Research Article

A Microbial Bioremediation Approach: Removal of Heavy Metal Using Isolated Bacterial Strains from Industrial Effluent Disposal Site

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ABSTRACT

Bioremediation is the simple and eco-friendly mechanism to solve this environmental issue. This process employs microorganisms capable of degrading toxic contaminants. In present bioremediation study, two heavy metal resistant microbial strains were isolated from industrial disposal site and on the basis of morphological, cultural and biochemical characteristics were tentatively identified as *Enterobacter* sp. and *Klebsiella* sp. These lead resistant isolates were used for removal of lead (Pb^{2+}) form synthetic aqueous solution in batch experiments. To maximize the adsorption efficiency, several factors were optimized, such as, incubation time, temperature and pH. The result showed a significant removal (%) of lead ions by both the isolates. Both the significant isolates, *Enterobacter* sp. and *Klebsiella* sp. showed excellent ability to reduce divalent chromium to non toxic monovalent lead, i.e. 78 % and 85 % at 31 °C, at pH 4.0 after 48 h of incubation. This indicates that these potential bacterial isolates can be effectively employed for removal of Pb^{2+} from industrial effluents containing higher concentration of this heavy metal.

Keywords: Bioremediation, adsorption, Lead, *Enterobacter* sp., *Klebsiella* sp.

INTRODUCTION

Heavy metals, in particular are a group of pollutants (mostly from industrial, agricultural and domestic activities) of major concern in the aquatic environment due to their toxicity[1]. The uncontrolled discharges of large quantity of heavy metal-containing wastes create huge economical and health care burden particularly for the people living near that area (since the effluents of the industries excreted into the environment and through food chain, affect humans and animals from various anthropogenic sources such as industrial wastes, automobile emissions, mining activity and agricultural practices as well). The important toxic metal pollutants like lead, nickel and cadmium enter to the water bodies through industrial wastewater treatment plants[2]. Among the heavy metals, Lead is a nonessential heavy metal and general toxicant. It is a multimedia pollutant that causes pollution of soil, water and atmosphere[3]. Lead enters into the environment and human food chain due to its usage in lead-based gasoline, paints, gunshot, batteries and alloys. Adults absorb 5-15% of interest lead and usually retain approximately 5% of what is absorbed. The entrance of lead at levels > 0.5-0.8 µg/ml into blood causes various abnormalities. Lead is classified as a 2B carcinogen by the IARC. Because of very toxic effects, lead measurement for exposure monitoring is very important[4].

Conventional techniques commonly applied to remove heavy metals from waste water and contaminated soil includes chemical (precipitation, neutralization) or physical (ion exchange, membrane separation, electro dialysis and activated carbon adsorption) methods[5]. Moreover, these processes may be non–viable at low concentrations. Further, these processes are expensive and not ecofriendly[6,7]. Bioremediation technology has provided an alternative to conventional methods for remediating the metal-polluted soils[8]. Bioremediation can be defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition[9]. Since microorganisms have developed survival strategies in heavy metal polluted habitats, their different microbial detoxifying mechanisms such as bioaccumulation, biotransformation, biomineralization or biosorption can be applied either ex situ or in situ to design economical bioremediation processes[10-13]. In the biosorption mechanisms, the complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The biosorption mechanisms are various; they may be classified according to various criteria. According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into: Metabolism dependent; and Non -metabolism dependent. According to the location where the metal removed from solution is found, biosorption can be classified as: (1) Extra cellular accumulation/ precipitation; (2) Cell surface sorption/ precipitation; and (3) Intracellular accumulation[14]. In this paper an effort has been made to isolate and identify heavy metal resistant bacteria from industrial disposal site and using these strains to remove heavy metals.

MATERIALS AND METHODS

Collection of Sample

Samples were collected from the zone of industry, Nagalkeni (Latitude 12°57'39" N; Longitude 80°8'13" E), Chennai, Tamil Nadu; from different places such as drainage canal at 2-3 cm depth of soil surface. Samples were in the form of untreated soil from which heavy metal resistant bacteria were isolated.

All the samples were collected in sterile polythene bag and preserved at 4 °C in refrigerator and samples were tested within 24 h of collection.

Synthetic feed solution preparation

All the chemicals were procured from Merck, India and were of A.R grade. Experimental metal used in the study was Pb (II) in the form of its metal salt solution.

A synthetic standard stock lead nitrate $Pb(NO_3)_2$ was prepared with concentration (1000 mg/l).

Then this solution is diluted as required for batch adsorption experiments with the use of distilled-deionized water.

Isolation and screening of heavy metal resistant bacteria

The soil samples were serially diluted upto 10^{-8} dilutions, in saline and 1 ml sample from 10^{-3} to 10^{-7} were pour plated in Nutrient Agar (NA) (Hi-Media, India) plates.

The plates were kept for incubation at 37 °C for 24 h in an inverted position.

Well grown bacterial colonies were picked and further purified by streaking. Isolated morphologically distinct bacterial isolate were screened by streaking it in nutrient agar supplemented with lead nitrate i.e. heavy metal salts at concentration (20 mg/100 ml).

The plates were incubated for 48 h at 37 °C. The well grown cultures on nutrient agar plates supplemented with heavy metal resistant strains were maintained in nutrient agar slants in pure form for further work.

Biochemical identification of active isolates

Selected isolates were grown on nutrient agar (Hi-Media, India) plates.

Based upon the staining reactions, physiological and biochemical characteristics[15] of the isolates were identified according to Bergey's Manual of Determinative bacteriology[16] and the organism was identified upto genus level.

Bioremediation activity assay

The bioremediation potential of lead tolerating bacterial isolates for removing heavy metal was assessed by batch experiment process. Bacteria were cultured in nutrient broth supplemented with lead metal salt (lead nitrate) and the concentration of the metal salt was maintained at 20 mg/100 ml of the medium. Cells were inoculated in nutrient broth (100 ml/flask) and kept under agitation in a rotary shaker, at 180 rpm, for 48 h at 35 ± 2°C. Cells to be used in bioremediation experiments were separated by centrifugation.

Samples taken at predetermined intervals were centrifuged and supernatant were analyzed. The analysis of Pb (II) ions were carried out by UV-vis Spectrophotometer at 600 nm. Finally the % removal of lead was calculated for each run as follows.

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

where, C_i – initial concentration of lead in the solution

C_f – final concentration of lead in the solution.

Optimization for heavy metal removal

Incubation time, temperature, pH are the factors which affects the adsorption process. Particularly, pH[17] on bioremediation experiments was investigated by optimization process. The bacterial isolates were inoculated into flask containing 100 ml of nutrient broth medium amended with 20 mg/100 ml of Pb (II) ions. Percentage removal of lead was determined for different time interval (24, 48, 72, 96 and 120 h). To find out the optimum temperature for maximum adsorption of this toxic heavy metal, strains were incubated with a wide range of temperature (21, 29, 31, 39 °C). The pH was varied from 4 to 8 (4, 5, 6, 8) by adjusting the medium supplemented with Pb (II). These culture conditions were maintained for the optimum growth of microorganisms in the batch culture. All the tests were performed in triplicates.

RESULTS AND DISCUSSION

Pollution is a serious environmental concern and interest in bacterial resistance to heavy metals of practical significance. The strong biosorbent behavior of certain types of microbial biomass toward metallic ions is a function of the chemical makeup of microbial cells[18]. In this study, we have demonstrated that microbes might be used to remediate metal contamination by removing metals from contaminated water or waste streams by biosorption of metals.

Screening and identification of heavy metal resistant bacteria

Numerous studies have revealed a number of bacterial species which are capable of removing metals from aqueous environment. In the present study, a total of seven bacterial strains were isolated from the soil by pour plating method.

The screening was done by plating all seven isolates on nutrient agar supplemented with heavy metal salts Pb (II) of concentration 20 mg/100 ml. From these, two bacterial strains (HM1 and HM2) were screened for further heavy metal removal study.

Result of Table 1 revealed that the isolate HM1 is a gram-negetive, rod shaped bacterium with Catalase, Voges-Proskauer positive and Methyl red, Indole negative reactions and HM2 is a gram-negative, facultative anaerobic, rod shaped bacterium with Catalase, Citrate utilization positive and Oxidase, Indole negative reactions. Based on the physiochemical, morphological characteristics, the isolate HM1 and HM2 were identified as *Enterobacter* sp. and *Klebsiella* sp., respectively.

Table 1: Morphological, physiological and biochemical characteristics of the isolated strain HM1 and HM2

Characters	Isolate HM1	Isolate HM2
Morphology	Rod shaped, gram -ve, facultative anaerobic and without endospore	Rod shaped, gram -ve, facultative anaerobic and without endospore
Motility	+ve	-ve
Catalase	+ve	+ve
Oxidase	-ve	-ve
Methy red	-ve	-ve
Voges- Proskauer	+ve	-ve
Indole production	-ve	-ve
Citrate utilization	+ve	+ve
Nitrate reduction	+ve	+ve
Hydrolysis of starch	-ve	+ve
Fermentation with Glucose, Lactose, Sucrose, Mannitol	+ve	+ve

Measurement of biosorption

Both the isolates, *Enterobacter* sp. and *Klebsiella* sp. were used separately for the adsorption of Pb (II) and the optimized conditions (incubation time, temperature, pH) were determined for maximum lead removal.

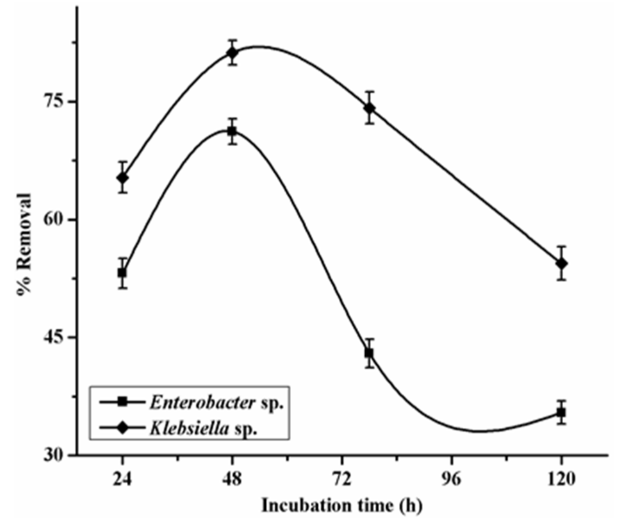

Figure 1: Effect of time on bioremediation of Pb (II) ion by *Enterobacter* sp. and *Klebsiella* sp.

Effect of adsorption time

The results for the effect of adsorption time on biosorption of Pb (II) ion as shown in Figure 1 for *Enterobacter* sp. and *Klebsiella* sp. Graph shows that for the both cases, removal of Pb (II) ions increases at optimum incubation time of 48 h after that it decreases. For *Enterobacter* sp. and *Klebsiella* sp., there was a progression in the rate of adsorption of lead ion maximum at 48 h of 71 % and 81 %, respectively. This may be due to the effect of optimum incubation with microbial growth related with optimum biomass at which high level of heavy metal adsorbed.

Effect of adsorption temperature

The temperature of the solution is the most critical parameter for metal sorption as it influences both the bacterial growth chemistry as well as solution chemistry of soluble metal ion. Figure 2 shows effect of incubation temperature on metal adsorption. Here optimized incubation time was used for both the species with a temperature range 21-39 °C. Maximum % removal of metal ion was observed at 31 °C. Removal efficiencies were 73 %, 83 % for Pb (II) using *Enterobacter* sp. and *Klebsiella* sp. respectively.

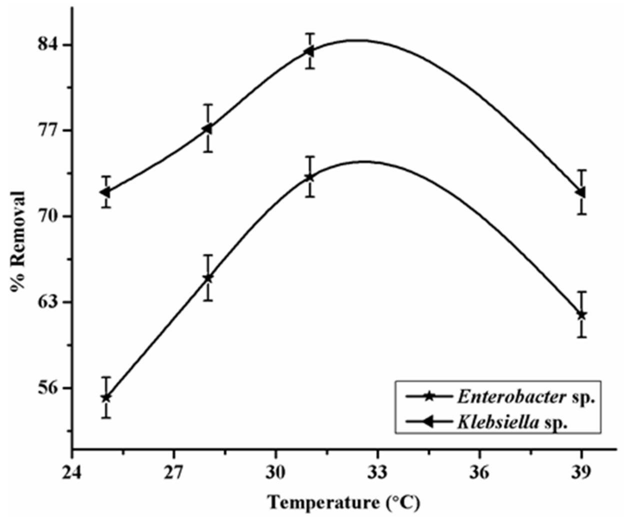

Figure 2: Effect of temperature on bioremediation of Pb (II) ion by *Enterobacter* sp. and *Klebsiella* sp.

Effect of pH

The adsorption of the metal ions using living cells is sensitive to pH. The cell surface metal binding sites and availability of metal in solution are affected by pH. At low pH, the cell surface sites are closely linked to the H^+ ions, thereby making these unavailable for other cations. However, with an increase in pH, there is an increase in ligand with negative charges which results in increased binding of cations[19]. The increase of pH resulted in an increased negative charge on the surface of the cell which favored electrochemical attraction and adsorption of metal[17] (Gourdon). In the pH range studied (4 to 8) with optimized time and temperature, all the heavy metal resistant bacteria isolates growth were increased gradually and decreased at increased pH (Figure 3). *Enterobacter* sp. and *Klebsiella* sp. both have the ability to adsorb maximum Pb at pH 4 of 78 % and 85 % respectively.

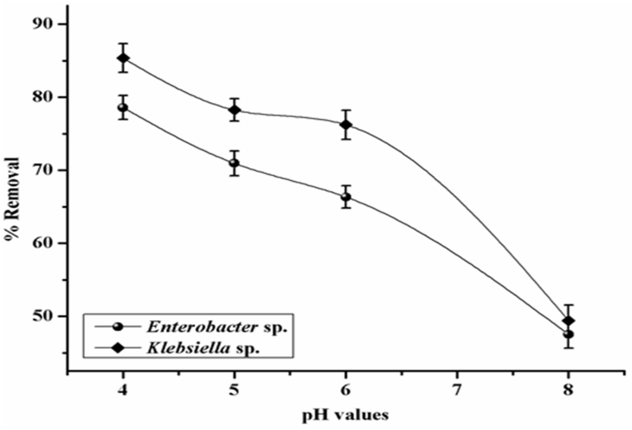

Figure 3: Effect of pH on bioremediation of Pb (II) ion by *Enterobacter* sp. and *Klebsiella* sp.

CONCLUSION

Heavy metal toxicity increases sharply along with industrial progress. To stop heavy metal pollution and to prevent metal-toxicity there is a clear need for an overall waste treatment strategy with the goal of elimination of priority pollutants at source. This can be achieved by indigenous microorganisms found in various industrial effluents which can be used as effective and economical bioremedial tool. Here two lead resistant strains were isolated and used for bioremediation study. They showed significant removal percentage of lead ions after optimization process. This study has clearly indicated the effectiveness of these stains which can be used as cost-effective biological adsorbents.

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