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Biosorption of lead by *Bacillus licheniformis* isolated from E-waste landfill, Hyderabad, Telangana, India.

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Abstract: Soil, the basic resource for the life on earth is getting polluted because of the release of different contaminants into it. So, the reduction of soil pollution is the main thrust of most researchers. The contaminants include different components released from different industries and the waste is getting accumulated in the soil because of improper processing. Electronic waste is the most up growing waste in the world. As the electronic industries are progressing the waste that is produced after the usage of the products is also increasing day by day. As a result, the heavy metals which are the main components in electronic goods leach and accumulate in the soil because of informal processing procedures. Poisonous substances such as lead, tin, mercury, cadmium and barium which are the constituents of the electronic goods get discharged into the environment and cause serious health and pollution problems if the electronic waste is not processed properly. The present study focuses on biosorption of lead, an important component of many electronic goods by *Bacillus licheniformis* isolated from E-waste dump yard soil in Hyderabad, Telangana, India. The adsorption studies were carried out using Atomic adsorption spectrophotometer. The adsorption capability of *Bacillus licheniformis* with different metal concentrations ranging from 10ppm to 25ppm was analyzed and it was observed that the bacteria could reduce 74.94% of 10ppm, 78.9% of 15ppm, 83% of 20ppm and 89.39% of 25ppm lead from the medium. Temperature has a prominent role in metal adsorption by bacteria. At 31^{0}C and 37^{0}C the adsorption was high. The % of metal adsorbed at 16^{0}C was 30.56%, at 31^{0}C (Room Temperature) was 56.54 % at 37^{0}C was 58.79% and at 60 ^{0}C it was 36.31%. The present study is proposed to explore bacteria for the determination of their tolerance capacity in and around the areas of Hyderabad where heavy metal ions are leached and observe for their biotransformation capabilities.

Key words: E-Waste; Heavy metals; *Bacillus licheniformis;* Adsorption studies

Introduction

The advancement in electronic industry is posing a new environmental challenge in the form of electronic waste. The electronic devices after their disposal into the soil are not processed properly which leads to the accumulation of the toxic metals in the soil. The electronic devices contain plenty of components of which few may be toxic like heavy metals. Heavy Metals are the metallic chemical elements with high density and toxic at low concentrations[1]. The dumping of used off electronic goods in landfills without proper treatment will lead to environmental destruction[2]. The hazardous materials include heavy metals like lead, mercury, hexavalent chromium, Cadmium[3]. One of the important components in electronic goods with adverse effect on the flora and fauna in soil is Lead. The effect is dependent on the level and duration of exposure. High levels of exposure may damage kidneys, gastrointestinal tract, joints, reproductive system, and chronic damage to CNS[4]. Transforming or converting a toxic component to non-toxic form by biological systems like microorganisms is termed as Bioremediation[5].

Bioremediation eliminates both natural and most manmade pollutants through natural bioprocesses. Heavy industrialization and lack of safe procedures to detoxify the toxic substances released into the environment has made the natural bioremediation process inadequate. So, there is a need for alternate bioremediation method to solve this problem[6]. The conventional methods employed for metal recovery like chemical oxidation, reduction, precipitation ion exchange are restricted due to technical constraints[7]. Therefore, there is a requirement of an effective and affordable biological method for the removal of lead from the electronic waste[8]. Now-a-days biosorption technique is receiving attention in the removal of toxic metals from waste[9] as it is non-polluting, highly selective, more efficient and cost effective for treatment of large volumes of wastewaters containing low metalconcentrations[10,11]. Biosorption is the ability of biological materials like bacteria, algae, fungi, yeasts to adsorb heavy metals from waste through uptake[12]. In bacteria, the negatively charged cell wall facilitates the binding of metals through the carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups present within the cell wall[13]. The metals bind to the phosphate groups and teichoic acids of negatively charged cell walls of Gram positive bacteria and are thus removed from the waste[14]. In biosorption of lead *B. licheniformis* is proved to be 65-70% efficient[15]. In the present study, *Bacillus licheniformis* isolated from the E-waste soil was investigated for metal adsorption under various

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conditions like the substrate concentration, contact time and the temperature employed.

Materials and Methods

Bacterial Culture: The bacterial isolate employed in the present study was isolated from E-waste dumping yard soil, Maheshweram Mandal, Hyderabad, Telangana, India. Four isolates were isolated from the soil out of which one isolate which could tolerate very high concentrations of lead up to 10000ppm was used for the study. The isolate was identified and confirmed as *Bacillus licheniformis* (Accession no. CP000002. 3) based on 16S rDNA data. The isolate was maintained by sub culturing on nutrient agar media and stored at 4^{0}C.

Study on biosorption capacity of *Bacillus licheniformis:*

Metal Solution: A stock solution of lead (100ppm) was prepared by dissolving 0. 01g of lead acetate in 100ml distilled water, and then leaving it to stand for 24h to obtain complete dissolution. Stock solution was diluted with distilled water to obtain the necessary concentrations. The initial lead concentration was measured at the beginning of all experiments carried out using an Atomic Absorption Spectrophotometer (Model: ICE 3000, Make: Thermofisher Scientific)

Effect of substrate concentration on adsorption: 1ml suspension of 24h old *Bacillus licheniformis* was inoculated in 100ml minimal broth medium containing different concentrations of lead (10,15,20,25ppm) and incubated at 37^{0}C for 48h. The metal concentration was determined by Atomic Absorption Spectrophotometer after the incubation period.

Effect of incubation temperature: Minimal broth medium (100 ml) containing 0. 1% of lead was inoculated with 1ml aliquot of *Bacillus licheniformis suspension* (24h old) in Erlenmeyer flask. Flasks were incubated at different temperatures (16^{0}C,31^{0}C,37^{0}C, 60^{0}C). Heavy metal concentration in the digested supernatant was measured as described earlier.

SEM micrographic analysis and EDS: The Scanning Electron Microscope (SEM) (Carl Ziess EVO 18) equipped with energy dispersive analysis of X-rays (Oxford EDAX) was used to determine the morphology and atomic composition. The EDS spectrum of bacterial cells before and after interaction with lead has been studied.

Results

Effect of substrate concentration on adsorption: The adsorption capability of an organism towards a metal depends on the concentration of the substrate present in the media. *B. licheniformis* CP000002. 3 (0. 5ml) when inoculated into minimal broth (100ml) with different concentrations of lead (10,15,20,25ppm) there was a considerable reduction in the metal concentration. The biosorption efficiency was calcualted as R (%) = 100×(Ci-Cf)/Ci where Ci is the initial concentration, Cf is the final concentration of the metal. The initial and final concentrations of metal was analyzed using Atomic Absorption Spectrophotometer and the % reduced was 74.94% of 10ppm, 78.9% of 15ppm, 83% of 20ppm and 89.39% of 25ppm in comparision with minimal broth with respective concentrations of lead without the organism which was taken as control (Fig. 1). From the results, it can be inferred that the percentage of lead removal increased with increased concentrations of lead (10>15>20>25ppm).

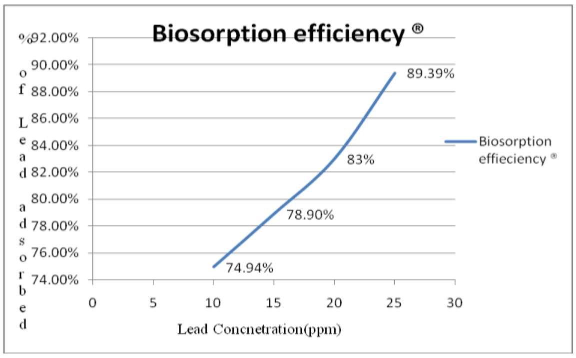

Figure 1: Effect of Lead concentration on adsorption by *B. licheniformis* CP000002. 3

Effect of Incubation temperature on biosorption: *B. licheniformis* CP000002.3 (0.5ml) inoculated into minimal broth (100ml) containing 10ppm Lead and incubated at different temperatures (16^{0}C, 31^{0}C, 37^{0}C and 60^{0}C) for 48hrs. The results (Fig. 2) revealed that the maximum adsorption of lead was observed at 31°C and 37°C. The room temperature was 31°C at the time of study. Lead biosorption by the organism decreased when incubated at low temperature i. e., at 16°C and at high temperature i. e., at 60°C. The % of metal adsorbed at 16^{0}C was 30.56%, at 31^{0}C (Room Temperature) was 56.54 % at 37^{0}C was 58.79% and at 60 ^{0}C it was 36.31%. The observations reveal that the biosorption efficiency by *B. licheniformis* CP000002. 3 was optimum between 31^{0}C to 37^{0}C.

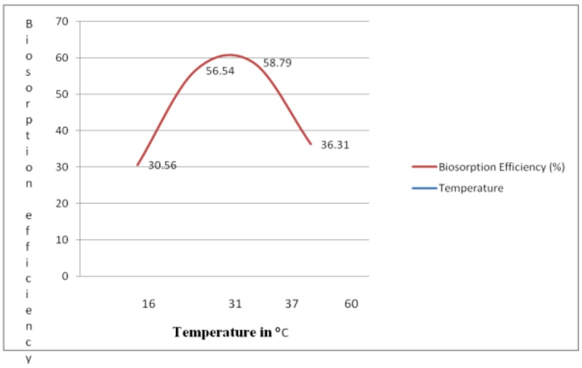

Figure 2: Effect of Incubation Temperature on biosorption

SEM micrographic analysis and EDS: The morphological characteristics of *B licheniformis* in absence and in presence of lead was depicted in figure3a and figure 3b. In presence of lead the size of the bacteria decreased. The EDX ((Energy Dispersive X-Ray) elemental analysis carried on *B. licheniformis* cells in absence and in presence of lead confirms the adsorption of the metal onto the cells as depicted in fig4a and fig4b.

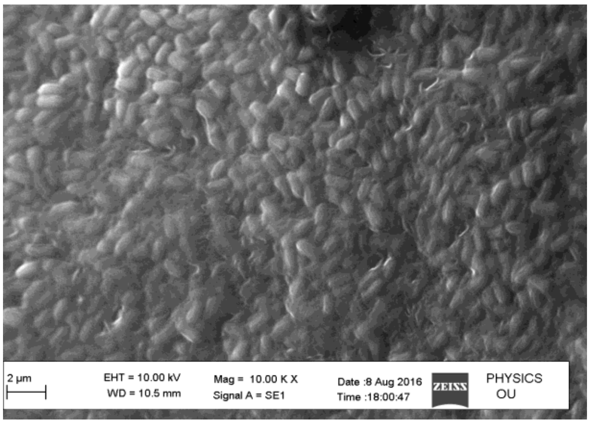

Figure 3a: SEM micrograph of *B. licheniformis*

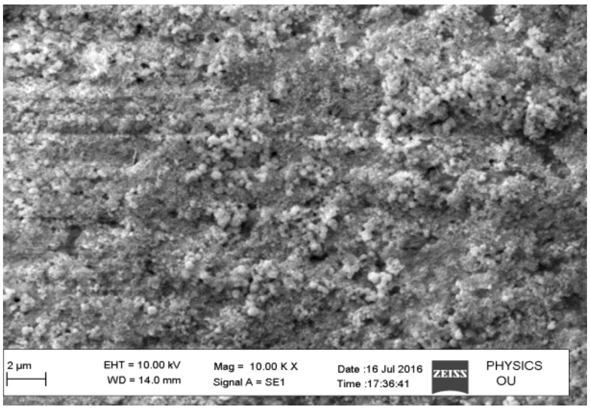

Figure 3b: In presence of Lead

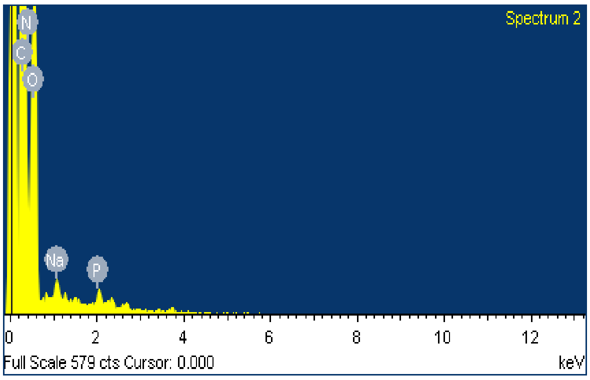

Figure 4a: EDS spectrum of *Bacillus licheniformis*

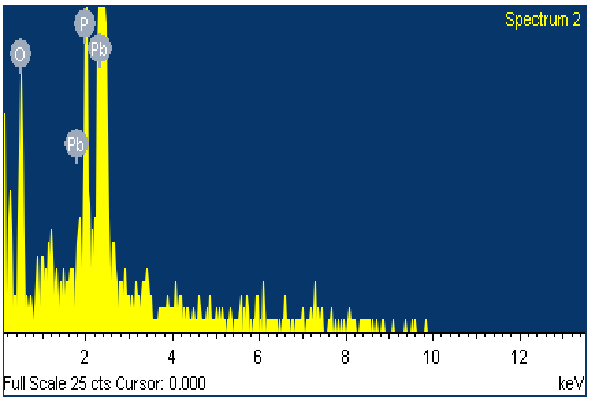

Figure 4b: In presence of Lead

Discussion

Rapid growth of electronic industry is posing a challenge to the environment in the form of improperly processed electronic waste. The heavy metals and other constituents leach into the soil and the damage the flora and fauna on the earth. Microorganisms surviving in those dumping yards can be used to remove toxic heavy metals as they adopt to that environment and become metal resistant. Different approaches like bioaccumulation and bioadsorbtion, oxidation and reduction, methylation and demythylation[16]can be employed. Microbial approach for metal removal is efficient and economical method than other methods[17]. It was reported that by Biosorption method certain types of microbial biomass could retain relatively high quantities of metal ions[18]. The high surface area to volume ratio of microbes facilitates large contact area for surrounding environmental materials.

Microorganisms become metal resistant by either preventing the entry of metal ions into the cell or eluting the metal ions from the cell[19]. Bacteria like *Thiobacilli*. and fungi like *A. niger*, *P. simplicissimum* were able to grow in the presence of electronic scrap and mobilize metals from electrical and electronic waste materials[20]. It was also observed that biosorption of lead (IV) ion by *Bacillus subtilis* is shown to be an effective bacterial bioremoval process[21]. *Bacillus cereus* were used for lead adsorption from aqueous solutions[22] and *Bacillus licheniformis* isolated from E-waste dumping yard was used for lead(II) adsorption[23]. Lead removal by *Bacillus licheniformis* revealed that as the concentration of lead increased the percentage removal of lead also increased. This increase in adsorption could be because of enhanced electrostatic interactions of the cell with metal ions[24,25]. The cells might uptake the metal ions and deposit them intracellularly by a process referred to as Bioaccumulation and has been reported for many metals including lead [26]. In the present study, the metal uptake by *Bacillus licheniformis* CP000002. 3 was influenced by metal concentration, contact time and incubation temperature. Temperature is an important parameter in biosorption of metals as changes in temperature will influence the microorganism –metal complex stability and also the cell wall configuration[27]. The study revealed that 31°C and 37°C temperatures are optimum for maximum lead uptake by *Bacillus licheniformis*. Kamsonlian *et al.,* (2011) reported that metal adsorption onto the biosorbent was dependent on temperature and an increase in percentage biosorption of metal ions from 25 to 40°C was observed[28].

The stress caused by the presence of lead on the structure of the organism was emphasized by the SEM results. There was a change in the shape of bacteria in presence of lead. The actual mechanism of biosorption onto bacterial cells was confirmed by EDS results. Hence as per the results *Bacillus licheniformis* CP000002. 3 can be used as best adsorbant in lead contaminated areas.

Conclusion

The results from the present study conclude that *Bacillus licheniformis* has the capability to adsorb lead which makes a way to use this organism as an agent to remove lead metal polluted areas. Further studies on the enhancement of rate of adsorption by preparing the composites which enables in removing the accumulated toxic metals in soils thereby cleaning up the environment can be the future work.

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