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Composite Biosorbents of Metal Ions Based on Yeast Cells and Diatomite

K.B. Korzhynbayeva[1*], S.M. Tazhibayeva[1], K.B. Musabekov[1], I. Dekany[2], M.M. Burkitbaev[1],
A.A. Zhubanova[1], and A.B. Orazymbetova[1]

[1] al-Farabi Kazakh National University, Al-Farabi av. 71, 050040, Almaty, Kazakhstan
[2] University of Szeged, Aradi vertanuk tere 1, H-6720, Szeged, Hungary

Abstract

The possibility of removing Cu^{2+} and Pb^{2+} ions from solutions by the yeast cells *Rhodotorula glutinis* and diatomite (natural mineral) was studied. It is shown that at the concentration of $CuSO_4$ and $Pb(NO_3)_2$ 10^{-3} mol/l the removal of metal ions by yeast cells was 59.1 and 72.4% for the ions of Cu^{2+} and Pb^{2+}. The yeast cells surface includes amino, hydroxyl, phosphate and carboxyl groups which activates sorption ability, because these groups can bind metal ions by ion exchange, donor acceptor and electrostatic interactions. The removal degree of metal ions by diatomite under the same conditions was for Cu^{2+} 91.6% and for Pb^{2+} 94.7%. To increase the removal degree of metal ions from solutions, the yeast cells were immobilized on the surface of diatomite. In order to attach the negatively charged cells of microorganisms with negatively charged surface of the mineral, the surface of diatomite was modified by polyethylenimine (PEI). As a result, the immobilization degree of *Rhodotorula glutinis* to the surface of diatomite at the concentration of PEI 0.02 base-mol/l increased from 62 to 88%, which is explained by the existence of electrostatic contacts between the negatively charged functional groups of the cell surface and amino groups of PEI, that has covered the surface of diatomite by PEI. It is shown that the obtained composite biosorbent removes 97.8% of Cu^{2+} ions and 99.4% of Pb^{2+} ions.

Introduction

Nowadays, water purification, especially industrial wastewaters, is an actual problem. The industrial wastewater contains a great quantity of heavy metal ions [1], which can have a negative impact on the environment and human health. One of the ways against water and soil pollution with heavy metal ions is analysis of effective sorbents. Researchers are greatly interested in removing metal ions from suspension using the cells of microorganisms as they differ in a variety of functional groups on their surface.

The advantage of biosorbents over mineral sorbents is their selectivity which is provided by specificity of interaction of functional groups with metal ions [2]. However, the problem for a wider use of biosorbents for water purification is in the difficulty of separation of microorganisms cells from the suspension. As it is known, cells are natural highly liophylic system that are impossible to separate from suspension by the precipitation method [3]. A convenient way to facilitate the separation of microorganism cells from the suspension is immobilization of cells of microorganisms on the surface of solid adsorbents.

The aim of this work is to optimize the conditions for extraction of Cu^{2+} and Pb^{2+} ions from solutions using cells of microorganisms immobilized on diatomite.

Materials and Methods

Solutions of Cu^{2+} and Pb^{2+}

Solutions of Cu^{2+} and Pb^{2+} salts were prepared from analytically grade copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) and lead nitrate ($Pb(NO_3)_2$). Concentration of salts in the range from 10^{-5} mol/l to 10^{-1} mol/l was used.

Sorbents

Yeast cells of *Rhodotorula glutinis* with the size (2.5-3.0) × (6.0-6.5) μm were used as biosorbents of metal ions.

Natural mineral diatomite was used as a carrier of cells. Diatomite is a natural highly porous material, which is deposited in large quantities in the Aktobe region, Kazakhstan. Chemical composition of diatomite from Aktobe deposits was determined by X-ray

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[*] Corresponding author. E-mail: kuralay12@mail.ru

phase analysis on diffractometer DRON - 4-07. The specific surface area determined by low temperature adsorption of nitrogen on AccuSorb made up 46 m^2/g.

Electrophoresis

The electrophoretic mobility of diatomite particles was measured on the equipment for electrophoresis.

Electron Microscopic Images

Electron microscopic images of the surface of diatomite were obtained by a scanning electron microscope SEI Quanta 3D 200 I (USA).

Adsorption

Adsorption of Cu^{2+} and Pb^{2+} ions on the surface of diatomite and biosorbents was studied under static conditions. For this, samples of diatomite (or cells) weighing 1 g were mixed with 10 ml solutions of $CuSO_4$ and $Pb(NO_3)_2$. Then the mixture was left to stand for 2 hours. Then, the solutions were separated from the sorbent and analyzed for the content of metal ions.

Analysis of solutions for Cu^{2+} and Pb^{2+} ions was carried out by spectrophotometer UV-7504 Shimadzu (Japan). The amount of adsorption of metal ions was calculated according to:

$$A = (C_1 - C_2) \cdot V/m,$$

where C_1 and C_2 are initial and equilibrium concentrations of metal ions, mol/l; m - is mass of diatomite, g; V - is volume of solutions of salts, l.

pH of diatomite suspensions and cells of microorganisms was regulated with 0.1N HCl and NaOH. The value of pH of solutions was regulated by pH meter 827 pH Lab, Metrohm (Switzerland).

Immobilization of Cells of Microorganisms on Diatomite

For immobilizing cells of microorganisms, the surface of diatomite samples initially was modified by polyethyleneimine (PEI) solution.

Results and Discussion

Adsorption of Cu^{2+} and Pb^{2+} Ions on the Surface of Rhodotorula Glutinis

Walls of microorganisms cells have a complex structure which is made of proteins, lipids and fat acids, alcohols, carbohydrates. This, in its turn, conditions the presence of hydroxyl, carboxyl, amino, ester, sulfhydryl, carbonyl and phosphate groups on them. Each of these groups can bind metal ions [4].

When removing metal ions from solutions with the help of biosorbents, many researchers use different initial concentrations of metal ions [5, 6] from $2 \cdot 10^{-5}$ mol/l for algae to several mol/l for biomasses such as Palm flower, Pomegranate peel, Rice bran and others [7-9]. So, to determine the sorption ability of *Rhodotorulo glutinis* cells, a high range of concentrations was used.

Experiments on the removal of Cu^{2+} and Pb^{2+} ions by yeast cells *Rhodotorula glutinis* in the range of concentrations 10^{-5}-10^{-1} mol/l showed that they adsorbed metal ions well (Table 1). At the concentration of $CuSO_4$ and $Pb(NO_3)_2 10^{-5}$ mol/l after a 24 hour contact of cells with suspensions the removal degree of Cu^{2+} and Pb^{2+} ions is 95.2-98.3%.

Table 1

Removing of ions of Cu^{2+} and Pb^{2+} from solution with yeast cells

C_0, mol/l	Removal degree (R), %	
	Cu	Pb
10^{-5}	95.2	98.3
10^{-4}	64.0	79.1
10^{-3}	59.1	72.4
10^{-2}	54.7	61.3
10^{-1}	50.1	59.7

The cells of microorganisms are good sorbents of metal ions [4], but the time for them to reach adsorption equilibrium differs depending on the nature of sorbents and the conditions of adsorption. In this relation, the experiments on determination of adsorption of Cu^{2+} and Pb^{2+} ions on the surface of yeast cells *Rhodotorula glutinis* depending on the contact time were performed.

The studies on the effect of the contact time of Cu^{2+} and Pb^{2+} ions with the concentration of 64 and 203 mg/l with the cells of microorganisms showed that curves of the removal degree for Pb^{2+} ions after 30 minutes and for Cu^{2+} ions after 60 minutes reached a plateau (Fig. 1). This is not surprising, as many biosorbents reach maximum removal degree of metal ions from the suspension for 30-60 min [5, 10]. So, the contact time of 2 hours was chosen by us for further studies as the time sufficient for the onset of adsorption equilibrium in the system.

Adsorption curves of Cu^{2+} and Pb^{2+} ions on the surface of cells *Rhodotorula glutinis* after 2 hours of contact are shown in Fig. 2. The peculiarity of adsorption curves of Cu^{2+} and Pb^{2+} ions on the yeast cells is the presence of an expressed plateau on the adsorption curves of Cu^{2+} ions corresponding to saturation of the surface. In case of Pb^{2+} ions, the value of adsorption is much higher, however, it becomes constant at higher concentrations of Pb^{2+} ions. It indicates differences in the mechanism of binding of Cu^{2+} and Pb^{2+} ions with the surface of yeast cells.

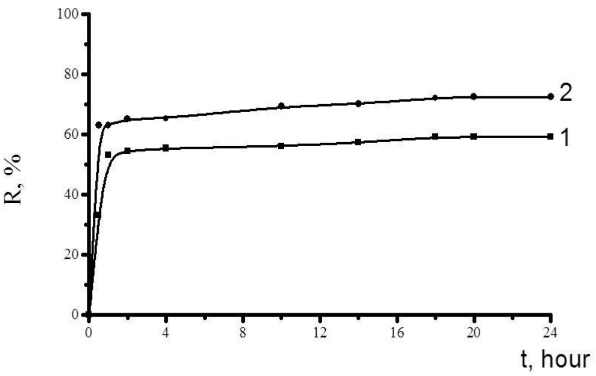


Fig. 1. The effect of the contact time of Cu^{2+} (1), and Pb^{2+} (2) ions with cells on the removal degree of metal ions. $C_0 = 1.10^{-3}$ mol/l.

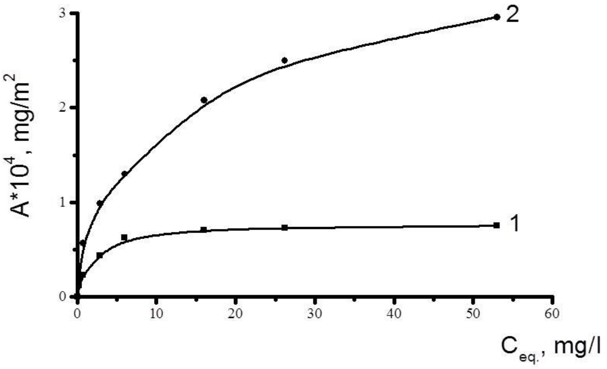


Fig. 2. Adsorption curves of ions of Cu^{2+} and Pb^{2+} on the surface of cells after 2 hours of contacts.

Adsorption of Metal ions on Diatomite

The studies on removal of Cu^{2+} and Pb^{2+} ions from $CuSO_4$ and $Pb(NO_3)_2$ solutions by diatomite have shown that the increase in concentrations of salts from 10^{-5} mol/l to 10^{-1} mol/l results in the decrease of the removal degree of Cu^{2+} ions from 92.1 to 15.6%, and for ions of Pb^{2+} from 95.1 to 20.3% (Table 2). Moreover, the most complete removal of metal ions is observed in the range of concentrations from 10^{-5} to 10^{-3} mol/l.

Obviously, the concentration range of 10^{-2}-10^{-1} mol/l is excessive relatively to the surface of diatomite and further studies of the adsorption process in this area of concentrations are not expedient. Therefore, for further experiments, concentrations (0.2-1.0)•10^{-3} mol/l were chosen.

Table 2

Dependence of ions of Cu^{2+} and Pb^{2+} removal degree with diatomite from their initial concentrations in solutions

C_0, mol/l	R_{Cu}, %	R_{Pb}, %
10^{-5}	92.1	95.6
10^{-4}	91.7	94.8
10^{-3}	91.6	94.7
10^{-2}	61.5	70.0
10^{-1}	15.6	20.3

Adsorption curves of Cu^{2+} and Pb^{2+} ions on the surface of diatomite are shown in Fig. 3. As is shown in the figure, the adsorption isotherm of Pb^{2+} ions is slightly above the adsorption curves of Cu^{2+} ions, which indicates the great affinity of Pb^{2+} ions for the surface of diatomite.

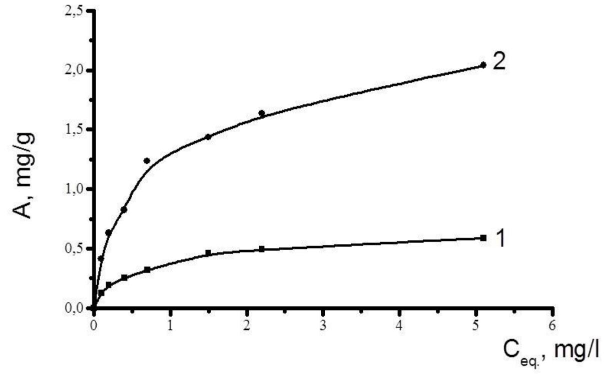


Fig. 3. Adsorption isotherms of Cu^{2+} (1) and Pb^{2+} (2) ions on diatomite. T = 298 K.

The value of maximum adsorption A_{max} is calculated by the method of processing of adsorption curves according to Langmuir. The value of A_{max} for Cu^{2+} ions is 0.56 mg/g and for Pb^{2+} ions is 2.19 mg/g, which also proves the preference of adsorption of Pb^{2+} ions on the surface of diatomite.

For comparison of adsorption values of Cu^{2+} and Pb^{2+} ions on cells *Rhodotorula glutinis* and on diatomite one can transform A_{max} of diatomite, measured in mg/g, into mg/m^2 by taking into account the specific surface of mineral. The calculated in this way values of A_{max} of Cu^{2+} and Pb^{2+} ions were $1.2\cdot10^{-2}$ mg/m^2 and $4.7\cdot10^{-2}$ mg/m^2. In case of microorganisms cells, these values are $0.7\cdot10^{-4}$ mg/m^2 and $2.9\cdot10^{-4}$ mg/m^2. A higher sorption ability of diatomite compared with that of microorganisms cells can be explained by its porosity [11].

To determine the mechanism of adsorption of these metal ions on diatomite, we will consider the compositions of diatomite (Table 3).

Table 3
Chemical composition of diatomite

Chemical composition, %	Diatomite	Chemical composition, %	Diatomite
SiO_2	65.83	MgO	1.01
Al_2O_3	8.66	Na_2O	0.75
Fe_2O_3	2.98	TiO_2	0.49
K_2O	1.16	CaO	0.47
		Cl	0.56

As is shown in Table 3, the main component of diatomite is SiO_2. It is natural to assume that interactions of silanol groups with metal ions are determining in the system. Apparently, adsorption in the system is conditioned by ion exchange processes, which can be expressed as follows:

$$2 \equiv Si - O - H + Me^{2+} \rightarrow \equiv Si - O - Me^{2+} - O - Si \equiv$$

As it is known [12], silanol groups of the surface of clay minerals depending on the pH of the medium can be in dissociated, non dissociated and protonated states:

$$\equiv SiOH + H^+ \rightarrow \equiv SiOH_2^+ \text{ (acidic medium)}$$
$$\equiv SiOH + OH^- \rightarrow \equiv SiO^- + H_2O \text{ (alkaline medium)}$$
$$\equiv SiOH - \text{(neutral medium)}$$

According to the electrophoretic data, the electrokinetic potential of the native surface of diatomite is -21.0 mV (pH=5.7) (Fig. 4). Acidification of the medium leads to higher values of ζ, which is related to the increase in the proportion of protonated silanol groups. Introduction of sodium hydroxide to the suspension of diatomite improves the negativeness of the surface due to dissociation of the silanol groups.

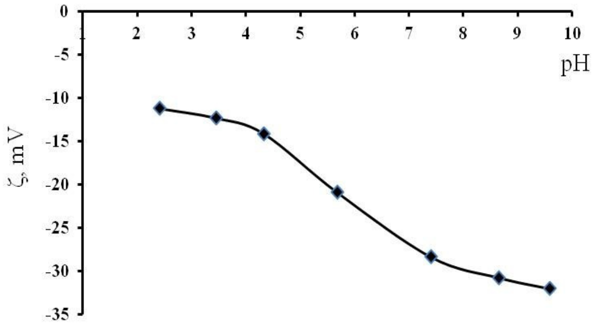


Fig. 4. The effect of pH on ζ-potential of the surface of diatomite particles.

Although higher pH of the medium favors dissociation of silanol groups, and experiments on the adsorption of metal ions in this area is limited due to the possibility of formation of hydroxides.

As is shown in Fig. 5, acidification of the medium leads to the decrease in the removal degree of metal ions due to the competition H^+-ions with metal ions for interaction with the silanol groups. The presence of silanol groups in the form of SiOH and SiO^-- groups creates conditions favorable for the procedure of the ion exchange reaction between them and the metal ions; and the electrostatic attraction between them.

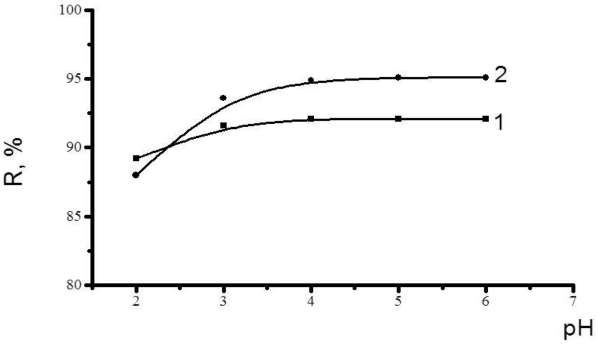


Fig. 5. The effect of pH of the medium on the removal degree of Cu^{2+} (1), and of Pb^{2+} (2) ions from the solutions. $C_0 = 1 \cdot 10^{-3}$ mol/l.

Therefore, experiments on the studies of influence of pH of the medium on the removal degree of Cu^{2+} and Pb^{2+} ions from suspensions using diatomite were carried out only in an acidic medium.

Thus, the maximum removal degree of ions of Cu^{2+} and Pb^{2+} from solutions with concentration 10^{-3} mol/l by diatomite makes up 91.6 and 94.7%. Such removal degree cannot solve the problem of wastewater purification, as the residual concentration of metal ions exceeds the maximum allowable concentration, which is 0.1-4.0 mg/l for ions of Cu^{2+} and 0.2-1.0 mg/l for ions of Pb^{2+} [13].

The Composition Based on Yeast Cells, Diatomite and PEI as a Biosorbent

Currently, to obtain materials with the designed properties, the methods of obtaining compositions of different substances are widely used. Diatomite used by us as a sorbent of metal ions is a highly porous mineral with a high mechanical strength, and considerable sorption ability. An important factor is that this mineral is a relatively cheaper mineral raw material.

The cells of microorganisms are also optimal sorbents of metal ions, which, in response to the negative effect of the environment, can transform on its surface additional functional groups capable of binding metal ions [3]. This is indicated by results of Ulberg's work [3], which shows the abnormal increase in the negative value of zeta potential with the increase in concentration of introduced metal ions [14]. Nevertheless, the experiments on the removal of metal ions using diatomite and microorganisms' cells show that complete removal of Cu^{2+} and Pb^{2+} ions from solution is achieved only at the initial salt concentration of $1·10^{-5}$ mol/l (Tables 1 and 2). An effective way to increase the degree of purification of water is to increase the amount of useful adsorbents. However, in real conditions this implies overconsumption of the adsorbent. A convenient way to solve the problem of increasing the degree of purification may be production of compositions of diatomite and microorganisms cells, immobilization of cells on the surface of diatomite. The known methods for immobilization of cells on the surface of solid carriers is based on the adsorption of cells due to covalent and noncovalent interactions [15]. Non-covalent immobilization of microorganisms cells is most widely used in practice due to its simplicity of existence and low cost.

It is difficult to use the method of immobilization of the yeast cells *Rhodotorula glutinis* on the surface of diatomite because cells and carrier are negatively charged. Therefore, it is necessary to preliminary modify the surface of diatomite. Polyethyleneimine was chosen as a surface modifier. In an aqueous solution, imine groups of PEI are protonated by ions of H^+ of water to $-NH_2^+$, imparting a positive charge to the polymer macromolecules. So, aminogroups of PEI can electrostatically be attracted both to the surface of diatomite and to the surface of cells.

However, the results of experiments on cells immobilization on the surface of diatomite, treated with polyethyleneimine (PEI) showed that the use of the polymer is not always efficient for immobilization of cells. As is seen in Fig. 6, immobilization of cells *Rhodotorula glutinis* on the surface of diatomite is maximum at the concentration of PEI equal to 0.02 base-mol/l. The further increase in the concentration of PEI reduces the degree of immobilization of cells. This can be explained by the formation on the surface of mineral particles of an adsorption layer of PEI of a greater thickness covering pores and projections.

The results of studies on immobilization of yeast cells *Rhodotorula glutinis* on the surface of the modified diatomite showed (Fig. 7), that up to 11.8·106 *Rhodotorula glutinis* cells can be adsorbed on the surface of 1 g of diatomite. High immobilizing ability of diatomite in regard to microorganisms cells may be due to the presence on the surface of microorganisms cells of a large amount of negatively charged functional groups capable of electrostatic interaction with amino groups of PEI. It is known [16] that one cell has in average 107-108 negative charges and 106-107 positive charges. Also, of importance is the possibility of penetration of some part of cells into the pores of diatomite. According to [17], the pore size on the surface of diatomite varies from 60 nanometers to 1 micron.

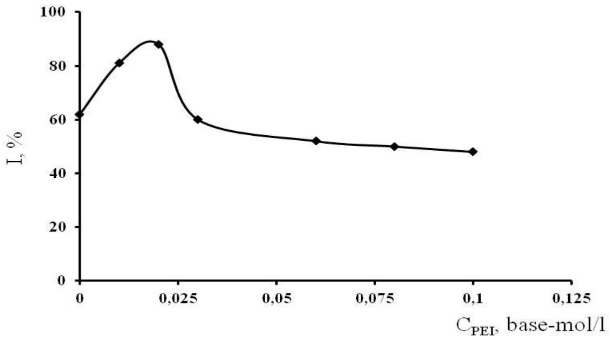


Fig. 6. The dependence of immobilization degree of the yeast cells *Rhodotorula glutinis* on concentration of polyethyleneimine.

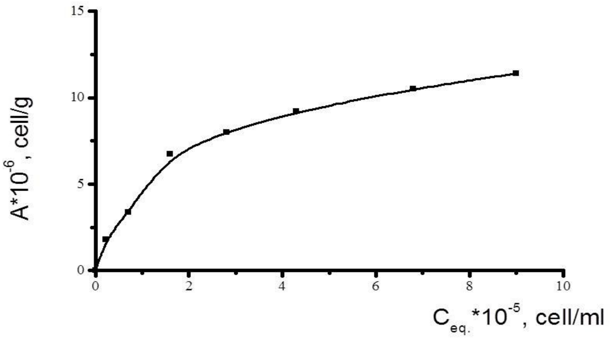


Fig. 7. Dependence of immobilization of cells *Rhodotorula glutinis* on the surface of diatomite on equilibrium concentration of cells.

As a result of adsorption, recharging of the diatomite surface occurs on the surface of polymer and on the surface of diatomite. When the surface of the mineral particles becomes positively charged, it creates conditions for adsorption of negatively charged yeast cells on it (Fig. 8).

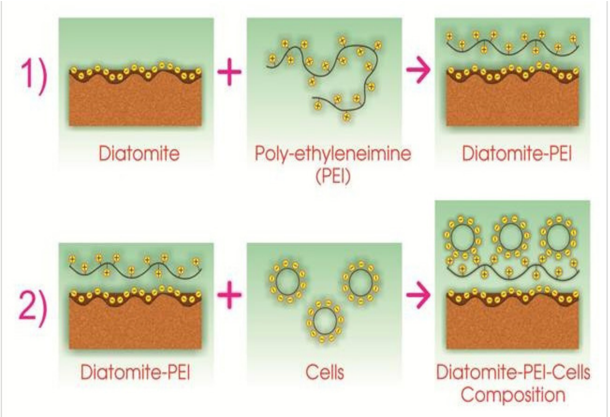
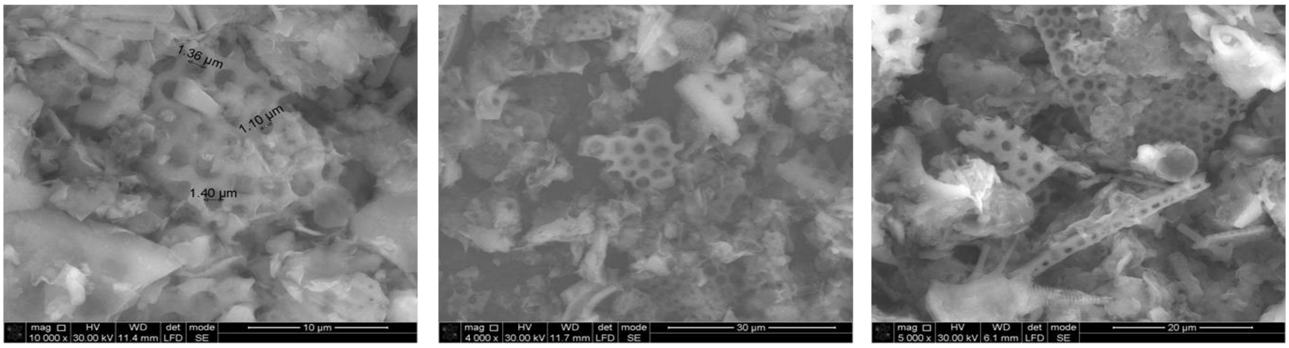


Fig. 8. Schemes of modification of the surface of diatomite by PEI (1) and immobilization of yeast cells on the modified surface of diatomite (2).

Figure 9 presents electron micrographs of diatomite without PEI (a), with PEI (b) and Diatomite-PEI-Cells compositions (c). Covering of diatomite's surface with polymer decreases the sharpness of graphs relief of mineral surface, at the same time some separate cells are seen on the surface of modified diatomite.



a b c

Fig. 9. Electron micrographs of the surface of native diatomite (a), diatomite modified by PEI (b) and Diatomite – PEI – Cells Compositions (c).

Experiments on removal of Cu^{2+} and Pb^{2+} ions from solutions by the composition diatomite-PEI-cells were performed (Fig. 10). It is shown that the synthesized biosorbent removes in the first 0.5 hour up to 97.8% of Cu^{2+} ions and 99.4% of Pb^{2+} ions.

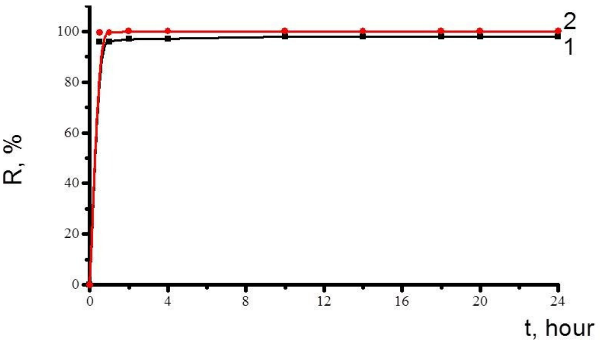


Fig. 10. Effects of the contact time of composition diatomite-PEI-cells with suspensions of salts on the removal degree of ions of Cu^{2+} (1), and ions of Pb^{2+} (2). $C_0=1\cdot10^{-3}$ mol/l.

The high sorption ability of the synthesized composite biosorbent is explained by the fact that all components are potential sorbents of metal ions. The sorption ability of the yeast cells Rhodotorula glutinis and diatomite was assessed in this study. At the concentration of salts 10^{-3} mol/l, cells of Rhodotorula glutinis removes 59.1% of Cu^{2+} ions, and 72.4% of Pb^{2+} ions. PEI complexation ability is well known, too [18]. Complexation of PEI with ions of Cu^{2+} is based on formation of a donor-acceptor bond between nitrogen atoms of the amino groups of the polymer and d-elements. It is necessary to take into account the fact that complex of PEI with ions of transition metals is soluble in water. So, the process of complexation of PEI with ions of transition metals is studied in solutions by the methods of potentiometry and spectrometry. For removing them from solutions, they should be isolated into a separate phase. In studies [19, 20], the possibility of using reactions of complex formation of PEI with transition metal ions for removing metal ions from solutions was shown [20]. Also, for removing metal ions from solutions it is necessary to add anionic polymers and surfactants. The coordination complex of PEI/Me^{2+} acquires the charge of metal ions and anionic polymers and surfactants neutralize this charge and hydrophobize the complex with non-polar sites of macromolecules or hydrocarbon radicals. The removal degree of mentioned metals by diatomite under the same conditions makes up 91.6% for Cu^{2+} ions and 94.7% for Pb^{2+} (Table 2).

Conclusion

Thus, immobilization of yeast cells Rhodotorula glutinis on diatomite in the presence of PEI was carried out. The highest degree of attachment of yeast cells to the surface of diatomite is observed at the concentration of PEI 0.02 base-mol/l. Immobilization of negatively charged cells on the negatively charged surface of diatomite is due to the modifying effect of PEI on the surface of diatomite, whereby the surface of particles of diatomite acquires a positive charge. Acquisition of a positive charge by the surface of diatomite provides electrostatic attraction of negatively charged yeast cells to it. The synthesized composition removes up to 97.8% of Cu^{2+} ions and 99.4% of Pb^{2+} ions from the suspensions with the concentration of 10^{-3} mol/l. In addition, the maximum removal degree of metal ions by biosorbent is reached in the first 0.5 hour.

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