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Column studies of heavy metal biosorption by immobilized *Spirulina platensis-maxima* cells

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ABSTRACT

The adsorption capacities of alginate, chitosan and immobilized *Spirulina platensis-maxima* cells were studied in a continuous packed-bed column, under dynamic condition. The composition of alginate and chitosan beads was optimized. Various operating conditions were applied; the effect of flow rate, column height and initial metal ion concentration were investigated. The alginate beads have the highest adsorption capacity for Cu(II), Cd(II) and Pb(II) ions, 3.4, 2.3 and 3.1 mmol g^{-1} per dry weight, respectively. The alginate-*Spirulina* system has slightly decreased adsorption capacity 3.1, 1.9 and 3 mmol g^{-1}. The uptake of Cu (II), Cd(II) and Pb(II) by chitosan and chitosan-*Spirulina* beads is 0.8, 0.9, 0.7 and 1, 0.8, 0.6 mmol g^{-1}. The Thomas and Yoon–Nelson models have been applied to analyse the breakthrough data. The results indicate feasible reuse of the beads at least in five sorption cycles.

Keywords: Heavy metal; Alginate; Chitosan; Packed-bed column; Modelling

1. Introduction

The growing industrial production is one of the main causes of the environmental pollution nowadays. Common and dangerous pollutions are caused by heavy metals, such as Cu, Zn, Pb and Cd. However, Cu and Zn are required as nutrients in trace amounts, but at high concentrations they become toxic. Lead and cadmium are toxic elements even at low levels. Main sources of the heavy metal pollution are automotive industry, electroplating, microelectronics, battery manufacturing, metallurgical processing and tanneries, which lead to an increase of heavy metals content in water and soil [1].

The removal of toxic heavy metals from wastewater is of great importance from environmental point of view. Environmental decontamination has attracted much attention for biotechnological processes, like biosorption and bioaccumulation. Studies of biosorption using a variety of biomass [2–6] have already

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given some promising results for removal of inorganic and organic pollutants from aqueous medium, however, information on interaction of biosorbents and pollutant compounds for technological application under dynamic condition is still limited [3]. Biosorbents can be used for the treatment of high-volume complex wastewaters having low heavy metal concentration (1–100 mg L^{-1}) [7,8]. In this concentration range, many traditional treatments are not feasible due to precipitation or extraction difficulties. Also, treatments, e.g. reverse osmosis, membrane filtration or ion exchange resins are very expensive and difficult to perform [9]. Some chemical methods have long reaction time and can be detrimental due to forming secondary metabolites and impurities [10]. As a consequence, the development of alternative technologies has become more important in the last two decades [11].

For this study, the blue-green algae (cyanobacteria), *Spirulina platensis-maxima* were chosen. The cells of these microscopic algae can form a perfect spiral. The cell wall of algal cells is surrounded by a porous three-dimensional macromolecular network. Important cell wall components are peptidoglycan, teichuronic acid, teichoic acid, polysaccharides and proteins [12], which display mainly charged carboxylic, hydroxyl, phosphate and amine groups [13]. The presence of anionic and cationic sites gives the algal cell wall amphoteric properties and depending on the pH, the groups are either protonated or deprotonated [14]. The chemical composition of the cell wall, the presence and availability of metal-binding sites are not only associated with microbial species, but depend also on the viability and concentration of the cells [3]. *S. platensis-maxima* algal cells have large sorption capacity, they are applicable in a wide pH range and their sorption is rapid for heavy metals.

The cardinal question of micro-organism application as biosorbent is how to separate the material from the medium containing impurities. With immobilization methods, the utilization of free cells during column operations can be promoted. Some advantages of immobilization against the biomass suspension are its better reusability and minimal clogging [15]. The main goal of this process is to utilize the high adsorption capacities of micro-organisms and immobilizing agent (for example, natural polymers).

Natural polysaccharides: alginate and chitosan were selected for cell immobilization. Alginate is one of the most widely used immobilizing agents. It is a linear polyuronate obtained from brown marine algae, which contains variable amounts of D-manuronic acid and L-guluronic acid. It can be cross-linked using calcium ions. Ca^{2+} ions (and other divalent cations) can bind selectively between sequences of polyguluronosyl residues [16]. Chitosan made by alkaline deacetylation of chitin is considered to be a very promising agent for immobilization [4]. Chitin is the biopolymer that is mostly found in crab and shrimp shells. It is a heteropolymer containing glucosamine and acetyl glucosamine units [17]. The amine groups can react with metal ions, as a free electron doublet is located on the nitrogen atom in the amino group. The mechanism that was involved during the adsorption process was explored both for alginate [18] and chitosan [19] using spectroscopic studies. Both natural polysaccharides are available worldwide. They are of low price and can react with metal cations in chelation or ion exchange mechanisms, to reach high sorption capacity.

The biosorption properties of—Ca-alginate and chitosan immobilized—*Spirulina platensis-maxima* cells for Cd(II), Cu(II) and Pb(II) under dynamic conditions were investigated in this study. Optimization of bead preparation and column parameters were necessary in order to achieve the maximum efficiency. The size, immobilizing agent and algal cell concentration of beads were tested in a batch system. During continuous experiments, the preconditioning of the column, the column length, the flow rate, the effect of different initial heavy metal concentrations and regeneration of the column were varied.

2. Materials and methods

2.1. Materials and adsorbent

S. platensis-maxima cells were purchased (Czech Academy of Sciences) in dried form. Na-alginate is a product of Sigma-Aldrich (Germany), and high-density chitosan was made by Molekula (VWR, USA). The heavy metal test solutions containing Pb(II), Cd (II) and Cu(II) ions were prepared from reagent—grade metal of hydrated $Pb(NO_3)_2$, $Cd(NO_3)_2$ and $CuCl_2$ (Fluka, Germany).

2.2. Analysis of metal ions

The concentration of heavy metals in supernatants was evaluated by atomic absorption spectrometry (Perkin-Elmer 2380) at 283.1 nm for Pb(II), at 228 nm for Cd(II) and at 324.8 nm for Cu(II).

2.3. Immobilization of S. platensis-maxima cells

The S*pirulina* cells were immobilized by the entrapment method in 10, 20, 30 g L^{-1} Na-alginate and in 10, 20 g L^{-1} chitosan solution.

Different amounts (2, 5, 10, 15, 20 g L^{-1}) of lyophilized alga cells were suspended in hot distilled water and mixed with equal volume of 20, 40 and 60 g L^{-1} of Na-alginate solution. The gel was dropped into the 0.2 M $CaCl_2$ solution and allowed for 2 h at 4°C to harden. The diameters of the beads were 2.0 ± 0.2, 3.5 ± 0.2 and 5.0 ± 0.3 mm. Pure alginate (20 mg L^{-1}) beads were also prepared [20].

Chitosan suspension (10, 20 g L^{-1}) was prepared from high viscosity chitosan powder in distilled water. The suspension was agitated for 12 h (200 rpm) and then gelled into 5 wt.% acetic acid solution. The beads were prepared by two methods: (1) the gel was dropped into 0.1 M NaOH solution. The hardened beads were washed with 0.1 M HCl solution and distilled water [21]. (2) The gel was dropped into 0.05 M $Na_2P_4O_7$ solution. The solidified beads were washed with 1 M phosphate buffer pH 7.4 [22]. Beads were prepared with and without algal cells. The algal cells were added to the chitosan powder, their concentration in the prepared beads was 1–10 g L^{-1}.

2.4. Optimization of column parameters

The optimized beads were used as column packing material. Solutions containing Cu(II) ions were pumped through the beads. The preconditioning of the column was tested. The effect of distilled water, HCl and H_2SO_4 solutions on the adsorption capacity of column material was examined. The pH of each solution was between 5 and 6, the temperature was 25°C during the experiments. The influence of flow rate, column size and heavy metal ion concentration on bead adsorption performance was studied. The flow rates varied 2, 5 and 10 mL min^{-1}, the column length of 10, 20 and 30 cm and heavy metal concentrations of 50, 100 and 150 mg L^{-1}.

2.5. Column studies

Before each measurement, the column filled with alginate or alginate-*Spirulina* beads was washed with distilled water for 60 min. Breakthrough curves were determined using 100 mg L^{-1} of Pb(II), Cd(II) and Cu (II) solutions, 2 mL min^{-1} flow rate, 30 cm column height and 2 cm inner column diameter (ID). The composition of beads was 20 mg L^{-1} for alginate and 40 mg L^{-1} for chitosan. *Spirulina* concentration in alginate-*Spirulina* and chitosan-*Spirulina* beads was 1 g L^{-1}. The diameter of beads was 2 mm. The column capacity (q_c in mg) for a given inlet concentration and flow rate is equal to the area under the plot of the adsorbed metal ion concentration C_{ads} ($C_{ads} = C_0 - C_e$), where C_0 and C_e are the influent and effluent metal ion concentrations (mg L^{-1}), respectively, vs. time (min) and is calculated as follows (Eq. (1)) [23,24]:

$$q_c = \frac{Q \cdot A}{1000} = \frac{Q}{1000} \int_{t=0}^{t=t} C_{ads}\, dt \tag{1}$$

where Q is the flow rate (mL min^{-1}), A is the area over the breakthrough curve (mg L^{-1} min^{-1}) and t (min) is the flow time, respectively. The amount of metal ions (m in mg) entering the column can be calculated from the following equation (Eq. (2)):

$$m = \frac{C_0 \cdot Q \cdot t}{1000} \tag{2}$$

The biosorption capacity q_e, the mass of metal ions adsorbed per mass of biosorbent (mg g^{-1}) can be calculated with Eq. (3):

$$q_e = \frac{q_c}{m_{ads}} \tag{3}$$

where m_{ads} is the mass of the biosorbent in the column (g).

2.6. Modelling of column processes

To describe the column breakthrough behaviour obtained at different bed heights, flow rates and initial metal ion concentrations, the Thomas and Yoon–Nelson models were used. The Thomas model assumes Langmuir kinetics of adsorption-desorption, no axial dispersion and a second-order reversible reaction kinetics for the rate driving force. The Thomas equation is (Eq. (4)):

$$\frac{C}{C_0} = \frac{1}{1 + \exp\left(\frac{k_{Th}}{Q}(q_0 m_{ads} - C_0 V_{eff})\right)} \tag{4}$$

where k_{Th} is the Thomas model rate constant (mL mg^{-1} min^{-1}), q_0 is the maximum concentration of the solute on the solid phase and V_{eff} (mL) is the volume of the effluent ($V_{eff} = Q\,t$) [25].

The Yoon–Nelson model is based on the assumption that the rate of decrease in the probability of adsorption for each sorbate molecule is proportional to the probability of sorbate sorption and the probability of sorbate breakthrough on the sorbent. The Yoon–Nelson equation (Eq. (5)):

$$\frac{C}{C_0} = \frac{\exp(k_{YN}t - \tau k_{YN})}{1 + \exp(k_{YN}t - \tau k_{YN})} \quad (5)$$

where V_{eff} is the volume of metal solution passed into the column (L), k_{YN} is the Yoon–Nelson model rate constant (1 min^{-1}) and τ is the time required for 50% adsorbate breakthrough (min) [25].

2.7. Regeneration of the biosorbent

A plastic column (2 cm ID and 30 cm height) was packed with 60 g of beads with optimized composition for continuous biosorption experiments. Cu(II) solution (100 mg L^{-1}) was pumped through the column (2 mL min^{-1}). For the regeneration of beads containing Cu(II) ions different methods were tested. In case of alginate beads, 16-h adsorption cycles were used. Four procedures were compared. In the first procedure, the eluent was distilled water which was used for 60 min. During the second, third and fourth method elution with 0.1, 0.01 and 0.005 M HCl solutions for 15 min was followed with distilled water for 45 min. The chitosan beads were regenerated in 8-h adsorption cycles: three procedures were compared. In the first procedure, the eluent was distilled water which was used for 60 min. In the second method, elution with 0.01 M H_2SO_4 solution for 15 min was followed by distilled water for 45 min. In the third method, elution 0.01 M H_2SO_4 solution was used as eluent. The results of five sorption cycles were summarized.

3. Results and discussion

3.1. Optimization of alginate beads

In order to achieve the efficiency of biosorption, the composition and size of the beads were optimized. Gel beads with a diameter of 2, 3.5 and 5 mm were prepared. The beads with 2 mm diameter were chosen for column studies because beads with larger diameter can easily be deformed during manufacturing. Beads with 3.5 and 5 mm diameters resulted in decreased adsorption capacity for heavy metal ions compared to beads with 2 mm diameter (detailed data are not shown). With increasing concentration of algae cells in beads, remarkable leakage can be observed, which would have a negative effect in a real application system.

Three different concentrations of alginate were used: 10, 20 and 30 g L^{-1}. The alginate concentration of 10 g L^{-1} resulted in beads with low mechanical strength and the leakage of algal cells from beads was also significant. The alginate beads with 30 mg L^{-1} concentration were rigid, which made the preparation and homogenization difficult. These beads adsorbed significantly reduced amount of heavy metal ions (detailed data are not shown), compared to the beads prepared with 20 g L^{-1} concentration. For this reason, this alginate concentration was applied in preparation of beads.

The concentration of algal cells in beads was varied between 1 and 10 g L^{-1}. Unfortunately, the increase of *Spirulina* cell concentration in beads led to a strong leakage of cells from beads to solution with the exception of 1 g L^{-1} algal cell concentration. Higher than 1 g L^{-1} algal concentrations were previously applied by other research groups [26–28]. On the basis of our experimental results, however, using higher than 1 g L^{-1} algal concentration was not possible without leakage of *Spirulina* cells to the solution and contamination of the aqueous system. The evaluation of the adsorbed heavy metal amounts by higher algal cell concentration was not relevant due to this process.

Thus, in further experiments the most effective parameters were chosen, the diameter of gel beads was 2 mm, the concentration of Ca-alginate was 20 g L^{-1} and that of the *Spirulina* cells was 1 g L^{-1} in column measurements.

3.2. Optimization of chitosan beads

Chitosan gel beads of 2 mm diameter were prepared. It was not possible to form beads with larger size because they were amorphous and fibrous. Chitosan concentrations of 20 and 40 g L^{-1} were used. Two different methods were used to create beads. In the first procedure the gel was dropped into a NaOH solution. In this way, it was possible to form only very small and fragile beads. However, beads containing chitosan of 40 g L^{-1} produced from Na-pyrophosphate (second procedure) proved to be an adequate preparation method for getting promising column material. Raising the algal concentration above 1 g L^{-1} resulted in a significant leakage of algal cells from the beads again. In further experiments, chitosan gel beads of 40 g L^{-1} with the size of 2 mm and *Spirulina* concentration of 1 g L^{-1} were used.

3.3. Optimization of column parameters

The effect of column preconditioning was tested using 100 mg L^{-1} Cu(II) solution. The columns filled with alginate beads were washed with distilled water before biosorption. In this way, they could adsorb 10%

more Cu(II) amount than without preparation. Washing the columns with HCl solution resulted in 50% less adsorption capacity for metal ions by the alginate beads, compared to the preconditioning with distilled water. The columns filled with chitosan beads were preconditioned with 0.01 M H_2SO_4 solution, which resulted in 15% increase of Cu(II) adsorption as opposed to washing the columns with distilled water. Consequently, columns filled with alginate beads were preconditioned with distilled water, and columns filled with chitosan beads were preconditioned with 0.01 M H_2SO_4 solutions before each measurement.

In packed-bed column studies, breakthrough curves were studied at different operating conditions followed by modelling. The effect of column height, flow rate and initial heavy metal concentration were investigated. Three different column heights: 10, 20 and 30 cm, flow rates: 2, 5 and 10 mL min^{-1} and initial concentrations: 50, 100 and 150 mg L^{-1} were applied. The obtained breakthrough curves are depicted in Fig. 1, where Q is the flow rate (mL min^{-1}), l is the bed height (cm) and C_0 is the initial influent heavy metal concentration (mg L^{-1}).

The amounts of adsorbed Cu(II) are summarized in Table 1, where q_e is the total amount of the adsorbed Cu(II) per mass of biosorbent (mg g^{-1}), $q_{e,dm}$ is the adsorbed Cu(II) amount per dry mass content (mmol g^{-1}) and t_r (min) is the residence time in the column.

The beads adsorb high amounts of heavy metal ions with all of the flow rates studied. The breakpoint time and adsorbed metal ion concentration decreased with increasing flow rate. The main reasons for this behaviour are the adsorption equilibrium, the structure of the beads and the diffusion of the solute into the pores of biosorbent. The adsorption equilibrium between Cu(II) and biosorbent occurred very rapidly when 5 and 10 mL min^{-1} flow rates were used in a 30 cm long column, and also when 2 mL min^{-1} flow rate was used in a 10 cm long column, while the concentration was kept the same (100 mg L^{-1}). This fact can be explained by physical adsorption processes. The residence time of the solute in the column was not long enough for the adsorption equilibrium, and the metal solution left the column before equilibrium when the 20- and 30-cm long columns were used with 100 and 150 mg mL^{-1} concentrations, respectively. Thus, the contact time of the ions with biosorbent was very short. As a consequence, adsorption concentrations decreased with increasing flow rates. Also, the breakpoint time and the adsorbed metal ion concentration decreased with increasing inlet concentrations. This relationship is illustrated in Fig. 1. At lower inlet concentrations, the breakthrough occurs later and the surface of the adsorbent also needs more time to be saturated with metal ions. Higher amounts of metal ions can be removed by reducing the flow rate and the initial concentration and by increasing the column height. Based on the breakthrough curves with the working parameters of 2 mL min^{-1} flow rate, 30 cm column length, 50 and 100 mg L^{-1} initial metal ion concentrations, we obtained valuable experimental data and information for evaluation. The average residence time in the column can be used to model the processes in technological applications. For further experiments 2 mL min^{-1} flow rate, 30 cm column height and 100 mg L^{-1} initial metal ion concentration were chosen.

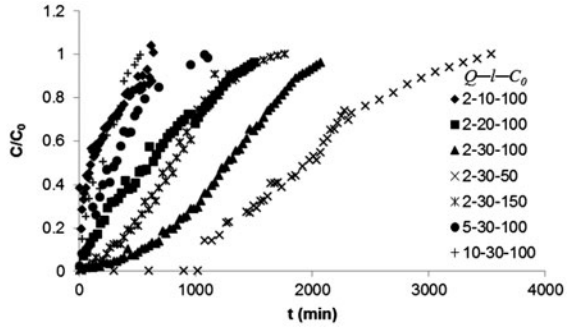

Fig. 1. Breakthrough curves of alginate-*Spirulina* beads obtained by different column heights, flow rates and initial Cu(II) concentrations. Alginate concentration: 20 mg L^{-1}, *Spirulina* concentration: 1 g L^{-1} (Q: flow rate in mL min^{-1}, l: column length in cm, C_0: concentration in mg L^{-1}).

3.4. Packed-bed column studies

Column operations do not have sufficient contact time for attainment of equilibrium. Hence, in addition to equilibrium studies, there was a need for performing biosorption studies using a column. The columns were packed with alginate and chitosan gel beads bearing the optimized composition and they were rinsed with heavy metal solutions by adjusting selected parameters. The specific amounts of absorbed Pd(II), Cd(II) and Cu(II) ions by hydrogel beads are summarized in Table 2.

The alginate gel beads adsorbed more of the heavy metal ions than the chitosan gel beads. In comparison with the alginate beads, the adsorption capacity of alginate-*Spirulina* beads was slightly reduced in each case. Chitosan-*Spirulina* beads can remove more Cu(II) and less Pb(II) and Cd(II) ions than the chitosan beads. The additionally adsorbed amount of Cu(II) by chitosan-*Spirulina* beads was 0.25 mmol g^{-1} per dry

Table 1
The adsorption capacities of alginate and alginate-*Spirulina* beads obtained by different column parameters (Q: flow rate in mL min^{-1}, l: column length in cm, C_0: concentration in mg L^{-1}, q_e: the total amount of the adsorbed Cu(II) per mass of biosorbent, $q_{e,dm}$: the adsorbed Cu(II) amount per dry mass content, t_r: the residence time in the column)

Q–l–C_0	2–10–100	2–20–100	2–30–50	2–30–100	2–30–150	5–30–100	10–30–100
q_e (mg g^{-1})	3.9	3.1	5.5	4.1	4.0	2.8	3.3
$q_{e,dm}$ (mmol g^{-1})	2.9	2.3	4.1	3.1	3.0	2.1	2.4
t_r (min)	5	17.5	30	30	30	12	6

Table 2
The adsorption capacities of alginate, alginate-*Spirulina*, chitosan and chitosan-*Spirulina* hydrogel beads (q_e is the total amount of the adsorbed Cu(II) per mass of biosorbent (mg g^{-1}), V_b: breakthrough volume, V_e: exhaustion volume)

Metal	Pb(II)			Cd(II)			Cu(II)		
Biosorbent	q_e (mg g^{-1})	V_b	V_e	q_e (mg g^{-1})	V_b	V_e	q_e (mg g^{-1})	V_b	V_e
Alginate	13	3,480	10,200	5.2	1,800	5,160	4.3	480	4,140
Alginate-*Spirulina*	12.9	5,640	9,760	4.5	600	4,480	4.1	120	4,140
Chitosan	5.7	820	8,280	4.2	1,080	5,160	2	300	4,320
Chitosan-*Spirulina*	4.8	1,320	2,580	3.6	300	5,040	2.7	600	5,520

mass compared to chitosan beads. The increased adsorption capacity is due to the modified structure of the chitosan beads when containing *Spirulina* cells.

3.5. Modelling of breakthrough curves

Breakthrough curves were determined in column experiments, which can be used to simulate and predict adsorption processes. The Thomas and Yoon–Nelson models were applied to evaluate the data. The results of fitting of the Thomas model to the breakthrough curves for Pb(II) adsorption on alginate, alginate-*Spirulina*, chitosan and chitosan-*Spirulina* gel beads are presented in Figs. 2 and 3. The fitted parameters for each metal ions and both models are shown in Tables 3 and 4.

In case of alginate and alginate-*Spirulina* beads both models can be fitted very well. The maximum adsorbed quantities calculated by Thomas model were in good agreement with the experimentally determined values. The breakthrough times of Yoon–Nelson model (τ) were exactly the same as the 50% breakthrough time in the experiments.

The Thomas model was the most appropriate to describe the processes in chitosan and chitosan-*Spirulina* beads-packed columns. The calculated maximum

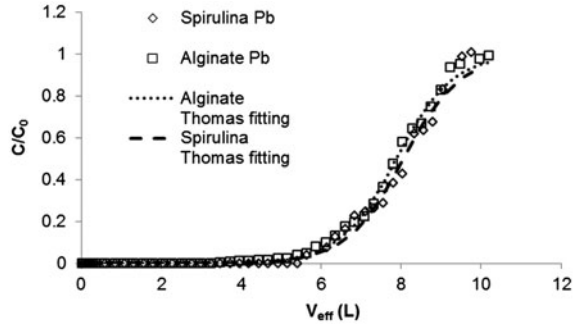

Fig. 2. Thomas model fitted to the experimental data for Pb(II) adsorption on alginate (□) and alginate-*Spirulina* beads (◇).

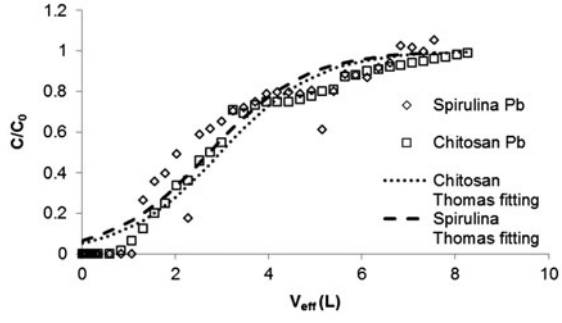

Fig. 3. Thomas model fitted to the experimental data for Pb(II) adsorption on chitosan (□) and chitosan-*Spirulina* beads (◇).

Table 3
Fitted parameters of Thomas and Yoon–Nelson models by alginate and alginate-*Spirulina* beads for Pb(II), Cd(II) and Cu(II) adsorption

	Thomas model			Yoon–Nelson model		
Biosorbent/Metal	k_{Th} (mL mg^{-1} min^{-1})	q_e (mg g^{-1})	R^2	k_{YN} (min^{-1})	τ (min)	R^2
Alginate Pb	3×10^{-2}	13.1	0.99	3×10^{-3}	3,939	0.99
Alginate-*Spirulina* Pb	3×10^{-2}	13.4	0.99	3×10^{-3}	4,027	0.99
Alginate Cd	4×10^{-2}	5.3	0.99	4×10^{-3}	1,590	0.99
Alginate-*Spirulina* Cd	3×10^{-2}	4.7	0.99	3×10^{-3}	1,409	0.99
Alginate Cu	4×10^{-2}	4.4	0.99	3×10^{-3}	2,467	0.93
Alginate-*Spirulina* Cu	3×10^{-2}	4.3	0.99	3×10^{-3}	1,275	0.99

Table 4
Fitted parameters of Thomas and Yoon–Nelson models by chitosan and chitosan-*Spirulina* beads for Pb(II), Cd(II) and Cu(II) adsorption

	Thomas model			Yoon–Nelson model		
Biosorbent/Metal	k_{Th} (mL mg^{-1} min^{-1})	q_e (mg g^{-1})	R^2	k_{YN} (min^{-1})	τ (min)	R^2
Chitosan Pb	2×10^{-2}	5	0.97	2×10^{-3}	1,470	0.82
Chitosan-*Spirulina* Pb	2×10^{-2}	4.6	0.92	5×10^{-4}	4,858	0.77
Chitosan Cd	2×10^{-2}	4.3	0.95	2×10^{-3}	1,288	0.95
Chitosan-*Spirulina* Cd	2×10^{-2}	4.5	0.94	2×10^{-3}	1,339	0.94
Chitosan Cu	4×10^{-2}	2.5	0.92	6×10^{-4}	2,986	0.68
Chitosan-*Spirulina* Cu	4×10^{-2}	1.7	0.95	6×10^{-4}	3,559	0.7

adsorbed quantities by the beads were close to the values measured during the experiments. The 50% breakthrough time can be calculated by the Yoon–Nelson model for Cd(II) adsorption, but for Pb(II) and Cu(II) adsorption the model was not suitable to describe the examined system.

3.6. Regeneration of gel beads

The desorption of bound metal ions resulted in recovered metal ions and regenerated biomass. The elution process enables the reuse of biomass in repeated sorption cycles. Due to the weak nature of metal binding to the biosorbent, it is possible to elute the adsorbed metal ions from the beads. Mineral acids can be used with high efficiency [28,29]. Five adsorption-desorption cycles of 100 mg L^{-1} Cu(II) solution were tested.

Before the first cycle, columns packed with alginate and alginate-*Spirulina* beads were preconditioned with distilled water. The effectiveness of four methods was investigated. From the second cycle, the packed-bed columns were washed with 0.1, 0.01 and 0.005 M HCl solutions for 15 min, then with distilled water for 45 min. After 16-h adsorption, one-hour desorption was applied. The results are summarized in Table 5. The efficiency of the elution with hydrochloric acid-distilled water combination was higher than that of the washing with pure distilled water. There was no significant difference in the adsorbed amounts of Cu (II) using various concentrations of hydrochloric acid solution. The regeneration of alginate beads can be easily investigated by washing with 0.005 M HCl solution for 15 min, then with distilled water for 45 min.

As the chitosan gel beads dissolve in solutions containing chloride ions [22], regeneration with distilled water and H_2SO_4 solution were tested. The chitosan beads were agitated in sulphuric acid solution for 24 h to examine their stability. Sulphuric acid solution of 0.01 M concentration did not dissolve the chitosan beads, so this concentration was applied to elute the metal ions from chitosan beads. Three different methods were applied to choose the best conditions: (1) washing with distilled water in each cycle; (2) washing with distilled water in the first cycle, and in the subsequent cycles with 0.01 M sulphuric acid solution for 15 min, followed by washing with distilled water for 45 min and (3) washing in each cycle with 0.01 M

Table 5
Regeneration of alginate and alginate-*Spirulina* hydrogel beads with distilled water and hydrochloric acid-distilled water combination

Eluent	Water	0.1 M HCl + water	0.01 M HCl + water	0.005 M HCl + water
q_e (mg g^{-1})/SD	2.9 ± 1.2	4.2 ± 0.1	4.1 ± 0.4	4.2 ± 0.4

Table 6
Regeneration of chitosan and chitosan-*Spirulina* beads with distilled water and hydrochloric acid-distilled water combination

Eluent	Water	0.01 M H_2SO_4 + water	0.01 M H SO_4
q_e (mg g^{-1})/SD	1.2 ± 0.3	1.4 ± 0.4	1.6 ± 0.3

sulphuric acid solution for 15 min then with distilled water for 45 min. Combination of 8-h adsorption and 1-h desorption was investigated. The results are summarized in Table 6. The effectiveness of the washing with sulphuric acid in each cycle is better than that of both of the other methods, therefore sulphuric acid proved to be the best candidate for regeneration.

4. Conclusions

Biosorption experiments by studying the breakthrough curves for Pb(II), Cd(II) and Cu(II) showed that alginate, alginate-*Spirulina*, chitosan and chitosan-*Spirulina*-packed hydrogel beads possess high biosorption capacity. The alginate beads have higher adsorption capacity than the chitosan beads. Alginate and alginate-*Spirulina* beads can remove nearby the same amount of Pb(II), Cd(II) and Cu(II) ions from aqueous solutions. The uptake of chitosan-*Spirulina* beads is greater than that of chitosan beads for Cu(II) biosorption, and less for Pb(II) and Cd(II) biosorption. The chitosan-*Spirulina* beads adsorb additionally 0.25 mmol g^{-1} Cu(II) per dry mass compared to chitosan beads. Efficient regeneration of gel beads is possible using mineral acids and water.

Column processes can be modelled with the Thomas model; the estimation of maximum adsorbed quantities is very accurate. In many cases, the 50% breakthrough time can be calculated with the Yoon–Nelson model.

The combination of micro-organisms and natural polysaccharides as immobilizing agent to utilize their high adsorption capacities is very promising. According to this study, the natural polysaccharides encapsulate several micro-organisms. The adsorption capacity of free cells for metal ions has been lost during the immobilization process. The active sites of these microorganisms and also those of alginate and chitosan seem to be partially covered in immobilized form resulting in a decreased adsorption capacity and reduced porosity. Against the numerous experimental studies, efficient adsorption system can rarely be constructed applying these methods for technical purposes.

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References

[1] A. Saeed, M. Iqbal, Immobilization of blue green microalgae on loofa sponge to biosorb cadmium in repeated shake flask batch and continuous flow fixed bed column reactor system, World J. Microbiol. Biotechnol. 22 (2006) 775–782.
[2] J.S. Chang, R. Law, C.C. Chang, Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21, Water Res. 31 (1997) 1651–1658.
[3] L.E. de-Bashan, Y. Bashan, Immobilized microalgae for removing pollutants: Review of practical aspects, Bioresour. Technol. 101 (2010) 1611–1627.
[4] N. Mallick, Biotechnological potential of immobilized algae for wastewater N, P and metal removal: A review, Biometals 15 (2002) 377–390.
[5] A. Mudhoo, V.K. Garg, S.B. Wang, Removal of heavy metals by biosorption, Environ. Chem. Lett. 10 (2012) 109–117.
[6] K. Vijayaraghavan, Y.S. Yun, Bacterial biosorbents and biosorption, Biotechnol. Adv. 26 (2008) 266–291.
[7] J. Wang, C. Chen, Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review, Biotechnol. Adv. 24 (2006) 427–451.

[8] J.L. Wang, C. Chen, Biosorbents for heavy metals removal and their future, Biotechnol. Adv. 27 (2009) 195–226.
[9] F. Veglio, F. Beolchini, Removal of metals by biosorption: A review, Hydrometallurgy 44 (1997) 301–316.
[10] S.V. Gokhale, K.K. Jyoti, S.S. Lele, Kinetic and equilibrium modeling of chromium (VI) biosorption on fresh and spent *Spirulina platensis*/*Chlorella vulgaris* biomass, Bioresour. Technol. 99 (2008) 3600–3608.
[11] M.A. Hashim, S. Mukhopadhyay, J.N. Sahu, B. Sengupta, Remediation technologies for heavy metal contaminated groundwater, J. Environ. Manage. 92 (2011) 2355–2388.
[12] S. Schiewer, M.H. Wong, Ionic strength effects in biosorption of metals by marine algae, Chemosphere 41 (2000) 271–282.
[13] Z. Aksu, Determination of the equilibrium, kinetic and thermodynamic parameters of the batch biosorption of nickel(II) ions onto *Chlorella vulgaris*, Process Biochem. 38 (2002) 89–99.
[14] A. Esposito, F. Pagnanelli, F. Vegliò, pH-related equilibria models for biosorption in single metal systems, Chem. Eng. Sci. 57 (2002) 307–313.
[15] I. Kaur, A.K. Bhatnagar, Algae-dependent bioremediation of hazardous wastes, Prog. Ind. Microbiol. Biotransform. Biorem. Technol. Health Environ. Prot. 36 (2002) 457–516.
[16] Y.M. Lu, E. Wilkins, Heavy metal removal by caustic-treated yeast immobilized in alginate, J. Hazard. Mater. 49 (1996) 165–179.
[17] M. Rinaudo, Chitin and chitosan: Properties and applications, Prog. Polym. Sci. 31 (2006) 603–632.
[18] J.P. Chen, L. Wang, S.-W. Zou, Determination of lead biosorption properties by experimental and modeling simulation study, Chem. Eng. J. 131 (2007) 209–215.
[19] W.S.W. Ngah, S. Fatinathan, Pb(II) biosorption using chitosan and chitosan derivatives beads: Equilibrium, ion exchange and mechanism studies, J. Environ. Sci. 22 (2010) 338–346.
[20] M.F. Ahmad, S. Haydar, T.A. Quraishi, Enhancement of biosorption of zinc ions from aqueous solution by immobilized *Candida utilis* and *Candida tropicalis* cells, Int. Biodeterior. Biodegrad. 83 (2013) 119–128.
[21] B. Aguilar-May, M.D. Sanchez-Saavedra, Growth and removal of nitrogen and phosphorus by free-living and chitosan-immobilized cells of the marine cyanobacterium Synechococcus elongatus, J. Appl. Phycol. 21 (2009) 353–360.
[22] V.M. Kaya, G. Picard, Stability of chitosan gel as entrapment matrix of viable *Scenedesmus bicellularis* cells immobilized on screens for tertiary treatment of wastewater, Bioresour. Technol. 56 (1996) 147–155.
[23] J. Cruz-Olivares, C. Pérez-Alonso, C. Barrera-Díaz, F. Ureña-Nuñez, M.C. Chaparro-Mercado, B. Bilyeu, Modeling of lead (II) biosorption by residue of allspice in a fixed-bed column, Chem. Eng. J. 228 (2013) 21–27.
[24] R. Sharma, B. Singh, Removal of Ni (II) ions from aqueous solutions using modified rice straw in a fixed bed column, Bioresour. Technol. 146 (2013) 519–524.
[25] K. Vijayaraghavan, D. Prabu, Potential of *Sargassum wightii* biomass for copper(II) removal from aqueous solutions: Application of different mathematical models to batch and continuous biosorption data, J. Hazard. Mater. 137 (2006) 558–564.
[26] S.V. Gokhale, K.K. Jyoti, S.S. Lele, Modeling of chromium (VI) biosorption by immobilized *Spirulina platensis* in packed column, J. Hazard. Mater. 170 (2009) 735–743.
[27] R. Vannela, S.K. Verma, Co^{2+}, Cu^{2+}, and Zn^{2+} accumulation by cyanobacterium *Spirulina platensis*, Biotechnol. Progr. 22 (2006) 1282–1293.
[28] R. Vannela, S.K. Verma, Cu^{2+} removal and recovery by SpiSORB: Batch stirred and up-flow packed bed columnar reactor systems, Bioprocess. Biosyst. Eng. 29 (2006) 7–17.
[29] M.S.A. Hameed, Continuous removal and recovery of lead by alginate beads, free and alginate-immobilized Chlorella vulgaris, Afr. J. Biotechnol. 5 (2006) 1819–1823.