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# *Brevundimonas vesicularis*:A Novel Bio-sorbent for Removal of Lead from Wastewater

**Resmi, G .*, Thampi, S. G . and Chandrakaran, S.**

Department of Civil Engineering, NIT Calicut, Kerala, 673 601, India

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**ABSTRACT:** In this study, a bacterial species which could remove lead from wastewater was isolated from lead contaminated soil prepared in the laboratory. Based on the biochemical and morphological characterization the bacteria were identified as *Brevundimonas vesicularis.* The bio-sorption potentiel of powdered dry biomass of B. *Vesicularis* was investigated by batch adsorption experiments. It was found that, the bio-sorption capacities were significantly affected by the pH and initial concentration of the solution, bio-sorbent dosage and contact time. Batch and isothermal studies were carried out at optimum pH of 4. The rate of bio-sorption was found to be fast during the initial 10min and it reached equilibrium by 60min. Langmuir isotherm model was suitable for describing the bio-sorption of lead by B. *Vesicularis.* The results indicated that, the dry biomass of B. *Vesicularis* is suitable as an efficient bio-sorbent for the removal of lead from wastewater.

**Key words:** Wastewater, Bacterial species, Heavy metal, Biomass, Bio-sorption

## INTRODUCTION

Contamination of soil and ground water with various pollutants has become a major environmental problem worldwide. The extensive production and use of heavy metals and organic compounds makes them the most prevalent pollutants in the subsurface and at waste disposal sites. Because many of these pollutants are known as potential threat to public health and environment, there is an urgent need to understand their fate in the environment and to develop cost-effective methods for their control (Lewandowski and DeFilippi, 1998).

Lead is among the most toxic heavy metal ions, affecting the environment. Main sources of lead are the manufacture of storage batteries, pigments, leaded glass, mining, metal electroplating, painting, smelting, petrochemical, plumbing, fuels, photographic materials, matches and explosives. Apart from these, lead is also used in insecticides, food, beverages, ointments etc for flavoring and sweetening. These industries may discharge lead into the environment without adequate treatment. Untreated effluents from these industries have an adverse impact on the environment and aquatic life (Qaiser *et al.,* 2009). Lead exposure has the tendency to cause adverse health effects. Exposure to lead can cause damage to brain, kidney and can cause anemia (Goel *et al.,* 2005). Conventional physicochemical processes which are being used for lead removal include chemical oxidation, reduction, precipitation, adsorption, solidification, electrolytic recovery and ion exchange (Sanjay and Sugunan, 2006). Application of such methods is sometimes restricted because of technical and economical constraints.Bio-sorption is highly preferred and advantageous as this method is less expensive and effective (Abbas *et al.,* 2008; Bossrez *et al.,* 1997). Apart from that, bio-sorption is non-polluting, selective and easy to operate (Bossrez *et al.,* 1997; Galvan *et al.,* 2008; Zubair *et al.,* 2008).Bio-sorption is the passive process of sequestration of metals by the interaction with live and dead biological matter. It is at present the most practical and widely used approach for bio-remediation of metals and radionuclide. Bio-sorption involves accumulation of metals on the surface of the cells or cell fraction by adsorption or ion exchange.

*Corresponding author E-mail: rgcednss@yahoo.com

Bacteria cells are excellent bio-sorbent materials, because of their high surface to volume ratio and high content of potentially active chemisorptions sites such as techoic acid in their cell wall (Hu *et al.*, 1996). Bacterial cell wall consists of negatively charged groups like phospholipids and lip polysaccharides which can attach metals (Segers *et al.*, 1994). It is reported that, *Pseudomonas aeruginosa* - a genetically unaltered bacterial strain could bind dissolved hexavalent uranium (Hu *et al.*, 1996) to its cell wall. This biomass was reported to adsorb significantly more uranium than certain novel patented bio-sorbent prepared from algal and fungal biomass (Churchill *et al.*, 1995). The aim of this work was to study the utility of *Brevundimonas vescularis*, which is a closely related strain to *Pseudomonas aeruginosa* as an adsorbent for Pb (II) removal from aqueous solution by batch experiments. The bio-sorption potential of these bacteria to remove heavy metals from aqueous phase has not been reported elsewhere. The effect of experimental parameters such as solution pH, initial concentration, contact time and temperature was investigated. Furthermore, the sorption mechanism was investigated by fitting the experimental data to suitable isotherm model.

## MATERIALS & METHODS

Bacteria used in the present study were isolated from artificially contaminated soil. Soil was collected from Chathamangalam, Calicut District, Kerala, South India and it was kept artificially contaminated with lead nitrate solution of 1000 mg/L for 50days. From this, 10 g of soil was taken and mixed with 90 ml of bacteriological saline water followed by 10 times dilutions by serial dilution (Pelczer *et al.*, 2004). Sterile conditions were maintained throughout the procedure. This was followed by plating the dilutions in nutrient agar plates (Hi-media). Plates were then incubated at 37°C for 12 hours.

Biochemical and morphological characterizations were carried out as per Bergey's Manual of Determinative Bacteriology (1994). Bacterial cultures were grown on nutrient agar plates and were examined based on gram reaction.A series of selective medias were used for characterization which included Macconkey agar and Blood agar. Motility test was also performed. Biochemical test for utilization of D-glucose, D-galactose, Salicin, Maltose, Iisobuyrate, L-histidine, L-Rhamnose, test for enzymes like *Oxidase, Catlase, DNase, Lysine Decarboxylase* and *Ornithine Decarboxylase* were done.

The tested bacteria were maintained in nutrient agar slants. The stock cultures were transferred weekly and stored at 4°C in a refrigerator. Biomass of the tested bacteria was grown in nutrient broth at pH 7 and at a temperature of 37°C for 18 h on a rotary shaker at 120rpm. Cells were harvested by centrifugation at 10000 rpm for 10min. Harvested cells were washed twice with de-ionized distilled water and desiccated in an oven at 80°C for 48 hours. To assess complete death of the bacteria, samples of the dried cells were inoculated on Petri–dish containing blood agar. Absence of colony formation showed that the bacteria were dead. The dried cells were then ground in a porcelain mortar to get a fine powder (0.2mm) and stored at 5°C, until further use.Stock solution of lead was prepared by dissolving AR grade lead nitrate salt in de-ionized distilled water (AWWA, 1998). This was followed by shaking for 15min and then keeping it for 24 h for absolute dissolution. pH value was adjusted in the range of 2 -10 by adding 0.1M NaOH or 0.1M HCl. The concentration of lead was measured using an Ion meter with selective electrode for determination of lead (Thermo – Orion U.S.A).In batch adsorption experiments, different doses of bio-sorbent (50 mg to 500 mg) was added into several 250ml Erlenmeyer flasks, each containing 50 ml of lead nitrate solution. The flasks were then shaken at 120rpm in a rotary shaker for 24 h. The effect of contact time (10 to 130min), concentration (80 to 600 mg/L), solution pH (2–10) and adsorbent dose (50 mg to 500 mg) was studied at 25°C. Isotherms were obtained by adsorbing different concentrations of Pb(II) ions. After prescribed contact time, the solutions were centrifuged at 10000rpm for 10min, the supernatant was filtered and the concentrations of Pb(II) ions were determined. All the experiments were carried out in duplicates and the average values were used for further calculations.

Metal adsorbed by tested dried cells (mg/g) was calculated as per the procedure given by Yang and Volesky, (1999), which is expressed as,

$Q = (C_i - C_f)V/W$
Q = Specific lead uptake (mg/g)
V = Volume of lead solution (l)
$C_i$ = Initial concentration of lead in the solution (mg/L)
$C_f$ = Final concentration of lead in the solution (mg/L)
M = Mass of the powdered dried cells (g)

To test the effect of storage on the efficiency of bio-sorbent material, the dried powdered cells of bacteria used in the study were stored at room temperature for 150 days.100mg of dried powdered cells after different storage period were contacted with 50ml aliquots of lead solution of 550 mg/L concentration at pH 4 for 60min. The residual lead content was measured at 2, 5, 10, 25, 50, 100, 125 and 150$^{th}$ day.

## RESULTS & DISCUSSION

The bacteria isolated from contaminated soil were tested for its biochemical and morphological characterization. Based on these studies conducted, the bacterial species was identified as *Brevundimonas vesicularis.* It is a gram negative bacterium. It hydrolyzes Esculin. These bacteria can assimilate D-Glucose, Maltose, L-Rhanose and acetate. Growth parameters were also observed. The bacteria have aerobic respiration and grow in blood agar and Mac Conkey agar. It contains enzymes like *oxidize* and g*lucose oxidizer*. B. *Vesicularis* is rod shaped and has motility. Bre*vundimonas vesicularis* belongs to the sub class Proteobacteria. This subclass contains bacteria like Pseudomonas which were reported to be very effective in bio-sorption (Hu *et al.,* 1996).

The effect of pH on bio-sorption of lead metal ions is shown in Fig. 1. The lead bio-sorption was studied in a pH range of 2 to 10. An increase in percentage removal with increase in pH of the medium was observed for the lead ion adsorption up to pH value of 4. The maximum bio-sorption was obtained at a pH range of 3 to 5. It is observed that the maximum specific lead uptake of 45 mg/g was obtained at pH 4. With increase in pH (beyond 4), the bio-sorption declines sharply. Therefore, the pH value of 4 is the optimum and taken for further studies. Senthilkumaar et al. (2000) has reported that bio-sorption of a metal is dependent on the initial pH of the aqueous solution. Investigations by other researchers (Jalali *et al.,* 2002; Leung *et al.,* 2000) also reported a similar trend in bio-sorption studies. At high acid pH a negative network of charges is formed on the surface of bio-sorbing materials. Apart from this, a lower pH will affect the physiochemical process and hydrolysis of the metal (Leung *et al.,* 2000).

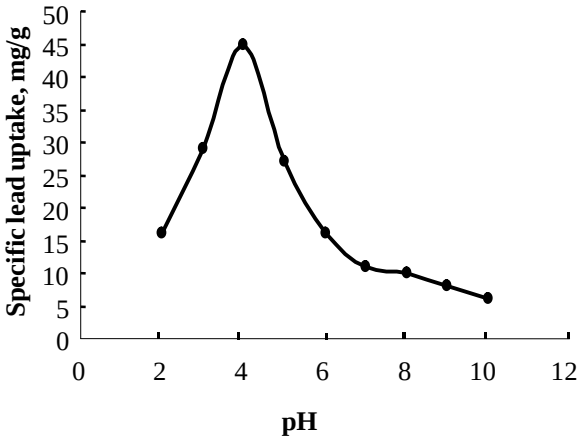


**Fig. 1. Effect of pH on Specific lead uptake**

The tolerance of the bacteria to most metal in low pH media probably results from effective competition by $H^+$ ions for negatively charged sites at the bacterial surface. These results, suggest that the bio-sorption of lead ions to the biomass occurs mainly due to oppositely charged ionic attraction. Therefore, as pH decreases the cell surface becomes more positively charged, reducing attraction between biomass and metal ions (Hossain *et al.,* 2006). In contrast, higher pH results in facilitation of metal uptake, since the cell surface is more negatively charged. An optimum pH of 4 for the adsorption of lead ions was found in the present studies which indicate that, at pH of 4 (optimum value), neutralization of positive and negative ions was occurred (Hossain *et al.,* 2006).

The effect of initial lead concentration on bio-sorption of lead ions from wastewater is shown in Fig. 2. The initial lead concentration was varied from 80 to 600 mg/L in the studies conducted. Analysis of the figure indicates that specific metal uptake increases by increasing initial lead concentration. Specific lead uptake was 18.15 mg/g when the initial lead concentration was 80 mg/L. It got increased to 42.3 mg/g when the initial

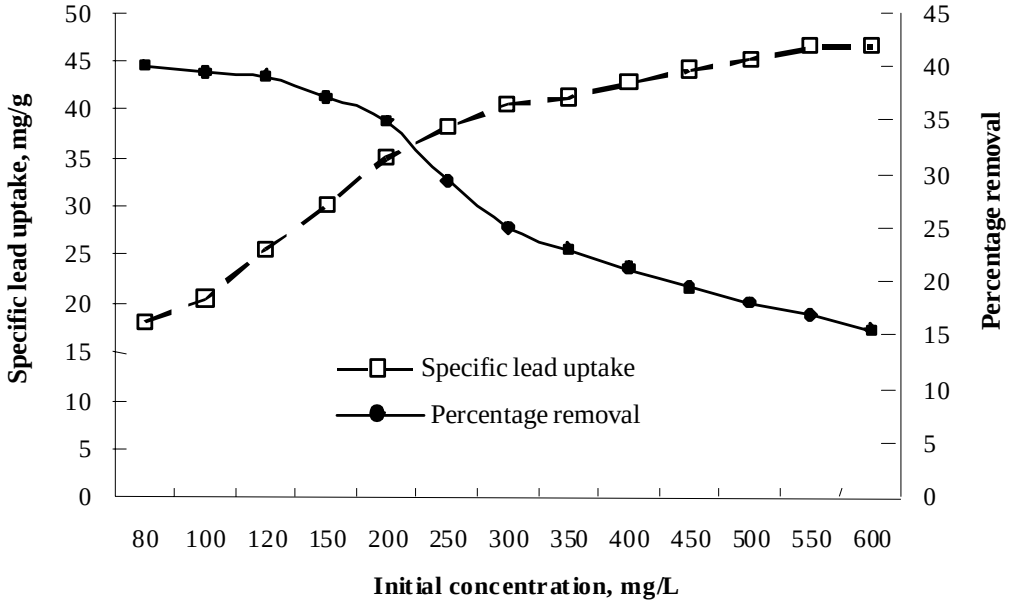


**Fig. 2. Effect of initial lead concentration on specific lead uptake**

lead concentration was increased to 550 mg/L. When the initial lead concentration was increased to 600 mg/L, no further increase in metal uptake was observed indicating that the binding sites were completely saturated by lead ions.

Al-Asheh and Duvnjak (1995) reports that, the increase in lead sorption may be due to the increase in electrostatic interactions. Dead cells of B. *Vesicularis*, used in the present studies, consist of 5-20% of peptidoglycan and lipo-polysaccharides on its cell wall, which shows affinity to cation adsorption. Bio-sorption by dead cells of B. *Vesicularis* is due to negative charged groups like carboxy and amine groups on the bacterial cell wall. Along with these, the cartenoid pigments present on its cell wall collectively increase the bio-sorption capacity of the material (Puranik and Pakniker, 1999). However the percentage removal of lead from the solution was found to be decreasing as the initial lead concentration increased in the solution. The percentage removal was decreased from 44.8 to 15.5 when the initial lead concentration was increased from 80 to 600 mg/L. Fig. 2 shows the combined graph of effect of initial lead concentration on specific lead uptake and percentage removal of lead.

The effect of bio-sorbent concentration on the uptake of lead is shown in Fig. 3. The amount of dried powdered cells of B.*Vesicularis* was varied from 1 to 10 g/L to determine the effect of dosage of dry biomass on lead removal.It is observed that, with the increase in dosage of bio-sorbent the percentage removal was increased, while the specific lead uptake was decreased. The increase in percentage removal of lead can be attributed to the increase in adsorption surface area and the availability of free adsorption sites (Bueno *et al.*, 2008). The percentage removal of lead was observed as 18% at a bio-sorbent dosage of 1 g/ L. There was a regular increase in percentage removal of lead and as the mass of dried cells increased from 1 to 10 g/L, the percentage removal was increased from 18 to 40. Further increments in bio-sorbent dose led to a decrease in the percentage removal of lead. This can be explained by the formation of aggregates during bio-sorption, which takes place at high biomass concentrations causing a decrease of the effective adsorption area as depicted by Ekmekyapar et al. (2006) or due to interference between the binding sites at higher concentration (Rome and Gadd, 1987).

The specific lead uptake was found to be maximum (65 mg/g) at lower biomass concentration (1 g/L). Further increase in dosage of bio-sorbent led to a decrease in the specific lead uptake. This optimum dose of 1 g/L which gave maximum specific lead uptake was fixed as the bio-sorbent dosage for other bio-sorption

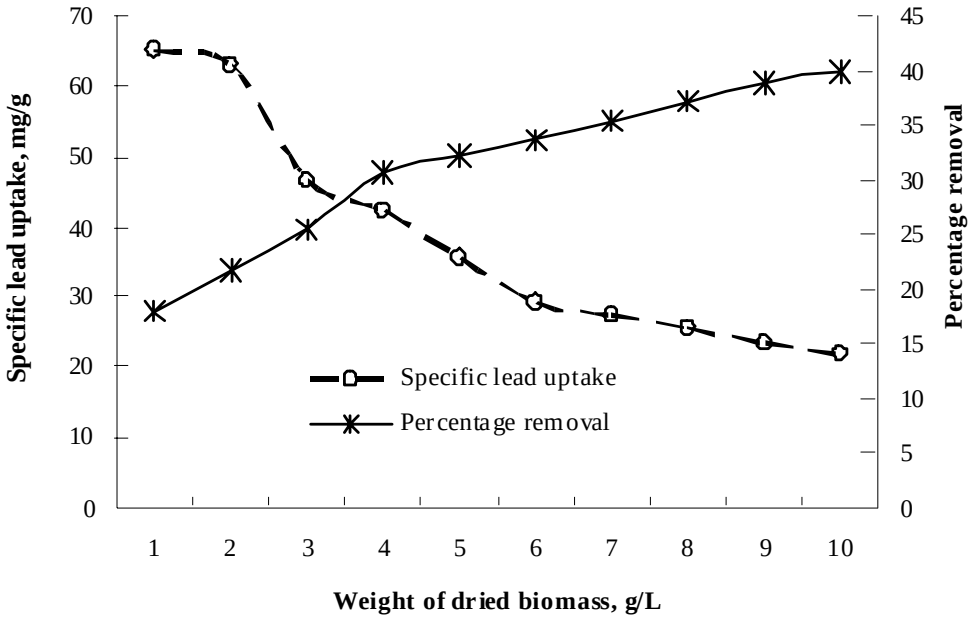


**Fig. 3. Effect of weight of dried biomass on specific lead uptake**

studies conducted. There are reports on reduction of zinc uptake by *Rizopus arrhizus* with increase in biomass concentration, which was attributed to an inadequacy of metal ions in solution, with respect to available binding sites (Fourest and Roux, 1992).

Similar kinds of observations were reported by other workers (Al-Asheh and Duvnjak, 1995; Bueno *et al.,* 2008). At lower concentration of dried cells of B.*Vesicularis* an increase in metal to bio-sorbent ratio exists which decreases upon increase in concentration of dried cells (Puranik and Paknikar, 1999).To analyze the effect of contact time on the lead bio-sorption mechanism, 100 mg of dried powdered cells of *B. Vesicularis* were exposed to 50 ml of lead solution with an initial concentration of 550 mg/L in 250ml conical flask. Fig. 4 shows the effect of contact time on bio-sorption of lead. Based on the experimental results it can be seen that, a rapid specific lead uptake occurs during the initial 10 min when the lead concentration was 550 mg/L with 1 g/L dried cells.

Time required for attaining equilibrium was observed as less than 70 min. Mohanty et al. (2008) reported that, metal uptake was influenced by factors affecting the mass transfer from bulk solution into binding site. As per Al-Garni (2005) kinetics of the process was determined by 3 steps. Initially, bulk transport of metal ion takes place from the solution phase to surface of binding site, which is regularly fast because of mixing and advective flow (Geoffrey *et al.*, 1992). It is followed by film transport, which involves diffusion of metal through a hydrodynamic boundary layer around the bio-sorbent surface and finally actual adsorption of metal ion takes place into the active sites of the biomass (Mohanty *et al.,* 2008). In the experiments with dead cells of B. *Vesicularis*, the system partially suppressed the first and second step and hence a steep increase in adsorption was observed in the initial 10min. It was previously reported that bio-sorption of lead by the fungus *Phanerochaete chrysosporium* was rapid in first 15 min and equilibrium was attained after 3 h (Al-

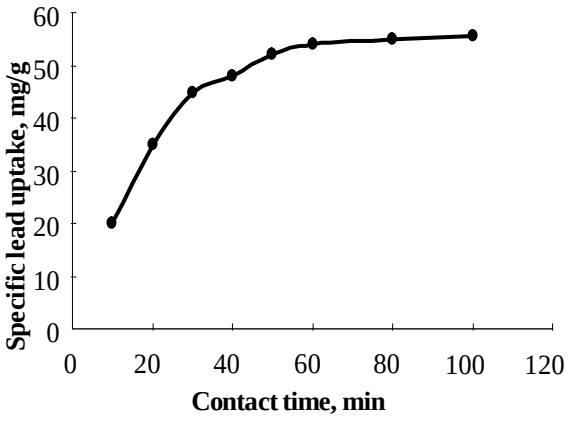


**Fig. 4. Effect of contact time on bio-sorption of lead**

Garni, 2005; Ceribasi and Yetis, 2001). In the present study the steady equilibrium state was reached after 60 min. Based on these results, the optimum contact time for the batch studies were fixed as 60min.

Practical effectiveness of bio-sorbents increases when it can be stored for future use. So the effect of storage of dried cells on bio-sorption of lead was studied. The results obtained from Fig. 5. indicate that there is no significant change in specific lead uptake due to storage time. For 150days at room temperature (25°C), the dried cells of B. *Vesicularis* showed no change in the bio-sorption capacity. This finding can be highly advantageous as it increases the scope of dried cells of B. *Vesicularis* as a potential bio-sorbent over the conventional methods for lead removal from solutions.

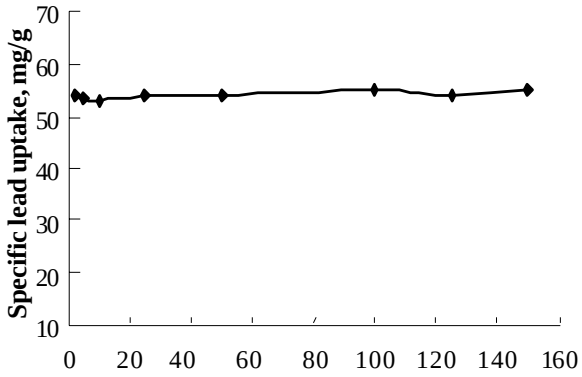


**Fig. 5. The effect of storage time on specific lead uptake**

Application of the bio-sorption technique on a commercial scale requires quantification of the sorption equilibrium for process simulation. The sorption equilibrium data for lead on the bio-sorbent (B. *Vesicularis*) was analyzed using Langmuir and Freundlich isotherm models. The Langmuir model fitted to the experimental data reasonably well (Fig. 3). The Langmuir model can be expressed as

$$\frac{C_e}{q_e} = \frac{1}{bq_m} + \left(\frac{1}{q_m}\right) C_e$$

where b and $q_m$ are Langmuir coefficients representing the equilibrium constant for the adsorbate–adsorbent equilibrium and the monolayer capacity.

The linear Langmuir plot was obtained by plotting $\frac{C_e}{q_e}$ vs $C_e$ respectively, from which the adsorption coefficients were evaluated. The constant $q_m$ and b are calculated from the y-intercept and slope of the linear plot. The value obtained for $q_m$ signifies the adsorption capacity (mg/g) and b is related to the energy of adsorption (mg/L). The Langmuir isotherm obtained in the present study is shown in Fig. 6.

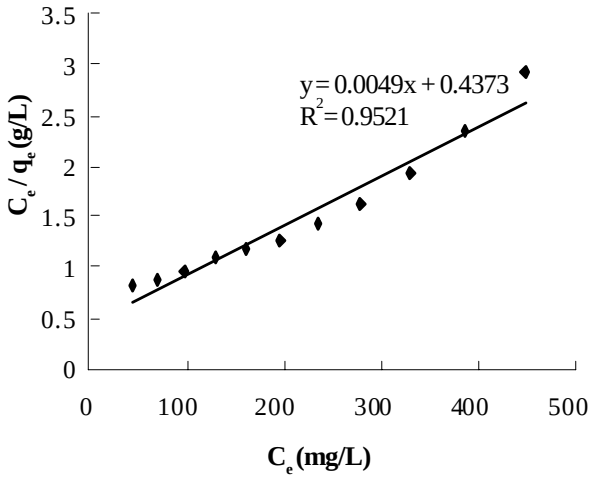


**Fig. 6. Langmuir isotherm**

From the plot (Fig. 6). the value of maximum bio-sorption capacity of the biomass was found to be 72.93 mg/g. As the experimental results gave good fit to Langmuir model, it can be interpreted that, the adsorption takes place to finite number of identical sorption sites and it is based on monolayer coverage on the bio-sorbent.

## CONCLUSION

The dried biomass of *Brevundimonas vesicularis*, gram negative bacteria isolated from lead contaminated soil was tested for its lead adsorbing potential. It has been observed that the powdered dried cells of B. *Vesicularis* could adsorb lead from synthetically prepared lead contaminated water. Lead bio-sorbtion capacity of the organism was optimum at pH 4, initial lead concentration of 550 mg/L and most favorable amount of sorbent was 1 g/L and most advantageous contact time was 60min. However

storage of dried cells of B. *Vesicularis* has no detectable influence in bio-sorption efficiency. Langmuir isotherm fitted well to the experimental data obtained for bio-sorption studies conducted at optimum conditions, which indicated that bio-sorption takes place on finite number of sorption sites.Using this model, the maximum bio-sorption capacity of the biomass was predicted as 72.93 mg/g.The present study indicates that B. *Vesicularis* can be effectively utilized as a potential sorbent for removing lead from wastewater. The finding will have much application in industrial wastewater treatment processes, especially in the design of bio-filters for removing lead from effluents.

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