



## Lead biosorption by resting cells of *Bacillus cereus*

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### Abstract

A study on the lead biosorption by resting cells of lead resistant bacteria, isolated from industrial effluents, was carried out to ascertain their biosorption capacities. The strain showing highest MIC (minimum inhibition concentration) for lead was selected for the study and identified as *Bacillus cereus*. Lead biosorption studies on *Bacillus cereus* pretreated with physical (heat and oven dried) and chemical (Sodium azide) methods showed improved lead biosorption with the exception of heat treatment in comparison to live biomass. Among the pretreatment methods, azide treatment showed maximum lead biosorption followed by oven drying and heat treatment. The lead biosorption capacity of *Bacillus cereus* can be exploited for the bioremediation of heavy metal contaminated industrial effluents.

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## Introduction

Water pollution, caused by heavy metals discharged through industrial effluents is a serious worldwide problem due to their harmful effects on human health even at low concentration in the environment. Many industries, especially plating and those manufacturing batteries, pigments, and ammunition, release heavy metals such as lead, cadmium, and zinc into wastewaters (Puranik and Paknikar, 1997; Puranik *et al.*, 1995). Though lead has no known biological function, it is highly toxic and accumulates in humans. The current EPA and WHO drinking water standard for lead is  $0.05 \text{ mg L}^{-1}$  and  $10 \text{ } \mu\text{g L}^{-1}$ , respectively (Hussain *et al.*, 2009). Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, and reproductive and nervous systems. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behavior disorders (Soltan *et al.*, 2008). It is therefore, essential to remove lead from wastewater before disposal.

The conventional approaches for removing dissolved heavy metals, such as precipitation, oxidation/reduction, ion exchange, filtration, electrochemical processes, membrane separations, and evaporation, all exhibit several disadvantages, such as high cost, incomplete removal, low selectivity, high energy consumption, and generation of toxic slurries that are difficult to eliminate (Celaya *et al.*, 2000). These conventional approaches are only practical and cost-effective when applied to wastes with high heavy metal ion concentrations. Low strength heavy metal containing wastewaters generally cannot be treated successfully with such methods (Wilde and Beneman, 1993).

New separation methods are required that can reduce heavy metal concentration to environmentally acceptable levels at affordable cost. Bio-removal has the potential to contribute to the achievement of this

goal (Volesky and Holan, 1995). The uptake of metal occurs actively only within living cells through bioaccumulation or passively at the surface of both living and dead cells (Klimmek *et al.*, 2001). This passive mechanism is called "biosorption". An important aspect of biosorption is that it can be carried out either with metabolically active or inactive cells. Passive biosorption is metabolism independent and proceeds rapidly by any one or a combination of the following metal binding mechanisms: coordination, complexation, ion exchange, physical adsorption (e.g. electrostatic) or inorganic microprecipitation. Metal uptake by non-living cells is mainly in passive mode. (Veglio *et al.*, 1997). The functional groups involved in the binding of heavy metals to microbial cells are phosphates, carboxyl, hydroxyl groups. Complexation of metals by these ligands is by physio-chemical adsorption on the surface. (Rama *et al.*, 2005). The phenomena of adsorption has been described in a wide range of living biomass like fungi (Matheickal and Yu, 1997), bacteria (Öztürk and Ayar, 2004; Chang *et al.*, 1997), yeast (Zhang *et al.*, 1998), moss (Low and Lee, 1991), aquatic plants (Schneider, 1995) and algae (Yu *et al.*, 1999; Kaewsarn, 2002). Both chemical pretreatments, such as contacting cells with acids, alkali, and organic compounds and physical pretreatments, such as heat treatment, autoclaving, freeze drying, and boiling (Das *et al.*, 2007), showed enhancement in metal biosorption by microorganisms.

Numerous studies have identified a number of potential bacterial species capable of accumulating metals from aqueous environment. Among the bacteria, *Bacillus* sp. has been identified as having a high potential for metal sequestration and has been used in commercial biosorbent preparation (Nilanjana *et al.*, 2008).

This study describes the biosorption of lead by a nonliving biomass of gram positive bacteria identified as *Bacillus cereus*. The live cells of *Bacillus cereus* were

inactivated by physical and chemical pretreatment using heat and sodium azide respectively.

### Materials and methods

#### *Microorganisms and culture media*

The Vrishabhavathi River, flowing through Peenya Industrial area, Bangalore, (Karnataka, India) which has dried up and now carries industrial effluent and urban sewage, was selected for sample study. Sixty bacterial strains were isolated from the water samples and were subjected to MIC studies for lead. The MICs of sixty isolates for lead concentrations (50,100,150,200,300,400 and 500 mg/l) revealed that five out of sixty isolates were able to tolerate a high concentration of lead (500mg/l) and one isolate could tolerate 1000mg/l of lead. The isolate showing highest MIC was identified and confirmed as *Bacillus cereus* (GenBank Accession Number: EF488087) at Bangalore Genei, Bangalore based on 16S rDNA data. The isolate was maintained by weekly sub culturing on tryptone glucose extract agar and stored at 4°C.

#### *Metal solution*

A stock solution of lead (100,000 mg/L) was prepared by dissolving 16mg of lead nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>] in deionised distilled water, shaking it for 15 min and then leaving it to stand for 24 hrs to obtain complete dissolution. Stock solution was diluted with deionised distilled water to obtain the necessary concentrations. Solutions were adjusted to desired pH values with 0.1 M sodium hydroxide and 0.1 M nitric acid. The initial lead concentration was measured at the beginning of all experiments carried out using an Atomic Absorption Spectrophotometer (Electronic Corporation of India Ltd., model ElementAS AAS4139).

#### *Bacterial suspension preparation*

Freshly grown (24 hrs) single colonies of the isolates were picked up with an inoculation loop, stirred into 10ml deionized water in a test tube and maintained as suspension stock for inoculation experiments.

#### *Lead uptake experiment*

To monitor lead biosorption, the isolate was grown in nutrient broth at 37°C with 150 rpm shaking for 24 hours. After 24 hours culture was harvested and centrifuged at 13,000 x g for 10 minutes. The pellet was washed twice with sterile deionized water and stored at 4°C. Five different initial lead [Pb(NO<sub>3</sub>)<sub>2</sub>] concentrations (100, 200,300, 400 and 500 mg/l) were used. The inoculum was prepared as follows: i) one gram of fresh cell pellet was oven dried at 60°C for 48 hours (oven dried cells), ii) the same amount of pellet was taken and treated with sodium azide for 1 hr (metabolism inhibited) iii) the same amount of pellet was suspended in deionized distilled water and autoclaved at 121°C for 20 minutes iv) same amount was used untreated (live cell mass). All experiments were conducted in 250 ml conical flask containing 100 ml of metal solution. Before adding the bacteria, the pH of the metal solution was adjusted to the required value with 0.01 NaOH. The bacterial cell suspension was added to metal solutions and incubated at required temperature with shaking. At regular time intervals samples were taken out aseptically and were centrifuged at 10,000 rpm for 10 minutes at 4°C. After centrifugation both supernatant and pellet (three times washed with NaOH 1N) were digested with HNO<sub>3</sub> 67% and H<sub>2</sub>O<sub>2</sub> (30% v/v), and metal concentration was determined by atomic absorption spectrometry (Electronic Corporation of India Ltd., model ElementAS AAS4139). All experiments were conducted in triplicates.

#### *Effect of oven dried cell on lead biosorption*

The isolate was grown in Nutrient Broth at 37°C for 48 hrs on a shaker (120 rpm). Cells were harvested by centrifugation and washed with distilled water three times. The washed cells were dried in an oven at 60°C for 48 hrs. To assess complete death of the dried cells, samples of the dried cells were inoculated to Petri-dishes containing nutrient agar medium, absence of any growth indicating positive results. The dried cells were then ground in a porcelain mortar to obtain a fine

powder and stored at 4°C, until use. Aliquots of 0.025 g of dried biomass were added to Erlenmeyer flasks containing different concentrations of lead solution (100, 200,300, 400 and 500 mg/l). Erlenmeyer flasks were shaken (120 rpm) for 90 min at 30°C and then suspensions were centrifuged and both supernatant and pellet were collected. Heavy metal concentration in the digested supernatant was measured as previously described.

#### *Effect of azide treatment on lead biosorption*

The isolate was grown in Nutrient Broth at 37°C for 48 hrs on a shaker (120 rpm). Cells were harvested by centrifugation and washed with distilled water three times. Metabolism inhibited cells were prepared by suspending the cells in Nutrient Broth containing 1mM sodium azide (NaN<sub>3</sub>) for 1 hr. Then the cells were washed twice with deionized water to remove azide and suspended in Erlenmeyer flasks containing different concentrations of lead solution (100, 200,300, 400 and 500 mg/l). Erlenmeyer flasks were shaken (120 rpm) for 90 min at 30°C and then suspensions were centrifuged and both supernatant and pellet were collected. Heavy metal concentration in the digested supernatant was measured as previously described.

#### *Effect of heat treatment on lead biosorption*

The isolate was grown in Nutrient Broth at 37°C for 48 hrs on a shaker (120 rpm). Cells were harvested by centrifugation and washed with distilled water three times. The washed cell suspension was autoclaved at 121°C for 20 minutes. The heat killed cells were washed twice with deionized water and suspended in Erlenmeyer flasks containing different concentrations of lead solution (100, 200,300, 400 and 500 mg/l). Erlenmeyer flasks were shaken (120 rpm) for 90 min at 30°C and then suspensions were centrifuged and both supernatant and pellet were collected. Heavy metal concentration in the digested supernatant was measured as previously described.

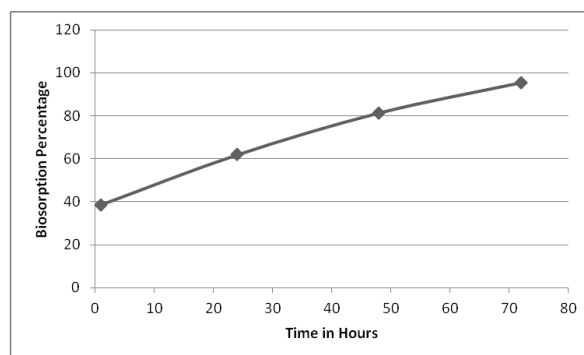
#### *Effect of live cells on lead biosorption*

The isolate was grown in Nutrient Broth at 37°C for 48 hrs on a shaker (120 rpm). Cells were harvested by centrifugation and washed with distilled water three times. The cells were suspended in Erlenmeyer flasks containing different concentrations of lead solution (100, 200,300, 400 and 500 mg/l). Erlenmeyer flasks were shaken (120 rpm) for 90 min at 30°C and then suspensions were centrifuged and both supernatant and pellet were collected. Heavy metal concentration in the digested supernatant was measured as previously described.

## Results

#### *Effect of dried cell on lead biosorption*

The effect of dried cells on lead biosorption by *Bacillus cereus* was studied by incubating the culture with different concentrations of lead (100, 200,300, 400 and 500 mg/l) at 30°C and the lead concentration in the digested supernatant was measured. The result (Fig.1) revealed that *Bacillus cereus* attained its maximum lead biosorption of 95.52% after 72 hrs of incubation.

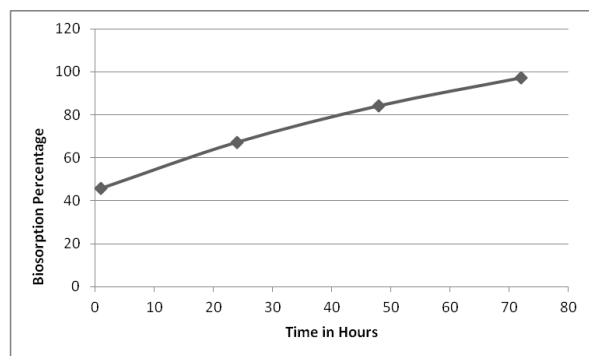


**Fig. 1.** Effect of dried cell on lead biosorption on *Bacillus cereus*.

#### *Effect of azide treatment on lead biosorption*

The effect of azide treatment on lead biosorption by *Bacillus cereus* was studied by incubating the culture with different concentrations of lead (100, 200,300, 400 and 500 mg/l) at 30°C and the lead concentration in the digested supernatant was measured. The results have (Fig.2) revealed that *Bacillus cereus* attained its

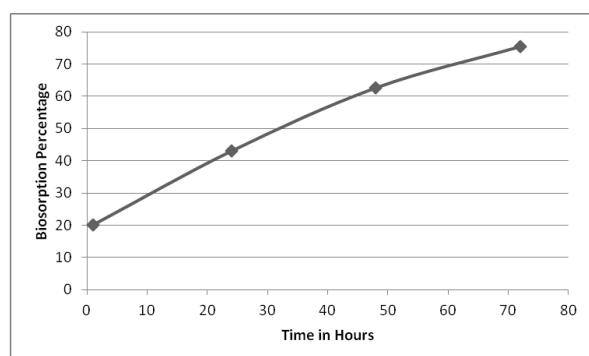
maximum lead biosorption of 97.23% after 72 hrs of incubation.



**Fig. 2.** Effect of azide treatment on lead biosorption on *Bacillus cereus*.

#### Effect of Heat treatment on lead biosorption

The effect of heat treatment on lead biosorption by *Bacillus cereus* was studied by incubating the culture with different concentrations of lead (100, 200,300, 400 and 500 mg/l) at 30°C and the lead concentration in the digested supernatant was measured. The results (Fig 3) have revealed that *Bacillus cereus* attained its maximum lead biosorption of 75.5% after 72 hrs of incubation.

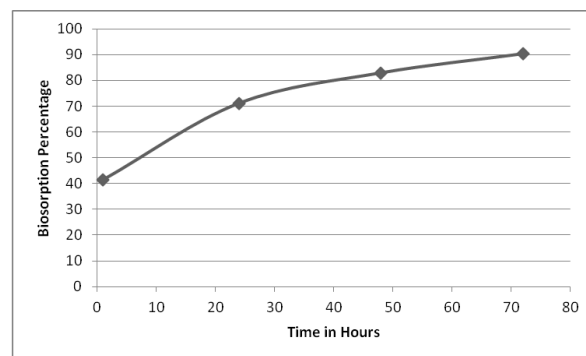


**Fig. 3.** Effect of heat treatment on lead biosorption on *Bacillus cereus*.

#### Effect of Live cells on lead biosorption

The effect of live cells on lead biosorption by *Bacillus cereus* was studied by incubating the culture with different concentrations of lead (100, 200,300, 400 and 500 mg/l) at 30°C and the lead concentration in the digested supernatant was measured. The results have (Fig.4) revealed that *Bacillus cereus* attained its

maximum lead biosorption of 90.3% after 72 hrs of incubation.



**Fig. 4.** Effect of live cells on lead biosorption on *Bacillus cereus*.

#### Discussion

The results obtained on the effect of physical and chemical pretreatments on the Lead biosorption capacity of *Bacillus cereus* have revealed that there was an increase in lead removal capacity except for heat treatment (Fig. 1-4). Pretreatment of live cells of *Bacillus cereus* using oven drying and sodium azide showed an increased lead uptake by 6% and 8 % respectively in comparison with live cells. On the other hand, a decrease of 16% was noticed in case of heat treated cells of *Bacillus cereus*. Improved lead sorption capacity of *Bacillus cereus* by oven drying could be attributed to an increase in the availability of binding sites or to the removal of polysaccharides that presumably block the access of metals to the binding sites. It can also be due to a change of permeability on the cellular wall, in the case of bioaccumulation (Hsuan *et al.*, 2004). Whereas, a reduced lead sorption capacity of *Bacillus cereus* when autoclaved can be attributed to the loss of effective metal binding sites. This observation is in good agreement with what has been reported by Hu *et al.* (1996) and Puranik and Paknikar (1999).

Sodium azide is a useful probe reagent, mutagen, and preservative. In hospitals and laboratories, it is a biocide; it is especially important in bulk reagents and stock solutions which may otherwise support bacterial

growth where the sodium azide acts as a bacteriostatic by inhibiting cytochrome oxidase in bacteria a characteristic similar to antibiotic resistance. Since sodium azide is a metabolic inhibitor, this result suggests that the lead uptake by *Bacillus cereus* is metabolic- independent. This is in accordance with previous work carried out by Wong and So (1993) on copper accumulating *Pseudomonas putida*.

The present study has revealed that lead biosorption capacity of *Bacillus cereus* could be improved by using resting cells (treated with oven drying and sodium azide) in place of live cells. It may be advantageous to use resting cells since living cells are likely to be more sensitive to metal ion concentration and adverse operating conditions of pH and temperature. Furthermore, a constant nutrient supply is required for using living cells. Recovery of metals and regeneration of biosorbent is complicated for living cells. In addition, resting cells frequently exhibits a higher affinity for metal ions compared with live cells probably due to absence of competing protons produced during metabolism. So, resting cells are preferred over live cells (Das *et al.*, 2007). Thus the resting cells of *Bacillus cereus* can be used as a potent biosorbent for lead removal from industrial effluents.

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