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Bacillus subtilis on Pb^{2+} ions removal from aqueous solution by Biosorption

P Sethuraman, M Dharmendira Kumar*

Department of Chemical Engineering, A C Tech Campus, Anna University, Chennai-600 025, India

ABSTRACT

The biosorption of Pb^{2+} ions from aqueous solutions using live & dead *B.subtilis* were studied. Batch experiments were conducted to determine the factors affecting adsorption and kinetics of the biomass and the maximum adsorption capacity of Pb^{2+} ion was observed. The equilibrium data satisfied the Langmuir and Freundlich isotherms. Kinetic studies revealed that Pb^{2+} adsorption about 90% or more when first 04-12 hrs of contact time. The kinetics data follows the pseudo second order model over pseudo first order with a correlation coefficient of 0.9813. Fourier transform spectrometer (FTIR) analysis confirms the chemical modification process and indicates that COOH groups on the ground wheat stems are one of the main active groups in Pb^{2+} ions removal process. The changes in texture on the surface of biosorbent were screened by using Scanning electron microscopy (SEM).

Keywords: Biosorption; Pb^{2+} removal; Kinetics; Isotherms; *Bacillus subtilis*-dead and live; waste water treatment

Corresponding author

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INTRODUCTION

Industrialization has led to increased amounts of heavy metals being dumped into the environment [5], and it has contributed to the release of toxic heavy metals in to water streams. Mining, electroplating, metal processing, textile and battery manufacturing industries are the main sources of heavy metal ion contamination. Metals such as Lead, cadmium, copper, arsenic, nickel, chromium, zinc and mercury have been recognized as hazardous heavy metals. The pollution of the environment with toxic heavy metals is reaching hazardous levels and spreading through the world along with industrial progress [2-4]. These stable heavy metals are persistent environmental contaminants since they cannot be degraded or destroyed, and therefore tend to accumulate in the soil, seawater, freshwater, ground water industrial and even treated wastewaters has emerged as a potential alternative method to conventional techniques [5]. Heavy metals are known to have adverse effects on the environment and human health. Among them Pb^{2+} is extremely toxic and can damage the nervous system, brain, kidneys, gastrointestinal track, liver, reproductive system causing infertility and abnormalities in pregnant women and sickness even death [6]. It is therefore, essential to remove Pb^{2+} from wastewater before disposal.

Pb^{2+} is the most toxic heavy metal ion affecting the environment [7]. It comes into water through the combustion of fossil fuels and the smelting of sulphide ore, and into lakes and streams by acid mine drainage. Storage battery manufacturing, metal plating and finishing, insecticides, plastics pipes, food, beverages, ointments and medical concoctions for flavoring and sweetening are prime source of Pb^{2+} pollution. The Pb^{2+} ion concentration standard in drinking water as formulated by the EPA & WHO is 0.05 mg/l & 10 µg/l. Many physico-chemical methods such as chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment membrane technologies, adsorption on activated carbon and evaporative recovery have been developed for heavy metal ions removal from aqueous solutions [8]. However, all these methods involve high operating costs and may produce large volumes of solid wastes [9, 10]. These techniques have significant disadvantages including incomplete metal removal, need for expensive equipment and monitoring systems, also high energy & reagent requirement for generation of toxic sludge & other waste products that have to be disposed [11]. They also are ineffective when metal ion concentration in aqueous solution is as low as ppm levels.

To overcome the above disadvantage is used. Biosorption uses various natural materials of biological origin, including bacteria, fungi, yeasts, algae, molds and composting materials [12]. In the past decades biosorption was extensively used for the treatment of waste water having high volume and low concentration complexes [13]. Indeed, some types of potential biomaterials, which are very effective in accumulating heavy metals, with different metal-binding capacity have been investigated [14, 15]. The presence of polysaccharides, proteins or lipid on the surface of their cell walls containing some functional groups such as amino, hydroxyl, carboxyl and sulphate, act as binding sites for metals [16, 17]. A complete understanding of the mechanism of interaction between the metal ions and the sorbent

material, as well as the parameters that affect metal biosorption is necessary to effectively apply these materials in the removal of Pb^{2+}.

Bacteria (*Bacillus subtilis*), fungi (*Rhizopus arrhizus*), Marine algae (*Sargassum natans*), yeast (*Saccharomyces cerevisiae*) are unwanted biomass resulting from the fermentation process and from some food industries which are potential heavy metal biosorbents [11 and 18]. Bacteria has commercial application as biosorbent in major fields. At first *B.subtilis* is easy to cultivate on large scale using unsophisticated fermentation techniques and inexpensive growth medium secondly the biomass of *B.subtilis* can be obtained from various food, beverage and pharmaceutical industries. Thirdly *B.subtilis* is a commercial commodity and considered safe. Therefore, biosorbent made from *B.subtilis* may be easily accepted by the public when applied in practice as it can be used on a large scale at very low cost, especially for treating large amounts of wastewater containing heavy metal in low concentration. Other than being a biosorbent *B.subtilis* can also be used as an ideal model organism to identify the kinetics of the biosorption in metal ion removal & to investigate the interactions of metal microbe at molecular level [18].

The objective of the study was to investigate biosorption studies for the removal of Pb^{2+} metal ion from aqueous solution by live and dead *B.subtilis* in batch system. The effects of metal concentration, pH, varying dosage and contact time were examined. The equilibrium biosorption data obtained from batch process was applied to Freundlich and Langmuir isotherm models in addition to the metal-biosobent interactions evaluated by AAS, FTIR and SEM analysis.

MATERIALS AND METHODS

The bacterial culture of *B.subtilis* (MTCC-121) species was obtained from Microbial Type Culture Collection (MTCC). Nutrient broth culture media was prepared as per the guideline of MTCC. All chemicals used in this study were of analytical grade obtained from Ranbaxy Fine Chemicals Ltd., India. Stock solution of Pb^{2+} was prepared using Pb^{2+} nitrate ($Pb(NO_3)_2$) in purified double distilled water. Pb^{2+} solution of different concentrations were obtained by diluting the standard stock solution of Pb^{2+} (1000 mg/l). The analysis of Pb^{2+} solutions were done by using an atomic absorption spectrophotometer Perkin Elmer AAnalyst 800, (USA) at a wavelength of 283.3 nm. JEOL JSM-6360, (Japan) were used for scanning electron microscopy. Infra red spectrophotometers, Tensor 27, Bruker Optic GmbH (Germany) samples were recorded on the region of 4000-600 cm^{-1}.

Batch adsorption studies

The equilibrium kinetics data of the biosorbent *B.subtilis* were obtained by performing batch experiments. The experiments were carried out in 250 ml flasks to which 100 ml of Pb^{2+} solution and l ml of biomass (exponential phase) were added. The mixture was stirred at 150 rpm at room temperature & 1 ml of sample was collected and centrifuged at 6000 rpm for 10 min in a centrifuge. The remaining concentration of Pb^{2+} in residual solution was analyzed by

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the atomic absorbance spectrophotometer. The pH of the solution was approximately 6.0 and the temperature was approximately 35-38 ^{o}C. The final reading for each solution was taken after about 24 hrs. Each experiment was carried out thrice & the mean values were reported.

RESULTS AND DISCUSSION

Effect of Growth curve

The growth rate of *B.subtilis* in Nutrient medium was studied in aseptic condition. It can be noticed from Fig. 1 the growth rate of bacteria has four distinct phases, such i) lag phase, ii) log phase, iii) stationary phase and iv) death phase. In lag phase, bacteria adapt to the environment to grow, in this phase, the bacteria are not yet able to divide. The bacterial growth cycle, synthesis of RNA, enzymes and protein molecules may occurs. Exponential phase or log phase is characterized by doubling of cell. During stationary phase, the growth rate slows down as a result of nutrient depletion and accumulation of toxic products. During death phase, lack of nutrients, unavailability of space and Oxygen lead to the decay of bacteria. The optical density recorded at 600nm taken for *B.subtilis* shown good exponential phase within 4 h. Maximum biomass growth in 20 h in *B.subtilis*, while in 8 h to 16 h good exponential growth occurred in *P.aeruginosa* and in *E.cloacae* within 8 h to 16 h. The biomass for bioaccumulation process for Cr(VI) removal were taken at the highest exponential phases for all the bacteria.

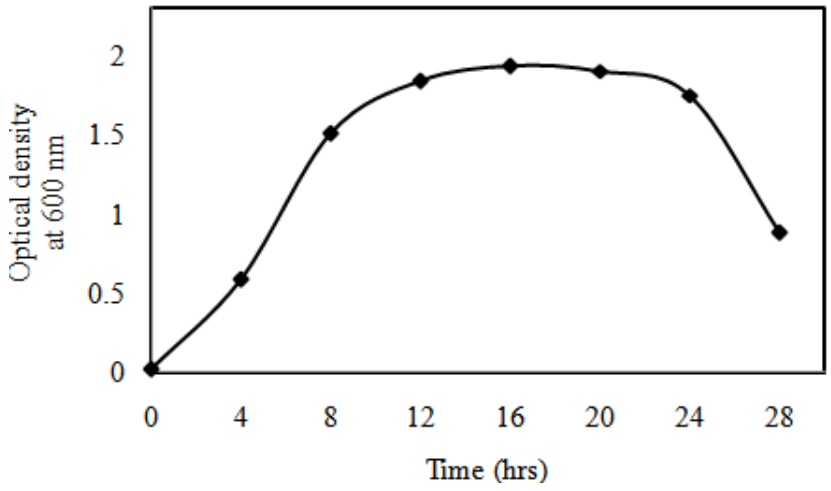

Fig. 1. Growth curve of live *Bacillus subitilis* in closed system optical density at 600 nm

Adsorption isotherms

The process of biosorption metal uptake can be quantitatively evaluated by experimental equilibrium isotherms. The graphical expression of isotherm is a plot of the metal uptake by the per unit weight of biosorbent against the residual metal ion concentration in the biosorption medium. There are two widely accepted and easily linearized adsorption isotherm models used in the literature, which are namely Langmuir and Freundlich models.

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Langmuir isotherm

The general Langmuir isotherm equation can be represented by the following expression

$$q_e = \frac{Q_0 b C_e}{1 + b C_e} \quad (1)$$

The equation of linearized as follows,

$$\frac{C_e}{q_e} = \frac{1}{Q_0 b} + \frac{C_e}{Q_0} \quad (2)$$

Position of q_e is the amount of metal ion removed (mg/l), C_e the equilibrium concentration (mg/l), Q_0 and b are the Langmuir constants related to adsorption capacity and affinity, [19] respectively. The Langmuir biosorption isotherms of Pb^{2+} are illustrated in Fig. 2 and the isotherm model parameters are tabulated in Table 2. The regression coefficients obtained for Pb^{2+} from the live and dead Langmuir models are 0.995 and 0.987 respectively.

Table 2. Langmuir and Freundlich isotherms constants for the biosorption of Pb^{2+} on live and dead of *B.subtilis.*

Lead	Langmuir constant			Freundlich constant		
	b	Q_0	R^2	n	K_F	R^2
Live	0.0009	3.133	0.9952	1.302	5.232	0.9882
Dead	0.0012	2.846	0.9868	1.245	4.185	0.9889

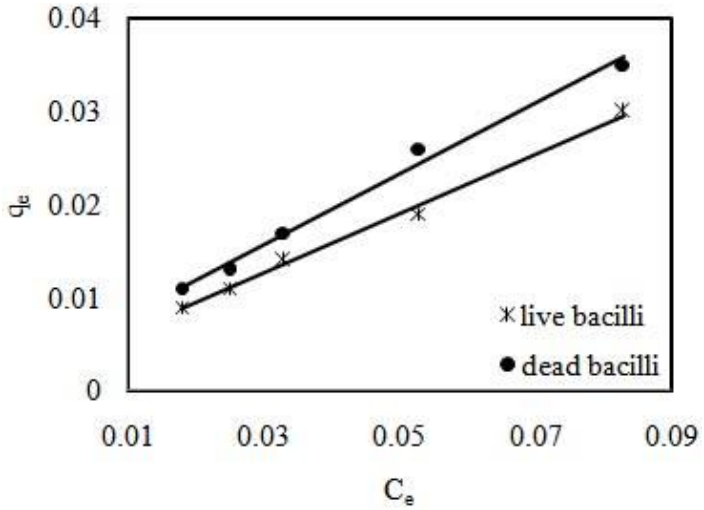

Fig. 2. Langmuir isotherms of Pb^{2+} ion on live and dead biomass of *B.subtilis*.

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Freundlich isotherm

The Freundlich model is based on the Pb^{2+}uptake capacity of q_e (mg/l) of biomass and the residual (equilibrium) metal ion concentration C_e (mg/l). The general Freundlich equation is as following expression

$$q_e = k_F C_e^{1/n} \quad (3)$$

the linearized form of this model is given below

$$\ln q_e = 1\ln k_F + \frac{1}{n}\ln C_e \quad (4)$$

where intercept, ln k_F, to measure the biosorbent capacity, and the slope, l/n, is the intensity of biosorption [20]. The Freundlich biosorption isotherm for Pb^{2+} are illustrated in Fig. 3 respectively, the calculated values of K_F and n which are given in Table 2.

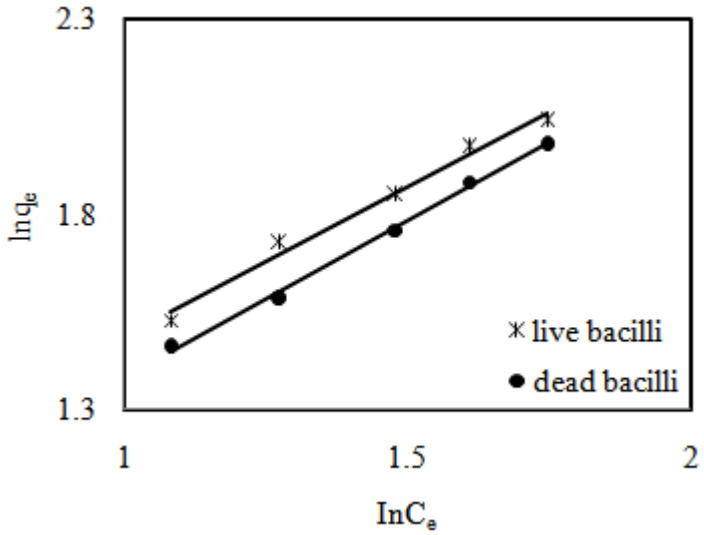

Fig. 3. Freundlich isotherm of Pb^{2+} ion on live and dead biomass of *B.subtilis*.

Biosorption kinetics models

Pseudo first order model

Pseudo first order kinetic model were used to evaluate the kinetics of the Pb^{2+} biosorption on the *B.subtilis* and it is expressed by the following equation [21].

$$\log(q_e - q_t) = \log(q_e) - \frac{K_1 t}{2.303} \quad (5)$$

where, q_t is the amount of adsorbed Pb^{2+} on the adsorbent at equilibrium, t time, and k_1 is the rate constant of first order adsorption, respectively. In pseudo first order kinetic model parameters is given in Table 3 respectively. The pseudo first order model failed to estimate q_e because the correlation coefficient in live (0.273) and dead (0.391) biosorbent on Pb^{2+}.

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Table 3. Kinetics data for the uptake by live and dead of *B.subtilis.*

Lead	Pseudo first order			Pseudo second order		
	q_e	K_1	R^2	q_e	K_2	R^2
Live	0.193	0.0126	0.273	31.055	0.040	0.9691
Dead	0.185	0.0274	0.391	29.155	0.067	0.9813

Pseudo second order model

The kinetic data was analyzed through the pseudo second order relation. The pseudo second order kinetic model is expressed as [22].

$$\frac{t}{q} = \frac{1}{k_2 q_e^{\ 2}} + \frac{t}{q_e} \qquad (6)$$

where, k_2 is the rate constant of second order adsorption. q_e equilibrium and k_2 can be determined from the slope and intercept of the plot, respectively.

In second order kinetics model, the values of correlation coefficient were extremely good, even more than 0.981.besides. The experimental q_e was comparatively close to Pb^{2+} biosorbent. The parameters values are given in Table 3 and Fig 4 respectively. In accordance with the pseudo second order reaction mechanism, the biosorption of Pb^{2+} onto *B.subtilis* maximum controlled by the chemical processes [23].

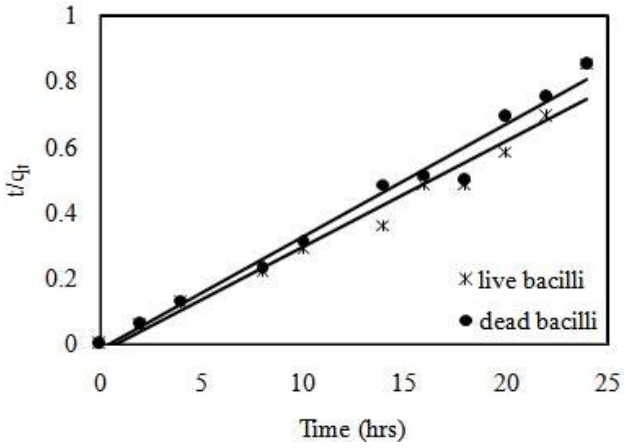

Fig. 4. Pseudo second order kinetic modeling of Pb^{2+} adsorption on biomass of *B.subtilis*.

Characterization of the biosorbent

The FTIR spectra of native and Pb^{2+} treated algal of biomass are presented in Fig. 5. The figure indicates that, presence of amino, carboxylic, hydroxyl and carbonyl groups. Display of strong broad O-H stretch carboxylic bands in the region 3408 cm^{-1} and carboxylic/phenolic stretching bands in the region of 2925 cm^{-1} was observed. Display of strong broad and intense peak at 3400-3700 cm^{-1} was assigned to the stretching of hydrogen-bonded hydroxyl group.

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This also coupled with stretching vibration of the NH_2 moiety is presented in Table 1. The very strong absorption at 1050 cm^{-1} in *B.subtilis*, is due to the stretching of C-O in polysaccharides [24]. The peaks appearing in the region 1652 cm^{-1} might be attributed to C= N, C= C and C= O stretch whereas the peaks appearing in the region 1538 and 1442 cm^{-1} might represent quinine O-H bonds.

Table 1. Assignments of infrared absorption bands

Wavenumber (cm-1)	Intensity shape	Assignment
3600-3750	Sharp	O-H stretching
3400-3550	Sharp	O-H stretching
3100-3500	Strong-broad	N-H stretching
2500-3400	Weak-broad	O-H stretching
2700-2950	Variable	C-H stretching
1400-1660	Variable	N-H bending
1280-1430	Variable	C-H bending
1160-1420	Variable	O-H bending
900-1350	Variable	C-N stretching
900-1380	Variable	C-O stretching
800-880	Medium-strong	N-H and C-H rocking

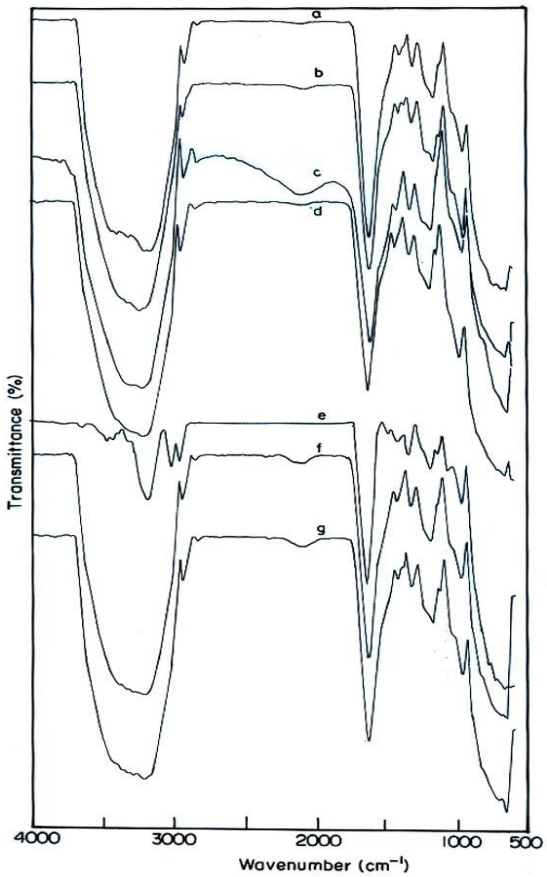

Fig. 5. Infra spectra of (a) live *B.subtilis* were treated with Pb^{2+} ions for different lengths of time namely (b) 0.0, (c) 0.2, (d) 0.12, (e) 0.24 hrs, (f) dead *B.subtilis* and (g) dead Pb^{2+} ion.

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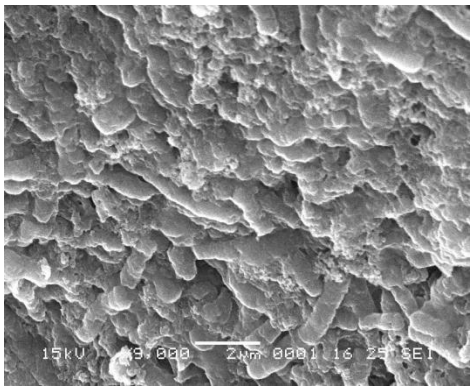
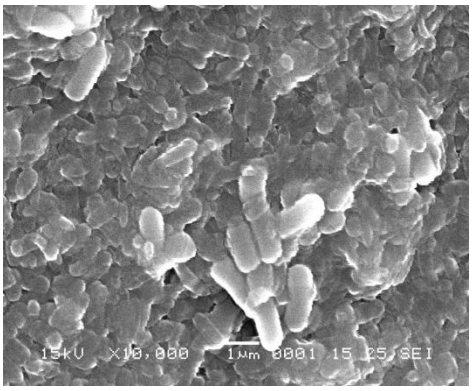
Live *B.subtilis* were treated with Pb^{2+} ions for different lengths of time namely 0.0, 2.0, 12, 24 hrs, live and dead bacilli Pb^{2+}. The IR spectra of this differently treated sample are shown in Fig. 5. The IR spectrum of all peaks shows a peak at 1276 and 1474 cm^{-1} where strong vibration has occurred. From the peaks of 2920-3000 cm^{-1} sharp stretching vibration has indicates participation of O-H and NH_2. The O-H stretching vibrations occur within a broad range of frequencies indicating the presence of free hydroxyl groups and bonded O-H bands of carboxylic acids [25]. The peaks appearing in the region 1353, 1078 and 1028 cm^{-1} represents N–H bending, –CH3 wagging and C–OH stretching vibrations, respectively, are due to the several functional groups present on the algal cell walls. As seen in Fig. 5, the absorbance of the peaks in the Pb^{2+} treated algal biomass is slightly lower than that in the native one. The analysis of the FTIR spectra showed the presence of ionisable functional groups (carboxyl, amino, amide and hydroxyl) able to interact with protons or metal ions. The above results obtained give an idea about the presence of functional groups on the bacterial cell surfaces. Membrane-bound transport enzymes in live *B.subtilis* help in intracellular interaction of metal ions with amino acid. As in dead *B.subtilis*, metal ions interact with hydroxyl and phosphate moiety in the form of live *B.subtilis*.

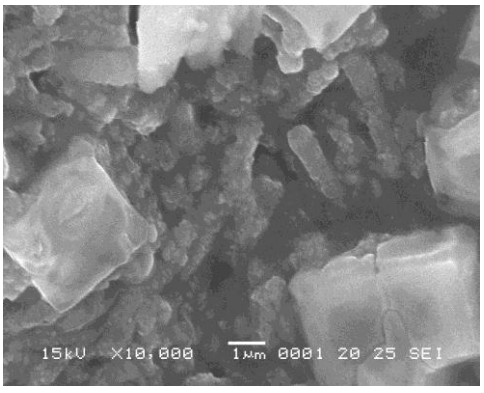
Scanning Electron Microscopy



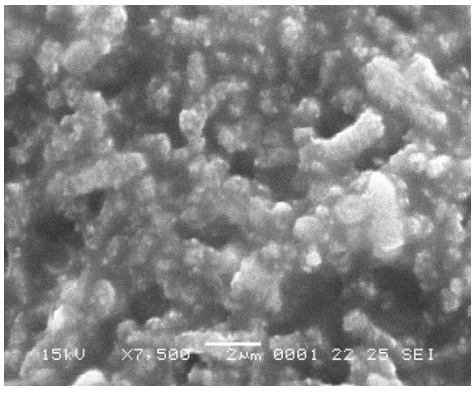
Live *B.subtilis*



Dead *B.subtilis*



Live Pb^{2+} in *B.subtilis*



Dead Pb^{2+} in *B.subtilis*

Fig. 6. Scanning electron micrograph of live and dead *Bacillis subtilis* and Pb^{2+} ions.

The Scanning electron micrograph is clearly presented in Fig. 6, the length and width of the filament of live *B.subtilis* varies from 1.0 to 2.5 µm and 0.3 to 0.8 µm and as expected the cell shrinks when it is dried. In dry species the length and width of the bacilli lies in the range of 0.6 to 1.8 µm and 0.1 to 0.4 µm. The cell surface of the bacteria becomes rough after metal uptakes. IR spectroscopy and kinetic studies show that live *B.subtilis* takes up more metal ions than the dead one. From the close observation of SEM micrographs shows the width of the cell of treated live biomass is more compared to the dried species. When treated with metal of Pb^{2+} ions there is the possibility of transport of Pb^{2+} ions through the cell membrane in live biomass that may be responsible for more uptakes in this *B.subtilis*.

CONCLUSION

The presented study elucidates significant information regarding biosorption of Pb^{2+} ions from was investigated by *B.subtilis* in terms of live and dead biomass. This study indicates that, *B.subtilis* is an effective biosorbent for Pb^{2+} removal.

The Langmuir and Freundlich adsorption model were used for the mathematical description of the biosorption of Pb^{2+} ions onto bacterial biomass and it was found that the adsorption equilibrium data fitted well to the Langmuir model.

The biosorption of Pb^{2+} ions on the bacterial biomass follows pseudo second order biosorption kinetics. The interactions between the metal ions and the functional groups on the cell surface of the biomass were confirmed by FTIR. The functional groups involved in metal ions biosorption included carboxyl, hydroxyl and amino groups.

SEM microscopic studies support the sorption data. Sorption for Pb^{2+} live and dead *B.subtilis* took up significant amount of Pb^{2+} ions in this live *B.subtilis* were found to be simple, fast and efficient. With the advantage of high metal biosorption capacity, the biomass of *B.subtilis* has the potential to be used as an efficient and economic biosorbent material for the removal of Pb^{2+}.

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