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Effect of pretreatment of *Bacillus subtilis* biomass on biosorption and its real time application

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The research study investigated the biosorption behavior of Pb(II) ions by treated and untreated biomass of *B. subtilis*. At initial biosorption conditions, the biosorption efficiency was found to be 36.75%. At the optimized experimental conditions, control biomass showed maximum biosorption efficiency of 58.04% where the biomass was treated with different chemicals. The biomass treated with formaldehyde showed the highest efficiency of 80.9% which was further optimized and attained maximum efficiency of 89.8% for Pb(II) ions. SEM (Scanning Electron Microscope) and EDX (Energy dispersive X- ray) analysis evaluates the structural and elemental changes that occurred as a result of biosorption. Functional groups that are involved in biosorption were revealed by FTIR (Fourier Transform Infrared spectroscopy). Kinetic data showed the best fit with the pseudo second-order model. Effective removal of lead ions from industrial contaminated water sources by pretreatment biomass of *B. subtilis* elucidates its potential use as biosorbent for metal remediation..

Keywords: Effluents, Formaldehyde, Heavy metals, Kinetics, Lead, Microscopic, Pollution.

INTRODUCTION

One of the global concerns in the environmental field is pollution occurred by heavy metals with the progress in industrial activities that discharges heavy metals into the water streams and poses harmful effects to humans and other forms of life[1]. These metals accumulate and become toxic when present in less concentration. Apart from other metal ions, lead was considered as hazardous and neurotoxic heavy metal[2]. Larger amounts of lead accumulation in various body parts due to industrial discharge results in several health problems like nervous disorders, gastrointestinal damage, sickness and sometimes even death[3]. Hence it is necessary to remove Pb(II) ions by bringing it to the permissible range of 0.01 mg/L as guided by WHO[4]. Although many conventional methods exist in removing these heavy metals they are associated with many disadvantages and can be less cost-effective in practical use[5].

Consequently, techniques that are cost-effective and efficient in removing metal ions have to be developed. In this regard, biosorption is possible another biological technique for heavy metal removal with added advantages like high metal-binding ability, environmentally friendly, and biosorbent regeneration with the possible recovery of metal[6, 7]. Recently, microorganisms were used as biosorbents for metal removal[8–10]. The occurrence of numerous active sites on microorganism surfaces makes there use as biosorbents in the remediation of metals from water resources in various studies [11, 12].

In recent years pretreatment of biomass by different methods was developed to increase biosorbent efficiency. Since biosorption implies the availability of metal-binding sites, pretreatment will modify the biosorbent surface features by increasing the available sites which as a result influences the increase in biosorption efficiency[13].

Hence, the study focuses on the utilization of *B. subtilis* as biosorbent for Pb(II) ion biosorption under optimized conditions. **The impact of pretreatment with various chemicals and optimization of biosorption conditions to increase the biosorption efficiency for pretreated *B. subtilis* was studied for the first time in literature**. Batch biosorption studies to determine the maximum biosorption efficiency of both control and treated biomass of *B. subtilis* for Pb(II) ions was evaluated. SEM-EDX, FTIR, and kinetic studies were done to evaluate the biosorption changes.

MATERIALS AND PREPARATION METHODOLOGY

Microbial strain and growth

Bacillus subtilis (1427) having lead reducing activity was obtained from Microbial Type Culture Collection (MTCC), Chandigarh. On the fresh slants of growth medium (beef extract 1.0 g, yeast extract 2.0 g, peptone 5.0 g, agar 15.0 g, and sodium chloride 5.0 g in 1.0 L of distilled water) bacteria was streaked and incubated at 37°C for 24–48 h. The bacterium slants were subculture at regular intervals every 30 days by maintaining at 4°C.

Inoculum preparation

The inoculum was prepared from the freeze slant by incubating at 25°C for 7 days. Then the slant was washed after sporulation into the culture medium by using autoclaved deionized water and resulted in suspension was filtered by using sterile cheese cloth (several layers). The spore density of the obtained inoculum suspension was adjusted to 2×10^8 numbers per mL and used for propagating the bacterium for further biosorption experiments.

Preparation of metal stock solutions

Weigh accurate quantity of lead nitrate (159.8456 mg) and dissolve in 0.1 L of double distilled water to attain standard stock solution of lead with a final concentration of 1000 μg/mL by diluting the stock, working concentrations were prepared.

Cultivation and Preparation of microorganism for biosorption

Into the growth medium (Nutrient broth) *B. subtilis* was inoculated and incubated at 37ºC for 5 days at 100 rpm. After incubation, the biomass obtained by centrifugation was purified by washing with sterile distilled water. At 60ºC the biomass was dried for 24 h and used for experiments.

Pre-treatment of biomass

The biomass of *B. subtilis* was treated with five different chemicals. The following chemical solutions (100 mL) were used for pre-treatment of biomass:

– 0.1 M oxalic acid;
– 0.1 M calcium chloride;
– 0.1 M sodium carbonate;
– 10% hydrogen peroxide solution and
– Commercially available formaldehyde solution.

To each of the prepared chemical solutions, 1 g of powdered *B. subtilis* biomass was treated for 30 min. After incubation, the biomass of each treatment was separated, washed and dried for use.

Biosorption experiments

Biosorption experiments for the control biomass of *B. subtilis* (without any pretreatment) were performed in 250 mL Erlenmeyer flasks by dissolving 1 g of biomass with lead ion concentration of 50 μg/mL and agitated with 40 rpm for 16 h at 35ºC after adjusting the pH to 6.5. To attain the maximum biosorption efficiency of control biomass, various experimental conditions (pH, temperature, incubation time, inoculum dosage and agitation speed) were optimized.

At the optimized biosorption conditions of control biomass (where it has shown the maximum biosorption capacity), biosorption experiments were conducted for *B. subtilis* after treated with different chemicals. Experimental conditions for the treated biomass which showed the highest biosorption were further optimized to attain the maximum biosorption efficiency.

After incubation at respective experimental conditions, the contents of the entire flasks were separated by centrifugation (4000 rpm, 3 min) and supernatants were collected to determine the residual lead ions by Inductive Coupled Plasma Optical Emission Spectrometry (ICP--OES). The optimized operating conditions of ICP-OES that are given the best results are RF power – 1100W, cool gas flow – 12.5 L/min, auxiliary gas flow – 0.9 L/min with a nebulizer gas flow of 0.265 L /min.

Determination of biosorption capacity (q_e) of biomass was done by applying the equation:

$$qe = \frac{(Ci - Ce)\,V}{m} \qquad (1)$$

Where q_e is the amount of metal biosorbed to the biosorbent (mg g^{-1}), C_i is the initial metal ion concentration in solution (mg L^{-1}), C_e is the residual metal concentration in the supernatant (mg L^{-1}), V is the volume of the medium (L), and m is the amount of the biomass used in the biosorption process (g).

The biosorption potential (%) was determined by the equation:

$$\%\ \text{of biosorption} = \frac{\text{Ci} - \text{Ce}}{\text{Ci}} \times 100 \qquad (2)$$

Scanning Electron Microscope and Energy Dispersive X-ray (SEM–EDX) analysis

Morphological and structural changes that occurred as a result of lead biosorption were evaluated by using CARL ZEISS SUPRA 55 GEMIN (German Technology Jena, Germany) Scanning Electron Microscope (SEM). For this, the samples of the biomass were applied on metal stubs after glutaraldehyde fixation and drying. The samples were then sputtered with gold (SC7620 'Mini' sputter coater) and observed. Mapping of metal ions (distribution) and elemental analysis were performed by Energy Dispersive X-ray analyzer. The X-ray spectrum of each loaded sample was acquired at 16 KeV acceleration voltages.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

Determine of functional groups and their chemical modifications occurred as a result of biosorption was analyzed by FTIR spectroscopy. For analysis, KBr discs containing dried biomass and potassium bromide in 1:100 ratios were prepared and analyzed by recording the spectra within 4000–500 cm^{-1} range and 4 cm^{-1} resolution.

Biosorption Kinetics

Pseudo first and second-order kinetic models were used for the prediction of mechanism and biosorption rate between metal-binding sites and metal ions.

The linear form of the pseudo first-order model is expressed by the following equation:

$$\log(q_e - q_t) = log q_t - \frac{k_1 t}{2.303} \qquad (3)$$

The linear form of the pseudo second-order model is expressed by the following equation:

$$\frac{t}{q_t} = \frac{1}{k_2 . q_e^2} + \frac{t}{q_e} \qquad (4)$$

Where q_e and q_t are the amount of metal ion biosorbed at equilibrium and at time t respectively, (mg/L), k_1 is the pseudo first-order rate constant (per min) and k_2 is the pseudo second-order rate constant (per min). The parameters of pseudo first and second-order kinetic models can be calculated from the linear plots of log (q_e–q_t) versus t, and t/q_t versus t, respectively.

Statistical analysis

Triplicate data were obtained for each experiment and statistical analysis was performed by one way Annova. Graph Pad Prism 5 was used for plotting the graphs expressing mean ± standard deviation values.

RESULTS AND DISCUSSION

B. subtilis is recognized as GRAS organism in recent years[14] and also pretreatment of biomass with different chemicals enhanced the biosorption efficiency which makes its use as an effective biosorbent in metal ion removal. Since scanty information is available regarding the use of treated biomass of *B. subtilis* in biosorption studies, hence its role is also discussed in the present study.

Biosorption studies

Based on ICP-OES result, at initial biosorption conditions, the biosorption efficiency of *B. subtilis* was 36.75%. Hence various experimental parameters were optimized to attain the maximum biosorption efficiency.

At the optimized experimental conditions (which was described below), the biosorption efficiency was increased to 58.05% with a lead ion concentration of 50 μg/mL, at pH 6.5 with biosorbent dose of 1.5 g incubated at 35°C for 16 h at 40 rpm. Similar results were obtained with other metal ions using *B. subtilis* as biosorbent with biosorption efficiency of 60% for Cr(VI)[15] and 63.73% for Zn(II) ions[16] at optimized conditions. Some studies reported that the highest biosorption efficiency of 98.4% and 90% was achieved for Pb(II) ions using *B. subtilis* and *B. cereus* as biosorbents respectively[17, 18]. Further to increase the biosorption efficiency the biomass of *B. subtilis* was treated with different chemicals. At optimized biosorption conditions of control biomass, biomass treated with formaldehyde (Fig. 1) showed the highest efficiency of 80.92% when compared with the other treatments and control (58.05%). Further optimization of experimental parameters was conducted to attain the maximum biosorption efficiency for the treated biomass.

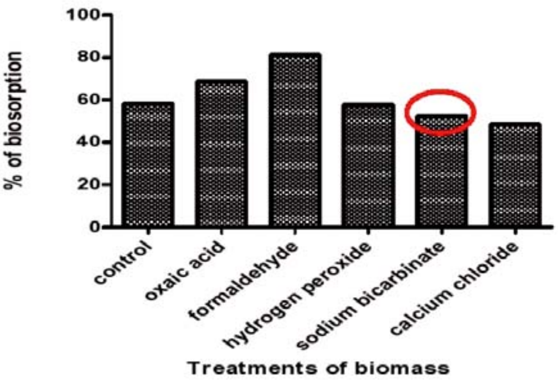


Figure 1. Effect of pretreatment on biosorption efficiency of B. subtilis for Pb(II) when treated with different chemicals

Optimization studies

Various experimental parameters were optimized for both control (untreated) and treated biomass of *B. subtilis* to increase the efficiency of biosorption.

Effect of pH

The study of pH is essential for the determination of the surface charge of biosorbent for metal ion biosorption. Figure 2a and b show the effect of pH for both control and treated biomass of *B. subtilis* within the range of 4–7.5. At acidic conditions, the efficiency of biosorption was low, reaches the maximum at pH 6.5 and declines in the alkaline conditions. The phenomenon is due to at low pH, more protons exist which competes with the existing metal ions thus decreasing the biosorption efficiency. With the increase in pH, protons get deprotonated making the biosorbent negatively charged which attracts the metal ions and reaches the maximum. At high pH values, metal hydroxides are formed due to the occurrence of more OH^- ions and results in decrement of biosorption efficiency. Hence pH 6.5 was considered as optimum with high biosorption efficiency of 62% and 89% for control and treated biomass of *B. subtilis* respectively. A similar effect of pH was observed with *B. pumilus* for Pb(II) ion biosorption with the highest efficiency at pH 6.0[19].

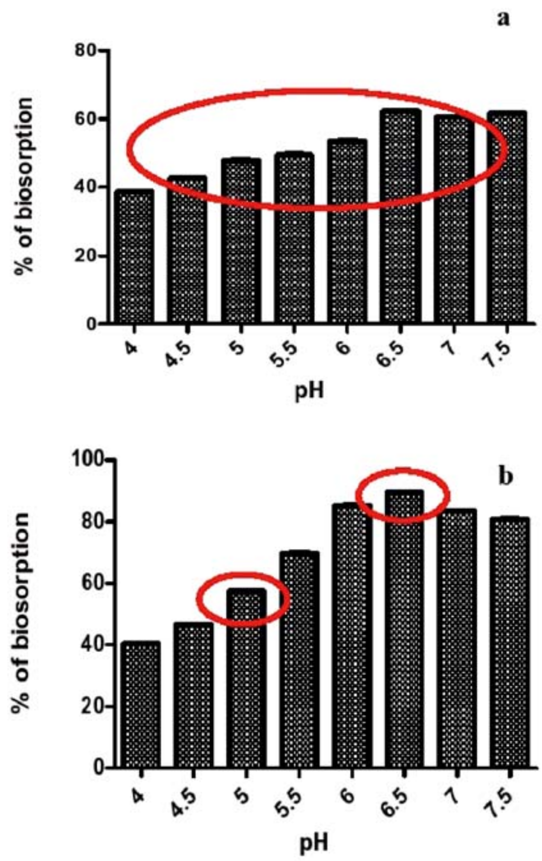


Figure 2. Effect of pH on biosorption of Pb(II) by control (a) and formaldehyde (b) treated biomass of *B. subtilis*

Effect of contact time

Figure 3a and b represents the Pb(II) ion biosorption with time for control and treated biomass of *B. subtilis*. From the results, the rate of biosorption was rapid and maximum within the first 16 h of contact time which is followed by a slower or saturated stage of biosorption with a further increase in time to 48 h. The reason for the highest efficiency is due to the availability of abundant metal-binding sites. Thereafter competition exists for occupying the available binding sites with the metal ion which results in a slower rate of biosorption. Also in practical applications, the treatment of biomass for longer periods will be uneconomical and power wasting[20] and hence contact time of 16 h was taken as optimum with maximum biosorption efficiency of 59.83% and 80.79% for control and treated biomass of *B. subtilis* respectively. A similar result was observed with *Pseudomonas aeruginosa* and *B. cereus* in biosorption of Zn(II) ions[21].

Effect of Biosorbent dose

Figure 4a and b shows the impact of biosorbent dose on Pb(II) ion biosorption with control and treated biomass. With the increase in biosorbent dose, the efficiency of biosorption was also increased up to 1.5 g/L. Afterward

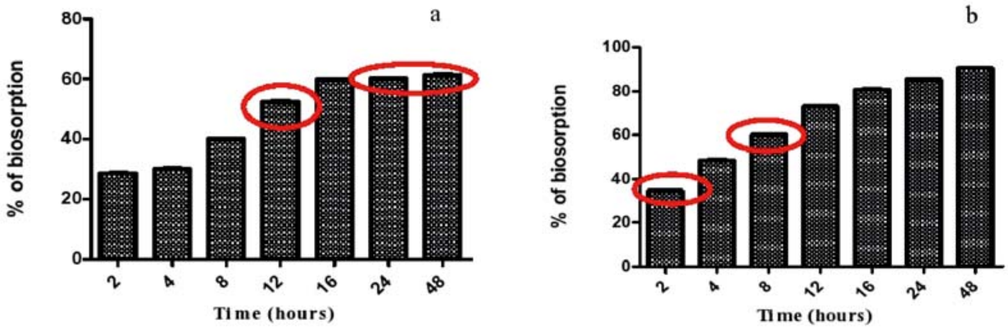


Figure 3. Effect of contact time on biosorption of Pb(II) by control (a) and formaldehyde (b) treated biomass of *B. subtilis*

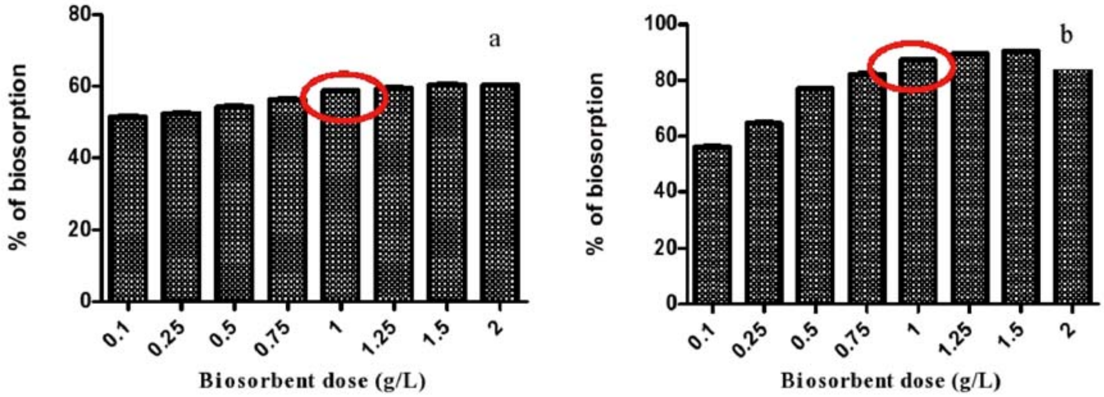


Figure 4. Effect of biosorbent dose on biosorption of Pb(II) by control (a) ans formaldehyde (b) treated biomass of *B. subtilis*

the biosorption efficiency was decreased or remains constant. An increase in biosorption at the initial biosorbent dose is due to the presence of a larger surface area that results in an increase of available active sites for metal ions. At higher biosorbent dose aggregate formation or overlapping of the biosorbent surface occurs that reduces the surface area for biosorption and results in lower biosorption efficiency. Hence biosorbent dose of 1.5 g was found to be optimum for control and treated biomass of *B. subtilis* with biosorption efficiencies of 60.4% and 90.4% respectively. Recent studies show that *Citrobacter freundii* and *Klebsiella pneumonia* achieved maximum biosorption efficiency for Pb(II) ions when biosorbent dose of 2 g/L was used[2].

Effect of temperature

Pb(II) ion biosorption by control and treated biomass of *B. subtilis* with temperature was given in Figure 5a and b. With the increase in temperature from 25°C to 60°C, the efficiency of biosorption decreased with the maximum at 35°C. An increase in temperature enhances the rate of biosorption due to increased solute kinetic energy and surface activity. Higher temperatures result in deactivation or damage to the metal active sites present on biosorbent surface which results in rupturing of the bonds and a decrease in biosorption efficiency. Also at high temperatures, it is expected that physical damage can occur to the biosorbent. Hence 35°C was considered as optimum with maximum biosorption efficiency of 51% and 89% for control and treated biomass of *B. subtilis* respectively. Similarly, the maximum biosorption efficiency of 92% for Fe(II) at 30°C and 94% for Cu(II) ions at 45°C was achieved by *Bacillus licheniformis*[22].

Effect of agitation speed

Figure 6a and b shows that the optimum agitation speed was 40 rpm with maximum biosorption efficiency of 58% and 87% for control and treated biomass of *B. subtilis*. Agitation speed is an important parameter for biosorption since it expands the interaction possible between the metal and biomass. Settling of biomass can be observed at low speed which results in the burying

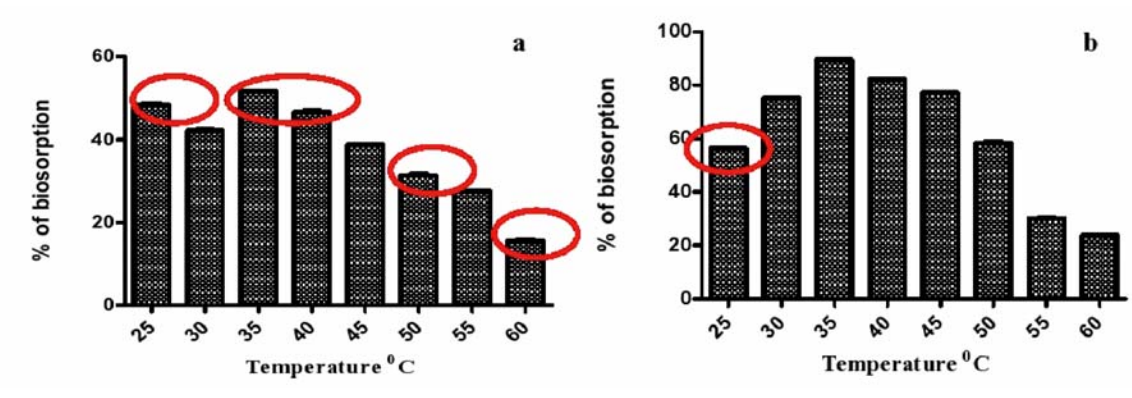


Figure 5. Effect of temperature on biosorption of Pb(II) by control (a) and formaldehyde (b) treated biomass of *B. subtilis*

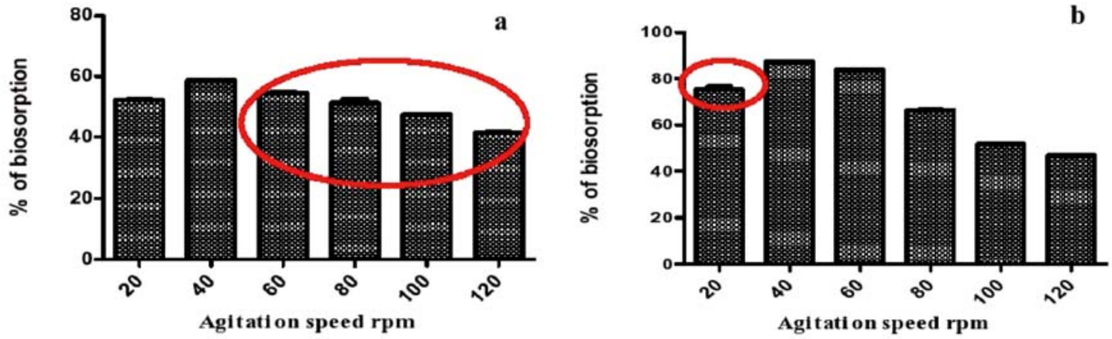


Figure 6. Effect of agitation on biosorption of Pb(II) by control (a) and formaldehyde (b) treated biomass of *B. subtilis*

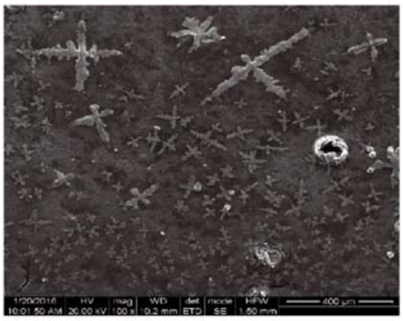
of metal-binding sites. Hence the sites available on the upper region of the biosorbent take part in biosorption process that results in lower efficiency. At high agitation speeds, due to the occurrence of random collisions between the biosorbent and biosorbate the time required to interact with the binding sites of biosorbent decreases which also results in low efficiency. Hence in the present study, the agitation speed of 40 rpm was recognized as optimum. Studies also showed that 80 rpm was optimum to attain a maximum efficiency of biosorption for Cd(II) ions by *Klebsiella* sp.[23].

Effect of pretreatment

At the optimized experimental conditions the biosorption efficiency of formaldehyde-treated *B. subtilis* biomass was increased to 89.8% with 50 μg metal ion, 1.5 g/L of biosorbent dose at pH 6.5 for 16 h incubated at 35°C with 40 rpm. Thus the data indicate that the pretreatment enhances the efficiency of biosorbent for lead ion biosorption. Studies reported that pretreatment with different chemicals modifies the surface of the biomass either by masking or removing the surface impurities[24] and rupturing of cell membrane that results in the availability of more metal binding sites[25] which are the reasons for the increased biosorption. No reports of formaldehyde treated bacterial biomass for lead biosorption were found. However, a maximum biosorption capacity of 774 mg/g for Pb(II) was observed with red marine algae *Jania ruben* when pretreated with formaldehyde compared to the raw biomass (696 mg/g)[26]. Also, the fungal biomasses of *Aspergillus* versicolor, *Metarrhizium anisopliae* var. *anisopliae* and *Pencillium verrucosum* showed biosorption capacity of 22, 12.4 and 5.4 mg/g for Pb(II) ions which was increased to 23.1, 18.1 and 18.7 mg/g after formaldehyde treatment respectively[27].

SEM-EDX analysis

Figure 7a and 8a evaluate the morphological changes of control and Pb(II) biosorbed *B. subtilis* biomass. SEM images reveal that in the control biomass smooth and elongated cells are visible in loosely bound form. Following biosorption, the cells became small and puffy due to lead deposition. The results are also in accordance with EDX analysis. By comparing the elemental composition (Fig. 7b and 8b), and EDX spectra (Fig. 7c and 8c) the changes in the percentage composition of the elements and a peak for Pb(II) in the treated biomass confirms that the Pb(II) ions were biosorbed on to *B. subtilis* by ion-exchange mechanism. Similar changes in morphology and EDX spectra due to biosorption with metal ions were observed by other bacterial biosorbents[28, 29].



Element	Wt %	At%
C	53.27	68.12
O	17.23	16.54
Na	11.10	07.42
Cl	17.21	07.46
K	01.20	00.47

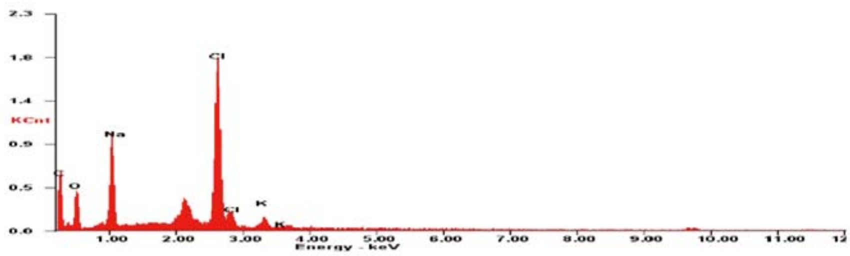


Figure 7. SEM image, elemental composition and EDX spectra of control biomass of *B. subtilis*

Element	Wt%	At%
C	41.15	73.47
O	16.06	21.53
Na	00.17	00.16
Cl	00.86	00.52
K	00.00	00.00
Pb	41.76	04.32

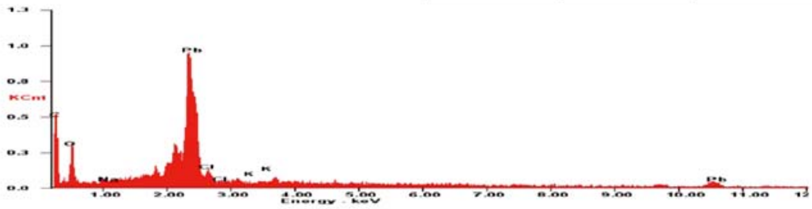


Figure 8. SEM image, elemental composition and EDX spectra of Pb(II) biosorbed biomass of *B. subtilis*

FTIR analysis

FTIR study enables one to examine the surface characterization of biosorbent as a result of chemical modification and biosorption and determination of functional groups that participate in biosorption process.

Figure 9a and b show the spectra of control (untreated) and treated biomass of *B. subtilis*. The bands at 3344.78 cm^{-1} (control) and 3857.72, 3748.40, 3332.58 cm^{-1} (treated) indicates the presence of –OH stretching of polymeric compounds and –NH stretching of proteins. The peaks at 2117.93 cm^{-1} (control) and 2919.87, 2115.77 cm^{-1} (treated) contributes to C-H stretching vibrations of aliphatic groups (CH_2 and CH). The peaks at 1636.43 (control) and 1632.59 cm^{-1} (treated) corresponds to C=C and C=O vibration respectively. The peaks at 1342.51 cm^{-1} (control) and 1325.8 cm^{-1} (treated) are due to phosphate group vibration. The peaks at 1038.09 cm^{-1} (control) and 1034.34 cm^{-1} (treated) correlate to C-O--C and O-H stretching vibration of polysaccharide like substance. The peaks at 720.77, 872.30, 668.3 cm^{-1} are indicative of finger print zone which contains phosphate and sulfur functional groups. Treated biomass shows an extra peak at 1150.64 cm^{-1} correspondings to –S=O stretching. The above comparison concludes that additional peaks are formed on the surface of *B. subtilis* biomass due to pretreatment which indicates the increase for biosorption efficiency.

Figure 10a and b shows the spectra after Pb(II) ion biosorption of control and treated biomass of *B. subtilis*. Comparing Figure 9a and 10a, a shift in the peak

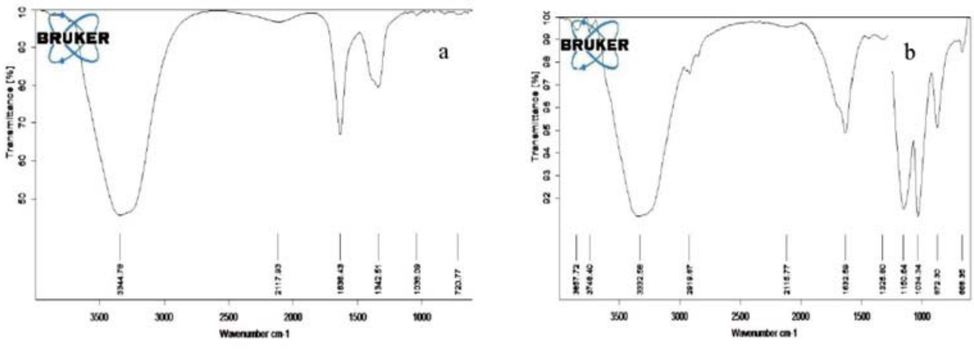


Figure 9. FTIR spectra of control (a) and formaldehyde (b) treated biomass of *B. subtilis*

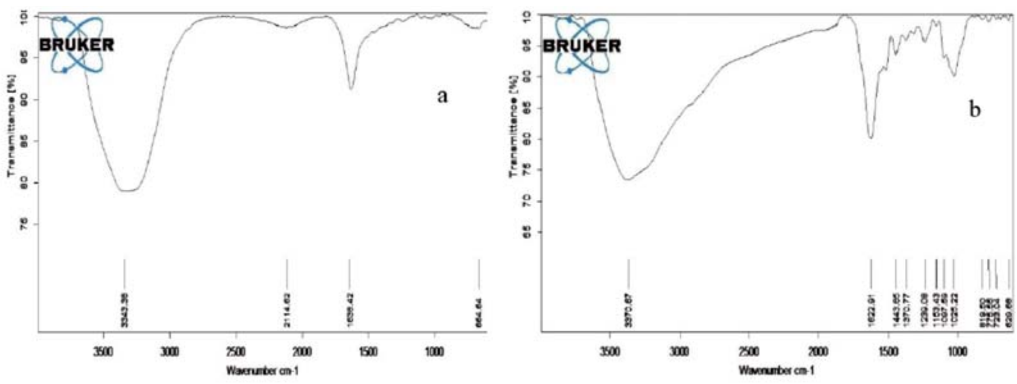


Figure 10. FTIR spectra of Pb(II) biosorbed control (a) and formaldehyde (b) treated biomass of *B. subtilis*

intensities to 3343.38, 2114.62, 1638.42, 664.64 cm^{-1} in the control biomass biosorbed with lead was noticed. Similarly, a shift to 3370.67, 1622.91, 1370.77, 1153.43, 1097.59, 10.25.22 819.50, 629.68 cm^{-1} in the treated biomass biosorbed with lead was observed (Fig. 9b and 10b). The shifts indicate that the functional groups present at these regions are involved in biosorption process to interact with metal ions. Appearance (1443.85, 1239.08, 775.55, 723.04 cm^{-1}) and disappearance (2919.87, 2115.77 cm^{-1}) of peaks in the treated biomass indicates the interaction with lead ions (Fig. 9b and 10b). Hence, it can be concluded that the functional groups hydroxyl, amino and carboxyl that are present on *B. subtilis* biomass surface take part in metal ion biosorption. This is in accordance with other results obtained by *Bacillus thuringiensis* for the biosorption of Cd(II), Cr(VI), Cu(II), Pb(II) and Ni(II)[30] and *Bacillus* sp. for the biosorption of Cr(VI) and Cu(II) ions[31].

Biosorption Kinetics

Evaluation of sorption kinetics describes the behavior of lead ion biosorption onto *B. subtilis* biomass. Table 1 summarizes the constants and correlation coefficients (R^2) obtained from the plots of kinetic models. Based on correlation coefficients values, it shows that the data were fitted well with the pseudo second-order kinetic model for both untreated (R^2 = 0.9753) and treated (R^2 = 0.9956) biomass of *B. subtilis*. Thus it indicates that the rate limiting step in biosorption is chemisorption with the involvement of covalent forces either by sharing or by the exchange of electrons between the metal ions and the surface functional groups of the biosorbent. Similarly, other studies show that the biosorption of Cu(II) ions by *Bacillus subtilis*[32] and Pb(II), Cr(III), and Cu(II) by *Rhodococcus opacus*[33] obeys the pseudo second-order kinetic model as the better fit.

Real time studies

Real water samples from streams and lakes near contaminated industrial sites located at Autonagar, Budampadu and Takkellapadu towns of Guntur district were collected for application of pretreatment biomass of *B. subtilis* for lead removal. Twenty samples labeled as RTS0-RTS20 were collected in wide mouth 1 L capacity bottles and stored at room temperature for analysis. Collected water samples were chemically analyzed for pH, temperature (°C), electric conductivity (μmos/cm), total alkalinity (mg/L) and turbidity according to standard methods of IS: 3015 [34] and summarized in Table 2. Metal concentration was determined by ICP-OES and the samples with a high concentration of metal were subjected to biosorption experiments by pretreatment biomass of *B. subtilis* at optimized experimental conditions. Figure 11 elucidates that the biomass of *B. subtilis* removed lead ions efficaciously in the range of 16.6% to 53% for

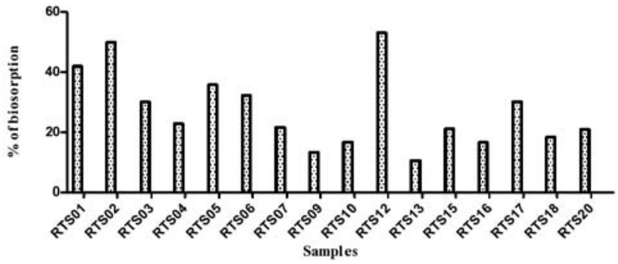


Figure 11. Percentage biosorption of lead ions from the contaminated wastewater samples by pretreated biomass of B. subtilis at optimized conditions

Table 1. Parameters of pseudo first and second order kinetic models for the biosorption of Pb(II) ions onto untreated (control) and treated biomass of *B. subtilis*

Treatments	Pseudo first order kinetic model			Pseudo second order kinetic model		
	q_e (mg/g)	K_1 (min^{-1})	R^2	q_e (mg/g)	K_2 (gmg^{-1} min^{-1})	R^2
Control biomass (Untreated)	28.33	0.173	0.9078	36.36	0.0058	0.9753
Treated Biomass	33.596	0.109	0.9888	50.50	0.0045	0.9956

Table 2. Physicochemical parameters of collected wastewater samples

S.No	Sample code	pH	Temperature in °C	EC in μmos/cm	Total alkalinity as mg/L	Turbidity in NTU	Initial lead concentration (mg/L)	Final concentration of lead (mg/L)
1	RTS01	8.5	24	2435	796	0.9	0.31	0.18
2	RTS02	8.4	26	2261	812	0.9	0.34	0.17
3	RTS03	8.9	25	2749	634	2.5	0.20	0.14
4	RTS04	8.7	23	2518	807	2.1	0.13	0.10
5	RTS05	7.9	22	2834	552	3.6	0.28	0.18
6	RTS06	7.8	26	2150	416	2.4	0.25	0.17
7	RTS07	8.8	27	2417	632	1.1	0.23	0.18
8	RTS08	9.5	22	2981	891	3.0	BDL	–
9	RTS09	8.6	23	2015	354	2.8	0.15	0.13
10	RTS10	8.2	27	2206	428	1.5	0.30	0.25
11	RTS11	7.1	25	2148	219	0.7	BDL	–
12	RTS12	7.9	24	2731	587	1.8	0.32	0.15
13	RTS13	8.0	23	2658	453	2.1	0.19	0.17
14	RTS14	7.4	22	1860	497	2.7	BDL	–
15	RTS15	7.9	27	2569	692	2.6	0.33	0.26
16	RTS16	8.4	25	2933	641	1.9	0.12	0.10
17	RTS17	8.6	26	2413	573	2.0	0.20	0.14
18	RTS18	7.5	24	1964	295	1.1	0.27	0.22
19	RTS19	7.6	22	1582	216	0.8	BDL	–
20	RTS20	7.3	24	1846	268	1.0	0.19	0.15

*BDL: below detection limit.

different water samples respectively. Thus it shows that the biomass of *B. subtilis* can be used as an efficient biosorbent for metal ion removal from real wastewater.

CONCLUSION

The present research evaluates that *B. subtilis* biomass is potential in removing Pb(II) ions from water sources. Pretreatment with different chemicals enhanced the biosorption efficiency of B. *subtilis* biomass. Among various chemical treatments, the biomass treated with formaldehyde showed the highest efficiency of 80.92%. The maximum biosorption efficiency obtained by the control and treated biomass of *B. subtilis* was 58.04% and 89.8% attained with the optimized experimental conditions: initial Pb(II) ion concentration of 50 μg/mL, biosorbent dose of 1.5 g, pH 6.5 at 35^{o}C with contact time of 16 h at 40 rpm. SEM-EDX analysis shows the bulging of cells and a peak for Pb(II) in the spectrum as a result of biosorption. FTIR evaluates that carboxyl, hydroxyl, and amino functional groups are involved in biosorption. Kinetic data were fitted better with the pseudo second-order model for both control and treated biomass of *B. subtilis*. From the results, we can conclude that the formaldehyde treated biomass of *B. subtilis* can be used as a cost-effective biosorbent for the removal of metal ions from contaminated water resources.

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