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Biosorption of Lead by Immobilized Biomass of *Brevundimonas vesicularis*: Batch and Column Studies

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The objective of the present study was to evaluate the feasibility of a strategy to remove lead [Pb(II)] from aqueous waste using *Brevundimonas vesicularis*, a bacterial species isolated from contaminated soil. Batch studies were conducted using free and immobilized biomass and the optimum conditions for removal of lead from solutions were determined. The maximum specific lead uptake of the dry biomass was found to be 12.4 mg g^{-1}. Column study was conducted using immobilized biomass and the maximum specific lead uptake was 74.8 mg g^{-1}. It is concluded that *Brevundimonas vesicularis* is a promising biosorbent to remove lead from contaminated wastewater.

Keywords biosorption; immobilization; isotherm; lead; Thomas model

INTRODUCTION

Incidents of pollution of the environment by toxic metals and radioactive wastes have been on the rise since the beginning of the industrial revolution (1). The Presence of heavy metals in reasonably high concentrations has been reported even in the treated effluents discharged from many industries (electroplating, mining, metal processing, etc.) (2) posing a serious threat to groundwater resources besides other ill effects. Also, uncontrolled release of many of the heavy metals into the environment is reported to have a toxic or an inhibitory effect on living systems (3). In some cases, these heavy metals exhibit toxicity in humans even at extremely low concentrations. Controlling heavy metal discharges and removing toxic metals from aqueous solutions have emerged as a serious challenge in the 21st century (4).

Among the various heavy metals, lead is a highly toxic element, exposure to which can cause adverse health effects (5). It is one of the most commonly found contaminants in wastewaters from industries manufacturing storage batteries, paints and pigments, dyes and leaded glass. It is reported that, unlike organic pollutants, most of which are susceptible to biological degradation, lead does not degrade into harmless end products and will accumulate in living organisms (6). As per the guidelines of the WHO, the maximum permissible level of lead in drinking water is 0.05 mg l^{-1}. Consumption of water having a higher concentration of lead can result in adverse health effects such as anaemia, encephalopathy, hepatitis, and nephritic syndrome (7). Severe exposure to lead has been associated with sterility, abortion, stillbirths, and neo-natal deaths (5). It is therefore essential to develop technologies for treatment of wastewater streams containing lead so that their concentrations can be brought down to acceptable levels before disposal.

Conventional treatment techniques such as chemical precipitation, ion exchange, membrane processes, etc. are not only costly but also not very effective when the metal concentration is less than 100 mg l^{-1}. In recent years, adsorption, particularly biosorption, using low cost and environmentally friendly materials, has emerged as a promising alternative for the removal of lead from wastewaters (5,6,8–12). Removal of toxic metals by biosorption using microorganisms has received a great deal of attention in recent years, not only as a novel treatment process, but also due to its potential for application in industry (5,11).

Metal accumulative bioprocesses generally are divided into two categories—biosorptive (passive) uptake by non-living biomass and bioaccumulation by living cells (13). Maintaining a viable biomass during the metal removal process may sometimes be very difficult. Use of non-living biomass has specific advantages over the use of living microorganisms. Killed cells may be stored or used for extended periods at room temperature. Also, these cells are not subject to metal toxicity and nutrient supply is not necessary for their maintenance (8,14). Therefore, biosorption using non-living biomass is considered to be a more competitive, effective, economical, and attractive method (14).

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The use of dead cells in powdered form as the biosorbent has been found to be successful in batch studies conducted by several researchers (8,10–12,15). However, the small particle size, low density, and rigidity can cause problems. Also, the separation of biomass from the treated effluent may create further issues during industrial application (2). Immobilization of the biomass in a suitable matrix (like, silica gel, alginate, or polyurethane) can overcome such problems (15–18). This technique provides mechanical strength, rigidity, and porosity to the biosorbent.

The adsorption capacity parameter obtained from a batch experiment is useful in providing information about the effectiveness of the sorbate-adsorbent system. But the data obtained under batch conditions are generally not applicable to most of the treatment systems (19), hence there is a need to perform dynamic column studies.

In the present study, biosorption of lead by a bacterial strain isolated from artificially contaminated clayey soil is investigated using batch as well as column studies. Several researchers have reported on the effectiveness of the immobilization of biomass in agar beads for enhancing the heavy metal sorption capacity (17,20). In the present study also, the biomass is immobilized in agar beads to evaluate its effectiveness in column applications. The biomass of a bacterial strain, *Brevundimonas vesicularis*, isolated from soil is used in this study. The use of the above-mentioned bacterial strain for removing lead from aqueous solutions has not yet been investigated by other researchers. Also, the use of agar immobilized cells of this bacterial strain for the removal of lead from wastewater has not been reported in literature. The effect of pH, contact period, initial concentration, and biosorbent dosage are investigated in batch studies. The effectiveness of agar immobilized beads for the removal of lead is evaluated using the adsorption isotherm. Pilot studies are conducted by packing the biobeads in a column to determine the suitability of the biosorbent in industrial applications.

METHODS

Isolation, Characterization, and Identification of the Bacteria

The bacterial strain used in the present study was isolated from artificially contaminated soil samples. The soil samples were collected from Chathamangalam, Calicut, Kerala, South India and they were artificially contaminated with lead nitrate solution (concentration of lead = 1000 mg l^{-1}) for 50 days. From this, 10 g of soil was taken and mixed with 90 ml of bacteriological saline (0.85% w/v NaCl) followed by 10 times dilution by serial dilution. Sterile conditions were maintained throughout this process. This was followed by plating the dilutions in nutrient agar plates (Hi-media). These plates were then incubated at 37°C for 12 hours. The stock cultures were transferred weekly and stored at 4°C in a refrigerator. Bacteria isolated from the contaminated soil were tested for its biochemical and morphological characteristics (Tables 1 and 2). Based on these studies, the bacterial species was identified as *Brevundimonas vesicularis* (B. *Vesicularis*).

Preparation of the Biosorbent

The isolated bacteria were grown in conical flasks containing nutrient broth. The flasks were kept at pH 7 for 18 h at a temperature of 37°C and at 120 rpm. The cells were harvested by centrifugation at 10000 rpm for 10 min.

TABLE 1
Biochemical characterization of B. *Vesicularis*

Biochemical characterization	
Cystine requirement	Negative
Hydrolysis of Esculin	Positive
Assimilation of	
Adonitol	Negative
D Mannose	Negative
D-Glucose	Positive
D-Galactose	Positive
Amygcladin	Negative
Arbutin	Negative
Salicin	Negative
Maltose	Positive
Trehalose	Negative
Propionate	Negative
Iso butyrate	Negative
N-capronate	Negative
Adipate	Negative
L-Rhanose	Intermediate
Pimelate	Negative
Suberate	Negative
Acetate	Positive
Growth parameters	
Aerobic	Positive
Facultative	Negative
Micro aerophilic	Negative
Growth on ordinary blood agar	Positive
Growth on Mac Conkey agar	Intermediate
Growth at 35–42°C	Intermediate
Growth at 6.5% NaCl	Negative
Enzymes present	
Oxidase	Positive
Catalase	Negative
Glucose fermenter	Negative
Glucose oxidizer	Intermediate
DNase	Negative
Lysine Decarboxylase	Negative
Ornithine Decarboxylase	Negative

TABLE 2
Morphological characterization of B. *Vesicularis*

Gram reaction	Negative
Bacillus	Positive
Presence of branching filament	Negative
Spore formation	Negative
Acid fast	Negative
Spirochete	Negative
Curved bacilli	Negative
Motility	Positive

The harvested cells were washed twice with de-ionized distilled water and desiccated in an oven at 80°C for 48 h. To ensure complete death of the bacteria, samples of the dried cells were inoculated on a petri dish containing blood agar. The absence of colony formation confirmed that the bacteria were dead. The dried cells were then ground in a porcelain mortar to a fine powder (0.2 mm) and stored at 5°C until further use. In order to prepare immobilized beads, 2 g of agar-agar was dissolved in 90 ml of distilled water and sterilized by autoclaving (121 °C, 20 min). 10 ml of the cell suspension was added after cooling to 50°C and mixed to obtain immobilized biobeads having $2\,g\,l^{-1}$ cell concentration. For beads, 10 ml of sterile water was added (17).

Preparation of the Metal Solution

Stock solution of lead was prepared by dissolving AR grade lead nitrate salt in de-ionized distilled water (21). This was followed by shaking for 15 min and then keeping it for 24 h for absolute dissolution. The pH was adjusted to be in the range 1–8 by adding 0.1 M NaOH or 0.1 M HCl. The concentration of lead was measured using an Ion meter with a selective electrode for determination of the lead concentration (Thermo – Orion U.S.A).

Batch Mode Studies

In batch adsorption experiments, known quantities of the biosorbent (0.025 g) were added to capped volumetric flasks each containing 50 ml solution of $10\,mg\,l^{-1}$ lead and shaken at 120 rpm for 90 min. The effect of pH on lead removal was determined by varying the pH from 1 to 8. For assessing the effect of contact time on lead removal, these experiments were continued till equilibrium was reached. The influence of initial concentration of lead was investigated by conducting biosorption studies with varying initial lead concentration (5 to $30\,mg\,l^{-1}$). In all these studies control flasks containing only agar beads were placed along with the flasks containing dry cells and immobilized cells (biobeads). All these flasks were maintained in identical conditions. The effect of biosorbent dosage was investigated by conducting the experiments at five different cell concentrations (0.01 g, 0.025 g, 0.05 g, 0.075 g, and 0.1 g). After the prescribed contact time, the solutions were centrifuged at 10000 rpm for 15 min, the supernatant was filtered, and the concentrations of Pb(II) ions were determined. All the experiments were carried out in duplicate and the average values were used for further calculations. The experiments were conducted at a temperature of about 25 ± 2°C. The equilibrium sorption capacity of the biosorbent for lead was calculated (22).

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (1)$$

q_e = Specific lead uptake ($mg\,g^{-1}$)
V = Volume of lead solution (l)
C_0 = Initial concentration of lead in the solution ($mg\,l^{-1}$)
C_f = Final concentration of lead in the solution ($mg\,l^{-1}$)
m = Mass of the dried cells (g)

Column Study

In the column experiment, a glass column (1.2 cm internal diameter and 50 cm long) was packed to 9.7 cm with immobilized biomass having 10 g dry cell concentration. A long column was selected to conduct the studies at various depth of packing such as 20 cm, 30 cm, etc. However, the study result of 9.7 cm packing is shown here. Lead nitrate solution (lead concentration = $100\,mg\,l^{-1}$) was allowed to flow from top to bottom of the column at a rate of $1.5\,ml\,min^{-1}$ using a peristaltic pump. The pH of the solution was maintained at 4. The effluent from the bottom of the column was collected at 10 min intervals and the residual lead concentration was measured after centrifugation and filtration. The column experiment was continued till the effluent concentration reached 99% of the influent concentration.

RESULTS AND DISCUSSION

Effect of pH

It has been reported that the pH of the solution plays a significant role in biosorption (5,23). In order to examine the effect of initial pH of the aqueous solution on lead biosorption, batch experiments were conducted at different pH values (1 to 8). The experiment was not performed at pH values in the alkaline range because of the possibility of precipitation of metal hydroxides (22,24). The results obtained are presented Fig. 1. Maximum biosorption was found to occur in the pH range of 3 to 5 for immobilized biomass and dry cells. After 1 h contact at pH 4, the immobilized biomass of B. *Vesicularis* showed about 79.4% lead removal. Lead removal by adsorption increased with increase in pH of the medium upto a pH of 4. With further increase in pH (beyond 4), removal by biosorption declined sharply. Therefore the optimum pH is 4 and this value was taken for further studies. Senthilkumaar et al. (2000) (25) have reported that biosorption of a metal is dependent

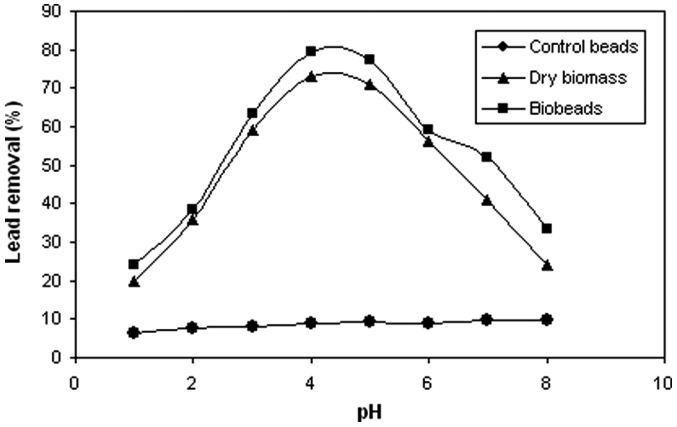


FIG. 1. Effect of pH on biosorption of lead.

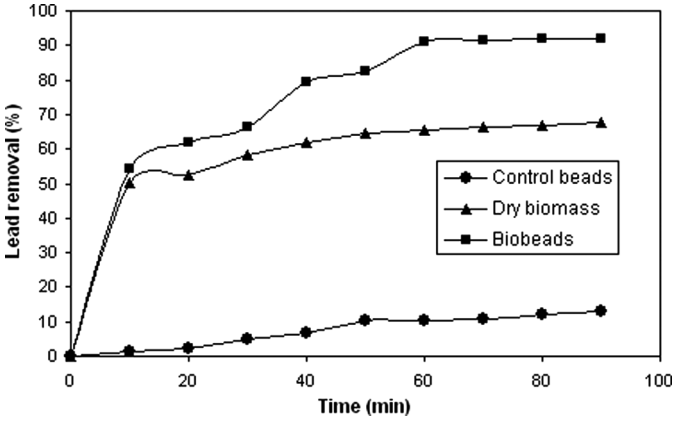


FIG. 2. Effect of contact time on biosorption of lead.

on the initial pH of the aqueous solution. A similar trend was observed by Leung et al. (2000) (26). At high values in the acidic range, a cluster of negative charge is formed on the surface of the biosorbing materials. Apart from this, a lower pH will affect the physiochemical process and hydrolysis of the metal (26). At a very high acidic pH (<3), lead ions compete with hydrogen ions for binding sites on the microbial cells and as a result removal by biosorption is reduced. However, at higher pH (>5), the solubility of lead ions is lowered and lead hydroxide gets precipitated (24). Deprotonation of carboxyl groups at pH 3–5 results in the increased binding of lead in this pH range (27). Influence of pH on the uptake of lead was negligible in the case of control beads prepared by agar alone. Percentage removal of lead by immobilized biomass (biobeads) was observed to be higher than that by dry powdered bacterial cells. Gourdon et al. (1990) (28) reported that the immobilization of microbial cells from activated sludge in sodium alginate matrix reduces the effect of pH on cadmium biosorption compared to free cells. A similar behavior was not observed in the present study.

Effect of Contact Time

Figure 2 illustrates the effect of contact time on percentage of lead removal, with three sorbents, agar beads (control beads), dry cells, and immobilized biomass. The initial lead concentration in the aqueous solution was 10 mg l^{-1} and pH was maintained in the range 3–5. It was observed that removal of lead was rapid during the initial 10 min in the case of dry cells and biobeads. The percentage removal is 50.1% with dry cells (contact time = 10 min). The corresponding percentage removal in the case of biobeads is 54.2%. However, rapid removal was not observed in the case of control beads. Removal achieved using biobeads was observed to be much greater than that by dry biomass and that observed for agar beads was nominal. After 60 min, equilibrium was reached; percentage lead removal by biobeads and dry biomass was observed to be 91.2% and 65.3% respectively. Rapid removal in the initial stages of biosorption experiments has already been reported (8,10). Rapid initial uptake of metals is important with reference to the application of biosorption in industrial wastewater treatment (24).

Effect of Initial Lead Concentration

Aqueous solutions of lead nitrate with initial lead concentrations 5, 10, 15, 20, and 30 mg l^{-1} were used to investigate the effect of initial lead concentration on the removal of lead. The immobilized biomass concentration was maintained at 1 g l^{-1}. Also, the pH of the solution was maintained at 4. Adsorption yield values were calculated as,

$$\text{Adsorption yield } (\%) = \left[\frac{C_0 - C_e}{C_0}\right] \times 100 \qquad (2)$$

where, C_e is the concentration of lead in the solution at equilibrium (mg l^{-1}) and C_0 is the initial lead concentration (mg l^{-1}). Results of this study are presented in Fig. 3. It

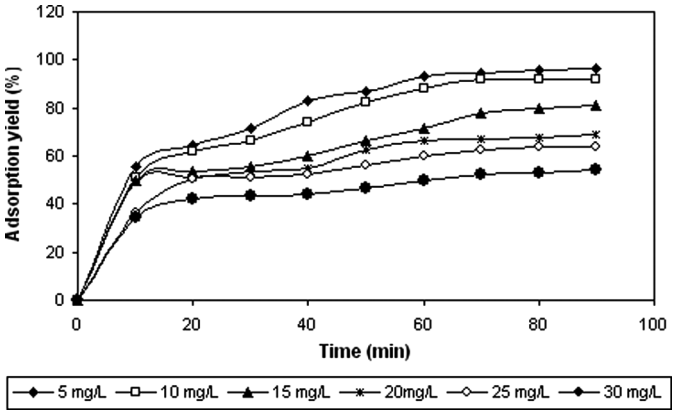


FIG. 3. Effect of initial lead concentration on biosorption of lead.

can be seen that lead removal from the solution is considerably influenced by the initial concentration of the solution. Adsorption yield by biobeads is maximum (96.4%) at an initial lead concentration of $5\,mg\,l^{-1}$. The specific lead uptake corresponding to this is equal to $0.025\,mg\,g^{-1}$. The adsorption yield was 54.4% at an initial lead concentration of $30\,mg\,l^{-1}$ and the corresponding specific lead uptake was $16.32\,mg\,g^{-1}$. From these observations it can be inferred that the adsorption capacity of the biosorbent is considerably affected by the initial lead concentration in the aqueous solution of lead nitrate.

Effect of Biosorbent Dosage

Studies on the effect of biosorbent dosage on specific lead uptake and percentage removal of lead from the aqueous solution of lead nitrate was studied at pH 4 and at an initial lead concentration of $10\,mg\,l^{-1}$. Experiments were performed at five different dosages of the immobilized biomass - 0.2, 0.5, 1.0, 1.5, and $2\,g\,l^{-1}$. It was observed that the specific lead varied from 15.5 to $4.8\,mg\,g^{-1}$ as the biosorbent dosage was varied from 0.2 to $2\,g\,l^{-1}$ (Fig. 4). The corresponding values of percentage lead removal ranged from 31 to 97.2%. At a fixed initial lead concentration, increasing the biosorbent dose provides greater surface area and larger number of sorption sites (29), thereby resulting in enhanced metal uptake. As the mass of the sorbent increases, the percentage removal of lead from the solution considerably increases, although the value of the specific uptake reduces.

Biosorption Isotherm

Application of the biosorption technique on a commercial scale requires quantification of the sorption equilibrium for process optimization. The sorption equilibrium data for lead on the biosorbent (B. *Vesicularis*) was analysed using various available isotherm models. The Langmuir model fitted reasonably well to the experimental data. The Langmuir model can be expressed as

$$\frac{C_e}{q_e} = \frac{1}{bq_m} + \left(\frac{1}{q_m}\right)C_e \qquad (3)$$

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

The linear Langmuir plot (Fig. 5) was obtained by plotting $\frac{C_e}{q_e}$ vs C_e respectively, from which the adsorption coefficients are evaluated. The constant q_m and b are calculated from the y-intercept and slope of the linear plot. q_m signifies the adsorption capacity and 'b' is related to the energy of adsorption. The values of q_m and b are found to be $12.4\,mg\,g^{-1}$ and $4.071\,mg^{-1}$ respectively. The maximum biosorption capacity obtained is found to be superior to that observed for *Saccharomyces cerevisiae* (2.7 mg/g) (30) and for *Pinus sylvestris* (11.38 mg/g) (31) obtained at similar laboratory conditions. As the Langmuir model fitted the experimental results, it can be concluded that the sorbent surface is homogenous. The model indicates that each binding site accepts only one lead [Pb(II)] ion; the sorbed molecules are organized as a monolayer since the biomass is not fully saturated; all sites are energetically equivalent and there is no interaction between the sorbed molecules (9).

Column Study

In order to describe the fixed bed column behavior and to scale it up for industrial application, suitable models have to be used to fit the experimental data. Successful design of a column process requires prediction of the concentration-time profile or breakthrough curve for the effluent (16). The maximum adsorption capacity of an adsorbent is also needed in design. The Thomas model is one of the most general and widely used methods to fulfill this purpose

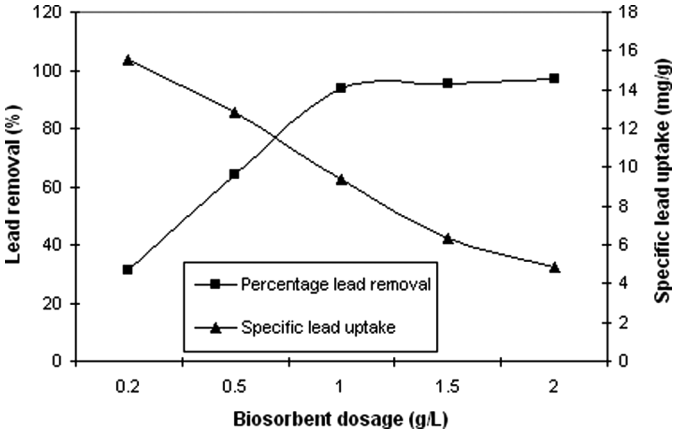


FIG. 4. Effect of biosorbent dosage on biosorption of lead.

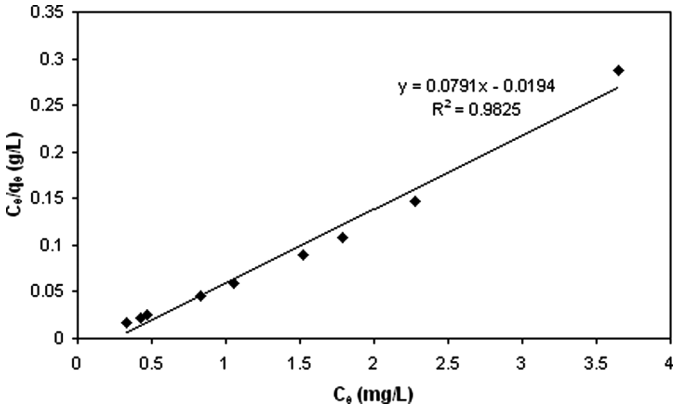


FIG. 5. Langmuir plot for the removal of lead.

and it is expressed as (19),

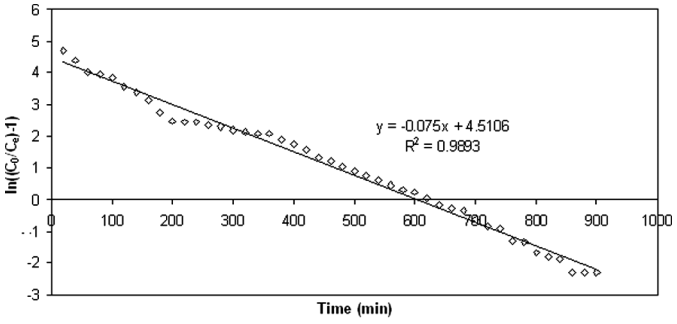
$$\frac{C_e}{C_0} = \frac{1}{1 + \exp(K_T(q_0 m - C_0 V)/\theta)} \quad (4)$$

where, C_e is the effluent solute concentration ($mg\,l^{-1}$), C_0 is the influent solute concentration ($mg\,l^{-1}$), K_T is the Thomas rate constant ($l\,min^{-1}\,mg^{-1}$), q_0 is the maximum adsorption capacity of the sorbent ($mg\,g^{-1}$), m is the mass of the adsorbent (g), V is the throughput volume (ml), and θ is the volumetric flow rate ($l\,min^{-1}$).

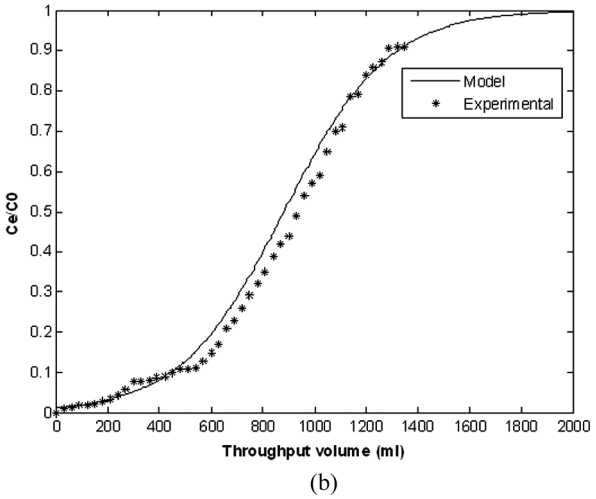
Application of the Thomas Model

The data from the column study were fitted to the Thomas model to determine the Thomas rate constant and maximum solid phase concentration. To determine these parameters, the linearized form of the Thomas model was used, which can be expressed as,

$$\ln\left(\frac{C_0}{C_e} - 1\right) = \frac{K_T q_0 m}{\theta} - \frac{K_T C_0}{\theta} V \quad (5)$$



(a)



(b)

FIG. 6. (a). Plot of Time vs. $\ln[(C_0/C_e) - 1]$ 6(b) Comparison of the experimental and predicted breakthrough curves according to Thomas model.

The Thomas rate constant, K_T and the adsorption capacity of the bed, q_0 can be determined from a plot of $\ln[(C_0/C_e) - 1]$ against time at a given flow rate (Fig. 6(a)). In the case of sorption using B. *Vesicularis*, the Thomas rate constant (K_T) and adsorption capacity (q_0) are obtained as $0.752 \times 10^{-4}\,l\,min^{-1}\,mg^{-1}$ and $74.8\,mg\,g^{-1}$ respectively. The experimental result with the ratio of effluent concentration to influent concentration was plotted against the throughput volume of influent passed through the column. The curve was plotted after passing 1390 ml of lead solution. The adsorption capacity of the biosorbent is obtained as the product of the area above the curve and influent concentration of the lead solution (32). Chang et al. (1997) (33) reported that, the maximum sorption capacity of *Pseudomonas aeruginosa* (a closely related strain of B. *Vesicularis*) is 79.5 mg/g and the result obtained in the present study shows a similar kind of performance for B *Vesicularis* also. The theoretical predictions based on the model parameters were compared with the observed experimental results (Fig. 6(b)). The input values used for the validation of model are $C_0 = 100\,mg\,l^{-1}$, $K_T = 0.752 \times 10^{-4}\,l\,min^{-1}\,mg^{-1}$, $q_0 = 74.8\,mg\,g^{-1}$, $m = 10\,g$, $V = 2000\,ml$, and $\theta = 1.5\,ml\ min^{-1}$. From the plot it was seen that the Thomas model fitted well to the experimental data.

CONCLUSIONS

This study has clearly brought out the effectiveness of dry cells and agar immobilized biomass of *Brevundimonas vesicularis* in the removal of lead from aqueous solutions by sorption. The bacterial strain was isolated from local soil and cultured in the laboratory. The biosorption process is pH dependent, the maximum sorption occuring at pH 4. Also, the sorbent and sorbate concentrations play a significant role in the biosorption process. The process is quite rapid in the initial 10 min and then the process slowly proceeds to an equilibrium state in about 60 min. The biosorption data from the column study fitted the Langmuir isotherm very well, indicating the homogenous nature of the sorption process. Immobilization of biomass in agar beads is found to be quite effective in enhancing the removal process in adsorption columns. *Brevundimonas vesicularis* is an ecofriendly, low-cost alternative biosorbent for the removal of lead from industrial wastewaters. Further research is needed to study the regeneration of the sorbent and recovery of the sorbed metal.

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