worldwide cause of death and diseases. [1, 2]. Recently their has been a concern about the quality of most tap and well water in United States of America for drinking due to heavy metals from industrial and environmental pollution. Heavy metals routinely penetrate and pollute natural water sources, while long term exposure can lead to liver damage, cancer and other serious conditions [2, 3]. The mechanisms of heavy metal toxicity inducing oxidative stress and interfering with protein folding and function [4, 5]. Heavy metals that commonly cited as being of the greatest public health concern are lead, cadmium, and mercury. Microbial produced surfactants (Bio-surfactants) have been successfully applied approach to remove metals from waste water streams, sewage sludge, industrial effluents, and mine water [6]. Their interaction with metals leads to separation of metal from the waste stream. The interaction of microorganisms with metals occurs through metal binding to the cell surface or within the cell wall, translocation of metal into the cell, volatilization of the metal as a result of a biotransformation reaction and the formation of metal precipitates by reaction with extracellular polymers or microbial produced anions such as sulphide or phosphate [7, 8]. Bio-surfactants are in general classified into lipopeptides, lipoproteins, glycolipids, polymeric, fatty acids, phospholipids and neutral lipids [9, 10]. The most known bio-surfactants are glycolipids. Glycolipids are carbohydrates molecules combined with long-chain aliphatic acids or hydroxyaliphatic acids. The three types of glycolipid are rhamnolipids, trehalolipids, and sophorolipids. Rhamnolipids are among the several bio-surfactants produced by microorganisms and have been applied for the remediation of contaminated soil and to enhance oil recovery [11, 12]. Rhamnolipids (Fig 1) are anionic bio-surfactants produced by the bacterium *Pseudomonas* species [16]. It consists of one or two rhamnose sugar, linked to one or two molecules of b-hydroxydecanoic acid. These molecules form films at the air-water interface, which determines their effectiveness for environmental applications[10, 13]. Interestingly it is well-known that microbial cells can complex metals from solution but there is a little information in the literature about the use of bio-surfactants to complex with the heavy metals [14, 15]. To this end, the goal of this study was to investigate the interaction between this bio-surfactant (Rhamnolipids) and different heavy metals usually present in water.

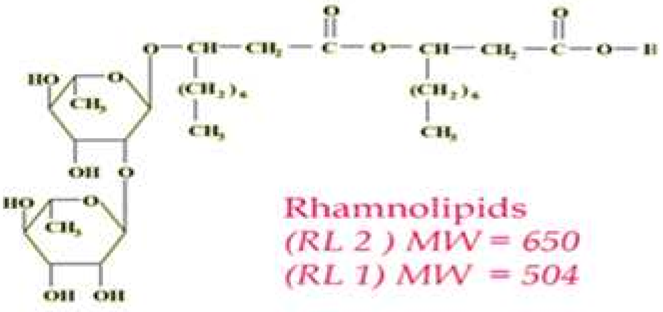

Fig 1: Structure of Rhamnolipids

EXPERIMENTAL SECTION

Materials

Heavy metals, (cadmium ($Cd(NO_3)_2 4H_20$), lead ($Pb(NO_3)_2$), nickel ($NiCl_2 6H_20$), barium (BaCl), zinc (ZnCl) and strontium (SrCl) were bought from the local pharmaceutical company, Libya. The rhamnolipids used in this study were biosurfactants from the glycolipid group made by *Pseudomonas aeruginosa*. Two major types of rhamnolipids, RLL(R1) and RRLL(R2), are mixed in the solution. R1 (C26H48O9) is L-rhamnosyl- β-hydroxydecanoyl-®-hydroxydecanoate. R2 (C32H58O13) is L-rhamnosyl-®-L-rhamnosyl-® hydroxydecanoyl-β-hydroxydecanoate. All chemicals used in the study were of analytical grade and used as received.

Production of Rhamnolipid

The *pseudomonas aeruginosa* was isolated and cultured period to incubation to produce rhamnolipid. Rhamnolipid (g/L) produced in the culture medium was then purified and quantified using the phenol sulfuric acid method as previously described [16]. Serial dilutions (20, 40 and 80 mg/L) of purified Rhamnolipid in water was made with pH adjusted to 7.2 using 0.1 N NaOH solution and a digital pH meter (Thermo Fisher Scientific Inc.).

Preparation of metal solutions

The protocol of samples were prepared by dissolving specified weight of metal salts (cadmium ($Cd(NO_3)_2 4H_20$), lead ($Pb(NO_3)_2$), nickel ($NiCl_2 6H_20$), barium (BaCl) zinc (ZnCl) and strontium (SrCl) in 1000 ml of deionized water to produce metal solution (500mg/L). The pH of each solution was measured using pH meter.

Experimental design

Aliquots of 10 ml samples (500 mg/L) of the metal solution were transferred into four test tubes. From each test tube, 1 ml sample was replaced by either 1 ml of deionized water as control or with 1 ml of 20ppm, 40ppm or 80ppm concentrations of Rhamnolipids solution. Samples were then incubated at room temperature for one hour, prior to filtration through whatman filter paper using bucchner funnel. The concentration of the heavy metal in the filtrate was measured using inductively coupled plasma optical emission spectrophotometer (ICP –OES vista –PRO, Libyan Petroleum Institute, Tripoli- Libya). All experiments were made in triplicate, and the average value was calculated and presented as a percentage of metal removed.

Calculation

The percentage of metal removed (η) was calculated based on the initial metal content (control) in the aqueous solution using the following equation:

$$\eta = CM\text{-}CMF/\ CM \times 100$$

Where; CM is the initial concentration of heavy metals (control, i.e. without treatment) and CMF is the final concentration of heavy metals (after treatment with Rhamnolipid).

Statistical analysis

The results were statistically analyzed and the differences between groups was examined using one-way analysis of variance (ANOVA) and post-tests carried out using Fisher's pair wise comparisons via the statistical package Minitab TM 13 windows. Statistically significant differences were set at $p < 0.05$.

RESULTS

Table 1 and Fig 2 show the effect of increasing concentration of bio-surfactant (Rhamnolipid) on the removal of heavy metals from aqueous solution.

Table 1: The effect of bio-surfactants (Rhamnolipid) concentration on heavy metals removal from aqueous solution

Heavy metal	Rhamnolipid concentration (ppm)			
	Control	20	40	80
Cadmium	390.53±12.4	330.80±17.6	254.03±9.8	191.67±5.5
Lead	355.13±10.3	322.53±15.6	294.30±6.9	160.67±15.3
Nickel	253.13±12.4	114.10±3.9	118.27±7.6	117.67±7.3
Barium	483.83±6.4	382.53±6.1	364.78±10.5	342.22±6.2
Zinc	124.77±8.0	109.33±6.5	105.58±4.9	103.60±6.3
Strontium	247.85±6.8	239.49±6.2	220.37±9.6	236.70±4.4

The results are presented as mean ±standard deviation of 3 measurements. ppm = Parts per ml

Compared to control, a significant reduction in the concentration of metal was observed after the addition of bio-surfactant (Rhamnolipid). A significant decrease in Cadmium and Lead concentration was high with rhamnolipid at 80ppm concentration compared to 20 and 40 ppm concentrations.

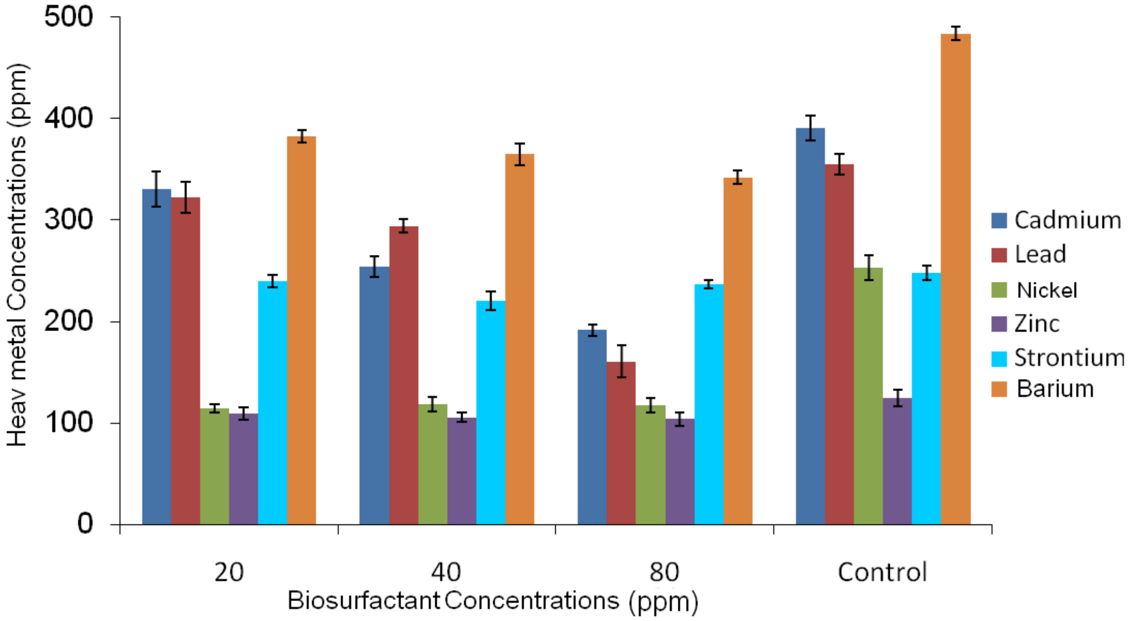

Fig 2. Effects of rhamnolipid concentration on the removal of heavy metals compared to control.

Rhamnolipid at 80 ppm was found to be a superior adsorbent in removing cadmium and lead from aqueous solution. Fig 3 shows the efficiency (%) of bio-surfactant in reducing metals contamination from water.

The desorption efficiencies achieved with rhamnolipid at 80ppm were 53% and 62% for cadmium and lead respectively. Rhamnolipid had also shown a significant effect in reducing the Nickel concentration. Rhamnolipid with concentration of 20 ppm, had lead to a desorption efficiencies of 58% for nickel, however, no significant change after concentration of rhamnolipid was increased to 40ppm and 80ppm. The desorption efficiencies of rhamnolipid on barium, zinc and strontium at concentration of 80 ppm were 28%, 20% and 7% respectively. In the case of zinc, no effect was shown probably due to the pH of metal solution (≤ 4) on rhamnolipid action that was less than the optimum pH of working rhamnolipid is 7.2. For barium, the highest effect was observed at rhamnolipid concentration of 80ppm while for strontium, the highest effect was observed at rhamnolipid concentration of 40 ppm.

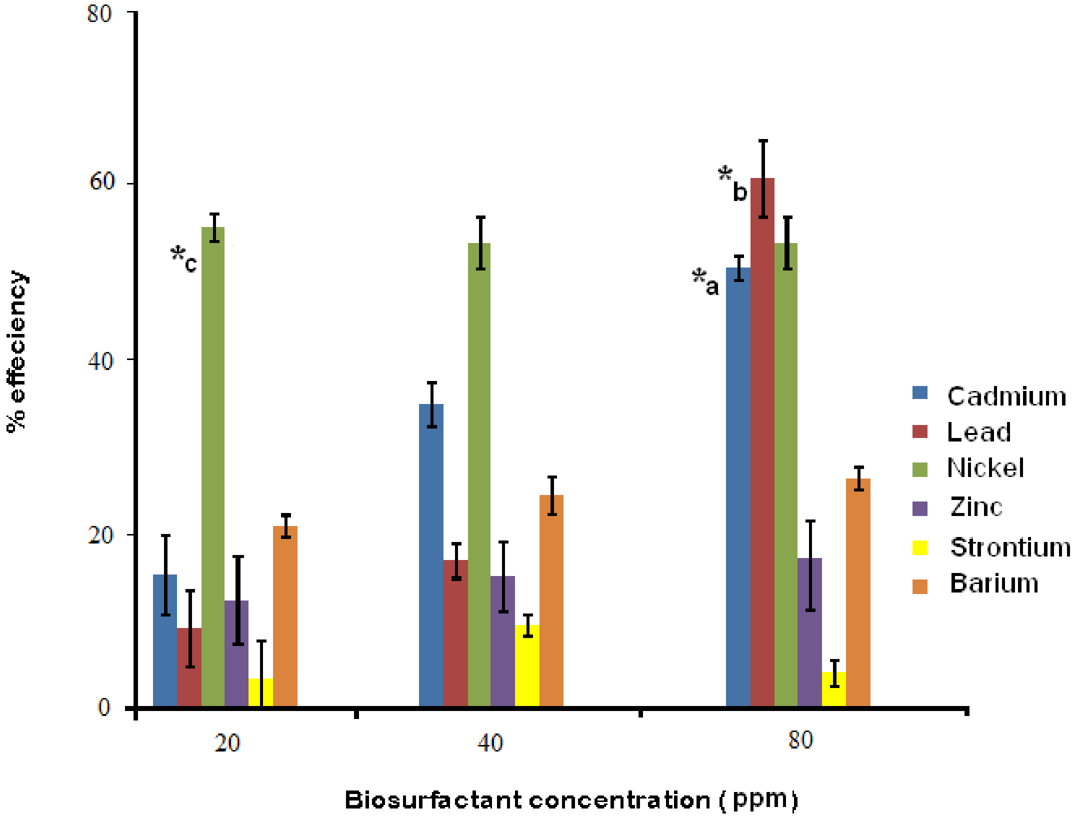

Fig 3. The desorption efficiency (%) of heavy metals by Rhamnolipid. *a and *b represent statistically significant difference ($p< 0.05$, Anova one way analysis of variance) relative to other concentration in each class, while *c represents statistically significant difference ($p< 0.05$, Anova one way analysis of variance) relative to other classes.

DISCUSSION

Bioremediation of industrial wastes containing heavy metals has been demonstrated by several biotechnology companies employing bioaccumulation [16-18]. Biosorption, bioprecipitation, and uptake by purified biopolymers derived from microbial cells provide alternative and/or additive processes for conventional physical and chemical methods. Intact microbial cells live or dead and their products can be highly efficient bioaccumulators of both soluble and particulate forms of metals [19, 20]. Various microbial species, mainly *Pseudomonas*, have been shown to be relatively efficient in bioaccumulation of uranium, copper, lead and other metal ions from polluted effluents, both as immobilized cells and in the mobilized state for example, Acinetobacter RAG-1 was found to bind up to 240 µg uranium (UO_2^{2+})/mg emulsion [21]. Similarly, a *Pseudomonas* exopolysaccharide bound up to 96 µg uranium/mg exopolymer [6] . The cell surfaces of all microorganisms are negatively charged owing to the presence of various anionic structures [8]. This gives bacteria the ability to bind metal cations [7, 8, 11]. A study of Cadmium-Arthrobacter exopolysaccharide complexation showed that cadmium binding (3.3 µg/mg exopolymer) was significantly less than that of uranium [22]. Other study of several marine *Pseudomonas sp*. exopolysaccharides showed complexation of copper, iron, lead, nickel, and zinc[23, 24] . There is a little information about the effect of bio-surfactant-metal interactions on metal structure. Our earlier work has concluded that the isolated bacteria from the soil of local site were found to have the ability of producing the biosurfactant (Rhamnolipid) in the form of biological molecules [16]. In this study presents experimental results that evaluate the capability of rhamnolipids and their ability on enhancing removal of heavy metals, in the water systems contaminated with heavy metals. The goal of the addition of a bio-surfactant may promote desorption of heavy metals from contaminated water through complexation of the free form of the metal residing in solution. This decreases the solution-phase activity of the metal resulting in direct contact between the bio-surfactant and the sorbed metal and, therefore, promotes desorption. Clearly, bio-surfactant structure size and charge will affect movement of bio-surfactant-metal complexes. In addition, structure size and charge will also affect the access of bio-surfactants to filter pores [25]. Therefore the rhamnolipid solution pH was optimized to minimize the size of the metal–ligand complex. The size of rhamnolipid aggregates is pH dependent and they are predominately small vesicles and micelles at pH > 6.0 [26]. The use of this technique allowed significant values of removal rates of cadmium, lead, and nickel. The removal efficiency of barium, zinc and strontium were not completely encouraging, may be due to some errors related to the use of rhamnolipid concentration. Hence, the evolution of rhamnolipid effective concentration in barium, zinc and strontium, the removal is a matter of investigation.

CONCLUSION

The conclusions drawn from this study that; Bio-surfactant addition to heavy metals contaminated water at concentrations above their CMC values generally enhance desorption of heavy metals. Using inductively coupled plasma optical emission spectrometer established that 80ppm of rhamnolipid can reduce up to 53 % of cadmium, 62 % of lead, and 58% of nickel respectively. Little effect (≤ 28% desorption efficiency) of rhamnolipid on barium, zinc and strontium removal was observed. The effect of rhamnolipid on barium, zinc and strontium suggest further research to be conducted.

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