Cadmium Uptake by Biomass of *Lysinibacillus sphaericus* KY203810 and *Enterobacter cloacae* KY203811 from Steel Company Dumpsite.

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ABSTRACT

Biosorption of cadmium (Cd^{2+}) was investigated using metal tolerant bacterial strains isolated from a steel company dumpsite. Various environmental conditions that can affect cadmium uptake were determined with the aim of optimizing its uptake using various isotherm models. Metal tolerant bacteria were screened using agar well diffusion technique. Isolates DNA were extracted, sequenced and identified using 16S rRNA phylogenetic approach. They were identified as *Lysinibacillus sphaericus KY203810* and *Enterobacter cloacae KY203811* after submitting the sequence to NCBI.

Effects of biosorption conditions: pH, biomass concentration, temperature and contact time were studied. Equilibrium isotherms were also determined. The result showed that optimum sorption of Cd^{2+} was achieved at pH 5.0, biomass dose of 1 g/L, temperature of 25 ^{o}C and contact time of 24 hours for *L. sphaericus.* Cadmium sorption by *E. cloacae* was at pH 9.0, biomass dose of 1 g/L, 35 ^{o}C and contact time of 12 hours. Adsorption data fitted isotherm models postulated by Langmuir and Freundlich. Maximum cadmium biosorption capacities for *L. sphaericus* and *E. cloacae* were 50.15 and 57.59 mg/g respectively, indicating higher biosorption efficiency of *E. cloacae* cells to heavy metals compared to *L. sphaericus*. This study showed that *Lysinibacillus sphaericus KY203810* and *Enterobacter cloacae KY203811* can effectively act as biosorbent for Cd^{2+} sorption from solution.

(Key terms: biosorption, environmental conditions, metal tolerant bacterial strains, cadmium ions, *Lysinibacillus sphaericus*, *Enterobacter cloacae*, equilibrium isotherm model, heavy metal)

INTRODUCTION

Modern agricultural practices and industrialization 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 by heavy metals in the environment is a major global concern because of their toxicity to humans and the environment Rajendran *et al.*, 2007; Ceribasi and Yetis, 2001). Metals are mobilized and carried into the food web as a result of leaching from waste dumps, polluted soils and water. Cadmium is one of the most toxic and common among the heavy metal pollutants of industrial effluents. Cadmium is introduced into the bodies of water from smelting, metal plating, cadmium-nickel batteries, phosphate fertilizer, mining, pigments, allay industries, and sewage sludge. Discharges containing cadmium, in particular, are strictly controlled due to the highly toxic nature of this element and its tendency to accumulate in the tissues of living organisms (Dianati-Tilaki *et al*., 2004).

Harmful effects of cadmium include a number of acute and chronic disorders, such as *itai-itai* disease, renal damage, emphysema, hypertension, and testicular atrophy (Leyva-Ramos *et al*., 1997). Cadmium is effectively bound by high molecular weight protein such as albumin and non-protein sulfhydryl group in the human body (Doshi, *et al*., 2007). Cadmium can affect the kidney, causing renal dysfunction, especially in the proximal tubular cells as it is the main site of cadmium accumulation. It can also cause bone demineralization, either directly by damaging the bones or indirectly as a result of

renal dysfunction (Blessy and Krishnamurthy, 2015; Bernard, 2008). Excess cadmium in the organisms can damage DNA sequencing and may cause genetic changes and cancer (Carmichael, 1994). There is an urgent need to find ways to remove dissolved cadmium from wastewater before it is released to the environment.

Conventional methods for removal of heavy metals from waste solutions are chemical precipitation, ion exchange, membrane processes, sorption, infiltration, and coagulation (Anielak *et al*., 2000). Application of such methods, however, is sometimes restricted because of technical or economic constraints, it is also time consuming and expensive (Al-Garni, 2005). Therefore there is a need for an effective and affordable biotechnological solution for removal of cadmium from the industrial effluents (Naik *et al*., 2012).

Biotechnological methods can be alternatives for conventional methods as they use natural properties of microorganisms to absorb and accumulate heavy metals. Processes that utilize biomass for removal of metals are referred to as bioaccumulation and biosorption (Chojnacka, 2009). The use of biological materials including living and non-living microorganisms to remove metal cations from industrial wastewaters has attracted much interest because of the performance, cost-effectiveness and eco-friendly nature of these sorbents (Kefala *et al.* 1999). Bioaccumulation is based on the incorporation of metals inside the living biomass, while biosorption is a metabolism-independent process and metallic ions remain at the cellular surface.

Biosorption is a physicochemical process that binds ions of metals (that are mostly present in the form of cations) by cell membranes (i.e., by compounds with negative charge that are present in cell membranes). Biosorption results from different mechanisms such as complex formation, ion exchange, coordination, adsorption and chelation (Hu *et al*. 1996; Velasquez, Dussan 2009). Dead cells are more advantageous for use in industrial applications because non-living biomass usually exhibits higher metal uptake capacities. Additionally, non-living biomass can be applied more easily and sometimes with higher selectivity, using existing treatment technologies (Tobin *et al*. 1994). The biosorption of metals is affected by many factors such as the pH, temperature, biomass concentration and type, and contact time, etc. (Özdemir *et al.* 2009).

A large number of microorganisms such as bacteria, fungi, yeasts, and algae have been reported to bind a variety of metals to different extents in solutions (Volesky, Holan 1995). Bacteria are preferred as biosorbents more than other microorganisms because of their high surface area to volume ratios, high content of potentially active chemosorption sites such as on teichoic acid in their cell walls (Beveridge 1989) and their abundance in all environments such as water, soil and air. They also can be easily propagated under laboratory conditions. The metal ions in solution are adsorbed on the bacterial surface through interactions with chemical functional groups such as carboxylate, amine, amide, imidazole, phosphate, thioether, hydroxyl and other functional groups found in the cell wall biopolymers (Gupta *et al.* 2000; Madrid *et al.* 1997; Vijayaraghavan and Yun 2008).

The pollution of the environment with metals has led to the appearance of metal-resistant microorganisms in the soil and water of industrial regions (Aleem *et al.* 2003). Biosorpton studies focused mostly on the selection of metal-resistant microorganisms from polluted environments. De Vicente *et al.* (1990) found a strong correlation between the extent of metal pollution and bacterial resistance to metals in aqueous environments. Microorganisms of metal-polluted soils have developed tolerance to the metals and probably increased metal biosorption capacities (Iqbal *et al.* 2005). They have more metal removal potential since they have adapted themselves to condition of growth in such environment. Therefore, it is important to explore microbes from such environments for use in metal biosorption.

Previous studies have well documented the occurrence and abundance of metal-tolerant microbes in metal-polluted water bodies (Abyar *et al.*, 2012; Jankowska *et al*. 2006; Wong *et al.* 2003). Bacterial species such as *Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis* and *Staphylococcus sp.* isolated from wastewater samples were found to be resistant to heavy metals (Filali *et al*., 2000). Similarly, cadmium-resistant *Klebsiella, Aeromonas caviae, Enterobacter* species, *Bacillus* and other bacteria have also been isolated from industrial effluents and soil samples (Alboghobeish *et al*., 2014; Karakagh *et al*., 2012;). These microorganisms develop a variety of resistance mechanisms to survive in different heavy metal concentrations, but the resistance is often specific to one or few

metals (Mejare and Bulow, 2001; Nies, 2003; Piddock, 2006). These heavy metal tolerant bacterial species may serve as an important and cost-effective bioremediation tool for the removal of heavy metals from wastewater and industrial effluents, preventing the contamination of water bodies (Jan *et al*., 2014).

Lysinibacillus species are potential candidates for heavy metal bioremediation. Some *Bacillaceae* strains have been successfully isolated from nickel contaminated soil (Abou-Shanab *et al*., 2007) industrial landfills (Desai *et al*., 2008), naturally metalliferous soils and a uranium-mining waste pile (Pal and Paul, 2004). In addition, native Colombian *Lysinibacillus* strains have been reported as potential metal bioremediators. The *Enterobacteriaceae* is a family of Gram-negative, non-spore-forming bacteria and is one of the most important groups of bacteria known to man. *Enterobacter sp.* has been reported by Zhang *et al*., 2013 to biosorb cadmium (46.2 mg g^{-1}).

The objectives of the study are isolation and identification of metal resistant bacteria from steel company dumpsite, evaluation of their cadmium biosorption potentials and optimization studies using various isotherms models.

MATERIALS AND METHODS

Collection of Sample

Soil samples were collected from a steel company dumpsite located on latitude 6.6764 and longitude 3.1999, South-west of Nigeria. The sample was collected at a depth of 5, 10, and 15cm in a sterile plastic container and transported immediately to the laboratory.

Determination of Heavy Metal Concentration in Soil Sample

Heavy metal concentrations were determined using Atomic Absorption Spectrophotometer (Shimazdu, Japan) as described by Zheljazkov and Nielson (1996). Nitric acid digestion method was used to digest the soil sample prior the toxic metal analysis. One gram of the soil sample was placed in a 250 ml digestion tube and 10 ml of concentrated HNO_3 was added. The sample was heated for 45 min at 90^{o}C, and then the temperature was increased to 150 ^{o}C at which the sample was boiled for at least 8 h until a clear solution was obtained. Concentrated HNO_3 was added to the sample (5 ml was added at least three times) and digestion occurred until the volume was reduced to about 1 ml. The interior walls of the tube were washed down with a little distilled water and the tube was swirled throughout the digestion to keep the wall clean and prevent the loss of the sample.

After cooling, 5 ml of 1% HNO_3 was added to the sample. The solution was filtered with Whatman paper. It was then transferred quantitatively to a 25 ml volumetric flask by adding distilled water. The concentrations of Cd, Cu, Pb, Co and Zn in the final solutions were determined by an atomic absorption spectrometer (AAS) (Shimadu European product).

Isolation and Identification of Heavy Metal-Resistant Bacteria from Soil Samples

Cadmium resistant bacterial were isolated from soil samples using nutrient agar (NA) medium. 1g of soil sample was suspended in 9 ml of sterile saline solution for 2 h, under shaken (150 rpm), and serially diluted to 10^{-6} with saline solution. Then, 1 ml of diluted suspension was plated on nutrient agar plates using standard pour plate method.

These Plates were incubated at 37°C for 24 h. The most frequent strains of the bacteria isolated were chosen for next experiments and stored onto nutrient agar at 4°C. These isolates were characterized morphologically (Gram staining). Biochemical characteristic like indole test, MR-VP, urease, catalase, citrate were also taken into consideration for characterization of these isolates (Cappucino and Sherman, 2002).

Preparation of Cd Solution

Standard solutions were prepared by dissolving the chloride salts in distilled water. Cd solution of 1000 mg l^{-1} was prepared with deionized water by dissolving 1.79g $CdCl_2.5H_2O$ salt in distilled deionized water. Solutions of varying concentrations (25, 50, 100, 200, and 400) were prepared by diluting the stock solution with deionized water.

Determination of Heavy Metal-Resistant Bacterial Isolates to Cadmium

Heavy metal resistant bacteria were determined by plate diffusion method (Hassen *et al*., 1998). Heavy metal salt solutions were prepared in different concentrations of 50, 100, 150, 200, 250 and 300 mg/L. Each plate was spread with overnight cultures of appropriate organisms. To each of the plate 0.5 ml of Cd metal salt solutions were added in each well of 10 mm in diameter. NA plates were incubated at 37°C for 24 h. After incubation, the zone of inhibition was measured. A zone size less than 1 mm scored as resistance strain.

16S rRNA Gene Amplification

After biochemical characterization, isolates were identified on the molecular basis using 16S rDNA sequencing for the accurate identification of bacterial isolates. First, the genomic DNA was extracted from the bacterial culture by Triton prep Method. The partial 16S rDNA was amplified by using the universal primers, 27 F (5'- AGA GTT TGA TCC TGG CTC AG – 3') and 1492R (5'- CGGTTACCTTGTTACGACTT- 3').

Polymerase chain reaction (PCR) was performed using thermal cycler (Applied Biosystem, USA) with 50 µl reaction mixture containing 1 µl (10 ng) of DNA extract as a template, each primer with a concentration of 10 picomole/µl, 25 mM $MgCl_2$, and 2 mM dNTPs along with 1.5U of *Taq* polymerase and buffer as per the manufacturer's recommendations (GCC Biotech, Kolkata, India).

The initial denaturation was performed at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 1 min, extension at 72°C for 1 min and 30 s, and final extension at 72°C for 10 min. The PCR products were analyzed by 1.5% w/v agarose gel electrophoresis (1× TAE) buffer with ethidium bromide (0.5 µg/ml).

Molecular Characterization

The PCR product was sequenced by capillary sequencing method using ABi 3500 Genetic Analyzer machine as per the manufacturer's instructions. The 16S rRNA gene sequences were compared with the known bacterial 16S rRNA gene sequences using NCBI ADVANCED BLAST so as to identify the most similar sequence alignment (Altschul *et al.*, 1997). Based on the scoring index, the most similar sequences were aligned with the sequences of other representative bacterial 16S rDNA regions by using multiple alignment software program, ClustalW (Thompson *et al*., 1994). A phylogenetic tree and a similarity index were generated and compared with the known sequences. The phylogenetic tree was constructed using the UPGMA method, and the tree reliability was tested by Bootstrap method using MEGA version 4 (Tamura *et al*., 2007).

Preparation of Bacteria Biomass for Biosorption

Cadmium resistant bacteria were cultured in nutrient broth medium for 48 h at 32°C, with shaking at 150 rpm, then harvested by centrifugation for 10 min at 8,000 rpm. The suspension was rinsed three times with sterile distilled water. Biomass was harvested from the medium by centrifugation at 8,000 rpm for 10min, heat killed in an hot air oven at 80 ^{o}C for 24 h, ground in a mortar and stored at 4 ^{o}C for further use.

Biosorption Studies

Biosorption studies were done using biomass as a function of various parameters such as pH, biomass concentration, temperature, contact time and initial metal concentration. Procedure for biosorption was performed according to Babak *et al*., 2012 and Ahalya *et al*., 2004. Biomass concentration of 0.05g was added to 20ml of cadmium salt solution (50mg/L) in 100ml Erlenmeyer flask. The flasks were placed on a shaker with a constant speed of 150 rpm. Samples were collected after 24 h, centrifuged and the amount of metal in the supernatant was determined using AAS at 228.8nm wavelength.

Effect of pH on Cadmium Biosorption by the Isolates

The impact of the pH on cadmium biosorption was investigated on biomass of *L. sphaericus* and *E. cloacae.* pH were conditioned to range from 3, 5, 6, 7, and 9.0 containing 20 ml of metal solution. The pH adjustment was done with the addition of either 0.1M NaOH or 0.1M HCl. Fifty

mg/L of cadmium salt solution was prepared from 1000 mg/L stock solution by dilution from $CdCl_2.5H_2O$. Biosorbent of 0.05 g was added to 20 ml of the cadmium solution of 50 mg/L. All flasks were maintained at different pH values for 24 h. Solutions were centrifuged and the supernatant analyzed for the residual concentrations of the metal ions using AAS.

Effect of Biomass Concentration on Cadmium Biosorption

Different weights of the biomass biosorbent ranging from 0.02 to 0.10g were added to 20ml metal solution containing the 50 mg/L metal concentration. The solutions were adjusted to the optimum pH (9.0 for *L. sphaericus* and *E.cloacae*) in which maximum biosorption of the metal ion occurred. Flasks were left to equilibrate for 24 h at 37 ^{o}C on a rotary shaker at 150rpm. The amount of cadmium biosorbed was determined using AAS.

Effect of Temperature on Cadmium Biosorption

Biosorption of cadmium was carried out at different temperature of 25, 35, 40 and 45 ^{o}C for each culture and kept on rotary shaker at 150 rpm. The solution were adjusted to the optimum pH of 9.0 and optimum biomass dose of 0.02g for both *L. sphaericus* and *E. cloacae*. The samples were allowed to agitate for 24 h. The sample were collected and analyzed for residual metal concentration using AAS.

Effect of Time on Cadmium Biosorption

Sorption process was carried out on constant volume of metal concentration at various contact time of 12 h intervals for 2 days. The cell pellet (0.02g) was dispersed in 20 ml metal solution (50 mg/L) and experiment was carried out at optimum pH of 9.0. Flasks were allowed to attain equilibrium on rotary shaker at 150 rpm. Samples were collected at regular time intervals. Centrifugation at 8000 rpm was done and the supernatant was analyzed for residual metal content using AAS.

Infrared Analysis of Biosorbent

Fourier Transform Infrared (FT-IR) absorption spectra of the biosorbent (*E .cloacae*) before and after it was loaded with metal solutions were analyzed by an FT-IR spectrophotometer (Shimadzu, Europe) using the KBr disk technique. This was performed to give a qualitative and preliminary characterization of the main chemical groups present on the cell wall responsible for heavy metal biosorption (Selatnia *et al*., 2004).

Determination of Cadmium Uptake by Isolates

Percentage removal and biosorption of Cd ions onto the biosorbent was determined using the following expressions:

$$\text{Removal (\%)} = \frac{Ci - Cf}{Ci} * 100 \ldots\ldots \quad \textbf{(1)}$$

$$\text{Metal uptake,} \quad q = \frac{V(C_i - C_f)}{m} \quad \textbf{(2)}$$

Where q is the amount of metals adsorbed onto the biosorbent, *Ci* and *Cf* (mg L^{-1}) are the initial and final concentrations of the metals in the solution respectively; V (liters) is the volume of the solution and m (grams) is the dry weight of the sorbent.

Optimization of Cadmium Uptake by *L. sphaericus* and *E. cloacae*

The optimum biomass (0.02g) for *L. sphaericus* and *E. cloacae* was dispersed in a desired concentration of metal salt ranging from 25, 50, 75 and 100 mg/L. In all these cases, the initial pH was adjusted to 9.0. The Flasks were incubated at 25^{o}C for *L. sphaericus* for 36h and 35^{o}C for *E. cloacae* for 12h after which the residual metal concentration was determined using AAS. Langmuir and Freundlich models were applied to interpret equilibrium values. Langmuir isotherm model was used in equilibrium interpretation (Langmuir, 1916) and its linear form of was expressed by the following equation:

$$\frac{Ce}{qe} = \frac{1}{qo.b} + Ce \quad \textbf{(3)}$$

Where Ce (mg l^{-1}) is the equilibrium metal concentration, Qe (mg g-1) the amount of metal ions adsorbed per unit mass of sorbent, Qo (mg g-1) is the maximum adsorption capacity and b is the Langmuir constant. A linear plot of Ce/Qe against Ce is created from which other constants will be determined.

Essential features of a Langmuir isotherm used to predict if an adsorption system is favorable or unfavorable is expressed by a dimensionless equilibrium constant, RL and is represented by the expression:

$$\frac{1}{RL} = \frac{1}{1+b.Co} \quad \textbf{(4)}$$

R_L value indicates the isotherm to be either unfavorable (RL> 1), linear (RL = 1), favorable (0 < RL< 1) or irreversible (RL ≤ 0).

Freundlich model is also used to describe surface sorption (Freundlich, 1906), with the linear form of this model expressed by the equation below:

$$Log\ qe = LogKF + \frac{1}{n} Log\ Ce \quad \textbf{(5)}$$

A plot of log qe against log Ce will give a linear curve from which values of the Freundlich constant, KF, and slope, 1/n, of the curve will be determined.

RESULTS AND DISCUSSION

Isolation of Toxic Metal Tolerant Bacteria

A total of forty (40) different bacteria strains were isolated based on their color, size and morphological differences. Different bacterial strains of *Bacillus* sp, *Staphyloccous* sp, *Enterobacter* sp, *Escherichia* sp, *Citrobacter* sp, *Proteus* sp, and *Klebsiella* sp were isolated from the sample. *Bacillus* sp had the highest occurrence of (55%) while *Klebsiella* sp recorded the lowest occurrence of 4% (Figure 1). Further screening was done on the basis of maximum metal tolerance for cadmium, a total of 12 bacterial isolates tolerant to high concentration of cadmium were selected.

Screening for Toxic Metal Degrading Bacteria

The isolated bacteria species screened for their metal degrading pattern indicated that most of the isolates showed resistance to one or more toxic selected metals. Two of the isolates that showed the highest metal-degrading properties as growth on medium with no zone of inhibition was observed after 24 h were used for biosorption studies. The two isolates are *Lysinibacillus sphaericus* and *Enterobacter cloacae.*

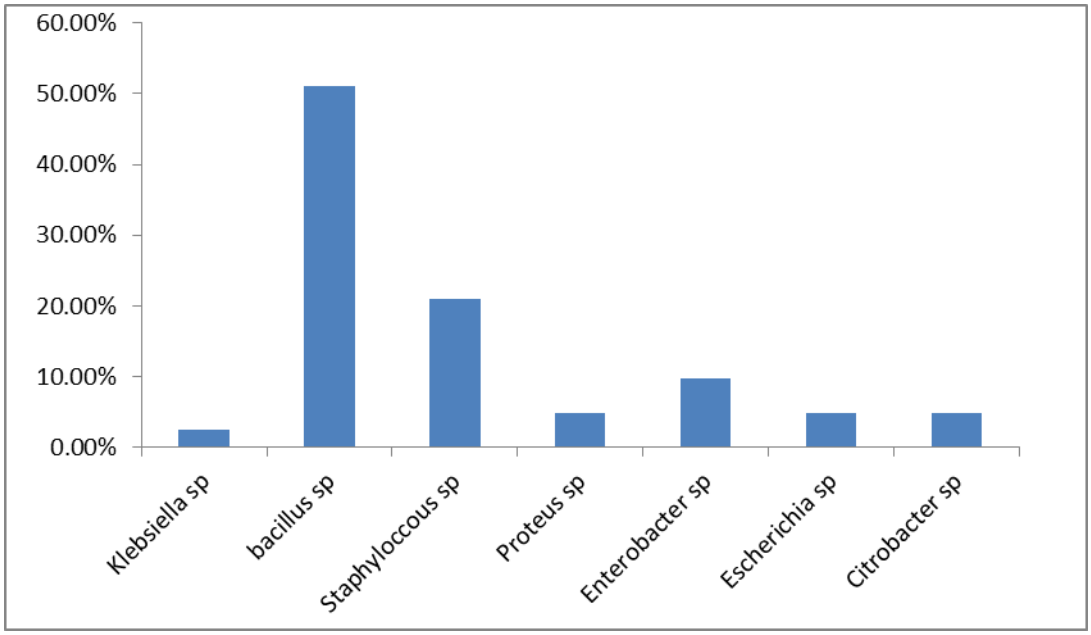


Figure 1: Occurrence of Bacterial Isolates in the Soil Sample.

Molecular Characterization of Toxic Metal Tolerant Bacterial Strains

Plate1 showed the gel electrophoresis of polymerase chain reaction which indicated that the DNA of the two strains were located at 1500 base pairs while Figure 2 showed phylogenetic relationships of *Lysinibacillus sphaericus* and *Enterobacter cloacae* with other strains of the same species retrieved from Gen Bank. The comparative analysis of the sequences of the two isolates used in this study with already available database using BLAST (Basic Local Alignment Search Tool) showed that the strains SAMPLE A is 94 % identical to *Lysinibacillus sphaericus* X-17 and sample B is 98 % identical to *Enterobacter cloacae* PR3.

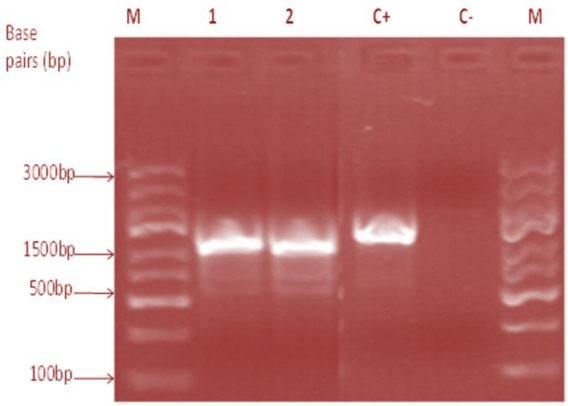


Plate 1: Gel Electrophoresis of Amplified PCR Products in 1 % Agarose Gel.

M = molecular marker, **1**= *Lysinibacillus sphaericus*,**2** = *Enterobacter cloacae*, **C+** = positive control, **C-** =negative control

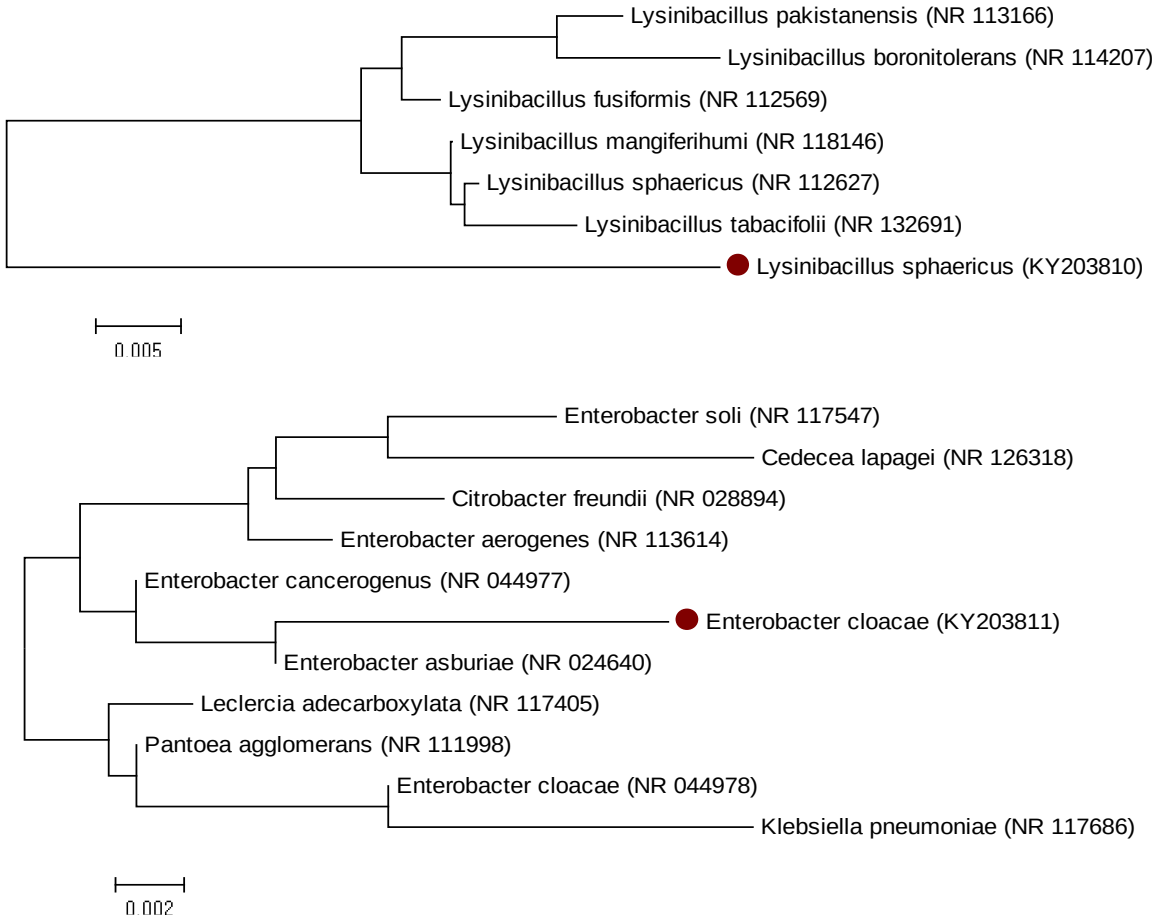


Figure 2: Phylogenetic Relationships of *Lysinibacillus sphaericus KY203810* and *Enterobacter cloacae KY203811* with other Strains of the Same Species Retrieved from Gen Bank.

Effect of pH

Influence of pH on the sorption of cadmium was investigated. Optimum pH for cadmium removal by *L. sphaericus* and *E. cloacae* was achieved at pH 5.0 and pH 9.0, respectively (Figure 3).

Results indicated that pH range 9.0 is optimum for cadmium uptake and metal uptake increases with increasing pH. This agreed with previous reports (Ahuja *et al*., 1999; Gong *et al*., 2005;) showing that at low pH, the cell surface sites are closely linked to the H^+ thus making these sites unavailable for other cations. However, with an increase in pH, there is an increase in negatively charged sites which results in increased binding of cations.

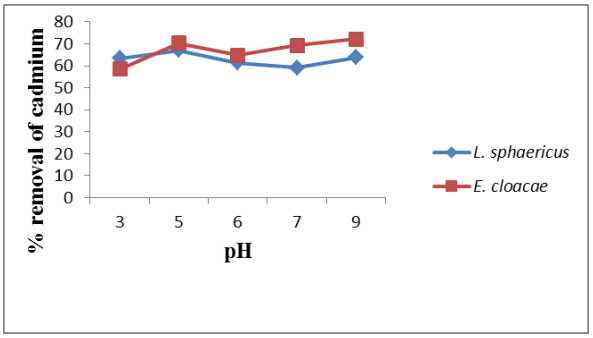


Figure 3: Effect of pH on Cadmium Biosorption.

Effect of Biomass Concentration

The biomass dose is known to affect the sorption rate as it determines the ability of the sorbent to interact with the sorbate (Adeogun *et al*., 2012). Biomass dose of 0.02g was observed as optimum for cadmium removal by *L. sphaericus* and *E. cloacae* (Figure 4). This denotes that the low dosage can be used to achieve high sorption rate. Result obtained was in accordance to what was reported by Gadd *et al*. (1998) who suggested that an increase in biomass concentration leads to interference between the binding sites. Various reasons, including limited availability of metal ions, increased electrostatic interactions, and interference between binding sites and reduced mixing at higher biomass densities, have been suggested to explain the decreased metal removal capacity above saturating biomass load (Fourest *et al*., 1994).

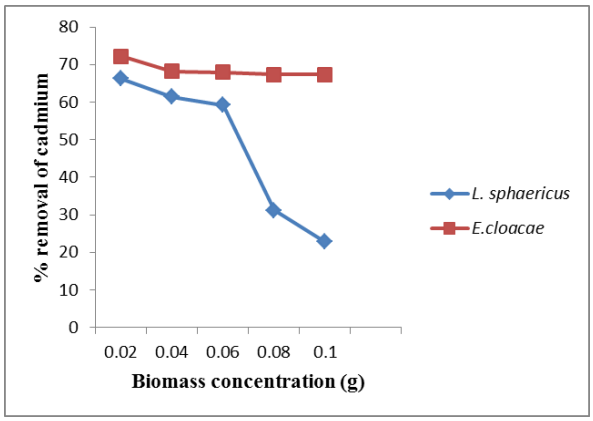


Figure 4: Effect of Biomass Concentration on Cadmium Biosorption.

Effect of Temperature

Optimum temperature for cadmium uptake was observed at 25 °C for *L.* sphaericus biomass and at 35^{0}C for *E. cloacae* (Figure 5). The temperature range observed in this study was in agreement with the study of Sag and Kutsal (1995) who reported that, maximum biosorption rates for Ni^{2+} and Cu^{2+} by *Zooglea ramigera* could be obtained at 25°C. Tohamy *et al*., 2006 and Jackson *et al*., 2011 also reported that *Arcanobacterium bernardiae* and *B. amylolikuefaciens* achieve their maximum capacity for lead uptaking at 35 °C. Decrease in sorption percentage with further increase in temperature was observed. This may be due to the shrinkage of cells at higher temperature.

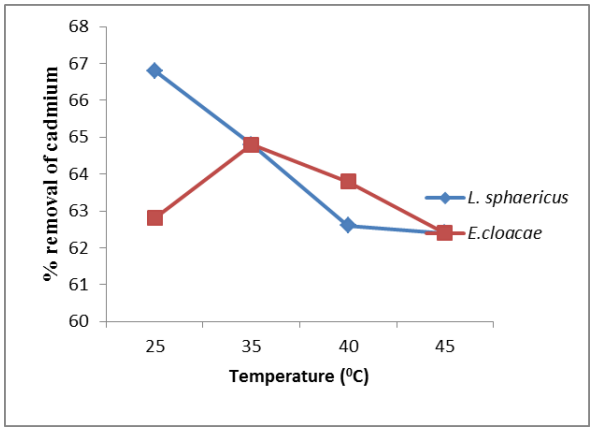


Figure 5: Effect of Temperature on Cadmium Biosorption.

Effect of Contact Time

The equilibrium time for *L. sphaericus* was observed to be 36 h for cadmium, *E. cloacae* attained equilibrium at 12 h (Figure 6). The sorption percentage of metal increased with increase in contact time until equilibrium was attained. High sorption rate at equilibrium may be attributed to high penetration and the accumulation of metals on to the high number of binding sites on biosorbent (Sangi *et al*., 2008; Gupta *et al*., 2011). The reduced sorption after equilibrium can be attributed to exhaustion of remaining binding sites due to presence of repulsive forces (Qaiser *et al*., 2009).

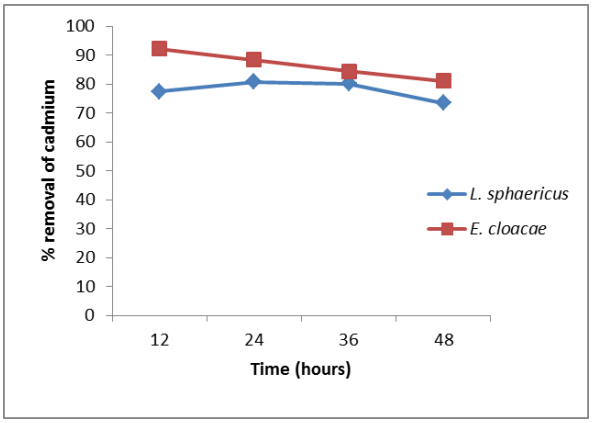


Figure 6: Effect of Contact Time on Cadmium Biosorption.

Adsorption Isotherm

The equilibrium data fitted well with the Langmuir and Freundlich adsorption isotherms for cadmium at various initial metal concentrations. Data obtained in this study showed that the Q_{max} (the maximum sorbate uptake) values obtained for cadmium uptake was 46.3mg g^{-1} by *L. sphaericus* and 57.2 mg g^{-1} by *Enterobacter cloacae*. The correlation coefficients (R^2) of Langmuir for cadmium by *L. sphaericus* and *E. cloacae* were found to be close to unity (≥ 0.95).

The Freundlich analysis determined that the biosorptive capacity (K_F) values for cadmium by *L. sphaericus* was 8.598. The values of K_F for cadmium by *E.cloacae* was 16.67. In general, these data indicated that the sorption capacity increased with increase in the initial metal-ion concentration for metal ions on the biomass surface. High Langmuir constant, b, observed indicates that there was high affinity of interaction between the biosorbent and metals (Holan and Volesky, 1994; Volesky, 2004), which is also indicated by the very low values of slope. Similarly, high Q_{max} values observed for the biomass present to be occupied by bacterial biomass and high amount of metal uptake, q, observed indicates that nearly all binding sites were indeed occupied (Volesky, 2004).

Favorable sorption was deduced for cadmium by *L. sphaericus* and *E. cloacae* with R_L values of 0.00347 and 0.00104 respectively. All the data on metal sorption of present study fitted better in Langmuir isotherm (R^2> 0.95) than the Freundlich as is evident from very high values of correlation coefficient for cadmium.

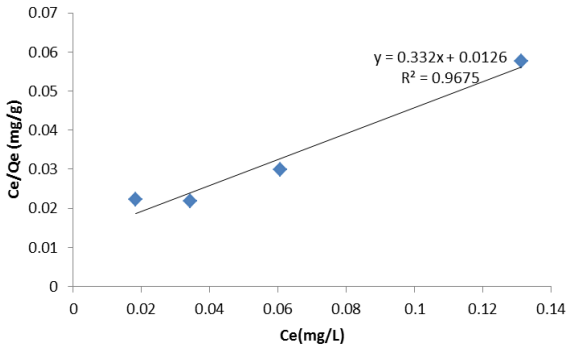


Figure 7a: Linear Langmiur Plot of the Sorption of Cadmium ions on *L. sphaericus* Biomass.

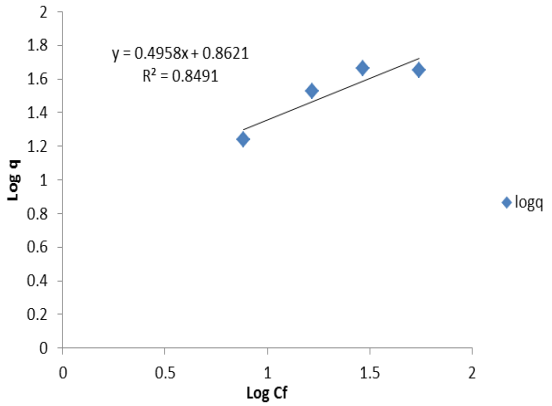


Figure 7b: Freundlich Model of Sorption Isotherm of Cadmium by *L. sphaericus*

Slope = 1/n = 0.495777; Intercept = 0.86211; Freundlich constant, K_F = 8.598; R^2 = 0.849

The value of slope 1/n was less than 1 for biosorption of cadmium using both biomasses. Fitting of data on metal adsorption of the present study in Langmiur model thus indicates homogenous assembly of binding sites at the cell surfaces.

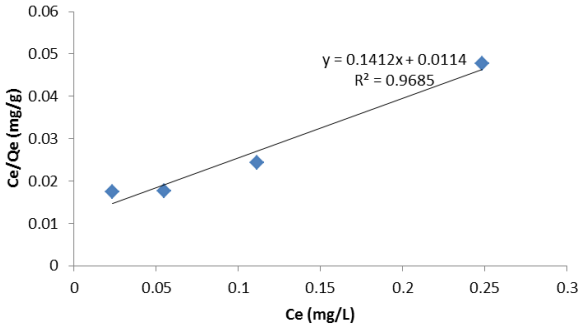


Figure 8a: Linear Langmuir Isotherm Plot of the Sorption of Cadmium Ions on *E. cloacae* Biomass.

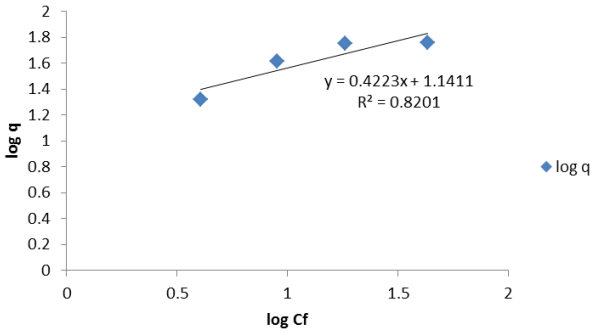


Figure 8b: Freundlich Curve for Cadmium Adsorption by *E. cloacae*.

Slope 1/n = 0.422, Intercept = 1.141, Freundlich Constant, K_F = 16.67, R^2 = 0.820

FTIR Spectral Analysis of Biosorbent

The FTIR spectra of the unloaded *Enterbacter cloacae* (control) (Figure 9) displayed major peaks at wave numbers from 3757.33 cm^{-1}, 3435.22 cm^{-1}, 2931.80 cm^{-1}, 2374.37cm^{-1}, 1643.35cm^{-1}, 1550.77cm^{-1}, 1400.32cm^{-1}, 1240.23cm^{-1}, 1076.28 cm^{-1}indicating the presence of hydroxyl, amine, amide, aromatic alkenes, nitro compounds, alcohol, carboxylic acids, esters, ethers, aliphatic amines and alkyl halides (-OH, N-H, C=O, N-O, C-O and C-N, C-C) functional groups on the surface of the organism.

These findings are similar to that reported by Volesky (2007) who concluded that the main functional groups responsible for a biosorption process are the hydroxyl, carbonyl, carboxyl, sulfonate, amide, imidazole, phosphate and phosphodiester groups, some of them are present on *Enterobacter cloacae*.

The IR spectra of *Enterobacter cloacae* biomass loaded with cadmium (Figure 10) revealed significant shifts in O-H band at 3921.28 cm^{-1}, N-H stretch(amines and amides) at 3425.58 cm^{-1}, N-H bend (amines) at 1643.35 cm^{-1}, C-N band at 1249.87 cm^{-1}(aliphatic amines) and 453.27 cm^{-1} band (alkyl halides).

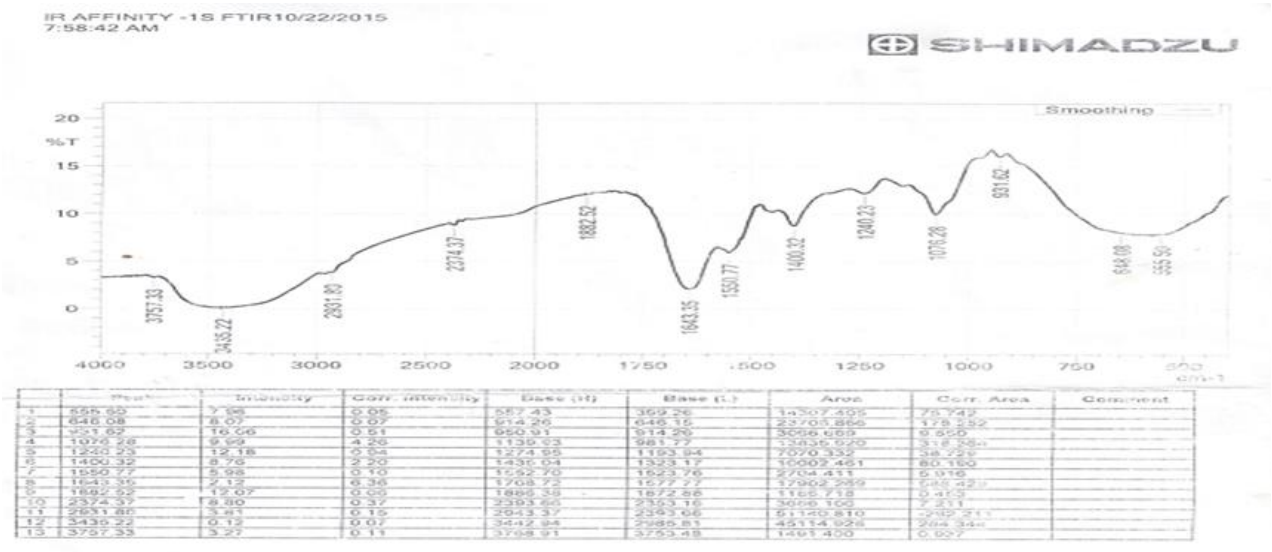
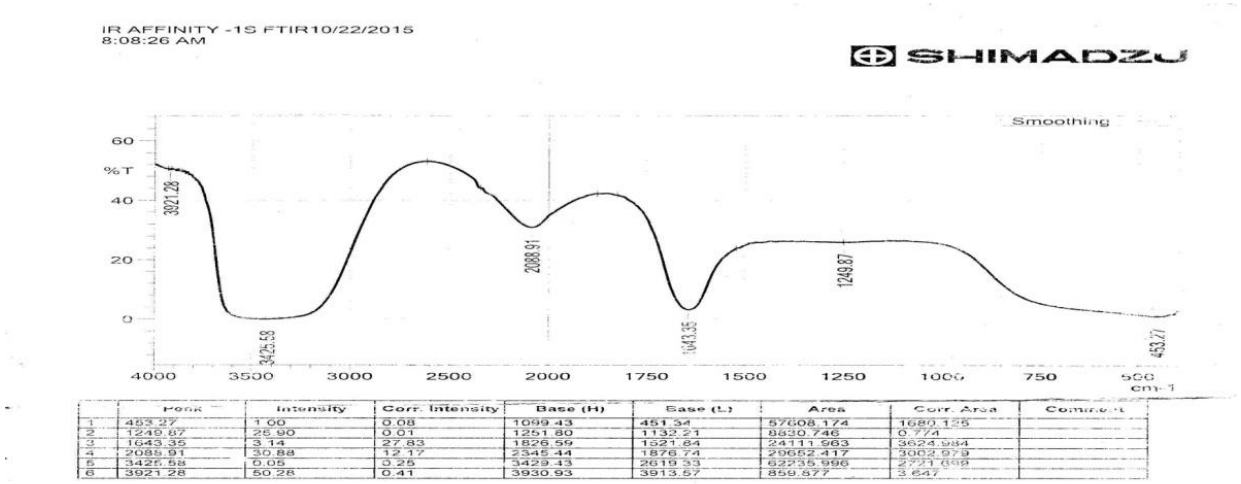


Figure 9: FT-IR Results for *Enterobacter cloacae* before Cadmium Biosorption.



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	453.27	1.00	0.08	1099.43	451.34	87608.174	1680.125	
2	1249.87	25.90	0.01	1251.80	1132.21	9830.746	0.774	
3	1643.35	3.14	27.83	1826.59	1521.84	24111.963	3624.984	
4	2088.91	30.88	12.17	2345.44	1876.74	29652.417	3002.979	
5	3425.58	0.05	3.25	3429.43	2619.33	62235.996	2721.668	
6	3921.28	50.28	0.41	3930.93	3913.57	859.877	3.647	

Figure 10: FT-IR Results for *Enterobacter cloacae* after Cadmium Biosorption.

These changes of the spectra clearly show the complexation/coordination of the metal ions during the biosorption process. Prominent and significant changes in peaks corresponding to hydroxyl, amides, amines and carboxylic groups were observed after metal (cadmium) adsorption on the biomass indicating possible involvement of these functional groups in metal removal.

CONCLUSION

This study showed that organisms with effective biosorbent capability can be isolated from environment contaminated with toxic metals. The adsorption equilibrium data fitted well into Langmuir and Freundlich models for metal ions in the studied concentration range.

The biosorption mechanism includes mainly ionic interactions, complexation and coordination processes between metal cations and active spots in the cell wall of bacterium. The results demonstrated that the bacterial isolate *Lysinibacillus sphaericus* X-17 and *Enterobacter cloacae* PR3 possess potential capability of bioremediating environments contaminated with cadmium.

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