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Utilization of Exo polymers secreted by *Bacillus licheniformis* for the Remediation of Lead

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Abstract

High levels of dissolved metals in the environment includes active and abandoned mine sites as well as industrial effluents also contribute to lead contamination in both water and soil. As compared to the conventional remediation techniques, biological methods are gaining prominence because of their potential in providing a cost effective technology for heavy metal remediation. Biopolymer produced by Bacillus licheniformis was investigated with regard to remediation of lead metal ion. Biopolymer production was optimum when grown in M9 medium, biopolymer from B licheniformis was then used for bio sorption of lead metal ion at neutral pH range, 95-98% of biosorption was observed in case of 10ppm and 50ppm lead metal ions, this study shows utility of biopolymers in bioremediation of lead metal ions.

Keywords: *Bacillus licheniformis*, biopolymer, lead, bioremediation.

Introduction

Heavy metal and their salts are considered as very important group of environmental pollutant which in small quantities may be essential nutrients that protect your health, yet in larger quantity it become toxic and dangerous to human being[1]. Environment pollution by lead is worldwide public problem, exemplified by an elevated blood levels among people living in the polluted areas. There are 35 metals that concern us because of occupational or residential exposure; 23 of these are the heavy elements or "heavy metals": antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc, which affect adversely if their level increases[2].

The free metal ion concentration not only depends on the total metal content in soils, but also on the pH of the soil. Metals can also be transported from soil into groundwater resulting in to soil contamination and inhibiting growth of plants. Metal contamination of agricultural soils by atmospheric deposition or by disposal of sewage sludge constitutes a risk of either leaching of metals into the groundwater or excessive accumulation in the top soil. They arrive in aquatic ecosystems as dissolved and solid waste from domestic, industrial, and agricultural runoff[3].

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 nonbiodegradable and toxic even at trace levels. Heavy metals can accumulate in living organism and cause various diseases[4].

Mining is an important economic activity in many countries all over the world. It is an essential human activity to provide rough materials for the society. Operations, whether small or largescale, are extremely disruptive and damaging to the environment, producing large quantities of waste that can have deleterious impacts for decades[5].

Microbial biopolymers promote adsorption by formation of bridges between metal ions. Biopolymers can be produced economically on a large scale and easily be recovered from culture broth. Therefore now they have wide applications in many sectors associated with textiles, detergents, clay removal, adhesives, oil recovery, metal removal and waste water treatment[6].

Predominant components of extracellular polymeric substances are polysaccharides, proteins, glycoproteins or nucleic acids[7, 8]. In recent years exo polymers have attracted wide attention because they are biodegradable, harmless and free of secondary pollution risk. Biopolymers could be used as potential replacements for organic synthetic chemicals which possess inherent drawbacks of being a source of carcinogenic monomers and being less biodegradable. Biopolymers have been widely used in wastewater treatment and in food-processing and fermentation industries[9].

A new biosorbent material from microbial biomass is an emerging trend and it is cost effective and eco friendly. In the recent years, several kinds of microorganisms which secret biopolymer have been screened and isolated from activated

sludge, soil, industrial effluent wastewater samples which are the major sources. Some microorganisms including algae, bacteria, actinomyces and fungi have been reported to produce biopolymer[10, 11].

Materials and Methods

Bacterial Culture: Pure culture of *Bacillus licheniformis* (MTCC 3037) used in this study was obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. It was subcultured in laboratory using Luria Bertani broth (LB) and growth kinetics were studied in M9 medium for production of exopolymers[12].

All reagents used in the present studies were of analytical reagent grade, deionised double distilled water was used in all the tests and 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. Change in pH of culture medium during growth was monitored at regular time intervals using Systronics digital pH meter, fully grown culture was later on subcultured in M9 medium for production of exopolymers.

Production of biopolymer: Bacteria was inoculated into a standard flask with 100 ml of Minimal medium[12] which includes (g/L) di-sodium hydrogen phosphate-64 g, potassium dihydrogen phosphate-15g, sodium chloride-2.5g, ammonium chlorid-5 g, magnesium sulphate-2mM, calcium chloride-0.1mM, glucose-5% were used at pH 7.2, the EPS (extra polymeric substances) produced under optimum conditions by incubation at 30^{o}C for 3 days in shaking condition at 200 rpm. Samples were taken at different time intervals and monitored for cell growth and cell number was determined by microscopic counting with Haemocytometer.

Extraction and partial purification of biopolymer: When the bacteria attained a stationary phase of 10^9 cells/mL in the broth medium, the culture was centrifuged at 10000 x g for 15 min to separate cell pellet, supernatant was poured into two volume of cold ethanol and kept for overnight at 4^{o}C to precipitate the biopolymer, resulting precipitate was collected by centrifugation at 10000 x g for 15 min and washed by re dissolving in distilled water. Repeat the ethanol precipitation was repeated for two more times to get crude bioflocculant, the biopolymer was dehydrated for 2 h to remove all water and ethanol and then crude extract was subjected to dialysis against distilled water to get pure exo polymers. The precipitate was collected by centrifugation at 10000 x g for 15 min and stored at 4^{o}C [13].

Characterization of partially purified biopolymer: The total protein concentration of polymers was determined according to Bradford method[14]. Total sugars were determined by Phenol-Sulphuric acid method using procedure of Carbohydrate Analysis[15]. After recovery of biopolymer by ethanol, resultant precipitate was found to be soluble in water but not in solvents, since biopolymer contained hydroxyl groups it has possibility of hydrogen bonding to one or more water molecules. Abundance of hydroxyl groups build up strong forces of attraction between biopolymer molecules resulting in relatively hard crystalline form, these forces are too great to be broken by organic solvents, so the purified biopolymer is insoluble in organic solvents[16].

Lead removal using exo polymers: Extracted exo polymers (2g/L) were dispersed in 100ml of 10ppm, 50ppm and 100ppm of lead nitrate solution at neutral pH, samples were kept in rotary shaker with 200 rpm at 30^{o}C after one hour of incubation, samples were centrifuged and supernatant was analyzed for residual concentrations of lead ions using Atomic absorption spectroscopy.

SEM micrographic analysis: SEM studies were carried out using an ESEM FEI Quanta 200, high resolution electron microscope.ion, high resolution electron microscope. The mineral particles were interacted with the bacterial cells collected by centrifugation and subjected to chemical fixation using 2.5% glutaraldehyde and dehydrated in grade series of ethanol. Detailed procedures are reported earlier[17].

Results and Discussion

Bacterial growth kinetics: Growth kinetics of *B. licheniformis* was plotted with optical density (OD) with respect to time as depicted in figure-1and it is evident that lag period of growth extends up to 4 hrs beyond which exponential growth phase can be observed up to 30 h after which a stationary phase is seen. Maximum optical density attained was 2.0.

After 70 hours death phase was observed. The production of biopolymer increased in parallel with cell growth during the exponential phase. Sharp decline in pH levels was observed from 7.5 to 6.5 with increase of cell growth in the first 30 h, which might have been due to the production of organic acids due to the presence of glucose. Beyond that point pH decreased up to 5.8 and it was nearly stable for the following 72 h.

SEM micrograph of *B licheniformis* is depicted in figure-2(a) to illustrate their morphological characteristics and figure-2(b) depicts the photograph of the extracted biopolymer.

Estimation of polysaccharides and proteins in exopolymers: Extracted exopolymers was subjected to Bradford test for proteins and Phenol-sulphuric acid estimation for polysaccharides. As depicted in table-1, the polysaccharide concentration was found to be 160.2 µg/ml and protein concentration was found to be 41.4 µg/ml.

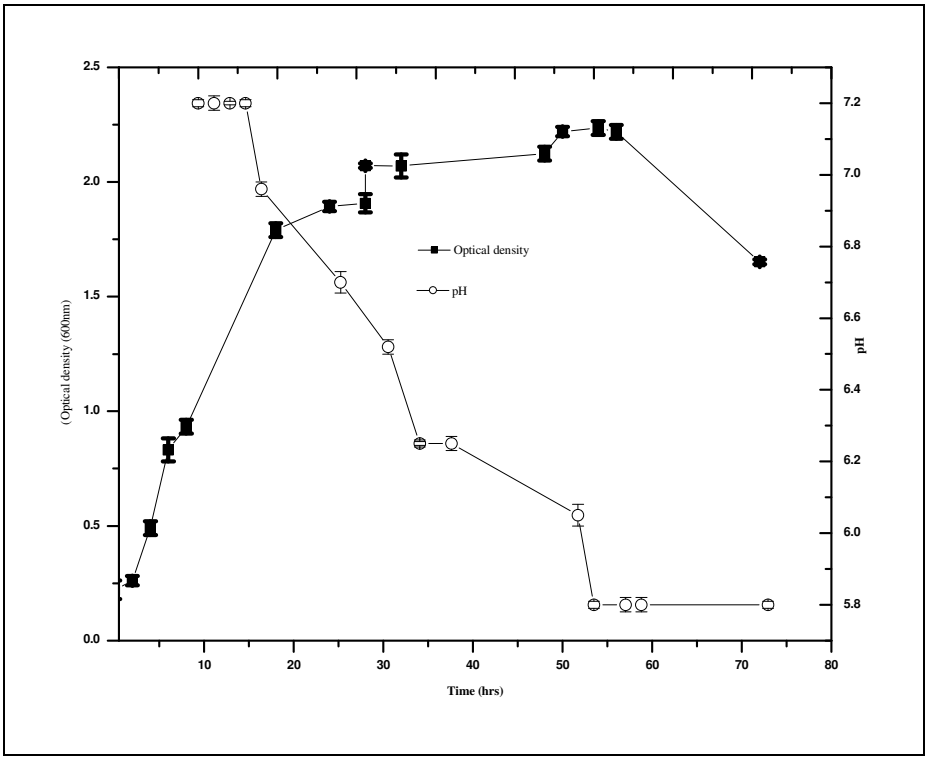

Figure-1
Optical density and pH as a function of time during growth of *Bacillus licheniformis*
(Note: Standard deviation varies between ± 0.005 and 0.05 for n = 3)

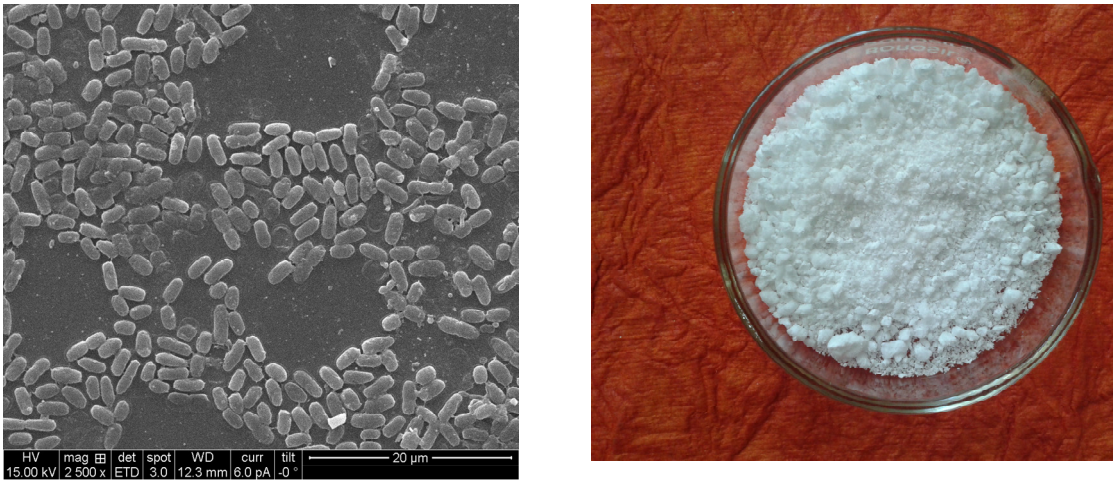

Figure-2
(a) SEM micrograph of *B licheniformis* and (b) Photograph of extracted biopolymer

Table-1
Estimation of Polysaccharides and Proteins secreted by *B licheniformis*

Organisms	Polysaccharides concentration (µg/ml)	Protein concentration (µg/ml)
B licheniformis	160.2	41.4

Characteristics of biopolymer: It can be seen from the UV spectrum of biopolymer that there are no nucleic acids (260 nm) and protein (280 nm) absorption peak, instead there is an absorption peak at 200 m characteristic for polysaccharide[8].

Percentage biosorption of lead metal ion by biopolymers: Extracted biopolymer was interacted with lead metal ion for 10min, 30min and 1 hour, percentage of adsorption had a

similar trend; hence one hour was maintained as standard for biosorption. As depicted in table-2, the biosorption efficiency was 98% for 10ppm, 95% for 50ppm and 47% for 100 ppm respectively.

Table-2
Efficiency (%) for biosorption of lead metal ion in one hour by exopolymers extracted from *B. licheniformis*

% of lead metal ion removed in one hour (pH 6.5-7.5)			
Biopolymer	10 ppm Pb	50 ppm Pb	100ppm Pb
	98%	95%	47%

Conclusion

Growth kinetics of *B. licheniformis* was studied; the exopolymers consisted of both proteins and polysaccharides. The exopolymers extracted from *B.licheniformis* proved to be 95-98% efficient in biosorption of lead metal ions; this study shows possibilities for development of eco friendly technologies for bioremediation.

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References

1. 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)**

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. Srinivas J., Purushotham A.V. and Murali Krishna K.V.S.G., The effects of Heavy metals on Seed Germination and Plant Growth on *Coccinia, Mentha* and *Trigonella* Plant Seeds in Timmapuram, E.G. District, Andhra Pradesh, India, *Int. Res. J. Environment Sci,* **2(6)**, 20-24 **(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. Kumar C.G., Joo H.S., Kavali R, Choi J.W. and Chang C.S., Characterization of an extracellular biopolymer flocculant from a haloakalophilic *Bacillus* isolate, *World J. Microbiol. Biotechnol,* **20(8),** 837-843 **(2004)**

7. Lazarova V. and Manem J., Biofilm characterization and activity analysis in water and waste water treatment, *Wat. Res*, 29, 2227-2245 **(1995)**

8. Gao J., Bao H.Y., Xin M.X., Liu Y.X., LiQ and Zhang Y.F., Characterization of bioflocculant from a newly isolated *Vagococcus*sp, W31, *J. Zhejiang Univ. Sci. B*, **7,** 186-192 **(2006)**

9. Wang S., Gong W., Liu X., Lin T., Yue Y. and Gao B., Production of a novel bio flocculant by culture of *Klebsiella mobilis* using dairy wastewater, *Biochem. Eng .J,* **36**, 81-86 **(2007)**

10. Takagi H. and Kodowaki K., Floccculant production by *Paecilomyces sp*. taxonomic studies and culture conditions for production, *A&c, Biol. Chem*., **49**, 3151-3157 **(1985)**

11. Huang W., Chang W. and Hu Y., Screening of bioflocculant producing strains by NTG mutagenesis, *J. Environmental sci* , **17,** 494-498 **(2005)**

12. Sambrook J., Fritsch E.F. and Maniatis T., Molecular cloning : A laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. **(1989)**

13. Ugbenyen A.M. and Okoh A.I., Flocculating Properties of a Bioflocculant Produced by *Bacillus sp*. Isolated from a Marine Environment in South Africa, *Chem. Biochem. Eng. Q.*, **27(4),** 511–518 **(2013)**

14. Bradford M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding, *Anal. Biochem*. **72,** 248-254 **(1976)**

15. Chaplin M.F., Kennedy J.F., Carbohydrate Analysis, Oxford University Press, New York, 55–57 **(1994)**

16. James K.C., Solubility and the Related Properties. *In*: Drugs and the Pharmaceutical Sciences, Vol. 28. Marcel Dekker, New York, USA, 91-125 **(1986)**

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