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Biosorption of Lead by *Bacillus cereus* Isolated from Industrial Effluents

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Research Article

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ABSTRACT

Aims: To study the biosorption of lead ions from solution using *Bacillus cereus* isolated from industrial effluents collected from Peenya industrial area, Bangalore, India and to determine the optimum conditions for biosorption.
Study design: Experimental study.
Place and Duration of Study: Department of Microbiology and Biotechnology, Bangalore University, Bangalore, Karnataka, India, between October 2008 and December 2009.
Methodology: Sixty bacterial strains were isolated from industrial effluents collected from Peenya industrial area. Among the sixty isolates only six were selected for further investigation due to their high minimum inhibition concentration for lead. Lead biosorption studies were carried out for all the six isolates using atomic absorption spectrometry. The optimum conditions (temperature, pH and culture age) for biosorption were determined for the isolate showing highest lead biosorption.
Results: The lead biosorption capability of all six isolates was studied at different concentrations of lead (100, 200, 300, 400 and 500 mg/l). The isolate 6 showed highest lead biosorption capability and was identified as *Bacillus cereus*. Studies on the control of environmental factors revealed that an optimum temperature of 30°C and pH 5, facilitates maximum biosorption of lead by 24hrs old culture of *Bacillus cereus*.
Conclusion: Biosorption is an alternative to traditional physicochemical methods for removing toxic metals from wastewaters. The results of this study are discussed in the light of the biosorption capacity of *Bacillus cereus* that could be exploited in the bioremediation of lead.

Keywords: Bacillus cereus; lead; biosorption; bioremediation; industrial effluent.

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1. INTRODUCTION

The growing industrialization and modern agricultural practices that have spread worldwide have adversely affected the ecosystem. These practices leave persistent toxic heavy metals like chromium, nickel, lead, zinc, cadmium and copper, which tend to accumulate and deteriorate the environment (Abbas et al., 2010). Contamination of heavy metals in the environment is a major concern because of their toxicity and threat to human life and environment (Rajendran et al., 2007; Boopathy, 2000). The high concentration of lead in the effluent causes a direct hazard to human and animals. Lead with no known biological function, is highly toxic and accumulates in humans (Williams et al., 1999; Hussaine et al., 2009; Soltan et al., 2008). Lead enters the food and water supply (Goyer and Chisholm, 1972) quite naturally and is absorbed by foodstuffs (vegetables and fruits) growing on soil contaminated with lead. Hu et al. (1998) have shown that lead adversely affects the concentration of sodium, potassium and calcium by decreasing the efficiency of ATPase pump as well as the activity of protein kinase, which maintain the concentration gradient of these ions in the cell. Further lead is shown to stimulate the formation of inclusion bodies in cells that may translocate metals into the nuclei and alter gene expression (Hu et al., 1998). There is an urgent need to find ways to remove dissolved lead from wastewater before it is released to the environment.

Bioremediation is the process of breaking down or transforming hazardous materials into simple nontoxic substances by biological treatments. It is a collective term used to describe the use of biological systems such as microorganisms to decontaminate polluted soil, water or air (Sivasubramanian, 2006).The basis for bioremediation has been the observation that over long periods of time even without human intervention, nature eliminates both natural and most man made pollutants through natural bioprocesses. Bioremediation is, therefore a naturally occurring bioprocess that harnesses microbial and geochemical process to degrade or transform contaminants to innocuous end products. Heavy industrialization, lack of safe procedures for effluent treatment and disposal of toxic substances have made the natural bioremediation process inadequate to reduce the quantity of toxic substances released into the nature. It has therefore become a necessity to augment the problem by the application of alternate bioremediation methods (Sivasubramanian, 2010).

Conventional physicochemical wastewater treatment processes such as chemical oxidation, reduction, precipitation, adsorption, solidification, electrolytic recovery, and ion exchange are being used for metal removal. Application of such methods, however, is sometimes restricted because of technical or economical constraints (Al-Garni, 2005). Therefore there is a need for an effective and affordable biotechnological solution for removal of lead from the industrial effluents (Naik et al., 2012). Recently biosorption has been receiving attention as a new technique in the removal of toxic metals from wastewaters (Karaca et al., 2010). Biological metal removal (biosorption) has distinct advantages over conventional methods: it is non-polluting and it can be highly selective, more efficient, easy to operate, and hence cost-effective for treatment of large volumes of wastewaters containing low metal concentrations (Puranik and Pakniker, 1999; Wilde and Beneman, 1993).

Biosorption can be defined as the ability of biological materials to adsorp heavy metals from wastewater through metabolically mediated uptake and/or physicochemical uptake (Fourest and Roux, 1992). The basis for biosorption is the metal binding abilities of various biological materials. Algae, bacteria, fungi and yeasts have been shown to be potential metal biosorbents. The cell walls of gram-positive bacteria naturally carry a negative charge because of their phosphate groups and teichoic acids that bind and regulate the movement

of cations across the membrane. Because the cell surface of bacteria carries a net negative charge due to the presence of carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups, it can adsorb appreciable quantities of positively charged cationic metals (e.g. Pb) (Parungao et al., 2007). The capacity of any biosorbent is mainly influenced by biomass characteristic, physiochemical properties of the target metals, and the micro environment of contact solution including pH, temperature and interaction with other ions (Hendawy et al., 2009).

Vrishabhavathi River which originates in the south western end of Bangalore has dried up and now carries industrial effluent and urban sewage. This river flows through Peenya industrial area, Bangalore, which houses industries like distilleries, electroplating and battery manufacturing units. Subsurface water samples were collected from this area and subjected to physico-chemical analysis. The results revealed that these industries release effluents mainly contaminated with heavy metals like Cu, Zn, Ni, Pb and Fe, and lead being the chief pollutant.

The removal of lead from contaminated wastewaters using bacteria (*Pseudomonas sp.*, *Chryseomonasluteola* and *Bacillus circulans*) has been reported by several authors (Leung et al., 2000; Khanafari et al., 2008; Azza et al., 2009). In this study, the biosorption ability and capacity of *Bacillus cereus* isolated from industrial effluent for lead was investigated under various conditions. Its lead uptake capacity was determined as a function of pH, temperature and culture age.

2. MATERIALS AND METHODS

2.1 Microorganisms and Culture Media

Sixty bacterial strains were isolated from industrial effluents released into Vrishbhavathi River flowing through the Peenya Industrial area, Bangalore (Karnataka, India). The minimum inhibition concentrations (MICs) of sixty isolates for lead concentrations (50,100,150,200,300,400 and 500 mg/l) revealed that six (PB1, 2, 3, 4, 5 and 6) out of sixty isolates were able to tolerate a high concentration of lead (500mg/l). The isolates were maintained by weekly subculturing on tryptone glucose extract agar and stored at 4°C.

2.2 Metal Solution

A stock solution of lead (100,000 mg/l) was prepared by dissolving 16mg of lead nitrate [$Pb(NO_3)_2$] in deionised distilled water, shaking it for 15 min and then leaving it to stand for 24 h 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).

2.3 Bacterial Suspension Preparation

Freshly grown 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.

2.4 Lead Biosorption Studies

Aliquots of 1 ml suspension of all the six bacterial suspension (24 h old) were inoculated in 100ml tryptone glucose extract broth medium containing different concentrations of lead (100, 200, 300, 400 and 500 mg/l). Before adding the isolates, the pH of the metal solution was adjusted to the required value with 0.01M NaOH. The Erlenmeyer flasks were incubated at 30°C in an orbital shaker at a speed of 120 rpm. The flasks were taken out on a regular basis i.e. after 1, 24, 48 and 72h of inoculation and were centrifuged at 10,000 rpm for 10 min at 4°C. After centrifugation the supernatant (three times washed with NaOH 1N) were digested with HNO_3 67% and H_2O_2 (30% v/v), and metal concentration was determined by atomic absorption spectrometry. All experiments were conducted in triplicate.

2.5 Effect of Incubation Temperature

Tryptone glucose extract broth medium (100 ml) containing 100 mg/l of lead was inoculated with 1 ml aliquot of *Bacillus cereus* suspension (24 h old) in Erlenmeyer flask. Flasks were incubated at different temperatures (20, 30, 40 and 50°C) in an orbital shaker at a speed of 120 rpm. All experiments were conducted in triplicate. Heavy metal concentration in the digested supernatant was measured as described earlier.

2.6 Effect of pH

Tryptone glucose extract broth medium containing 100 mg/l of lead was adjusted at different pH ranges (2, 3, 4, 5 and 6) using 0.1M HCl or 0.1 M NaOH at 30°C and heavy metal concentration in the digested supernatant was measured as previously described.

2.7 Effect of Culture Age

Bacillus cereus was grown in tryptone glucose extract broth medium, maintained at pH 7 and supplemented with 100 mg/l lead. The culture was incubated at 30°C for different incubation periods (12, 24, 36, 48 and 60h) and heavy metal concentration in the digested supernatant was measured following the methodology already described.

3. RESULTS

3.1 Lead Biosorption Studies

The lead biosorption capability of all six bacteria was studied at different concentrations of lead (100, 200, 300, 400 and 500 mg/l) and at different time intervals (1 h, 24 h, 48 h, 72 h). After 72 h interval there was no significant biosorption by all the six bacteria (data not shown) (Fig.1). All experiments were conducted in triplicate.

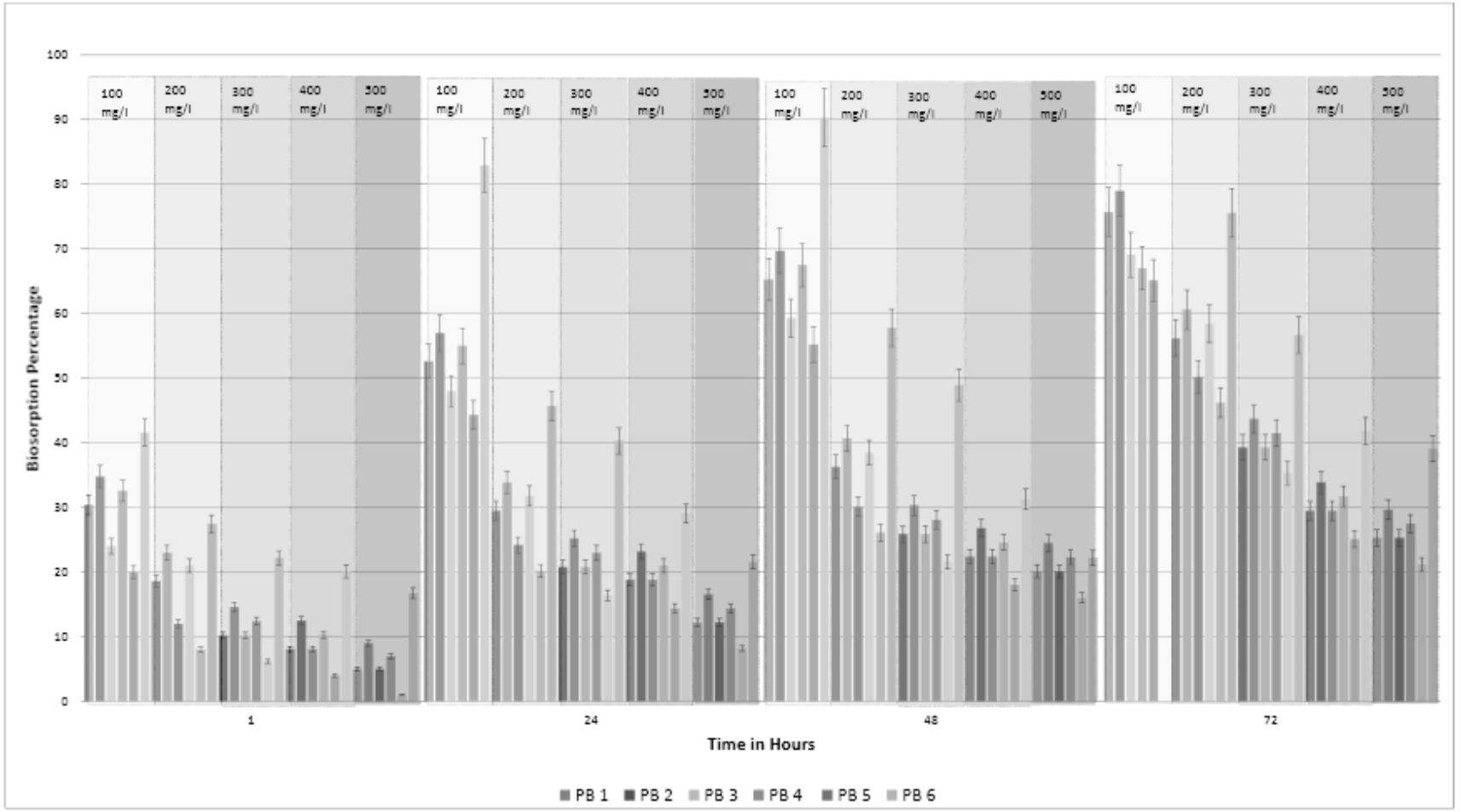

Fig. 1. Effect of different lead concentrations (100, 200, 300, 400, 500 mg/l) on biological removal by PB 1, 2, 3, 4, 5 and 6

PB 1 could efficiently remove lead from the medium. The bacteria could reduce 75.7% of 100mg/l lead, 56.2% of 200mg/l lead, 39.3% of 300mg/l, 29.5% of 400mg/l and 25.3% of 500mg/l from the medium after 72 h.

PB 2 showed remarkable ability to remove lead from the medium. The concentrations of lead 100mg/l, 200mg/l, 300mg/l, 400mg/l and 500mg/l was reduced to 79.0%, 60.6%, 43.7%, 33.9% and 29.7% respectively from the medium after 72 h.

PB 3 could efficiently remove lead from the medium. The isolate could reduce 69.1% of 100mg/l lead, 50.2% of 200mg/l lead, 39.3% of 300mg/l, 29.5% of 400mg/l and 25.3% of 500mg/l from the medium after 72 h.

PB 4 showed remarkable ability to remove lead from the medium, except 500 mg/l. The concentrations of lead 100mg/l, 200mg/l, 300mg/l, 400mg/l and 500mg/l was reduced to 67.0%, 58.4%, 41.5%, 31.7% and 27.5% respectively from the medium after 72 h.

PB 5 could efficiently remove lead from the medium. The isolate could reduce 65.1% of 100mg/l lead, 46.2% of 200mg/l lead, 35.3% of 300mg/l, 25.1% of 400mg/l and 21.2% of 500mg/l from the medium after 72 h.

PB 6 showed remarkable ability to remove lead from the medium. The concentrations of lead 100mg/l was reduced to 90.3% at 48 h interval and after 48 h PB 6 did not show significant biosorption (data not shown).Lead concentrations of 200mg/l, 300mg/l, 400mg/l and 500mg/l were reduced to 75.6%, 56.7%, 41.8% and 39.1% respectively from the medium after 72 h.

From the results it can be inferred that the percentage of lead removal by the bacterial isolates decreased with increasing concentrations of lead (100>200>300>400>500 mg/l). Amongst the six bacteria tested for lead uptake, PB 6 could remove maximum quantity of lead followed by PB 2, PB 4, PB 1, PB 3 and PB 5. PB 6 showed highest resistance to lead. The PB 6 was rod-shaped, Gram-positive, strict aerobe, motile, endospore former (single central spore) with positive catalase and oxidase activity. It grew over a wide range of pH (5-12), temperature (15°-55°C), NaCl concentration (0.0-10%), and was able to hydrolyze casein and gelatin. The strain was halotolerant as it grew in the presence of 0.0-10% NaCl, but did not require salt for its physiological activities. On account of morphological and biochemical characteristics, it was identified as Bacillus sp. according to the key of Bergey's Manual of Determinative Bacteriology (Kreig and Holt, 1984). The isolate was deposited to Bangalore Genei, Bangalore (GenBank Accession Number: EF488087). Analysis of 16 S rDNA sequence confirmed PB 6 as *Bacillus cereus*.SEM photographs and EDX signatures revealed that lead was mostly entrapped in the extracellular polymeric substances in *Bacillus cereus* (data not shown).

3.2 Effect of Incubation Temperature

The results on the effect of incubation temperature (Fig.2) revealed that the maximum adsorption of lead was observed at 30°C. At 30°C *Bacillus cereus* attained its optimum lead biosorption of 85.4% after 72 h of incubation. Lead biosorption by *Bacillus cereus* decreased in the order 30°C>40°C>20°C>50°C.

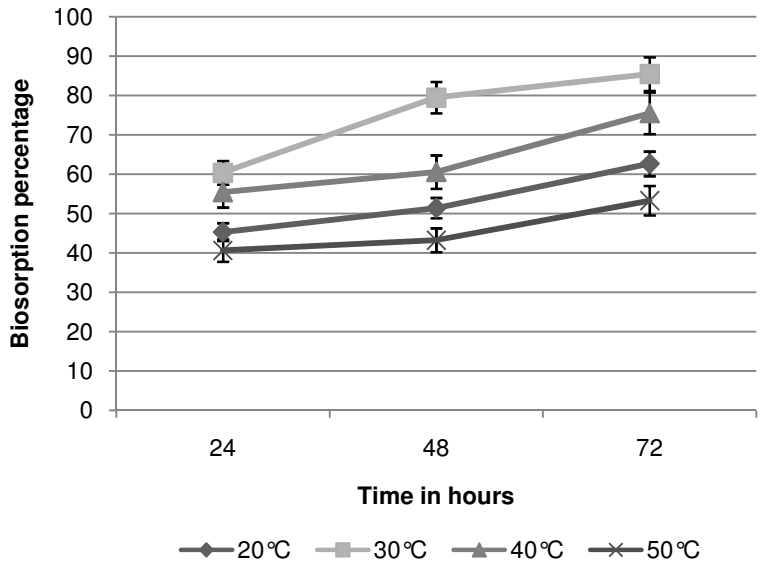

Fig. 2. Effect of different incubation temperatures (20, 30, 40, 50℃) on lead (100 mg/l) removal by *Bacillus cereus*

3.3 Effect of pH

The results on the effect of pH (Fig. 3) revealed that the maximum adsorption of lead was recorded at pH 5.0. At pH 5.0 *Bacillus cereus* attained its maximum lead biosorption of 75.6% after 72 h of incubation. Lead biosorption by *Bacillus cereus* decreased in the order 5 >6>4>3>2.

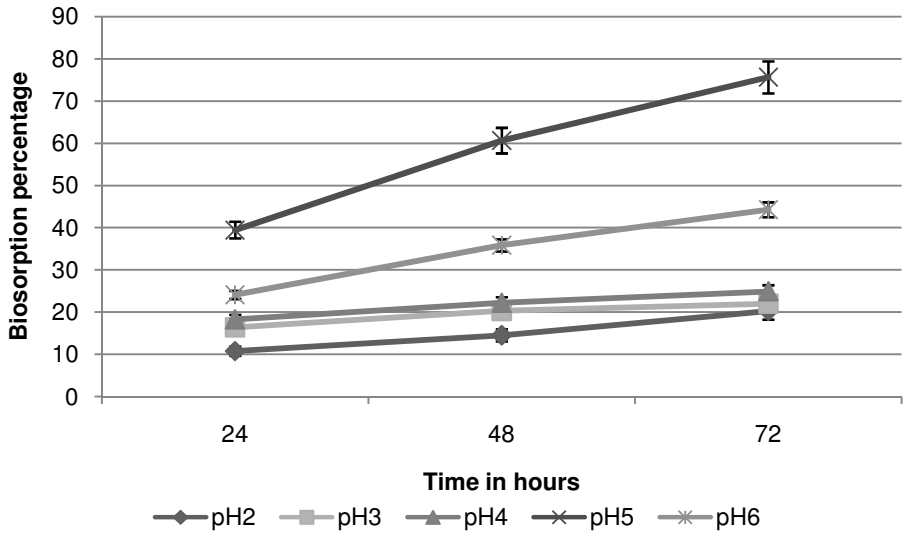

Fig. 3. Effect of different pH values (pH 2, 3, 4, 5, 6) on lead (100 mg/l) removal by *Bacillus cereus*

3.4 Effect of Culture Age

The results on the effect of culture age (Fig. 4) revealed that the maximum adsorption of lead was observed at 24 h. A 24 h old culture of *Bacillus cereus* attained its maximum lead biosorption of 89.3%. Lead biosorption by *Bacillus cereus* decreased in the order 24>36>12 >48>60 h culture age.

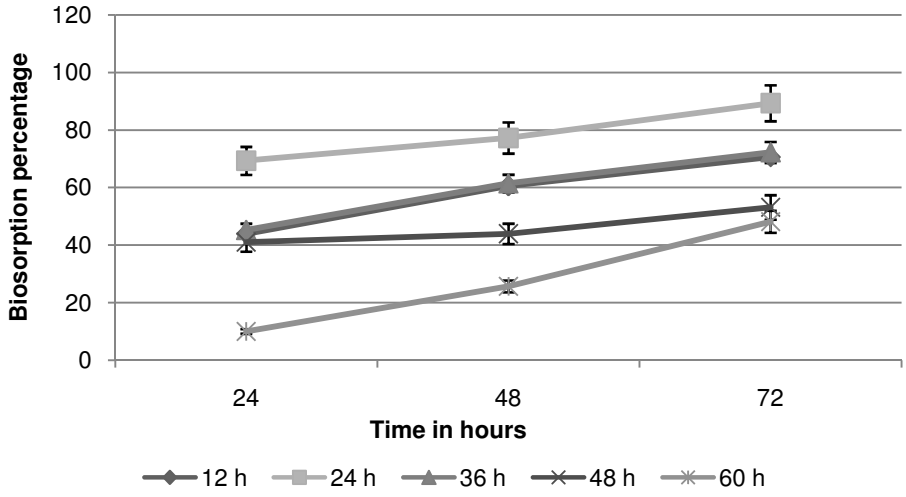

Fig. 4. Effect of different culture age (12, 24, 36, 48, 60 hours) on lead (100 mg/l) removal by *Bacillus cereus*

4. DISCUSSION

Increasing industrialization has resulted in an alarming increase in the discharge of heavy metals and other pollutants into the environment including water resources. Microbial populations in metal polluted environments contain microorganisms which have adapted to toxic concentrations of heavy metal and become metal resistant. These microorganisms can be used to remove heavy metals from the environment by various approaches like bioaccumulation and bioadsorbtion, oxidation and reduction, methylation and demythylation (Bolton and Gorby, 1995). The microbe based approach for removal and recovery of toxic metals from industrial effluents can be economical and more efficient in comparison to physicochemical methods for heavy metal removal (Gadd, 1992). Zouboulis et al. (2003) reported that certain types of microbial biomass could retain relatively high quantities of metal ions in a process known as biosorbtion.

Microorganisms have a high surface area to volume ratio because of their small size and therefore provide themselves with a large contact area that can interact with matter in the surrounding environment. The ability of the microorganisms to grow and survive under high metal concentrations is attributed to stress induced selection of these microbes in particular environments.

Various mechanisms have been postulated for the development of metal resistance in microorganisms (Hughes and Poole, 1989; Gadd, 1990; Silver, 1998). However in general,

all these strategies are found either to prevent the entry of metal ions into the cell or to actively pump out the metal ions from the cell (Roane et al., 1996).

The removal of lead by *Bacillus cereus* revealed that, the percentage of metal removal by the bacteria decreased with increasing concentration of lead. The enhancement in metal sorption could be due an increase in electrostatic interactions involving sites of progressively lower affinity for metal ions (Al-Asheh and Duvnjak, 1995; Puranik and Pakniker, 1999).

Bioaccumulation is the enhanced active uptake and intracellular deposition of metals, which has been reported for many metals including lead (Sakurai and Haung, 1995). This process is dependent on the metabolic activity of the cell referred to its intrinsic biochemical and structural properties, physiological and genetic adaptation, environmental modification of metal specification, availability and toxicity (Cha and Cooksey, 1991; Cooksey, 1993).

Factors such as organic and inorganic composition of the reaction mixture, pH and biomass density are important determinants of biosorptive process. In the present study the growth and metal uptake capability of *Bacillus cereus* was affected by the different environmental conditions such as incubation temperature, pH and culture age. Temperature plays a major role in the adsorption of heavy metals. Although, the magnitude of the heat effect for the biosorption process is one of the most important criteria for the efficient removal of heavy metals from the wastewater. Temperature changes will affect a number of factors which are important in heavy metal ion biosorption (AjayKumar et al., 2009). Some of the factors include: (i) the stability of the metal ion species initially placed in solution; (ii) the stability of microorganism-metal complex depending on the biosorption sites; (iii) the effect of temperature on the microorganism cell wall configuration; (iv) the ionization of chemical moieties on the cell wall (Sag and Kutsal, 2000). Studies on the effect of different incubation temperatures revealed that 30°C is the optimum temperature for maximum lead uptake by *Bacillus cereus*. Norris and Kelly (1977) reported that the temperature effects are confined to metabolism-dependent metal accumulation. Kamsonlian et al. (2011) reported that the adsorption of metal ions onto the biosorbent was dependent on temperature and there was an increase in percentage biosorption of metal ions from 25 to 40°C.

The results on the effect of pH revealed that the maximum adsorption of lead by *Bacillus cereus* was observed at pH 5.0 at 72 hrs incubation. The pH affects the network of negative charges on the surface of the biosorbing cells and the chemistry of the cell wall as well as physiochemistry and hydrolysis of the metal (Collins and Stotzky, 1996; Lopez et al., 2000). Puranik and Paniker (1999) reported that pH 4.5 was optimum for biosorption of lead by *Citrobactor* strain MCMB-181 and the pH between 3 and 5 resulted in lower biosorption efficiency of lead. The present results are in accordance with the findings of Chang et al. (1997) and Gadd (1992) who showed that pH beyond 5, lowers the solubility of lead.

The cell age is considered as an important microbial factor that affects metal accumulation. In the present study maximum lead uptake by *Bacillus cereus* is shown to occur after an incubation period of 24 hours. This is attributed to the increase in the presence of active enzymes at this metabolically active stage of growth. Some of these enzymes may be involved in complexing and binding with the metal.

5. CONCLUSION

The results from the present study clearly demonstrate that *Bacillus cereus* has strong potentialities to absorb lead and hence can be employed as a bio-agent for lead

detoxification from the contaminated effluents. Further, the findings of this study would help in exploring the biosorption capacities of biomass and develop appropriate technologies applicable in the treatment of industrial wastewater.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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