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Short Communication

The adsorption of copper(II) by *Z. ramigera* immobilized on Ca-alginate in packed bed columns: a dynamic approach by stimulus–response technique and evaluation of adsorption data by moment analysis

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Abstract

In this study, Cu^{II} adsorption by *Z. ramigera* immobilized on Ca-alginate was investigated in a packed bed column test circuit using a stimulus–response technique. The mathematical model was described and solved using "parameter estimation by cybernetic moment technique", and the adsorption rate constant of Cu^{II} ions on *Z. ramigera* immobilized on Ca-alginate was calculated. The Péclet numbers, which reflect the column flow characteristics in the cases of no diffusion and/or no adsorption, were calculated. The diffusional effects inside the pores of biomass immobilized on Ca-alginate matrices were investigated. The moment technique was used for evaluation of stimulus–response data of heavy metal–biosorbent interactions, to determine the process parameters.

Keywords: Cu^{II} adsorption; Immobilized *Z. ramigera*; Ca-alginate matrix; Stimulus–response analysis; Moment technique

1. Introduction

The use of microorganisms as biosorbents for heavy metals offers a potential alternative to existing methods for detoxification and recovery of toxic or valuable metals from industrial effluent. Many aquatic microorganisms such as bacteria and algae can adsorb dissolved heavy metals and radioactive elements from their surroundings.

Ca-alginate itself is a good adsorbent. When blended with other metal-binding biomass, it can also serve as an excellent matrix. High heavy metal adsorption and desorption yields were obtained in a packed column when immobilized *Z. ramigera* cells were used in it [1].

In the current study, we have proposed a novel approach, which is called the 'stimulus response technique', to analyse the dynamic metal–biosorbent interaction. Stimulus–response methods are well developed in chemical engineering processes for measuring rate and equilibrium parameters such as mass transfer coefficients, diffusivities and adsorption rate constants [2–5]. The moments of the response curves to pulse inputs have been extensively used in the analysis of several systems as well as in the packed bed systems [6–10].

In the present study, a modified bead column test using a stimulus–response approach was employed to investigate the dynamic interaction between copper(II) ions with Ca-alginate and *Z. ramigera* immobilized on Ca-alginate spherical particles. Fixed bed columns with an L/D ratio of 1 filled with the test beads, commercial polished stainless steel (PSS) beads (MKE, Turkey), Ca-alginate and immobilized *Z. ramigera* were subjected to a series of tests by the stimulus–response technique utilizing [^{99m}Tc]pertechnetate as inert tracer or copper(II) ions. The column was pulse stimulated and the response of the column was measured. The adsorption data are reported in the first part of this study [1].

The model equation for a packed column with radius r is written as follows:

$$\epsilon \frac{\delta C}{\delta t} = -U \frac{\delta C}{\delta x} + D_{\mathrm{L}} \frac{\delta^2 C}{\delta x^2} + \frac{D_{\mathrm{R}}}{r} \frac{\delta}{\delta r}\left(r \frac{\delta C}{\delta r}\right) - \rho_{\mathrm{P}} N_{\mathrm{A}}, \qquad (1)$$

This equation includes both axial and radial dispersion terms. If the particle diameter:column diameter ratio is lower than 1/10, radial dispersion can be neglected. Particles and column diameters were chosen as appropriate for this ratio. In this case, the radial dispersion term was eliminated from

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the equation, and the differential equation for a packed column with axial dispersion was obtained:

$$D_L \frac{\delta^2 C}{\delta x^2} - U \frac{\delta C}{\delta x} - \rho_p N_{A'} = \epsilon \frac{\delta C}{\delta t} \tag{2}$$

In this equation, $N_{A'}$ (mol g^{-1} s^{-1}) is the net flux to surface.

The boundary and initial conditions are based on Danckwerts' boundary conditions [11], so that, for a Dirac delta function pulse input, boundary condition I is

$$\text{at } \xi = 0, \quad M = J(s)_{\xi=0} - \left[\frac{1}{\mathrm{Pe}} \frac{\delta J(s)}{\delta \xi}\right]_{\xi=0} \tag{3}$$

and boundary condition II is

$$\text{at } \xi = 1, \quad \frac{\delta J(s)}{\delta \xi} = 0 \tag{4}$$

where M is the amount of tracer injected. For the inert tracer, substitute

$$N_{A'} = 0 \tag{5}$$

Eq. (2) was written in the Laplace domain as

$$\frac{1}{\mathrm{Pe}} \frac{\delta^2 J}{\delta \xi^2} - \frac{\delta J}{\delta \xi} - \frac{\epsilon L}{U} \frac{\delta J}{\delta t} = 0 \tag{6}$$

Eq. (6) can be solved in the Laplace domain for $J = f(\xi, s)$. Then, using the relationship

$$m_n = (-1)^n \lim_{s \to 0} \frac{\mathrm{d}^n \bar{J}_A}{\mathrm{d}s^n} \tag{7}$$

theoretical moment expressions were derived. For the zeroth moment, first absolute moment and second central moment for the column itself, the result is [9,12]

$$m_0 = M \tag{8}$$

$$M_1 = \frac{m_1}{m_0} = \frac{\epsilon L}{U} \tag{9}$$

and

$$M_2^{M\#} = \frac{M_2^M}{M_0^M} - \left(\frac{M_1^M}{M_0^M}\right)^2 \tag{10}$$

$$M_2^{M\#} = \frac{2}{\mathrm{Pe}} - \frac{2}{\mathrm{Pe}^2}(1 - \mathrm{e}^{-\mathrm{Pe}}) \tag{11}$$

For tracer which adsorb irreversibly on the column packing $N_{A'}$ can be written as

$$N_{A'} = k_A C_A \tag{12}$$

The equation becomes

$$\frac{1}{\mathrm{Pe}} \frac{\delta^2 J}{\delta \xi^2} - \frac{\delta J}{\delta \xi} - \frac{\rho_p k_A L}{U} J - \frac{\epsilon L}{U} \frac{\delta J}{\delta t} = 0 \tag{13}$$

where k_A (cm^3 g^{-1} s^{-1}) is the adsorption rate constant.

The zeroth absolute moment of Eq. (13) is [12]

$$M_0 = \frac{4M\beta_0 \, \mathrm{e}^{\mathrm{Pe}(1-\beta_0)/2}}{(\beta_0 + 1)^2 - (1 - \beta_0)^2 \, \mathrm{e}^{-\mathrm{Pe}\,\beta_0}} \tag{14}$$

where

$$\beta_0 = \left(1 + \frac{4 k_A L \rho_p}{U \, \mathrm{Pe}}\right)^{1/2} \tag{15}$$

Experimental values of the zeroth, first and second moments can be determined from the observed response peaks using the following equations [13]:

$$m_0^E = \int_0^a C \, \mathrm{d}t \approx \sum_{i=1}^{n} c_{\mathrm{av},i} \, \Delta t \tag{16}$$

$$m_1^E = \frac{1/2 \sum_{i=1}^{n} c_{\mathrm{av},i} (\tau_{i+1} + \tau_i)}{\sum_{i=1}^{n} c_{\mathrm{av},i}} \tag{17}$$

$$m_2^E = \frac{1/3 \sum_{i=1}^{n} c_{\mathrm{av},i} (\tau_{i+1}^2 + \tau_{i+1}\tau_i + \tau_i^2)}{\sum_{i=1}^{n} c_{\mathrm{av},i}} \tag{18}$$

2. Materials and methods

2.1. Microorganism and growth conditions

Z. ramigera was obtained from the US Department of Agriculture Culture Collection and was grown at 25 °C in agitated and aerated liquid media containing the following (grams per litre): glucose, 30.0; yeast extract, 1.0; bacteriological peptone, 1.0. The salts used in the growth medium were K_2HPO_4, KH_2PO_4, NH_4Cl, $MgSO_4 \cdot 7H_2O$ at 1.0 g l^{-1}, 0.5 g l^{-1}, 0.5 g l^{-1}, 0.5 g l^{-1} respectively.

In this study, Ca-alginate and *Z. ramigera* immobilized on Ca-alginate spherical beads were selected as the basic biosorbents for investigation of copper(II) adsorption in the fixed bed column.

2.2. Preparation of Ca-alginate beads

Ca-alginate itself adsorbs metals. Water-soluble sodium alginate was converted to water-insoluble Ca-alginate particles by using $CaCl_2$ solution. Sodium alginate (3%) was dropped into 100 mM $CaCl_2$ by means of a peristaltic pump.

2.3. Preparation of immobilized Z. ramigera beads

In the stationary phase of growth (120 h), *Z. ramigera* cells were centrifuged and resuspended with 3% sodium alginate. The ratio of sodium alginate to biomass was 3. Cell–

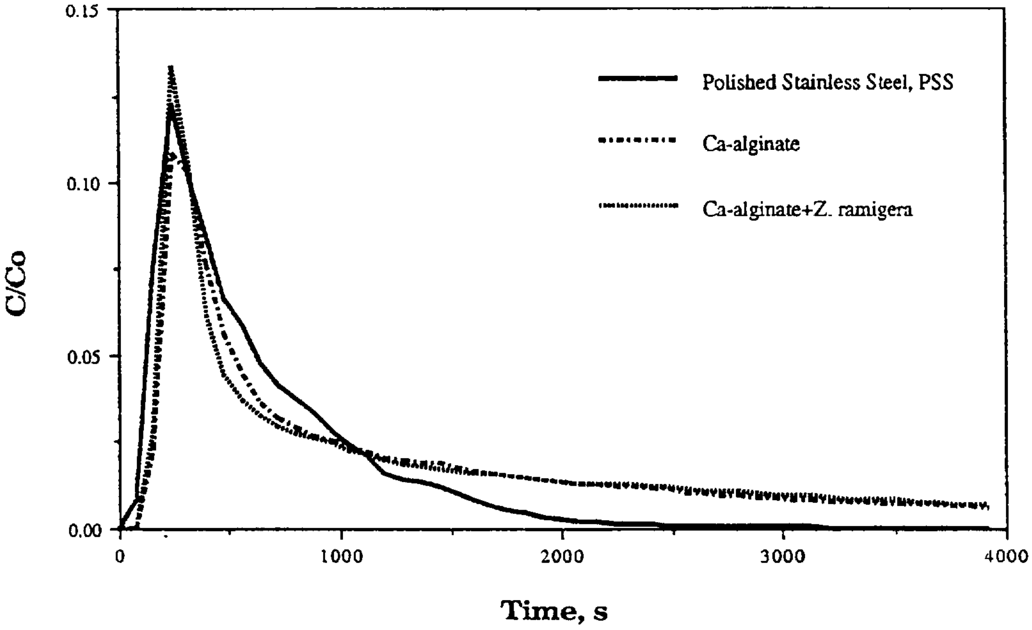

Fig. 1. Response ('C') curves of pulse-stimulated packed columns filled with PSS beads, Ca-alginate beads and *Z. ramigera* immobilized on Ca-alginate beads. C/C_0 denotes the ratio of instantaneous tracer concentration to initial tracer concentration. Inert tracer, [^{99m}Tc]pertechnetate.

Na-alginate mixture was dropped into the 100 mM $CaCl_2$ solution by a peristaltic pump. The drops of sodium alginate solution gelled into 4.6 ± 0.2 mm diameter spheres on contact with the $CaCl_2$ solution. The data were obtained by using Ca-alginate-immobilized *Z. ramigera* particles that had been stored in the $CaCl_2$ solution at 4 °C for at least 2 h prior to use. The details of this experimental procedure were given elsewhere [1]. Spherical beads with a diameter of 4.8 mm were used in the stimulus–response experiments.

2.4. Experimental set-up

A cylindrical Pyrex glass column (L = 48.0 mm) with a length:diameter ratio of unity was used. The column was filled with commercial PSS beads (MKE, Turkey) with a diameter of 4.8 mm as reference non-adsorbing surface or Ca-alginate or *Z. ramigera* immobilized on Ca-alginate spherical beads.

10 l of sterile distilled water were used to wash the system. Distilled water flow was maintained at a flow rate of 3.6 ml min^{-1}. This flow rate was determined as the optimum flow rate for copper(II) adsorption by Ca-alginate-immobilized *Z. ramigera* in the packed bed column [1].

2.5. Stimulus–response experiments

The experimental procedure was carried out in three stages. In the first stage, for investigation of dynamic behaviour of the column in the cases of no diffusion and no adsorption, the column was filled with PSS beads and the system was then 'pulse' stimulated by adding 0.1 ml of [^{99m}Tc]pertechnetate inert tracer to the eluate to determine the reference response of the column. In the second stage, for investigation of diffusional behaviour, under conditions of no adsorption, the column was filled with Ca-alginate or *Z. ramigera* immobilized on Ca-alginate and then 'pulse' stimulated by adding 0.1 ml of [^{99m}Tc]pertechnetate inert tracer. The column response, which is the so-called 'C curve', was determined by following the radioactivity of the eluent stream. In each case the radioactivity was detected by a γ scintillation counter (Berthold, BF 5300, Gammazint, Germany).

In the last stage of the study, the column was filled with immobilized *Z. ramigera* and then the system was 'pulse' stimulated with the addition of 10 000 mg Cu^{II} ions to the eluate to determine the adsorption rate constant of Cu^{II} ions on biosorbent. All the experiments were carried out at 25 °C. The column response was determined by following the amounts of copper on the eluent stream. In each case, the unadsorbed copper(II) ions in the effluent were determined spectrophotometrically. The coloured complex of copper(II) ion with sodium diethyldithiocarbamate was observed at 460 nm [14,15].

3. Results and discussion

Fig. 1 exemplifies the response ('C') curves obtained in the stimulus–response experiments in the case of no adsorption and diffusion utilizing PSS beads. The reference line on the graph represents the reference response of the column (no adsorption and no diffusion), where the system was 'pulse' stimulated by adding [^{99m}Tc]pertechnetate to the eluate. The area under this 'C' curve was calculated and normalized to be unity. In other words, the amount of [^{9m}Tc]pertechnetate adsorbed on the reference surface was equal to zero. It was decided that the 'C' curve obtained from the reference

Table 1
Column parameters: Péclet numbers, axial dispersion coefficients and the adsorption rate constant of Cu^{II} on *Z. ramigera* immobilized Ca-alginate

Tracer	Surface	Adsorption (%)	Moment values $M_2^{\#}$	Péclet numbers		Adsorption rate constant ($cm^3\,g^{-1}\,s^{-1}$)
				Pe_l	Pe_d	
[^{99m}Tc]pertechnetate	PSS	0	0.477131	2.7757	0.2776	–
[^{99m}Tc]pertechnetate	Ca-alginate	0	0.698375	1.1882	0.1188	–
[^{99m}Tc]pertechnetate	*Z. ramigera* immobilized on Ca-alginate	0	0.70980	1.1290	0.1129	–
Cu^{II} ions	*Z. ramigera* immobilized on Ca-alginate	76	16.38	1.1290	0.1129	0.6955

response of the column can be used for determining the Péclet number to investigate the effect of axial dispersion.

The observed moments were computed by numerical evaluation of the response peaks according to Eqs. (16)–(18). It is worth noting that the radioactivity detected by the γ scintillator is directly proportional to the concentration; it is not necessary to calibrate the scintillator to obtain the moments.

The Péclet number $Pe_l = UL/D$ which reflects the column flow characteristic in the cases of no diffusion and no adsorption was calculated from Eq. (11) and found to be 2.7757. To compare the results with the literature, the Péclet number $Pe_d = Ud_p/D$ in terms of particle diameter was also determined and found to be 0.2776, which agrees with the literature [4,16,17]. It is important to note that calculated column Péclet number in this stage indicated a large amount of axial dispersion at this Reynolds number and the value of the axial dispersion coefficient $D_L = 0.0332$ cm^2 s^{-1} found from this Péclet number directly characterized the effect of only axial dispersion in the column.

Diffusional effects in the column (or into the particles) were investigated in the second part. Response ('C') curves of pulse-stimulated packed columns filled with Ca-alginate or immobilized *Z. ramigera* are also shown in the same graph (Fig. 1). The areas under these 'C' curves were calculated and normalized to be unity. These values indicated no [^{99m}Tc]pertechnetate adsorption on the biosorbents.

By comparing 'C' curves for PSS, Ca-alginate and immobilized *Z. ramigera*, it can be seen that the residence time of the non-interacting tracer in the column increased substantially, because of the porous structure of the Ca-alginate matrix and the diffusion of the solute into the biosorbent's pores. Diffusion strongly affects the rate of dispersion in laminar flow [17]. At a flow rate of 3.6 ml min^{-1}, intraparticle diffusion promoted dispersion. The calculated values of axial dispersion coefficients for Ca-alginate and immobilized *Z. ramigera* matrices represent the combined effect of axial dispersion in the column and intraparticle diffusion. Copper(II) adsorption in the packed column system is strongly affected by solute diffusion into the pores of the Ca-alginate. The existence of dispersion in the column and the diffusion inside the pores cause a higher removal efficiency of copper(II) ions by Ca-alginate and immobilized *Z. ramigera* [1]. Results obtained from moment analysis are given in Table 1. The results of the first run show that there is no adsorption of [^{99m}Tc]pertechnetate on the PSS surfaces, and no intraparticular diffusion because of the rigid and nonporous structure of the steel. Therefore, the Péclet number obtained in this run gives the dynamic behaviour of the column that results from purely axial dispersion. The second run utilizes the same tracer, but the matrix, Ca-alginate, has a very porous structure. The change in the Péclet number was the result of intraparticular diffusion. However, it should be noted that Péclet number also includes the axial dispersion. In the third run, the matrix was converted to immobilized *Z. ramigera* on Ca-alginate matrix. Similar to the previous cases, the non-interacting tracer [^{99m}Tc]pertechnetate has not adsorbed on those surfaces. However, the Péclet number obtained in this case includes the dynamic behaviour of tracer due to axial dispersion and intraparticular diffusion in the heterogeneous matrix. The Péclet number $Pe_l = 1.1290$ obtained in the third run was utilized in the evaluation of the adsorption data of copper(II) ions on immobilized *Z. ramigera*.

The curve in Fig. 2 exemplifies the copper(II) adsorption on immobilized *Z. ramigera*. In the last stage of this study, to determine the adsorption rate constant of copper(II) ions on immobilized *Z. ramigera*, Eqs. (14) and (15) were solved with numerical results from Eq. (16) and are given in Table 1 with the related data. The calculated value of $Pe = 1.1290$ for immobilized *Z. ramigera* was directly used in Eqs. (14) and (15). It is assumed that different types of tracers do not affect the dispersion of the stream throughout the column. The immobilized *Z. ramigera* system was 'pulse' stimulated with addition of 10 000 mg Cu^{II} ions to the eluate. When the samples containing less than 10 000 mg Cu^{II} ions were injected into the column the 'C' curve was not obtained because all the copper(II) ions were adsorbed by the biosorbent. For this amount of Cu^{II} pulse stimulus, 76% of Cu^{II} ions injected into the column were adsorbed.

It is also interesting to note that the adsorption of copper(II) ions is considered as an irreversible process. The

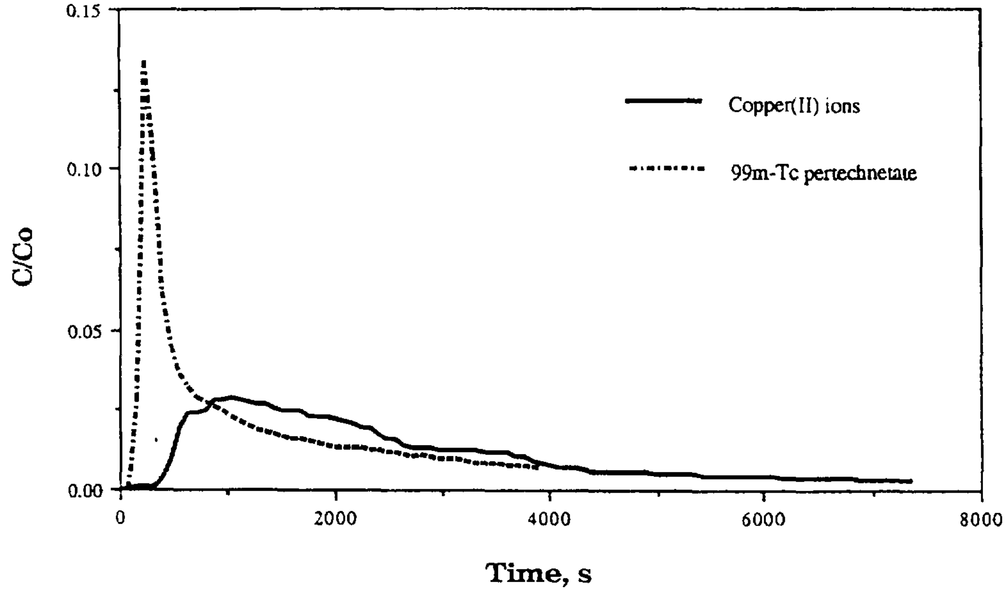

Fig. 2. Copper(II) adsorption on *Z. ramigera* immobilized on Ca-alginate beads. The system was "pulse" stimulated by adding 10 000 mg Cu^{II} ions to eluate. C/C_0 denotes the ratio of instantaneous tracer concentration to initial tracer concentration.

shape of the response curve of adsorbed tracer, Cu^{II} ions, is very similar to that found with non-adsorbed tracer [^{99m}Tc]pertechnetate. Only the areas under the response curves are different owing to adsorption by the column. In other words, no overshoots (extra peaks following the standard curve) were observed. This might be an indication that Cu^{II} ions are only adsorbed from the eluate but are not released back to it. The irreversible adsorption assumption in Eq. (12) was proved by the shape of the 'C' curve.

In conclusion, it can be said that the stimulus–response method may be considered as a deft dynamic approach to the investigation of heavy metal adsorption by biosorbents in the fixed bed systems. The evaluation of 'C' curves by cybernetic moment techniques gives the amount of adsorption in a single pass and also allows analysis of the kinetics of dynamic heavy metal ion–biosorbent interactions. Studies concerning the generalization of this dynamic test approach (different heavy metal–biosorbent systems, the effect of flow rate on the adsorption rate constant and reversible and equilibrium adsorption mechanisms) are still under investigation.

Appendix A: Nomenclature

C	tracer concentration (mol ml^{-1})
$c_{av,i}$	average concentration of *i*th species (mol cm^{-3})
D	axial dispersion coefficient (cm^2 s^{-1})
J	dimensionless concentration
k_A	adsorption rate constant (cm^3 g^{-1} s^{-1})
L	length of the packed bed (cm)
M	amount of tracer injected
M_0^M	model zeroth moment
M_1	m_1/m_0, first absolute moment (s)
M_1^M	model first moment (s)
M_2^M	model second moment (s^2)
$M_2^{\#}$	second central moment
m_0	zeroth moment
m_0^E	experimental zeroth moment
m_1^E	experimental first moment (s)
m_2^E	experimental second moment (s^2)
m_n	*n*th moment about the origin, Eq. (7)
$N_{A'}$	net flux to surface (mol g^{-1} s^{-1})
Pe	Péclet number
s	Laplace variable (s^{-1})
t	time (s)
t_0	injection time of pulse (s)
U	interstitial velocity (cm s^{-1})
z	axial length (cm)
β_0	coefficient in Eq. (14)
ϵ	void fraction
ξ	dimensionless length
ρ_p	particle density (g cm^{-3})

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