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Original Article

Removal of Strontium Ions by Immobilized *Saccharomyces Cerevisiae* in Magnetic Chitosan Microspheres

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ABSTRACT

A novel biosorbent, immobilized *Saccharomyces cerevisiae* in magnetic chitosan microspheres was prepared, characterized, and used for the removal of Sr^{2+} from aqueous solution. The structure and morphology of immobilized *S. cerevisiae* before and after Sr^{2+}adsorption were observed using scanning electron microscopy with energy dispersive X-ray spectroscopy. The experimental results showed that the Langmuir and Freundlich isotherm models could be used to describe the Sr^{2+} adsorption onto immobilized *S. cerevisiae* microspheres. The maximal adsorption capacity (q_m) was calculated to be 81.96 mg/g by the Langmuir model. Immobilized *S. cerevisiae* was an effective adsorbent for the Sr^{2+} removal from aqueous solution.

1. Introduction

Radiotoxic ion such as Sr-90 is one of the most hazardous metal ions in radioactive waste because of its high solubility, high transferability, easy accumulation in organisms, and long half-life [1,2]. Conventional methods for the removal of radiotoxic ions from aqueous solution include thermal treatment, chemical precipitation, membrane separation, solvent extraction, and ion exchange [3,4]. Adsorption is a physicochemical process that is economical and highly effective for removing radiotoxic ions from aqueous solution [5]. Different adsorbents have been examined for radiotoxic ions removal, such as bentonite [6], tobermorite [7], bone char [8], and Egyptian soils [9]. However, they possess low adsorption capacities in their natural forms and need to be modified to improve their adsorption characteristics.

The natural polymers and their derivatives can be utilized as economic and environmental-friendly materials for the

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removal of heavy metals and radionuclides from aqueous solution with high efficiency [5,10–12]. Chitosan is a suitable biopolymer for the removal of radiotoxic ions from aqueous solution since the $-NH_2$ and $-OH$ groups on chitosan can act as chelating groups [5,13]. The major application of chitosan and its derivatives is based on its ability to bind heavy metal ions. In addition, chitosan has many chemical and biological properties, such as biocompatible, bioactive, and biodegradable properties. It has been used in many biomedical and industrial applications [5,14].

To improve the adsorption capacity and to prevent the dissolution of chitosan in acidic medium, mechanical properties and various modified chitosan have been studied, respectively, for heavy metal removal [13,15–17].

Microbial immobilization using chitosan may also improve the adsorption capacity. Due to the liquid/solid separation problem, free yeast cells appear to be unsuitable for practical application [11]. Immobilization techniques have been used for the removal of heavy metal ions in recent years [18,19]. Chitosan is a suitable support material for microbial immobilization because of its characteristics, such as resistance to chemical degradation, improvement of mechanical strength, and antibacterial property. Microorganisms immobilized by polymers have many advantages, including easy separation from the reaction medium, continuous or repeated use, and enhancement in the stability of microorganism [19]. The advantages of magnetic chitosan for immobilization of *Saccharomyces cerevisiae* include that the magnetic performance will make the biosorbents easy to separate under the magnetic field; thus, after biosorption, the biosorbents can be easily separated, regenerated, and reused. Moreover, the addition of magnetic particles Fe_3O_4 will also enhance the mechanical strength of the biosorbents, which is very important for prolonging the lifetime of the biosorbents in a practical application.

Different kinds of microorganisms were used for the adsorption of Sr^{2+}, including *S. cerevisiae* [20–22], *Bacillus subtilis* [23,24], and *Pseudomonas mendocina* [25]. We used the waste biomass of *S. cerevisiae* produced from a local brewery for the adsorption of Pb^{2+}, Ag^{+}, Cs^{+}, and Sr^{2+} from aqueous solution [20]. Naeem et al. [21] examined the adsorption of protons, Cd^{2+}, Pb^{2+}, Sr^{2+}, and Zn^{2+} onto the fungal species *S. cerevisiae*. They measured the electrophoretic mobility of the cells as a function of pH and modeled the acid/base properties of the fungal cell wall by invoking a nonelectrostatic surface complexation model. The affinity of the fungal cells for the metal ions follows the following trend: $Pb^{2+} > Zn^{2+} > Cd^{2+} > Sr^{2+}$. Their results suggested that *S. cerevisiae* may be a novel biosorbent for the removal of heavy metal cations from aqueous waste streams. Peng et al. [22] prepared the immobilized *S. cerevisiae* on the surface of chitosan-coated magnetic nanoparticles and applied for removing Cu(II) from aqueous solution. They examined the effect of the initial pH, initial Cu(II) concentration, and contact time on the adsorption of Cu(II). Cu(II) adsorption followed the Langmuir model, and the maximal adsorption capacity was 144.9 mg/g. Fein et al. [23,24] conducted the metal adsorption experiments onto *B. subtilis* and determined the bacterial surface stability constants for Cd^{2+}, Cu^{2+}, Pb^{2+}, Al^{3+}, Co^{2+}, Nd^{2+}, Ni^{2+}, Sr^{2+}, and Zn^{2+}. Borrok and Fein [25] studied the effect of ionic strength on the adsorption of Cd(II), Pb(II), and Sr(II) onto the surfaces of *P. mendocina*.

The objective of this work was to prepare a novel biosorbent, i.e., immobilized *S. cerevisiae* by magnetic chitosan microspheres and use it for removing Sr^{2+} from aqueous solution.

2. Materials and methods

2.1. *Chemicals and reagents*

Chitosan with 90% deacetylation degree was provided by Sinopharm Chemical Reagent Co., Ltd. (SCRC; Beijing, China). All other chemicals used in this study were analytical grade and supplied by Beijing Chemical Plant. Fe(III) and Fe(II) used in this study were $FeSO_4 \cdot 7H_2O$ and $FeCl_3 \cdot 6H_2O$, respectively. $SrCl_2 \cdot 6H_2O$ was used to prepare the stock solution of 1,000 mg/L.

2.2. Preparation of immobilized *S. cerevisiae*

S. cerevisiae biomass was collected from a local brewery and immobilized using the following procedure: (1) chitosan (2 g) was completely dissolved in 100 mL of 2 wt% acetic acid in deionized water; (2) sodium alginate (1 g) was added and dissolved completely, and 20 g of *S. cerevisiae* were added; (3) the mixture was stirred for 30 minutes; and (4) Fe(III) and Fe(II) (0.02 mol:0.01 mol) were dissolved in the above solution, stirred for 30 minutes, and added into 300 mL of 10% (w/v) NaOH solution (containing 7.5 mL of ethyl acetate) drop-wise using a syringe to form magnetic chitosan beads.

2.3. Characterization of the immobilized *S. cerevisiae* microspheres

Scanning electron microscopy (SEM-EDX) was measured using FEI Quanta 200 FEG SEM instrument of FEI Company (Hillsboro, Oregon, USA). SEM-EDX was used to explore the properties of the immobilized *S. cerevisiae* microspheres.

2.4. *Adsorption experiments*

The radioactive wastewater produced in nuclear power plants at normal operation is usually relatively clean, which means that there are a few impurities with the exception of radionuclides. Therefore, no other cations were added for the adsorption experiments in this study.

For batch adsorption experiments, 15 mL of Sr^{2+} solution was mixed with 30 mg of adsorbents in 20-mL glass bottles. The pH values were adjusted using 0.1M HCl or 0.1M NaOH. For the adsorption isotherm experiments, the initial Sr^{2+} concentration varied from 5 mg/L to 300 mg/L at 30°C. After equilibration, the samples were taken and used for the determination of Sr^{2+} concentration.

2.5. *Analytical methods*

The concentration of Sr^{2+} was analyzed using atomic adsorption spectroscopy (AAS6 Vario, Analytik Jena AG, Jena, Germany). The equilibrium adsorption capacity was calculated using the following equation:

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$$q_e = (C_0 - C_e)V/W, \tag{1}$$

where q_e (mg/g) is the equilibrium adsorption amount, C_0 and C_e are the initial and equilibrium Sr^{2+} concentration (mg/L), respectively, V (L) is the volume of the Sr^{2+} solution, and W (g) is the weight of the dried adsorbent.

3. Results and discussion

3.1. Adsorption isotherms

For investigating the influence of the initial concentration, experiments were performed by changing the initial Sr^{2+} concentration from 5 mg/L to 300 mg/L at pH 8 and contact time of 5 hours. The adsorption capacity of Sr^{2+} at different initial concentrations has been illustrated (Fig. 1). The adsorption capacity of Sr^{2+} increased with increasing initial Sr^{2+} concentration. This was attributed to an increase in the driving force by the concentration gradient [26]. At higher concentrations, adsorption capacity got a stable state, which demonstrated the saturation of adsorption active sites on immobilized *S. cerevisiae* [17]. The cell wall of *S. cerevisiae* is composed of several functional groups, such as hydroxyl-, carboxy-, sulfhydryl-, phosphate-, and amino-. Sr^{2+} binding can be due to adsorption, ion-exchange, the formation of microprecipitation and crystallization of Sr and complexation processes occurring on the cell wall of *S. cerevisiae* [11]. The maximum adsorption amount was 77.53 mg/g for immobilized *S. cerevisiae*.

It is important to examine the equilibrium data, which can develop an equation that can be used to compare the different adsorbents under different conditions and to obtain the optimal conditions for the adsorption process [27]. In this study, the Langmuir and the Freundlich isotherms models were used to simulate the experimental data of Sr^{2+} adsorption.

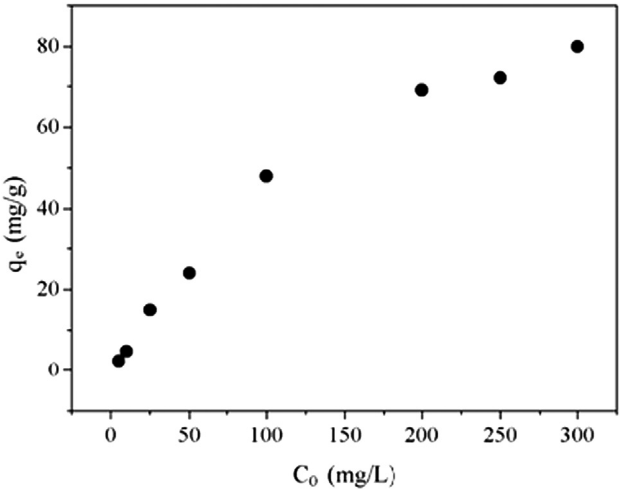

Fig. 1 – Effect of initial concentration on Sr^{2+} adsorption by immobilized *S. cerevisiae* magnetic chitosan (ISMC) microspheres. (W = 30 mg; t = 5 h; T = 30°C; shaking rate = 150 rpm; pH = 8.)

The Langmuir isotherm assumed that the adsorption of heavy metal ions on the adsorbent surface is monolayer coverage; a finite number of sorption sites with identical adsorption energy are on the adsorbent surface. The isotherm can be expressed by the following equation:

$$q_e = q_m bC_e/(1 + bC_e), \tag{2}$$

where q_e (mg/g) and C_e (mg/L) are the amount of adsorption of heavy metal ions and the residual concentration of heavy metal ions in the solution at an equilibrium, respectively; q_m (mg/g) and b (L/mg) are the maximum absorption capacity of heavy metal ions and a constant related to the affinity of the adsorption binding sites, respectively, which can be obtained by the linear plot of the logarithmic equation [28]:

$$C_e/q_e = C_e/q_m + 1/bq_m \tag{3}$$

By plotting C_e/q_e against C_e, it is possible to obtain the value of q_m and b. The Langmuir isotherm equation is applicable to homogeneous sorption and has the advantage of providing the maximum adsorption capacity q_m (mg/g).

The Freundlich isotherm is basically an empirical model and generally used to explain the sorption on heterogeneous surface, it can be expressed as:

$$q_e = K_F C_e^{1/n} \tag{4}$$

In order to obtain K_F and n, the linear form of the equation was used:

$$\ln q_e = \ln K_F + 1/n \ln C_e \tag{5}$$

where q_e is the amount of heavy metal ions per unit weight of absorbent(mg/g); C_e is the equilibrium concentration of heavy metal ions in solution (mg/L); and K_F and n are the Freundlich constants, which relates to absorption capacity and absorption intensity.

The Langmuir and Freundlich isotherm models were used to fit the adsorption of Sr^{2+} by immobilized *S. cerevisiae* magnetic chitosan microspheres (Fig. 2).

The Langmuir and the Freundlich parameters for Sr^{2+} removal by the immobilized *S. cerevisiae* microspheres were obtained by fitting the adsorption equilibrium data listed in Table 1. It can be seen that high correlation coefficients were obtained for both Freundlich ($R^2 = 0.9952$) and Langmuir ($R^2 = 0.9829$) models, indicating that the adsorption equilibrium data can be fitted in both Freundlich as well as Langmuir models. The maximum theoretical adsorption capacity (q_m) obtained by the Langmuir isotherm for Sr^{2+} adsorption was 81.96 mg/g, b = 0.10. The value of b represents the affinity of a given adsorbate to an adsorbent, which relates to the selectivity of the adsorbate. In this paper, b = 0.1, suggesting that the immobilized *S. cerevisiae* microspheres are highly selective to Sr^{2+}. The Freundlich constant (n) suggested whether the adsorption process is favorable or not. The value of $1/n$ is lower than 1, indicating that the adsorption intensity (the degree of the interaction between adsorbate and adsorbent) is suitable for the whole range of concentrations and the heterogeneous adsorption reaction happened [17]. A steep slope ($1/n > 1$) indicates that the adsorption intensity is suitable for the high concentration. In this study, the $1/n$ value for Sr^{2+} was 0.44, representing the suitable adsorption over the whole

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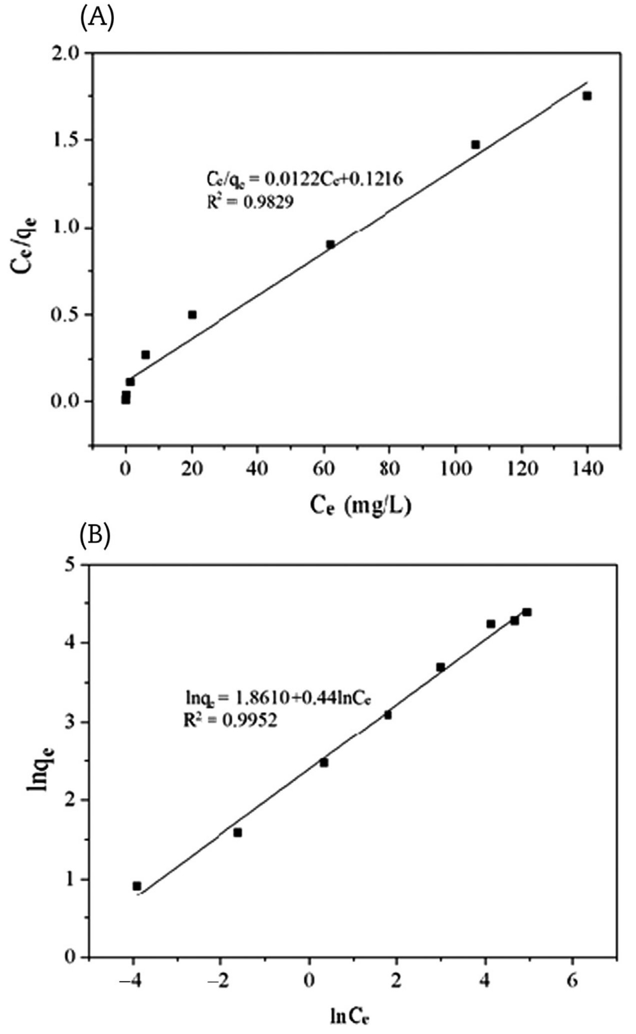

Fig. 2 – Isotherm models fitted for Sr^{2+} adsorption by immobilized *S. cerevisiae* magnetic chitosan (ISMC) microspheres. (A) The Langmuir model. (B) The Freundlich model.

range of concentrations. In comparison with the other materials, the value obtained in this study was higher than that reported by Chen and Wang [20]. "The immobilized *S. cerevisiae*" in this study represents "the immobilized *S. cerevisiae* in magnetic chitosan", which concludes that *S. cerevisiae*, magnetic particles Fe_3O_4, and chitosan played a role in the adsorption of Sr^{2+}; therefore, the higher q_m value (81.96 mg/g) in this study could be attributed to the involvement of *S. cerevisiae*, Fe_3O_4, and chitosan in the adsorption process.

Table 1 – Adsorption parameters of the Langmuir and the Freundlich isotherm models.

Models	Equations	Parameters
Langmuir model	$C_e/q_e = C_e/q_m + 1/bq_m$	$R^2 = 0.9829$ $q_m = 81.96$ mg/g $b = 0.10$
Freundlich model	$\ln q_e = \ln K_F + 1/n \ln C_e$	$R^2 = 0.9952$ $1/n = 0.44$ $K_F = 6.43$

3.2. SEM-EDX of immobilized *S. cerevisiae* microspheres

The SEM-EDX images of the microspheres before and after the adsorption of Sr^{2+} were investigated to confirm the adsorption of Sr^{2+} onto the immobilized *S. cerevisiae*.

The SEM pictures of the immobilized *S. cerevisiae* microspheres before Sr^{2+} adsorption are shown in Figs. 3A and 3B. Fig. 3A shows that the immobilized *S. cerevisiae* microsphere was sized about 1 mm in diameter. Fig. 3B shows that *S. cerevisiae* is physically entrapped within the network of chitosan beads because the pore size of beads is much smaller than the size of *S. cerevisiae*. Chitosan is a natural macromolecule, and it can form gels at proper conditions. In the magnetic chitosan microsphere of immobilized cells, a grid-like structure was observed; immobilized *S. cerevisiae* appeared slightly shrunk and was embedded in the grid-like structure. Fig. 3C shows the EDX spectra of the immobilized *S. cerevisiae* microspheres before adsorption of Sr^{2+}, which clearly indicates the peak of Fe (from Fe_3O_4), Mg and P (from biomass), thereby confirming that "immobilized *S. cerevisiae*" consists of Fe_3O_4, chitosan, and *S. cerevisiae*. Fig. 3D shows the EDX spectra of the immobilized *S. cerevisiae* microspheres after adsorption of Sr^{2+}, which clearly indicates the presence of Sr^{2+} peak, thereby confirming that Sr^{2+} adsorption occurred onto the microspheres. Fig. 3D provided the direct evidence of Sr^{2+} adsorption by the immobilized *S. cerevisiae* microspheres. Kousalya et al. [29] applied the EDX analysis to prove the presence of Cu(II) and Fe(III) after the adsorption of metal ions by modified forms of chitin. Ma et al. [30] used the EDX analysis to confirm the existence of Co^{2+}, Sr^{2+}, and Cs^{+} after the removal of Co^{2+}, Sr^{2+}, and Cs^{+} from aqueous solution by phosphate-modified montmorillonite.

3.3. *Comparison of Sr^{2+} sorption by different adsorbents*

Table 2 represents the adsorption capacity of Sr^{2+} by the immobilized *S. cerevisiae* microspheres and other adsorbents. It is necessary to state that most of these studies were conducted without adding the supporting electrolyte in the simulated solutions; hence, the comparison of the adsorption capacity of different adsorbents is reasonable.

For q_m obtained from the Langmuir isotherm, the adsorption capacity of Sr^{2+} by potassium tetratitanate whisker was the highest [31]. In comparison with other adsorbing materials, the adsorption capacity of Sr^{2+} by the immobilized *S. cerevisiae* microspheres was relatively high, much higher than the average value shown in Table 2.

S. cerevisiae is easy to cultivate at a large scale. The yeast can be easily grown using unsophisticated fermentation techniques and inexpensive growth media. Furthermore, the biomass of S. cerevisiae can be obtained from various food and beverage industries. *S. cerevisiae*, as a by-product, is easier to get from the fermentation industry, than other types of waste microbial biomass. *S. cerevisiae* is generally regarded as safe; therefore, the biosorbent made from *S. cerevisiae* can be easily accepted by the public when applied practically. Thus, the

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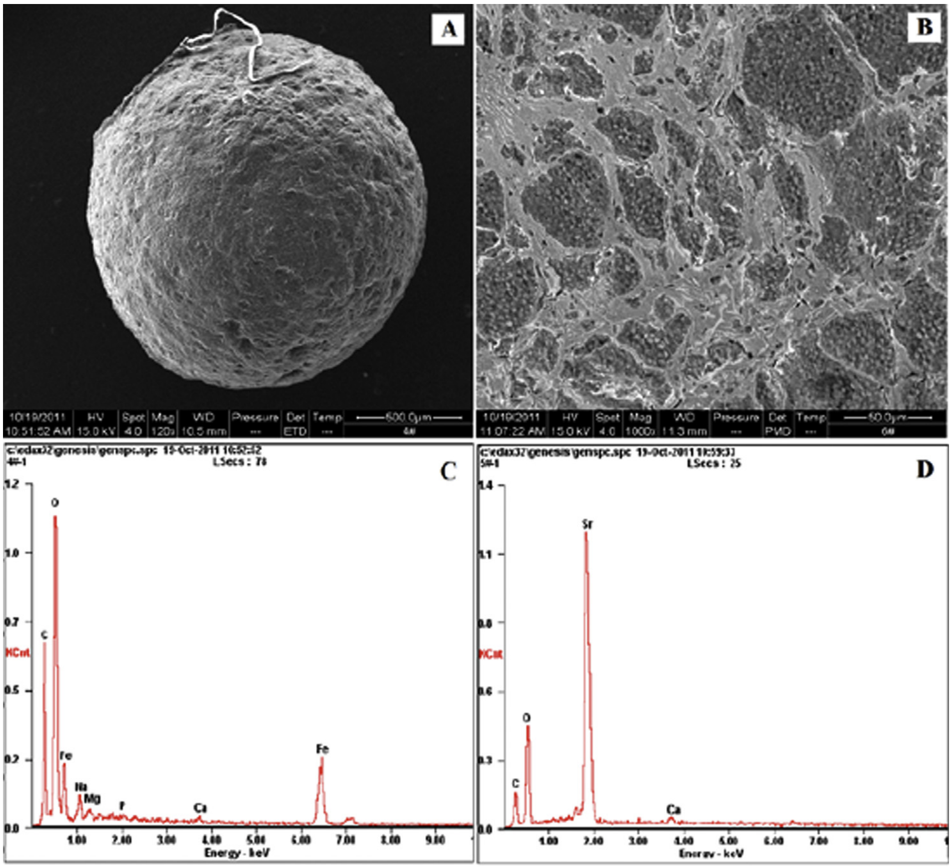

Fig. 3 – Scanning electron microscopy (SEM) images and energy dispersive X-ray spectroscopy (EDX) spectra of immobilized *S. cerevisiae* magnetic chitosan microspheres. (A) SEM image before Sr^{2+} adsorption. (B) SEM image after Sr^{2+} adsorption. (C) EDX spectra before Sr^{2+} adsorption. (D) EDX spectra after Sr^{2+} adsorption.

Table 2 – Comparison of Sr^{2+} adsorption by different adsorbents.

Absorbent	pH	Concentration (mg/L)	Adsorption isotherm	q_m/(mg/g)	References
Carbon nanotubes	7.0	10–75	Langmuir	6.62	[2]
Saccharomyces cerevisiae	4.0		Langmuir	7.97	[23]
Egyptian soils	7.8	8.8×10^{-3}–8.8	Freundlich	12.79	[9]
Ammonium molybdophosphate-polyacrylonitrile (AMP-PAN)	5.0	88–2640	Langmuir	15.77	[4]
Hydroxyapatite nanoparticles	7.0	88–2640	Langmuir	50.47	[25]
Hydroxypropyl methylcellulose phthalate (HPMCP)	4.0	–	Langmuir	83.30	[3]
Sodium trititanate whisker (STW)	6.0	10–500	Langmuir	95.2	[26]
Carboxymethylated chitosan (CMCts)	4.0	–	Langmuir	99.00	[3]
Potassium tetratitanate whisker (PTW)	6.0	10–500	Langmuir	104.2	[26]
Immobilized *Saccharomyces cerevisiae*	8.0	5–300	Langmuir	81.96	This study

immobilized *S. cerevisiae* could be an effective adsorbent for Sr^{2+} removal from aqueous solution.

4. Conclusion

The immobilized *S. cerevisiae* magnetic chitosan microspheres can be used as effective biosorbent for removing Sr^{2+} from aqueous solution. The adsorption capacity of Sr^{2+} increased with the increase in initial Sr^{2+} concentration at 30°C and pH of 8. The Freundlich isotherm model could be used to describe the Sr^{2+} adsorption onto immobilized *S. cerevisiae* microspheres. The results of SEM-EDX analyses confirmed the adsorption of Sr^{2+} onto the immobilized *S. cerevisiae* microspheres. The immobilized *S. cerevisiae* could be an effective adsorbent for Sr^{2+} removal from aqueous solution.

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Conflicts of interest

There is no conflict of interest.

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