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Lead Bioremediation with Respect to Mining and Industrial Effluents

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Abstract

Lead (Pb) is non-bioessential, persistent and hazardous heavy metal pollutant of environmental concern. Bioremediation has become a potential alternative to existing technologies for removal and/or recovery of toxic lead from waste waters before releasing it into natural water bodies for environmental safety. Bioremediation of lead metal ions was studied using Gram positive, heterotroph Bacillus licheniformis. The bacterium was grown in different concentrations of lead metal ion and it was found to be naturally tolerant up to 100 ppm of lead, above which bacterial growth decreased and also biosorption of lead metal ions was almost nil. Experiments were carried out at neutral pH range with cell count of 10^9 cells/ mL which gave optimum results 60-75 % of biosorption was observed in case of 10 ppm and 50 ppm lead metal ion. In case of 100 ppm lead the uptake was 32%. Size of bacteria also decreased due to stress caused by lead metal ions and uptake of lead by bacteria was confirmed by EDX spectroscopy.

Keywords: Lead, Bioremediation, Effluents, *Bacillus licheniformis*

Introduction

Pollution of environment is one of the major challenges of today's civilization[1]. Due to global industrialization and nuclear processes large amounts of toxic compounds have been released into biosphere. Environment pollution by lead is worldwide public problem, exemplified by an elevated blood levels among people living in the polluted areas. Lead is a well known non-biodegradable toxic metal in the environment and now, it has become a global health issue. More than 15 million children in developing countries are suffering permanent neurological damage due to Pb poisoning. Lead toxicity in children causes serious health hazards i.e. permanent brain damage, causing learning disabilities, hearing loss, and behavioral abnormalities and in adults causes hypertension, blood pressure problems, heart disease, etc. The elevated levels of Pb in blood of children (200 µg l -1) and dogs (250 µg l -1) of Indian megacities were reported[2]. Persistence of heavy metals in environment may pollute or contaminate soils and aqueous streams as both natural components or as the result of human activity. Today contamination of water by toxic heavy metals resulting from the discharge of industrial wastewater is a worldwide environmental problem. Many industries, particularly in metal processing operations and refineries, represent significant sources of heavy metal emissions. Unlike organic compounds, soluble heavy metals, such as copper, cadmium, lead, and chromium, are non-biodegradable and toxic even at trace levels. Heavy metals can accumulate in living organism and cause various diseases[3].

Lead may access into body through inhalation, by ingestion or by absorption through skin and mucous membrane. When women encountered to lead during pregnancy can cause a miscarriage, premature birth Low birth weight and it effects development of fatuous brain and growth of new born baby also retarded. Lead poses health risks for everyone, but young children and unborn baby more porn to lead toxicity which contributes to effect development of growing children and their behavior and learning ability[4].

Mining and metallurgical activities cause greater perturbation and devastation of both terrestrial and aquatic environments which has large scale ramifications. Due to improper planning and negligence of regulations, mining activities results in an appreciable damage, degradation and deterioration of the environment and ecological damage to water, air and soil occurs. The degradation of various environmental factors substantially would aggravate the health problems among the workers and the people living in the immediate vicinity of the mining area[5].

Bioremediation process in this regard is an option that offers possibility to destroy or render harmless various contaminants using plants and microbes. Microbial bioremediation includes removal of heavy metals by microorganisms (bacteria, fungi, yeast and algae) as sorbents. The soluble compounds of lead are poisonous. Microorganisms and microbial products can be highly efficient bioaccumulators of soluble and particulate forms of metals especially dilute external solutions. Microbe related technologies may provide an alternative or addition to conventional method of metal removal or metal recovery. Metal sequestering properties of certain types of microorganisms offer considerable promise.

A wide range of biological materials, mainly live and dead cells of bacteria, yeast, fungi and algae as well as cellular products such as polysaccharides have been extensively used for removal

of toxic heavy metals from aqueous solutions[6]. Biosorption and bioaccumulation processes have been reviewed for different metals with different microorganisms[7, 8].

Various lead resistant mechanisms employed by lead resistant bacteria include efflux mechanism, extracellular sequestration, biosorption, precipitation, alteration in cell morphology, enhanced siderophore production and intracellular lead bioaccumulation[9]. These mechanisms of removal of heavy metals includes efflux of metal ions outside the cell, accumulation and forms complex of metal ions inside the cell and later reduce toxic metal ions to a non-toxic state. The microorganisms involved in this process may belong to bacteria, fungi, yeast and algae [10]. Bioremediation may soon compete with chemical methods in efficiency and cost- effectiveness. Optimization of various conditions for removal of Pb was also carried out.

Material and Methods

Bacterial culture: A pure culture *Bacillus licheniformis* (MTCC 3037) utilised in this study was obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. It was subcultured in the laboratory using Luria broth medium (LB) and growth kinetics were studied[11].
Potassium chloride was used to maintain ionic strength, while sulphuric acid and sodium hydroxide were used as pH modifiers. All reagents used in the present studies were of analytical reagent grade. Deionised double distilled water was used in all the tests. Lead nitrate of analytical grade was used for the biosorption studies.

B licheniformis was cultured by inoculating 10 ml of pure strain to 90 ml of LB medium prepared in a 250 ml Erlenmeyer flask. This was incubated at 30ºC on Remi rotary shaker at 200 rpm. Cell concentration was determined with a microscope using haemocytometer. Variation in pH of culture medium during growth was monitored at regular time intervals using Systronics digital pH meter.

B licheniformis was grown in presence of 10, 50, 100, 200 and 500 ppm of lead metal ion at neutral pH range of 6.5-7.5. The biosorption studies were carried both in growing culture as well as in presence of bacterial cells.

Biosorption procedure of lead onto *B licheniformis:* Batch experiments are conducted to study the parameters such as contact time, concentration of lead ions and amount of biomass influencing bioremediation of heavy metal ion, fully grown bacterial culture was centrifuged at 10,000 x g for 08 min in a Remi make centrifuge, cell pellet was suspended in 10^{-3} M KCl. Bacterial cell number was enumerated under a microscope. In each experiment, desired aliquots of bacterial cells were added to a 250 ml conical flask containing 100ml of desired concentration of lead at neutral pH range and agitated at 200 rpm in Remi orbital shaker at 30ºC± 2ºC. Solutions were centrifuged at different time intervals of 10 to 60 min range and also at 24 hours, supernatant was collected through centrifugation and analyzed for the lead concentration using atomic absorption spectrophotometer (AAS), procured from Thermo Electron Corporation Ltd., Cambridge, UK, similar experiments were conducted in presence of growing culture for 24 hours and the readings were taken after 24 hours.

SEM micrographic analysis: SEM studies were carried out using an ESEM FEI Quanta 200, high resolution electron microscope. Bacterial cells were obtained by centrifuging them at 10,000 x g for 10 min, cell pellet was again suspended in double distilled water, a drop of the sample was placed on a glass cover slip and air dried, these samples were chemically fixed for a period of 24-96 h at room temperature using a final concentration of 2.5% glutaraldehyde, the samples were then rinsed in distilled water thrice to remove traces of glutaraldehyde, and later on the samples were dehydrated in grades series of ethanol and air dried under vacuum. Before doing SEM studies, samples were coated with a film of gold–palladium (60:40) using a vacuum coating device[12]. The EDS spectrum of bacterial cells before and after interaction with lead has been studied.

Results and Discussion

Growth curve of *B licheniformis* was plotted with cell number at a given time in various concentrations of lead metal ion as depicted in Figure 1, which shows that lag period of growth extends up to 4 hrs in case of bacteria grown in presence of 10 ppm and 50 ppm lead, after which the exponential growth phase can be observed up to 20 h after which a stationary phase was seen. Maximum growth attained was 1.0×10^9 cells/ml, after 40 hours death phase was observed and the pH remained almost same. *B licheniformis* was grown in presence of 100, 200 and 500 ppm of lead metal ion, with increase in metal ion concentrations drastic decrease in cell count was observed. Bacteria was naturally tolerant up to 50 ppm in higher concentration of metal ion, a significant decrease in cell count was observed and cells became smaller and cocci shaped in size due to stress produced in presence of toxic metal ions.

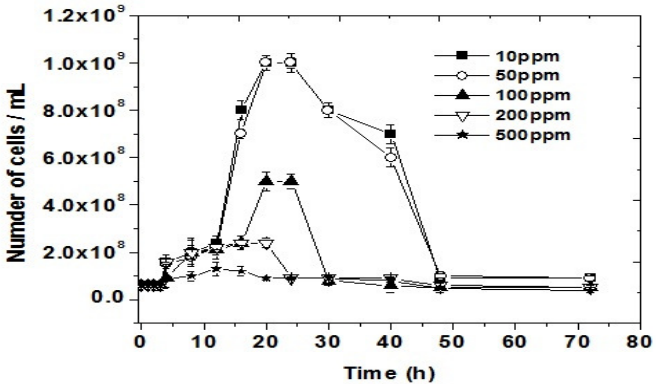

Figure 1 (a)
Optical density and pH as a function of time during growth of *Bacillus licheniformis* (Note: Standard deviation varies between ± $4x10^6$ and $3x10^7$ for n =3)

SEM micrograph of *B licheniformis* is depicted in figure 2a and figure 2b to illustrate their morphological characteristics. The bacteria when grown in presence of lead metal ion showed a visible decrease in their size.

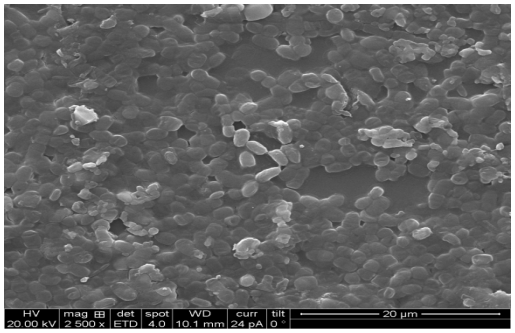

Figure 2
(a) SEM micrograph of *B. licheniformis*

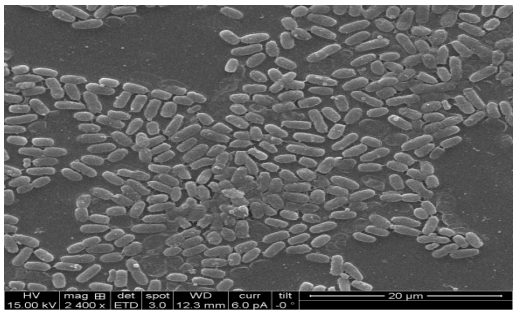

Figure 2
(b) *B. licheniformis* grown in presence of lead

Biosorption studies performed both in presence of bacterial cells and growing culture was almost same. Result of biosorption studies is mentioned in Table 1. 10^9cells/mL was suspended in 10^{-3} M KCl used as a background electrolyte at a pH of 6.5-7.5 range. Bacterial cells of *B licheniformis* were obtained by centrifugation from a fully grown culture. Bacterial cells were incorporated to 100 ml of lead solutions in 250 mL Erlenmeyer flasks were rotated in a rotary incubator shaker at 200 rpm at 30ºC± 2ºC for 10 min to 1hour. At the end of each time period studied, the solutions were centrifuged at 10000 rpm for 10 min in a Remi refrigerated centrifuge at 4ºC. The supernatant solutions were analyzed for lead in an M series atomic absorption spectrometer procured from Thermo Electron Corporation Ltd., Cambridge, UK.

Table-1
The efficiency (%) for the biosorption of lead metal ion in one hour by the cells from *B.licheniformis* (Note: Standard deviation varies between ± 0.05 and 0.2 for n = 3)

% of lead metal ion removed in one hour (pH 6.5-7.5)			
Cells of *B.licheniformis*	10 ppm Pb	50 ppm Pb	100ppm Pb
	70%	65%	32%

Percentage biosorption of lead metal ion by the bacterial cells: Cells were interacted with lead metal ion for 10min, 30 min and 1 hour. The percentage of adsorption had a similar trend; hence one hour was maintained as standard for biosorption. As depicted in table 1, the biosorption efficiency was 70% for 10 ppm, 65% for 50 ppm and 32% for 100 ppm respectively.

Possible mechanisms of biosorption includes exclusion by forming a permeable barrier, intra- and extracellular sequestration, active transport, enzymatic detoxification, dissolution of lead by acid production, chelation of lead, precipitation of lead through production of organic bases, extracellular metal precipitation and biotransformation reactions such as methylation, volatilization, oxidation and reduction[13].

Characterization studies: EDX (Energy Dispersive X-Ray) elemental analysis was carried out on bacterial cells as well as bacterial cells interacted with lead using an ESEM FEI Quanta 200, high resolution electron microscope. The bacterial cell samples were dried in a vacuum oven at 60ºC and were coated with a film (about 200 A thick) of gold–palladium (60:40) using a vacuum coating device were made conducting by gold sputtering coating using a JEOL ion sputtering device. Figure 3a depicts the EDS spectrum of pure bacterial cells and figure 3b shows the presence of lead metal ion.

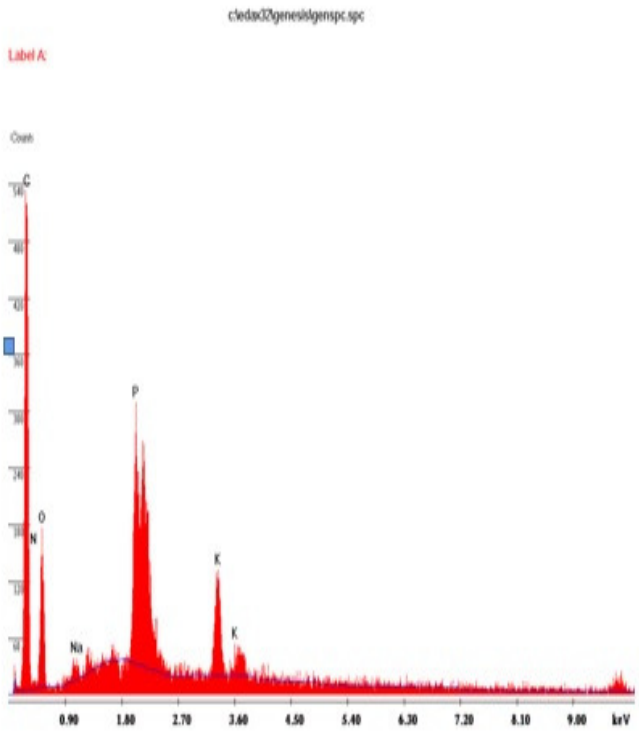

Figure-3 (a)
EDS spectrum of *Bacillus licheniformis*

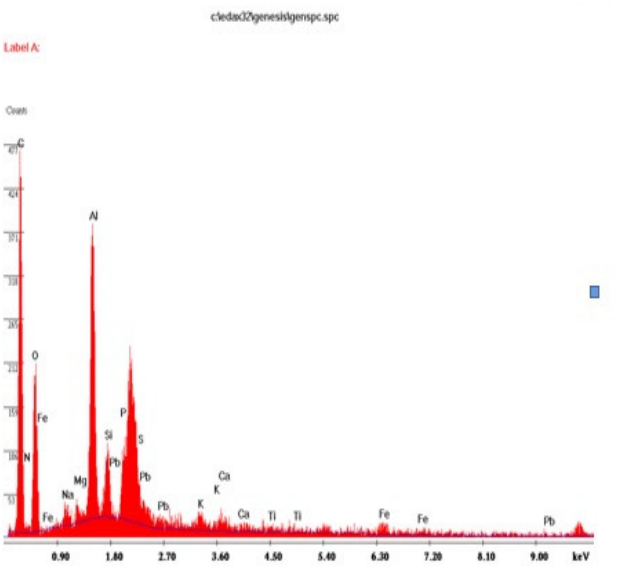

Figure-3 (b)
In presence of lead

Conclusion

Growth kinetics of *B.licheniformis* was studied. The cells of *B.licheniformis* proved to be 65-70% efficient in the biosorption of lead metal ions at 10 and 50ppm of lead metal ion. The SEM results show that size and shape of bacteria changed when grown in presence of lead due to stress caused by lead toxicity. EDS results further confirm biosorption of lead metal ion onto bacterial cells. In this regards, bioremediation process provides an effective innovative measures for treatment of a wide variety of contaminants. This study shows possibilities for development of eco friendly technologies for bioremediation.

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References

1. Kumar Praveen G.N. and Sumangala K.B., Fungal Degradation of Azo dye- Red 3BN and Optimization of Physico-Chemical Parameters, *ISCA Journal of Biological Sciences*, **1(2)** , 17-24 **(2012)**

2. Seema Tiwari and Tripathi I.P., Lead Pollution -An Overview, *Int. Res. J. Environment Sci*., **1(4),** 84-86 **(2012)**

3. Murhekar Gopalkrushna Haribhau and Rathod R. G., Trace Metals Contamination of Surface Water Samples in and Around Akot City in Maharashtra, India, *Res.J.Recent Sci*., **1(7)**, 5-9 **(2012)**

4. Kapoor Neeti., Tiwari Prakash and Hari Singh Gour., Effects of Heavy Metal Poisoning during Pregnancy, *Int. Res. J. Environment Sci*., **2(1)**, 88-92 **(2013)**

5. Ahanger Faroz Ahmad., Sharma Harendra K., Rather Makhmoor Ahmad and Rao R. J., Impact of Mining Activities on Various Environmental Attributes with Specific Reference to Health Impacts in Shatabdipuram, Gwalior, India, *Int. Res. J. Environment Sci*., **3(6)**, 81-87 **(2014)**

6. Wang J. and Chen C., Biosorbents for heavy metals removal and their future, *Biotechnology Advances*, **27**, 195-226 **(2008)**

7. Chojnacka K., Biosorption and bioaccumulation – the prospects for practical applications, *Environment International*, **36**, 299–307 **(2010)**

8. Gadd G.M., Heavy metal accumulation by bacteria and other microorganisms, *Experientia*, **46**, 834-840 **(1990)**

9. Naik M.M. and Dubey S.K., Lead resistant bacteria: lead resistance mechanisms, their applications in lead bioremediation and biomonitoring, *Ecotoxicol Environ Saf,* **98,** 1-7 **(2013)**

10. Vijayaraghavan K. and Yun Y.S., Bacterial biosorbents and biosorption, *Biotechnology Advances,* **26**, 266-291 **(2008)**

11. Sambrook J., Fritsch E.F. and Maniatis T., *Molecular cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, **(1989)**

12. Patra Partha and Natarajan K.A., Microbially-induced separation of chalcopyrite and galena, *Miner. Eng,* 21, 691–698 **(2008)**

13. Chen H. and Cutright T.J., Preliminary evaluation of microbial mediated recipitation of cadmium, chromium and nickel by rhizosphere consortium, *J Environmental Engineering*, **129**, 4-9 **(2003)**