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Studies on removal of lead(II) by Alginate Immobilized Bromelain (*AIB*)

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ABSTRACT

The work is based on augmentation of active sites of calcium alginate bead by immobilizing bromelain to remove lead(II) from simulated solution. The optimum immobilization condition as specified by response surface methodology is as follows: initial concentration of sodium alginate, bromelain, and calcium chloride are 20, 21.09, and 20 g/L, respectively, at pH 7 and 35°C and the sample, thus prepared, is termed as alginate immobilized bromelain (*AIB*). Maximum 99.5% lead(II) has been removed when 30 mL lead(II) solution having initial concentration of 0.0048 mmol L^{-1} has been treated with 5 g of *AIB* at pH 7 and 35°C. 77.9% lead(II) has been recovered when 1 g of spent adsorbent is stirred in 100 mL of solution having pH 2 for 30 min. The removal of lead(II) using *AIB* and, thereby, simultaneous inhibition of enzyme have been modeled both statistically and empirically.

Keywords: Enzyme immobilization; Bromelain; Lead; Response surface methodology; Empirical model

1. Introduction

Lead ranks second in the priority list of hazardous substances as prepared by Agency for Toxic Substances and Disease Registry. The effluents coming from different industries such as batteries, paint, leather, ceramic, and paper and pulp, etc. may contain lead(II) more than permissible limit. Due to its recalcitrant and non-biodegradable characteristics, it is bioaccumulated in the ecosystem causing severe health problem [1]. Thus, proper treatment of industrial effluent containing lead(II) is mandatory. Though conventional methods like simple filtration or precipitation, ion exchangers, or chelation can be used to abate lead (II) from wastewater, they are unable to reduce the concentration level to ppb level and may lead to secondary pollution [2]. Possibilities of application of bromelain for pollutant removal have not been explored. Alginate, a natural polymer, is a glycuronan extracted from brown algae and chemically is an unbranched copolymer consisting of residues of D-mannuronic acid and L-guluronic acid [3]. Several studies on lead(II)

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removal have been done with alginate bead, modified chemically with different reagents [4], or immobilized with micro-organism [5]. Alginate beads impregnated with nanoparticles have also been used in removal of lead(II). Idris et al. synthesized magnetic alginate beads by incorporating ferrofluids based on maghemite nanoparticles (g-Fe_2O_3) and sodium alginate [6]. In another study, Jiang et al. worked on assembly of polyaniline nanofibers on calcium alginate beads by competitive adsorption-restricted polymerization approach [7]. One more magnetic adsorbent (called magsorbent) was developed by Bée et al. by encapsulation of magnetic functionalized nanoparticles in calcium-alginate beads [8]. It is well known that synthesis of nanoparticles is capital intensive process and requires a great care at each stage of operation. Therefore, a low-cost natural chemical, suitable for modifying the surface of alginate bead in technically simple manner and capable of binding lead(II) from wastewater, is the need of the present hour.

Bromelain (EC: 3.4.22.32), a cysteine protease, is an extract obtained from stem of pineapple, *Ananas comosus*. It is readily available in bulk amount and low in cost in comparison with the other chemicals as used in previous studies. It can be used as phytotherapeutical drug, meat tenderizing agent, etc. [9]. Furthermore, it has a unique characteristic of metal binding due to the presence of sulfhydryl groups (–SH group) at its active site [10,11]. Shukor et al. [10] and Masdor and Said [11] used bromelain to develop a novel and robust protease inhibitive assay method for detecting heavy metals in agriculture and environment. Thus, methods for removal of lead(II) without causing secondary pollution are of great interest.

In the present study, bromelain has been selected as the said chemicals to be immobilized on calcium alginate bead. Bromelain binds to metal through its –SH group while calcium alginate beads also adsorb lead(II) from solution, it is expected that insertion of immobilized bromelain would enhance adsorption significantly. Process conditions for immobilization of bromelain on calcium alginate bead have been optimized using response surface methodology (*RSM*). Calcium alginate beads, on which bromelain has been immobilized will be referred to alginate immobilized bromelain (*AIB*). The amount of bromelain loading can be determined from the decrease in specific enzymatic activity (*SEA*) of the enzyme as on binding of metal on alginate, the enzyme loses its activity. For kinetic study, the percentage removal of lead(II) and *SEA* of used *AIB* have been used. An empirical model has also been proposed to correlate the percentage removal of lead(II) and percentage decrease in *SEA* at different values of other parameters. Afterward, the percentage removal of lead(II) has been expressed as a function of all the kinetic input parameters and percentage decrease in *SEA* as well. Such type of representation is probably a novel work in environmental engineering field.

2. Materials and methods

All the chemicals used were of AR grade unless otherwise stated. All the experiments were performed thrice and the mean value was reported.

2.1. Optimization of operating condition for immobilization of bromelain on calcium alginate beads using RSM and preparation of AIB

2.1.1. Experimental conditions

Bromelain (Xena Bioherbals Pvt. Ltd, India) was immobilized on calcium alginate beads by ionotropic gelation method [3,12]. Initially a mixture of sodium alginate (MERCK) and bromelain was prepared by dissolving these in 100 mL distilled water. The amount of the materials were different for each run and were determined corresponding to the compositions selected by *RSM* and given in Table 1. Magnetic stirrer (Remi Equipment Pvt. Ltd, Model No. 1 MLH) was used at a stirring rate of 300 rpm for 30 min to make the mixture homogeneous. Calcium chloride (MERCK) solution was prepared in 100 mL distilled water in the same manner and was cooled to 4℃. Then, 10 mL of alginate enzyme mixture was added dropwise by a syringe to the chilled calcium chloride solution, where the beads formed, entrapping bromelain and were left for 30 min for hardening. The size of the drops was controlled by adjusting the distance between the tip of the syringe and the surface of the solution and kept at 1 mm diameter size. Sodium alginate and calcium chloride undergo double displacement reaction and to form calcium alginate that entrap bromelain. The beads were then separated from calcium chloride solution and washed with distilled water. Temperature was kept at the lowest possible level, to maximize the loading of enzyme, as suggested in literature [3,12]. Finally the beads were stored in distilled water at 4℃ taken in 100 mL screw-capped tubes for further use. *SEA* of immobilized bromelain samples was measured using UV–VIS spectrophotometer (UV 2300, TECHCOM, GERMANY) at 280 nm following standard protocol [13]. Casein (MERCK) was used as substrate for such studies.

Table 1
Experimental design for preparation of *AIB*

Run	Initial concentration of sodium alginate (A_1, g/L)	Initial concentration of bromelain (B_1, g/L)	Initial concentration of calcium chloride (C_1, g/L)	pH (D_1)	$SEA \times 10^{-4}$ (g peptide formed)/(g $AIB \times$ h)
1	35.00	35.00	35.00	11.50	53
2	65.00	35.00	35.00	6.50	322
3	35.00	35.00	35.00	6.50	299
4	50.00	50.00	50.00	9.00	326
5	35.00	35.00	35.00	6.50	299
6	50.00	50.00	20.00	9.00	324
7	20.00	50.00	20.00	9.00	313
8	35.00	35.00	35.00	1.50	55
9	50.00	50.00	50.00	4.00	333
10	35.00	5.00	35.00	35.00	58
11	35.00	35.00	35.00	6.50	299
12	35.00	35.00	35.00	6.50	299
13	35.00	35.00	5.00	6.50	228
14	20.00	20.00	50.00	4.00	154
15	20.00	50.00	50.00	4.00	310
16	50.00	50.00	20.00	4.00	324
17	20.00	20.00	20.00	4.00	99
18	20.00	50.00	50.00	9.00	316
19	50.00	20.00	50.00	9.00	155
20	35.00	35.00	35.00	6.50	299
21	20.00	20.00	50.00	9.00	177
22	35.00	35.00	35.00	6.50	299
23	35.00	35.00	65.00	6.50	328
24	50.00	20.00	50.00	4.00	154
25	50.00	20.00	20.00	9.00	226
26	20.00	20.00	20.00	9.00	217
27	5.00	35.00	35.00	6.50	206
28	50.00	20.00	20.00	4.00	155
29	35.00	65.00	35.00	6.50	435
30	20.00	50.00	20.00	4.00	215

2.1.2. Response surface methodology

RSM was employed to optimize the immobilization condition using Design Expert Software 8.0.6. *RSM*, a combination of statistical and mathematical techniques has been exploited for development, improvement, and optimization process [14]. Four parameters viz., initial concentrations of sodium alginate (A_1), bromelain (B_1), calcium chloride (C_1), and pH (D_1) were selected as input variables and *SEA* (R_1) as output variable. Thirty experiments, as designed by the software, were carried out to cover up the total range of different combinations of input variables. The minimum (−1) and maximum (+1) levels of first three input parameters viz., initial concentrations of sodium alginate, bromelain, and calcium chloride were maintained at 20 and 50 g/L in each case and those of pH were kept at 4 and 9. The optimum condition for immobilization as specified by Design Expert software is: initial concentration sodium alginate: 20 g/L, initial concentration of enzyme: 21.09 g/L, initial concentration of calcium chloride: 20 g/L, and pH 7 at 35°C temperature. The immobilized sample obtained at this specified condition is termed as *AIB* and was used for the removal of lead(II) from simulated solution.

2.2. Characterization of AIB

2.2.1. FTIR study

FTIR study of *AIB* and *AIB*-lead(II) complex were performed by Fourier Transform Infrared machine (Nicolet iS10, Thermo Fischer Scientific, USA). Two milligrams of beads were mixed up with 40 mg of KBr (MERCK) for the preparation of crystal clear pellets for FTIR analysis.

2.2.2. Scanning electron microscopy and energy-dispersive X-ray spectrometry

Scanning electron microscopy (SEM) was performed for *AIB*, *AIB*-lead(II) complex, and *AIB* after recovery of lead(II) separately to obtain their surface topography using JEISS EVO 18, GERMANY. Energy-dispersive X-ray spectrometry (EDS) study was done to find the elemental analysis of *AIB*-lead (II) complex to verify the binding of lead(II) with *AIB* using EDS instrument (FEI Quanta 200, USA). Five grams of *AIB* were contacted with lead(II) solution having initial concentration 10 mg/L for 20 min at pH 7 and 35˚C temperature. *AIB* was then separated from treated solution and designated as *AIB*-lead(II) complex. One gram of *AIB*-lead(II) complex was contacted with solution having pH 2. Volume of solution and time of experiment were kept 100 mL and 30 min. At the end of the experimentation, *AIB* was separated from solution. SEM study was done of these *AIB* from which lead(II) has been desorbed. Gold coating was done on the samples. The acceleration voltage and working distance were 10 kV and 9 mm, respectively.

2.2.3. Determination of temperature optima and pH optima

The optimum temperature and optimum pH of *AIB* were found out by determining its *SEA* at various temperatures and pH conditions spectrophotometrically following the standard protocol. Five grams of *AIB* were used in each experiment and volume of solution was kept 30 mL. For the determination of temperature optima, *SEA* of *AIB* was measured at different temperatures (35, 50, and 70˚C) at constant pH 7, whereas for finding out pH optima, temperature was kept constant at 35˚C and *SEA* was measured at different pH conditions such as pH 5, 7, and 9.

2.2.4. Determination of temperature stability and pH stability

To determine temperature stability, 5 g of *AIB* was exposed to different temperatures such as 4, 35, 50, and 70˚C separately for 1 h. Similarly, to determine pH stability, 5 g of *AIB* was exposed at different pH conditions like pH 5, 7, and 9 separately for 1 h. In each case, volume of solution was kept 30 mL. Finally, the enzymatic assay of each *AIB* sample was carried out at 35˚C following standard protocol.

2.2.5. Determination of kinetic parameters of protein hydrolysis

The kinetic parameters of protein hydrolysis were determined in case of both free enzyme and *AIB* using varying amount of casein as substrate. 0.1 mL of 0.025% bromelain solution was taken for the determination of kinetic parameters in case of free enzyme, whereas 5 g of *AIB* was used for kinetic study in case of immobilized enzyme. *SEA* was determined. The loading of bromelain in *AIB* has been determined by Kjeldahl method.

2.3. Removal of lead(II) from simulated solution using AIB

2.3.1. Comparative study of removal of lead(II) using calcium alginate and AIB

0.016 g of lead acetate (MERCK) was dissolved in 100 mL of distilled water to prepare a stock solution of lead(II) having initial concentration 0.48 mmol L^{-1} (1 ppm = 0.0048 mmol L^{-1} in case of lead(II)). Thirty millliter simulated solution of lead(II) having initial concentration of 0.048 mmol L^{-1} was then prepared from stock solution by adding 3 mL of 0.48 mmol L^{-1} of lead(II) solution to 27 mL of distilled water. Initially, to establish the effectiveness of *AIB* over pure calcium alginate, 30 mL simulated solution of lead(II) having initial concentration of 0.048 mmol L^{-1} was contacted with 5 g of calcium alginate and *AIB* separately for 20 min at pH 7 and 35˚C temperature. The solution was then separated from adsorbents and analyzed for the residual metal ion concentration using Atomic Absorption Spectrometer (AAS) (JSM 6700F JEOL, Japan).

2.3.2. Kinetics study on removal of lead(II)

Kinetic study of lead(II) removal was done by contacting simulated solution of lead(II) with *AIB* in a batch contactor. Different concentrations i.e. 0.0048, 0.048, 0.14, and 0.24 mmol L^{-1}, of lead acetate solution were prepared by diluting the stock solution with definite amount of distilled water. Four parameters, namely initial concentration of lead(II), weight of *AIB*, pH, and temperature were varied in the range of 0.0048–0.24 mmol L^{-1}, 3–7 g and 5–7, and 25–45˚C in a systematic manner. In each case, volume of the lead(II) solution was kept 30 mL. The solution was stirred continuously. To keep the mass transfer resistance low; mixture was stirred continuously using magnetic stirrer at about 300 rpm. It is seen that up to this rpm, the alginate bead can retain its size and shape. The used adsorbent was separated from solution and analyzed for *SEA*. The residual metal ion concentration in solution was measured using AAS. Finally, the percentage removal of lead(II) (R_2) was determined using the data obtained from AAS analysis employing the equation:

$$R_2 = \left(\frac{C_0 - C_t}{C_0}\right) \times 100 \qquad (1)$$

where C_0 = Initial concentration of lead(II) in solution (mmol L^{-1}), C_t = Concentration of lead(II) in solution at time t (mmol L^{-1}).

On the other hand, percentage decrease in *SEA* (R_3) was measured using the data obtained from the analysis of used adsorbent as follows:

$$R_3 = \left(\frac{S_0 - S_t}{S_0}\right) \times 100 \qquad (2)$$

where S_0 = *SEA* of uninhibited *AIB* ((g peptide formed)/(g *AIB* × h)), S_t = *SEA* of *AIB* at time t after contacting with lead(II) solution ((g peptide formed)/(g *AIB* × h)).

2.3.3. Equilibrium study

To assess the extent of metal removal using *AIB*, equilibrium study was performed. Five grams *AIB* were contacted with 30 mL of lead(II) solution having different initial concentrations for 30 min at 35˚C and pH 7. The initial concentration of lead(II) varied in the range of 0.0048–0.14 mmol L^{-1}. The beads were separated from the solution and residual metal concentrations were measured using AAS.

2.3.4. Determination of optimum condition for removal of lead(II) by AIB using RSM

2.3.4.1. Experimental. Though kinetic study is efficient to elucidate the mechanism of removal of lead(II) from solution, it is unable to optimize the process condition for the same. Therefore, another set of batch studies were performed to optimize the process condition for removal of lead(II) using *AIB*. *RSM* was used once again for optimization. Three parameters viz., initial concentration of lead(II), weight of *AIB*, and pH were chosen as input factors, whereas percentage removal of lead(II) and percentage decrease in *SEA* were considered as responses. Since, in kinetic study, the percentage removal of lead(II) has been found to be the same, irrespective of the temperature of solution under the present range studied; temperature has not been considered as input parameter during optimization study. This has direct effect in reduction of cost of the process. Again, from kinetic study, it has been seen that after 20 min run time, the system reaches to its equilibrium value. Therefore, during optimization study, the run time was fixed to 30 min. Volume of solution was maintained at 30 mL in each case. The used adsorbent was separated from the solution and analyzed for *SEA*. Residual metal ion concentration in solution was measured by AAS.

2.3.4.2. Design of experiments. *RSM* was used to optimize the removal process and Design Expert software 8.0.6 was used for such study. The maximum (+1) and minimum (−1) levels of this process variables viz., initial concentration of lead(II), weight of *AIB*, and pH were kept 0.24 and 0.1 mmol L^{-1}, 8 and 3 g, and 10 and 4, respectively. Twenty experiments were carried out to cover up the total range of combinations of variables as designed by the software. ANOVA was employed to analyze the data for optimization of removal condition. The used adsorbent produced at the optimum removal condition was termed as spent alginate immobilized bromelain (*SAIB*) and used for recovery study of lead(II).

2.4. Recovery of lead(II) from AIB-lead(II) complex

The present investigation was performed to assess the recovery efficiency of the process. One gram *SAIB* was treated at different pH condition viz., pH 2, 5, 7, and 9. Initially, distilled water was taken to perform the experiment at pH 7. Later, 0.1 (N) NaOH and 0.1 (N) HCl were used to maintain the required pH. Volume of solution was maintained at 100 mL and time of experiment in each case was 30 min. At the end, solid was separated from solution. The final lead(II) concentration in solution was analyzed by AAS.

3. Theoretical analysis

3.1. Empirical modeling

During kinetic study, five input parameters viz., initial concentration of lead(II) (A_2), weight of *AIB* (B_2), pH (C_2), temperature (D_2), and time (E_2) have been varied and the efficiency of *AIB* has been judged in terms of two output variables such as percentage removal of lead(II) (R_2) and percentage decrease in *SEA* of immobilized bromelain (R_3). On binding of lead(II) with *AIB*, enzymatic activity of immobilized bromelain is decreased. In other words, lower enzymatic activity signifies the removal of lead(II) from solution. Thus, empirical models have been proposed to assess the effect of these input parameters on these responses.

At the first stage, each response has been expressed as the function of five input parameters individually.

$$R_2 = f_1(A_2, B_2, C_2, D_2, E_2) \tag{3}$$

$$R_3 = f_2(A_2, B_2, C_2, D_2, E_2) \tag{4}$$

Assuming Power Law form of equation in each case, the two output variables can be expressed as follows:

$$R_2 = \phi_1 A_2^{\alpha_1} B_2^{\beta_1} C_2^{\chi_1} D_2^{\delta_1} E_2^{\varepsilon_1} \tag{5}$$

$$R_3 = \phi_2 A_2^{\alpha_2} B_2^{\beta_2} C_2^{\chi_2} D_2^{\delta_2} E_2^{\varepsilon_2} \tag{6}$$

Using non-dimensional form of the input parameters, Eqs. (5) and (6) may be rewritten as follows:

$$R_2^* = \eta_1 A_2^{*\alpha_1} B_2^{*\beta_1} C_2^{*\chi_1} D_2^{*\delta_1} E_2^{*\varepsilon_1} \tag{7}$$

where

$$\eta_1 = ((\phi_1 A_{2,\max}^{\alpha_1} B_{2,\max}^{\beta_1} C_{2,\text{mean}}^{\chi_1} D_{2,\text{mean}}^{\delta_1} E_{2,\max}^{\varepsilon_1})/R_{2,\max}) \tag{7a}$$

Similarly,

$$R_3^* = \eta_2 A_2^{*\alpha_2} B_2^{*\beta_2} C_2^{*\chi_2} D_2^{*\delta_2} E_2^{*\varepsilon_2} \tag{8}$$

And

$$\eta_2 = ((\phi_2 A_{2,\max}^{\alpha_2} B_{2,\max}^{\beta_2} C_{2,\text{mean}}^{\chi_2} D_{2,\text{mean}}^{\delta_2} E_{2,\max}^{\varepsilon_2})/R_{3,\max}) \tag{8a}$$

where $A_2^* = \frac{A_2}{A_{2,\max}}$, $B_2^* = \frac{B_2}{B_{2,\max}}$, $C_2^* = \frac{C_2}{C_{2,\text{mean}}}$, $D_2^* = \frac{D_2}{D_{2,\text{mean}}}$, $E_2^* = \frac{E_2}{E_{2,\max}}$, $R_2^* = \frac{R_2}{R_{2,\max}}$, $R_3^* = \frac{R_3}{R_{3,\max}}$

where $A_{2,\max}$ = maximum initial concentration of lead(II) (mmol L^{-1}), $B_{2,\max}$ = maximum weight of *AIB* (g), $C_{2,\text{mean}}$ = mean pH, $D_{2,\text{mean}}$ = mean Temperature (°C), $E_{2,\max}$ = maximum time (min), $R_{2,\max}$ = maximum percentage removal of lead(II) (%), and$R_{3,\max}$ = maximum percentage decrease in *SEA* (%).

The values of $A_{2,\max}$, $B_{2,\max}$, $C_{2,\text{mean}}$, $D_{2,\text{mean}}$, $E_{2,\max}$, $R_{2,\max}$, and $R_{3,\max}$ are 0.24 mmol L^{-1}, 7 g, 7, 35°C, 20 min, 99.5, and 75%, respectively, and the values of all constants can be determined by non-linear regression analysis using the experimental data obtained during kinetic study.

In the present investigation, the abatement of lead(II) using *AIB* has been modeled in another way, where percentage removal of lead(II) has been expressed as a function of all five input parameters and percentage decrease in *SEA* as well. The logic behind this may be as follows: lead(II) can bind with the thiol (–SH) group present at the active site of bromelain and thereby enzymatic activity of immobilized bromelain is decreased. Decrease in *SEA* means the less availability of active group (–SH group) responsible for the binding of lead(II), which in turn decreases the percentage removal of lead(II). Thus, percentage removal of lead(II) is influenced by the five input parameters and percentage decrease in *SEA* as well.

Eqs. (3) and (5) have, therefore, been rearranged to express R_2 as a function of R_3.

$$R_2 = f_1'(A_2, B_2, C_2, D_2, E_2, R_3) \tag{9}$$

$$R_2 = R_3^{\theta} \times \frac{\phi_1}{\phi_2^{\theta}} A_2^{(\alpha_1-\alpha_2\theta)} B_2^{(\beta_1-\beta_2\theta)} C_2^{(\chi_1-\chi_2\theta)} D_2^{(\delta_1-\delta_2\theta)} E_2^{(\varepsilon_1-\varepsilon_2\theta)} \tag{10}$$

Using non-dimensional form of the input parameters, Eq. (10) may be re-written as

$$R_2^* = \psi R_3^{*\theta} A_2{}^{*(\alpha_1-\alpha_2\theta)} B_2{}^{*(\beta_1-\beta_2\theta)} C_2{}^{*(\chi_1-\chi_2\theta)} D_2{}^{*(\delta_1-\delta_2\theta)} E_2{}^{*(\varepsilon_1-\varepsilon_2\theta)} \tag{11}$$

$$\text{where} \quad \psi = \frac{\phi_1}{\phi_2^{\theta}} \times ((R_{3,\max}^{\theta} A_{2,\max}^{(\alpha_1-\alpha_2\theta)} B_{2,\max}^{(\beta_1-\beta_2\theta)} \times C_{2,\text{mean}}^{(\chi_1-\chi_2\theta)} D_{2,\text{mean}}^{(\delta_1-\delta_2\theta)} E_{2,\max}^{(\varepsilon_1-\varepsilon_2\theta)})/R_{2,\max}) \tag{11a}$$

Here ϕ_1, ϕ_2, θ, ψ = Constants.

The values of all exponents and constants have been determined by non-linear regression analysis using the experimental data obtained during kinetic study. Microsoft Excel 2007 has been used for such purpose.

4. Results and discussion

4.1. Optimum condition for preparation of AIB using RSM

To enhance active matter space required for better adsorption, bromelain has been immobilized on calcium alginate bead. Table 1 represents statistical experimental design made by the software for immobilization. ANOVA analysis suggests that the quadratic model is significant. The final equation in terms of coded factors is mentioned below:

$$R_1 = 0.03 + 1.78 \times 10^{-3} \times A_1 + 7.825 \times 10^{-3} \times B_1 + 1.05 \times 10^{-3} \times C_1 + 1.275 \times 10^{-3} \times D_1 + 6.8 \times 10^{-4} \times A_1 \times B_1 - 1.08 \times 10^{-3} \times A_1 \times C_1 - 1.125 \times 10^{-3} \times A_1 \times D_1 + 1.038 \times 10^{-3} \times B_1 \times C_1 - 7.25 \times 10^{-4} \times B_1 \times D_1 - 1.6 \times 10^{-3} \times C_1 \times D_1 - 4.29 \times 10^{-4} \times A_1^2 - 8.66 \times 10^{-4} \times B_1^2 - 7.9 \times 10^{-5} \times C_1^2 - 5.679 \times 10^{-3} \times D_1^2 \tag{12}$$

The higher values of R^2 (0.95) and R^2_{adj} (0.89) indicate the well fitting of experimental values. According to the software, conjugate effect of initial concentration of calcium chloride and pH on *SEA* has been found to be most significant.

Fig. 1 elucidates the combined effect of initial concentration of calcium chloride and pH on *SEA* at constant initial concentrtion of sodium alginate ($A_1 = 35$ g/L) and constant initial concentration of bromelain ($B_1 = 35$ g/L). *SEA* increases from 0.02 to 0.025 when initial concentration of calcium chloride changes from 20 to 50 g/L at pH 4, but *SEA* remains almost constant at pH 9 for the same range of initial concentration of calcium chloride. On the other hand, *SEA* changes from 0.02 to 0.026 and 0.025 to 0.024 when pH changes from 4 to 9 at constant initial concentration of calcium chloride 20 and 50 g/L, respectively.

For assessment of optimization condition for immobilization of bromelain on calcium alginate bead, the following criteria have been fixed: initial concentration of sodium alginate: "minimize", initial concentration of enzyme: "minimize", initial concentration of calcium chloride: "minimize" and pH: "equal to 7," and response i.e. Specific Enzyme Activity: "maximize". The intention for this is to prepare the bead at low cost and make the process cost effective. The optimum condition for immobilization as specified by software is: initial concentration sodium alginate: 20 g/L, initial concentration of enzyme: 21.09 g/L, and initial concentration of calcium chloride: 20 g/L, pH 7 at 35℃ temperature. The immobilized sample obtained at this specified condition is termed as *AIB* and used for further studies. The *SEA* of *AIB* (0.0185) matches quite well with predicted response (0.019).

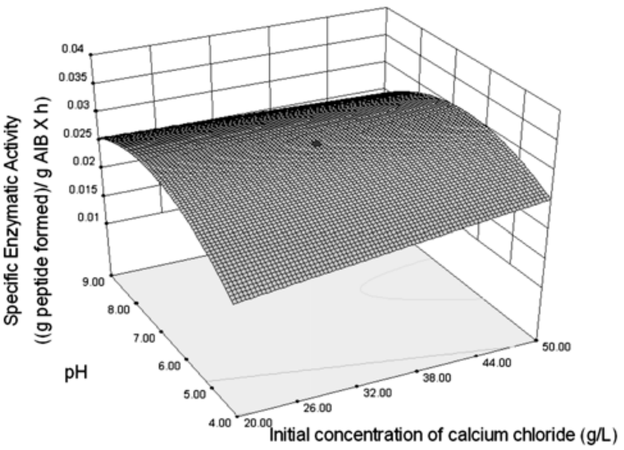

Fig. 1. Combined effect of initial concentration of calcium chloride and pH on *SEA*.

4.2. Characterization of *AIB*

4.2.1. FTIR study

Fig. 2(a) and (b) represent the FTIR spectra of *AIB* and *AIB*-lead(II) complex, respectively. The wave

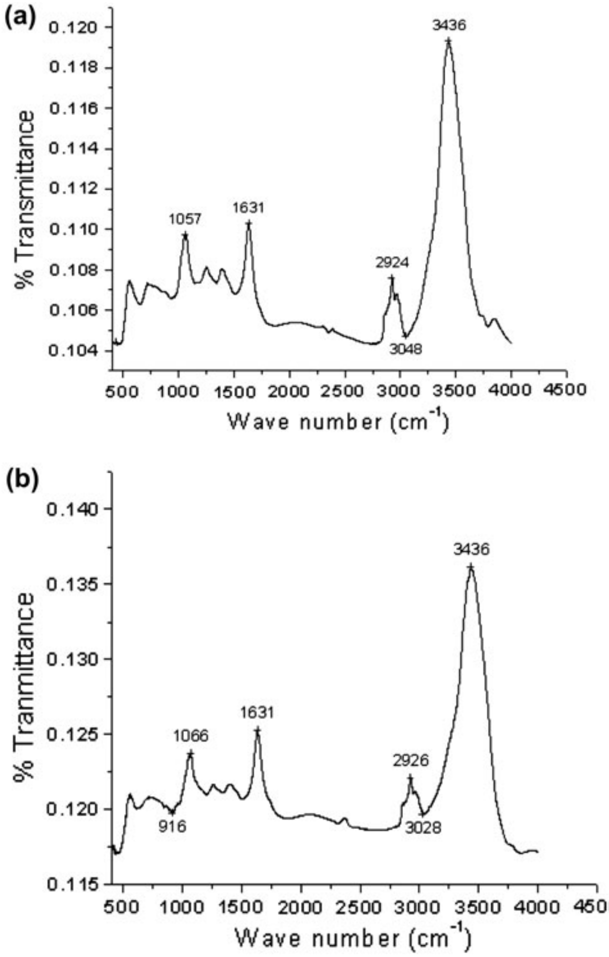

Fig. 2. (a) FTIR spectra of *AIB*, and (b) FTIR spectra of *AIB*-lead(II) complex.

number has been varied in the range of 500–4,000 cm^{-1}. Three peaks for C–N stretch, C–H stretch, and =C–H stretch in *AIB* are found at 1,057, 2,924, and 3,048 cm^{-1}, while in *AIB*-lead(II) complex, they are shifted to 1,066, 2,926, and 3,028 cm^{-1}, respectively. Since FTIR probes the bulk of the sample, it is not expected that there would be major changes in the spectrum for adsorbed materials. It can only be a preliminary investigation into the binding of lead(II) with *AIB* and not a confirmative test. Therefore, SEM and EDS techniques, which essentially probe surface topography and elemental composition near the surface, respectively, are employed in order to confirm the binding of lead(II) with *AIB*.

4.2.2. SEM and EDS

The SEM photographs of *AIB*, *AIB*-lead(II) complex, and *AIB* after recovery of lead(II) have been shown in Fig. 3(a), (b), and (c), respectively. After comparing the figures, it can be stated that the surface of *AIB* bound with lead(II) has become smoother than that of *AIB*. After the recovery of lead(II) from *AIB,* the surface becomes rough again. Fig. 3(d) represents the EDS study of *AIB*-lead(II) complex, which confirms the binding of lead(II) with *AIB*.

4.2.3. Determination of temperature optima and pH optima

For finding out temperature optima, experiments have been performed at three temperature viz., 35, 50, and 70℃ at pH 7. The optimum temperature of *AIB* has been found to be 50℃. Forty percent decrease in *SEA* has been observed both at 35 and 70℃ in comparison with that at 50 ℃. This may be due to the higher diffusion at 50 ℃ in comparison with that at 35 and 70℃. Again for finding out pH optima, *SEA* has been measured at three pH conditions such as 5, 7, and 9, keeping temperature constant at 35℃. The optimum pH of *AIB* has been found to be 5. Only about three percent *SEA* has been reduced both at pH 7 and 9.

4.2.4. Determination of temperature stability and pH stability

AIB has maintained its enzymatic activity in a wide range of temperature (4–70℃) when it is incubated at those temperatures separately for 1 h. Almost equal activity has been observed both at 4 and 35℃. *AIB* shows its highest activity at 50 ℃, while very low activity is observed at 70 ℃. Again, during assessment of pH stability, it is seen that *AIB* has retained its activity in a wide range of pH, viz., pH 5–pH 9, when it is incubated in buffer solutions having different pH at 35 ℃ for 1 h. Maximum activity is obtained at pH 9. Almost equal activity is found at pH 5 and pH 7. To make the removal process cost effective, the experimental work has been performed at room temperature i.e. 35 ℃ and at pH 7.

4.2.5. Determination of kinetic parameters of protein hydrolysis

The kinetic parameters of protein hydrolysis such as $V_{\max}$, K_m, and k_2 of free enzyme and *AIB* are found out using Lineweaver–Burk equation. From kinetic study it is observed that value of Michaelis–Menten constant K_m) is lower (2.05 g casein/L) in case of *AIB* than free enzyme (5.57 g casein/L) indicating the higher affinity of immobilized enzyme for the substrate over the free enzyme. The value of maximum reaction velocity ($V_{\max}$) has been seen to be decreased in case of *AIB* (0.1 g peptide formed/(g *AIB* × h)) than in free enzyme (0.5 g peptide formed/(g *AIB* × h)). The value of rate constant for enzymatic reaction (k_2) has also been seen to be reduced in case of *AIB* (1.95 g peptide formed/(g $AIB^2 \times$ h)) than in free enzyme (73.9 g peptide formed/(g $AIB^2 \times$ h)). The total enzyme content in *AIB* has been determined by Kjeldal procedure. 0.487 mg bromelain has been found to be immobilized in one gram of *AIB*.

4.3. Removal of lead(II) from simulated solution using AIB

4.3.1. Comparative study of removal of lead(II) using calcium alginate and AIB

To examine the metal binding capability of bromelain, a comparative study of removal of lead(II) has been done using calcium alginate and *AIB* separately as metal-removing agent. It has been observed that one gram of calcium alginate can remove 0.014 mg lead, whereas 1 gm of *AIB* is efficient to remove 0.812 mg lead from simulated solution under identical condition. Therefore, it can be stated that bromelain, on immobilization on calcium alginate is 5–6 times more effective for the removal of lead(II) from aqueous solution than that with only calcium alginate. Thus, bromelain immobilized on suitable solid matrix can be considered as an efficient tool for removal of lead(II) from its aqueous solution.

4.3.2. Kinetic study on removal of lead(II)

To investigate the efficacy of *AIB* as a means of lead(II) eradication from simulated solution, a systematic and thorough experimentation has been done by

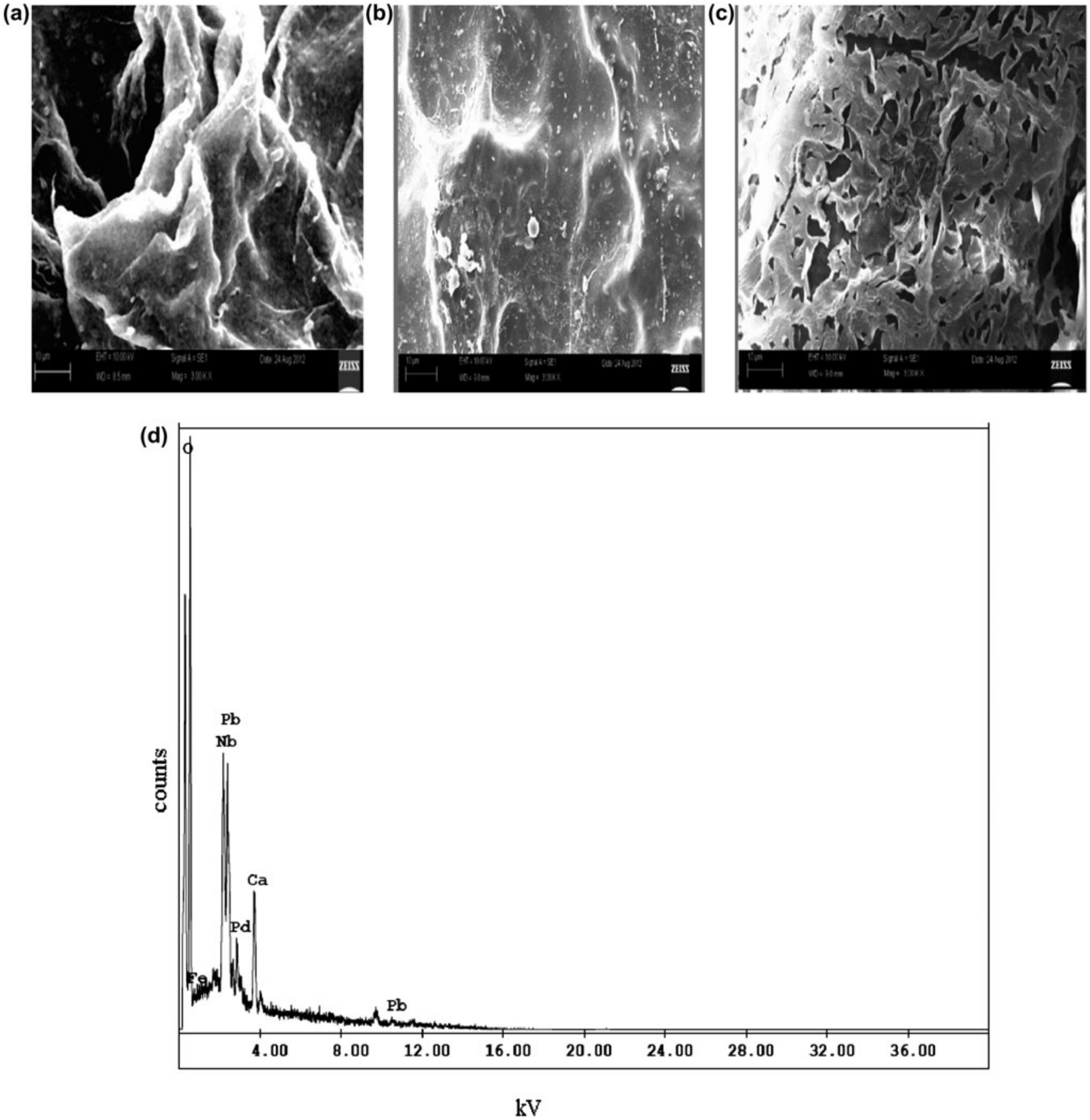

Fig. 3. (a) SEM of *AIB*, (b) SEM of *AIB*-lead(II) complex, (c) SEM of *AIB* after recovery of lead(II), and (d) EDS of *AIB*-lead(II) complex.

contacting the simulated solution of lead acetate with *AIB* in a batch contactor. Four operating parameters viz., initial concentration of lead(II), weight of *AIB*, pH, and temperature have been varied in the range of 0.0048–0.24 mmol L^{-1}, 3–7 g, 5–7, and 25–45˚C, respectively, in a systematic manner. During kinetic study, both solid *AIB* and solution have been analyzed at different time intervals to determine *SEA* of used *AIB* and residual lead(II) concentration, respectively. Finally, the variations of *SEA* as well as the percentage removal of metal ion with time have been represented. As far as known, no article has been published so far to show the interconnection between these two outputs and their relationship with time. Under this section, initially, the kinetics of metal removal has been discussed. Later, the variation in *SEA* with time has been elucidated. Finally, an empirical model has been proposed to establish the interconnection between the percentage removal of lead(II) and percentage decrease in *SEA*.

4.3.2.1. Kinetics of metal removal using AIB. Percentage removal of lead(II) from simulated solution at different values of four input parameters has been done. It is observed that in all cases, the initial rate of adsorption of lead(II) is very fast and major removal has been obtained within 2 min. In all cases, the curves become parallel to abscissa after 10 min, which designate the attainment of equilibrium value.

Fig. 4(a) shows the graphical representation of the percentage removal of lead(II) with time when initial concentration of lead(II) has been varied from 0.0048 to 0.24 mmol L^{-1}, keeping all other parameters viz., weight of *AIB*, pH, and temperature constant at 5 g, 7, and 35°C, respectively. Maximum 99.5% removal has been achieved at 0.0048 mmol L^{-1} initial concentration of lead(II). As the maximum removal has been obtained at lower initial concentration, the present removal process can be categorized to be kinetically driven rather than mass transfer driven. 91.5, 92.2, and 91.2% removal of lead(II) have been obtained when initial concentrations have been kept as of 0.048, 0.14, and 0.24 mmol L^{-1}, respectively. This implies that the binding sites of bromelain have not been saturated up to the present range of initial concentration investigated. Thus, it can be stated that bromelain has excellent capacity to bind with lead(II) and thereby usage of *AIB* can be a promising technology for treatment of lead(II) laden waste water.

Fig. 4(b) represents the percentage removal of lead(II), considering the weight of *AIB* as varying parameter. Initial concentration of lead(II), pH, and temperature have been kept constant at 0.048 mmol L^{-1}, 7, and 35°C, respectively, while weight of *AIB* varied from 3 to 7 g. It is seen that with increase in the amount of *AIB* from 3 to 7 g, the percentage removal of lead(II) from solution increased from 88.2 to 93.6%. This may be due to the availability of more amount of enzyme (bromelain) in the system with the increase in weight of *AIB*.

Fig. 4(c) represents the percentage removal of lead(II) considering pH as varying parameter. Initial concentration of lead(II), weight of *AIB*, and temperature have been maintained constant at 0.048 mmol L^{-1}, 5 g, and 35°C, respectively, and pH has been varied from 5 to 7. Maximum 98.7% removal has been obtained at pH 5, whereas 91.5% removal has been achieved at pH 7.

Fig. 4(d) represents the percentage removal of lead(II) with time at different temperature ranging from 25 to 45°C, keeping all other parameters viz., initial concentration of lead(II), weight of *AIB*, and pH constant at 0.048 mmol L^{-1}, 5 g, and 7, respectively. It has been observed that 90.5, 91.5, and 92.5% removal have been attained at 25, 35, and 45°C, respectively. Therefore, it can be stated that temperature has trivial effect on percentage removal. It will be valid only when bromelain remains equally active for the temperature range studied.

A comparative study has been made to judge the efficacy of *AIB* in removal of lead(II) against the other adsorbents used in previous studies [5,15–18]. The results are shown in Table 2. It is seen that the percentage removal of lead(II) using *AIB* is comparable with the other adsorbents.

To investigate the mechanism of adsorption process, three kinetic models viz., Lagergren model, pseudo-second-order model (PSOM), and Morris Weber model have been used [19,20]. The kinetic parameters as obtained by fitting the experimental data to those models are shown in Table 3**.** From Table 3, it is evident that experimental data fit most satisfactorily $R^2 = 0.99$) to the PSOM than any other model in all the cases. It signifies that the removal of lead(II) using *AIB* is of chemisorption in nature and the abatement process is kinetically controlled.

4.3.2.2. Variation of SEA with time during kinetic study. To investigate the inhibitory effect of metal on enzymatic activity of immobilized bromelain, the *SEA* of each immobilized enzyme sample obtained after contacting it with metal solution has been analyzed. The used *AIB* obtained during kinetic study of metal removal has been taken for analysis of *SEA*. To compare the extent of inhibition, the *SEA* of untreated *AIB* is also measured and considered as control. It has been found that *SEA*s of metal treated samples are lower than that of control.

Fig. 5(a) shows the variation of *SEA* of used *AIB* with time obtained by contacting *AIB* with metal solutions having different initial concentrations. The *SEA* of untreated *AIB* has also been shown in the same figure and designated as control. From Fig. 5(a), it is evident that *SEA* of each used *AIB* is less than its control value and it decreases with time. Therefore, it can be said that metal has inhibited the enzymatic action of *AIB*. This may happen due to the binding of metal ions with the sulfhydryl (–SH) groups present at the active site of the enzyme. After 8 min, the *SEAs* do not decrease and attain its equilibrium value. Fig. 5(a) also reveals that the extent of inhibition is different in each case and it depends on the initial concentration of

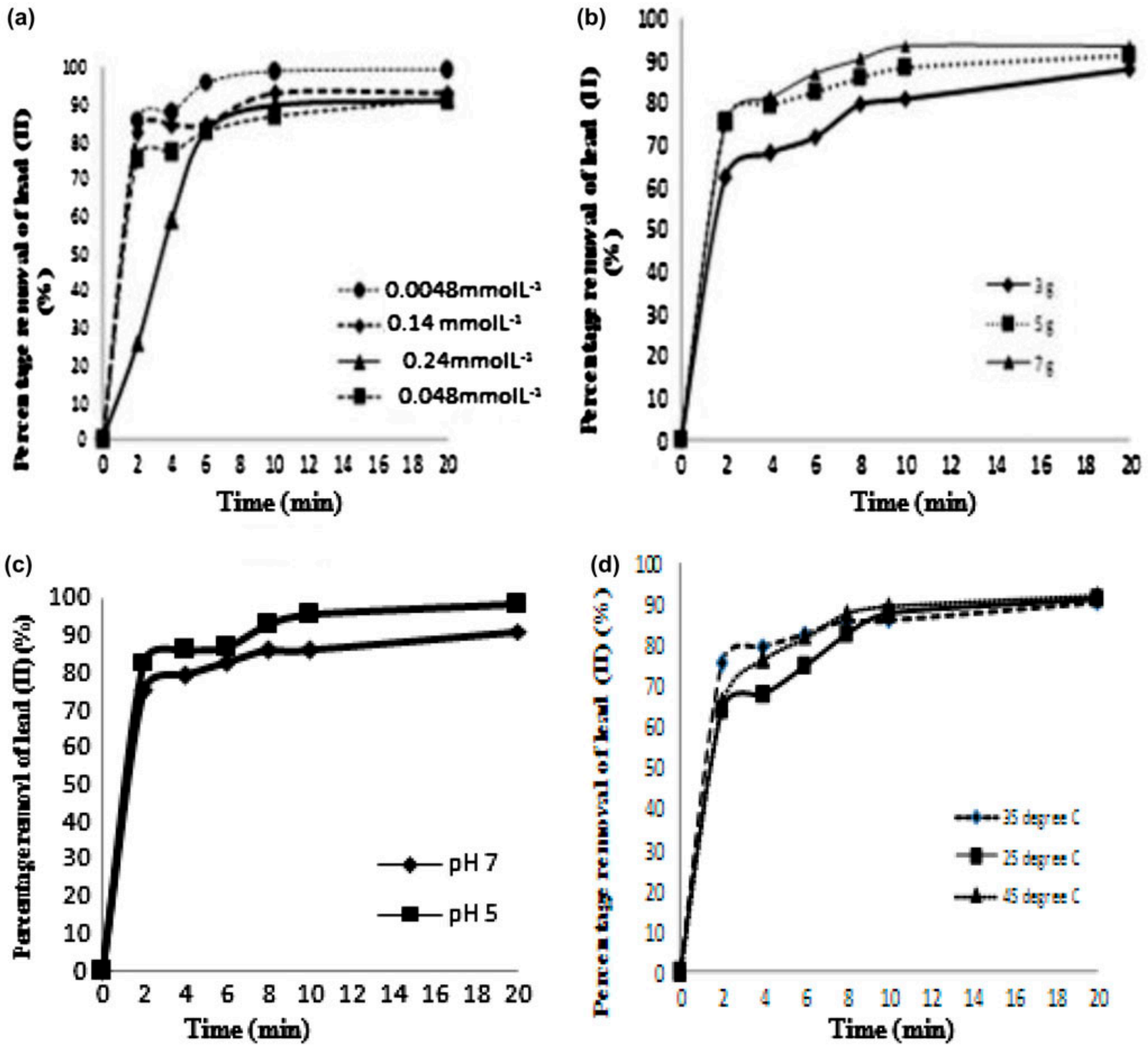

Fig. 4. Time histories of percentage removal of lead(II) when (a) initial concentration of lead(II) as parameter, (b) weight of *AIB* as parameter, (c) pH as parameter, and (d) temperature as parameter.

metal ion present in the solution. The rate of decrease in *SEA* has been found to be more in case of lowest initial concentration of solution i.e. 0.0048 mmol L^{-1}. It is also evident from Fig. 5(a) that the rate of decrease in *SEA* does not depend on initial concentration vis-á-vis mass transfer driving force expressed as the difference between bulk concentration and surface concentration and hence reaction-rate controlled nature of removal process has been reconfirmed.

Fig. 5(b) represents the variation of *SEA* of used *AIB* samples with time when different amounts of *AIB* have been treated with metal solution having initial concentration of 0.048 mmol L^{-1}. Result shows that *SEA* decreases more for 7 g *AIB* than the other cases. This implies the maximum removal with 7 g *AIB*.

Fig. 5(c) shows *SEA* of used *AIB* with time when *AIB* has been contacted with metal solutions having different pH. It has been observed that *SEA* of used *AIB* decreases with time in two cases. Metal has been attached with –SH group of bromelain and thereby a gradual decrease in *SEA* has been observed in two cases.

Table 2
Removal of lead(II) using various adsorbents

Adsorbent	Lead(II) concentration (mmol L^{-1})	Sorbent dose	Removal of lead(II) (%)	References
Alginate beads	0.1128 ± 0.013	10^4 beads/L	90 ± 2	[5]
Chlorella vulgaris cells, live	0.1094	3×10^{10} cells/L	88.6	[5]
Alginate immobilized *Chlorella vulgaris* cells, dead	0.1219 ± 0.0024	3×10^{10} cells	92 ± 3	[5]
Bed sediments	0.0096–0.12	5 g/L	90	[15]
Bagasse fly ash	0.024–0.336	10 g/L	96–98	[16]
Saw dust	0.024	40 g/L	98.8	[17]
Rice husk	0.12	2 g/L	98.15	[18]

Table 3
Values of kinetic parameters

		Parameters varied											
		Initial conc. of lead (II) (mmol L^{-1})			Weight of *AIB* (g)			pH			Temperature (°C)		
Model equation		0.024	0.048	0.24	3	5	7	5	7	9	25	35	45
Morris Weber model	k'_M	0.009	0.014	0.07	0.026	0.017	0.013	0.031	0.027	0.02	0.015	0.017	0.016
	R^2	0.31	0.16	0.79	0.66	0.04	0.04	0.56	0.61	0.48	0.7	0.45	0.61
Lagergren model	k'_L	0.6	0.47	0.25	0.41	0.47	0.71	0.09	0.14	0.11	0.24	0.29	0.14
	R^2	0.59	0.61	0.63	0.69	0.61	0.73	0.86	0.21	0.36	0.77	0.98	0.86
PSOM	k'_P	465.8	110.1	1.19	4.6	48.26	62.65	39.69	44.08	30.1	14.52	38.09	28.26
	R^2	0.99	0.99	0.97	0.94	0.94	0.99	0.99	0.99	0.99	0.98	0.99	0.99

The effect of metal binding on enzymatic activity at different temperatures has also been studied. Fig. 5(d) represents *SEA*s of used *AIB* obtained by contacting 5 g of *AIB* with metal solutions having initial concentration of 0.048 mmol L^{-1} at different temperatures against time. It is seen that *SEA* decreases gradually at 35°C, whereas the lowest *SEA* of used *AIB* has been obtained within 2 min at 45°C. This may be due to the higher diffusion of metal ion at 45°C. Metal ion can contact more with immobilized bromelain within first 2 min at this temperature. Thus, at 45°C, the maximum decrease in *SEA* has been observed during this period and it remains the same throughout the experiment.

4.3.2.3. Analysis of the equations obtained during empirical modeling. During empirical modeling, initially two responses have been expressed as the functions of four operating parameters and time individually using Power-law form equations. The values of exponents viz., $\alpha_1, \beta_1, \chi_1, \delta_1$, and ε_1 as used in Eqs. (5) and (7) and those of $\alpha_2, \beta_2, \chi_2, \delta_2$, and ε_2 as used in Eqs. (6) and (8) have been calculated by non-linear regression technique using the kinetic data obtained during experimentation. η_1 and η_2 have been evaluated by minimizing average "Sum of Error Square" i.e. average $\sum \text{error}^2$ with respect to both η_1 and η_2 separately by solving Eqs. (7) and (8) numerically. The values of all these exponents and constants are shown in Table 4. Similarly, in the second case, when percentage removal has been expressed in terms of all input parameters as well as percentage decrease in *SEA* (Eqs. (9), (10), and (11)), the value of θ has been measured by minimizing average "Sum of Error Square" i.e. average $\sum \text{error}^2$ with respect to θ by solving Eq. (11) numerically. The value is shown in Table 4. From the values of average $\sum \text{error}^2$, it is evident that percentage removal of lead(II) can be predicted in better way when it is expressed as a function of all input parameters as well as percentage decrease in *SEA* (Eq. (11): average $\sum \text{error}^2 = 0.02$ in comparison with other case, Eq. (7): average $\sum \text{error}^2 = 0.041$, and Eq. (8): average $\sum \text{error}^2 = 0.042$).

4.3.3. Equilibrium study

The data obtained during equilibrium study are fitted to different adsorption isotherm models viz., Langmuir model and Freundlich model. The data has

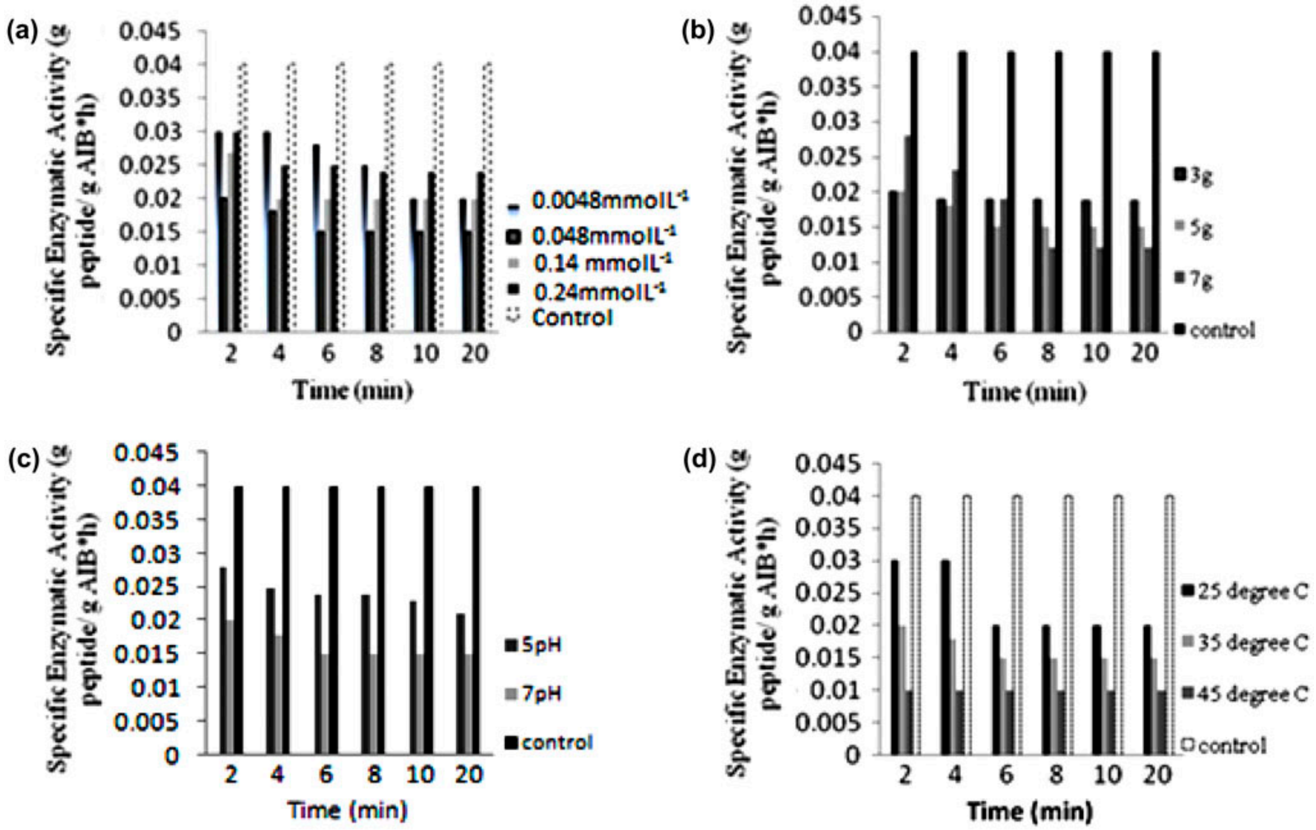

Fig. 5. Variation of *SEA* with time when (a) initial concentration of lead(II) as parameter, (b) weight of *AIB* as parameter, (c) pH as parameter, and (d) temperature as parameter.

Table 4
Values of constants and exponents as used in empirical modeling

Response	Exponents					Key parameters for minimizing average $\sum$ error^2	Average $\sum$ error^2
R_2^* used in Eq. (7)	$\alpha_1 = 0.039$	$\beta_1 = 0.189$	$\chi_1 = 0.2$	$\delta_1 = 0.26$	$\varepsilon_1 = 0.14$	$\eta_1 = 1.06$	0.041
R_3^* used in Eq. (8)	$\alpha_2 = 0.09$	$\beta_2 = 0.13$	$\chi_2 = 1.12$	$\delta_2 = 1.48$	$\varepsilon_2 = 0.167$	$\eta_2 = 0.94$	0.042
R_2^* used in Eq. (11)	$(\alpha_1 - \alpha_2\theta) = 0.019$	$(\beta_1 - \beta_2\theta) = 0.16$	$(\chi_1 - \chi_2\theta) = -0.14$	$(\delta_1 - \delta_2\theta) = -0.077$	$(\varepsilon_1 - \varepsilon_2\theta) = 0.102$	$\theta = 0.22$	0.02

been fitted most satisfactorily to Langmuir model. The values of correlation coefficients have been obtained as follows: for Langmuir model, $R^2 = 0.989$ and for Freundlich model, $R^2 = 0.774$. The Langmuir constants viz., solid phase concentration of adsorbate for complete monolayer formation (q^0) and adsorption equilibrium constant (K_L) have been found to be 0.812 mg lead (II)/g of *AIB* and 0.14 (L/mg adsorbate), respectively.

4.3.4. Determination of optimum condition for removal of lead(II) by AIB using RSM

The optimum condition for removal of lead(II) using *AIB* has been evaluated by employing *RSM*. Similar to kinetic study, both used *AIB* and solution have been analyzed to assess *SEA* and residual metal ion concentration, respectively. Percentage removal of lead(II) and percentage decrease in *SEA* have been considered as responses. These responses have been statistically modeled. Thus, knowing the values of input factors, one can predict both the responses and thereby their relationship can be assessed. As far the knowledge of present research group is concerned, no such study has been done so far. The variation of input factors as designed by the software and the corresponding responses have been shown in Table 5. According to the software, no transformation is required. Quadratic model has been suggested to fit the experimental data for both the responses. The final equations in terms of coded factors are given below:

$$R_2 = 89.9 - 2.65 \times A_2 + 2.3 \times B_2 - 16.33 \times C_2 - 0.35 \times A_2 \times B_2 - 1.5 \times A_2 \times C_2 + 0.38 \times B_2 \times C_2 - 0.28 \times A_2^2 - 2.52 \times B_2^2 - 7.21 \times C_2^2 \tag{13}$$

$$R_3 = 57.39 - 1.06 \times A_2 + 1.06 \times B_2 + 3.37 \times C_2 - 8.69 \times A_2 \times B_2 + 4.69 \times A_2 \times C_2 - 4.69 \times B_2 \times C_2 + 6.93 \times A_2^2 + 6.93 \times B_2^2 + 7.28 \times C_2^2 \tag{14}$$

The higher values of R^2 (0.9648 and 0.9684) and R^2_{adj}(0.9331 and 0.94) have been obtained for R_2 and R_3, respectively. This indicates the satisfactory fitting of experimental data to the model equations.

The interactive effect of initial concentration of lead(II) and pH on percentage removal of lead(II) at constant weight of *AIB* ($B_2 = 5.5$ g) has been shown in Fig. 6(a). The maximum removal has been obtained at pH 4 and it decreases uniformly with increase in pH. Percentage removal of lead(II) changes from 99.8 to 70.2% and 97.5–62.3% when pH changes from 4 to 10 at constant initial concentration of lead(II) at 0.1 and 0.24 mmol L^{-1}, respectively. The decrease of removal at higher pH may be due to some conformational change of bromelain.

The combined effect of initial concentration of lead(II) and pH on percentage decrease in *SEA* at constant weight of *AIB* ($B_2 = 5.5$ g) has been illustrated in Fig. 6(b). Percentage decrease in *SEA* has been changed from 73.5 to 62.3% when initial concentration of lead(II) changes from 0.1 to 0.24 mmol L^{-1} at constant pH 4. This may be due to the more blockage of active sites of immobilized bromelain molecules with lead(II) at higher initial concentration. Alternatively, percentage decrease in *SEA* remains almost the same at pH 10, while initial concentration of lead(II) has been changed from 0.1 and 0.24 mmol L^{-1}.

To optimize the lead(II) removal condition, the following criteria have been set: initial concentration of lead(II): "in range", weight of *AIB*: "in range", and pH: "equal to 7"; and response i.e. percentage removal of lead(II): "maximize". According to the software, 92.96% lead removal has been achieved when 6.81 g of *AIB* was treated with 0.1 mmol L^{-1} of lead(II) solution at pH 7 and 35°C. Under the present optimized condition, the predicted response (92.96%) matches well with the experimental observation (91.9%). At this condition, the corresponding predicted value of percentage decrease in *SEA* (72.39%) matches satisfactorily with experimental observation (71.5%). The used *AIB* obtained by removing lead(II) at optimum condition is termed as *SAIB* and is used for recovery study.

Table 5
Experimental design for removal of lead(II)

Run	Initial concentration of lead(II) (A_2, mmol L^{-1})	Weight of *AIB* (B_2, g)	pH (C_2)	Percentage removal (R_2, %)	Percentage decrease in *SEA* (R_3, %)
1	0.17	1.30	7.00	81.2	75
2	0.17	9.70	7.00	91.3	75
3	0.1	8.00	10.00	66.6	86
4	0.24	3.00	10.00	54.5	97.5
5	0.17	5.50	7.00	89.7	57.5
6	0.29	5.50	7.00	87.5	75
7	0.05	5.50	7.00	97.7	75
8	0.24	3.00	4.00	92.7	72.5
9	0.1	8.00	4.00	97.3	98.5
10	0.1	3.00	10.00	60.5	72.5
11	0.17	5.50	7.00	89.7	57.5
12	0.17	5.50	7.00	89.7	57.5
13	0.17	5.50	7.00	89.7	57.5
14	0.24	8.00	10.00	57.1	72.5
15	0.17	5.50	7.00	89.7	57.5
16	0.1	3.00	4.00	94.8	70
17	0.17	5.50	7.00	89.7	57.5
18	0.17	5.50	12.05	48.9	84.5
19	0.24	8.00	4.00	95.9	70
20	0.17	5.50	1.95	97.1	67.5

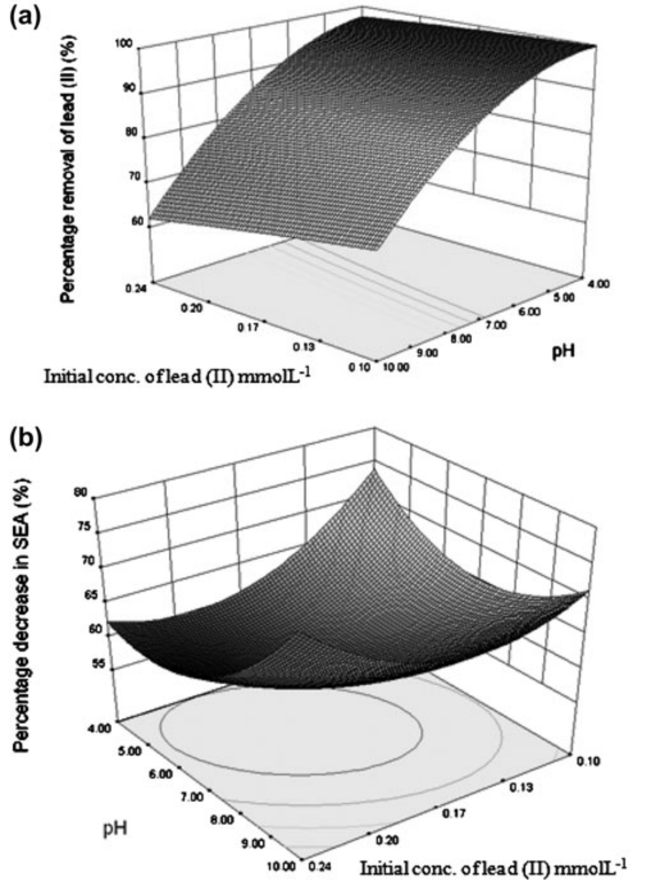

Fig. 6. Combined effect of initial concentration of lead(II) and pH on (a) percentage removal of lead(II), and (b) percentage decrease in *SEA*.

4.4. Recovery of lead(II) from AIB-lead(II) complex

Maximum 77.95% recovery of lead(II) has been obtained at pH 2 while negligible amount of recovery has been attained at pH 5, 7, and 9. The higher desorption of metal at very low pH may be due to blockage of sulfhydryl group with proton to bind with lead(II). At high pH, desorption is difficult due to strong binding of metal ion with deprotonated sulfhydryl group.

5. Conclusions

AIB, a novel adsorbent, has been used to remove lead(II) from simulated solution. Comparative study reveals that *AIB* can remove lead(II) 5–6 times more than native calcium alginate bead under identical condition. Percentage removal of lead(II) has been modeled empirically as a function of all input parameters and percentage decrease in *SEA*. Removal of lead(II) has also been optimized using *RSM*. However, it is a preliminary work and further development can be done encompassing a detailed parametric study with real wastewater.

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