Досліджено процес біосорбції катіонів міді Си²⁺ магнітокерованим біосорбентом, отриманим за допомогою багатовихрового магнітогідродинамічного перемішування дріжджів S. cerevisiae і нанорозмірних магнітних міток. Встановлено ступінь вилучення катіонів міді у залежності від параметрів магнітогідродинамічного перемішування. Оптимізовано параметри отримання магнітокерованого біосорбента, при яких його максимальна сорбційна ємність співпадає із сорбційною ємністю нативних дріжджів

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Ключові слова: магнітна рідина, магнітогідродинамічне перемішування, магнітокерований біосорбент, біосорбція, магнітна сприйнятливість

Исследован процесс биосорбции катионов меди Cu²⁺ магнитоуправляемым биосорбентом, полученным с помощью многовихревого магнитогидродинамического перемешивания дрожжей S. cerevisiae и наноразмерных магнитных меток. Определена степень извлечения катионов меди в зависимости от параметров магнитогидродинамического перемешивания. Оптимизированы параметры получения магнитоуправляемого биосорбента, при которых его максимальная сорбционная ёмкость совпадает с сорбционной ёмкостью нативных дрожжей

Ключевые слова: магнитная жидкость, магнитогидродинамическое перемешивание, магнитоуправляемый биосорбент, биосорбция, магнитная восприимчивость

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1. Introduction

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The development and use of magnetically operated biosorbents for biosorption of heavy metals is an urgent problem today. Numerous studies have been devoted to the research on a possible use of biosorption of heavy metal cations (for example, of copper, iron, and chromium) by the yeast *S. cerevisiae* [1–4] and by magnetically operated yeasts – with the attached nanoscale magnetic labels of Fe₃O₄ [5]. The mechanism of attaching magnetic labels to yeast cells has not been fully elucidated, but there have been studies on this problem, for example [6, 7].

The process of attaching magnetic labels to yeast cells and the process of biosorption of metal cations can be optimized by stirring. One of the methods of stirring is multivortical magnetohydrodynamic (MHD) stirring [8]. It has been found that, when using MHD stirring to obtain complexes of a yeast cell-magnetic labels, the nanomagnetite that is attached to the yeast cells virtually does not reduce the mass-transfer surface. It has also been found that the sorption capacity of magnetically labeled yeast cells obtained by MHD stirring is higher than the sorption capacity of magnetically labeled yeast cells obtained by mechanical mixing. The sorption capacity of magnetically labeled yeast cells obtained by MHD stirring is not reduced in comparison to the native yeast sorption capacity [9], and the value is approximately 25.5 mg Cu^{2+}/g of the dry mass under optimal biosorption conditions [10].

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THE DEVELOPMENT OF A MAGNETICALLY OPERATED BIOSORBENT BASED ON THE YEAST SACCHAROMYCES CEREVISIAE FOR REMOVING COPPER CATIONS Cu²⁺

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2. Literature review and problem statement

Yeast biomass modifications by magnetic labels have been researched for a long time. Studies have been conducted on the yeast S. cerevisiae cells being attached with micro-scale magnetite particles [11] or nanoscale magnetite particles [12], alone magnetic labels [13] or polymer coated ones [14]. Depending on the yeast condition, there have been studies of attaching magnetic labels without any prior modification of the yeast cell wall [15] or with some treatment to activate the surface [16]. The phenomenon of MHD stirring of electrolytes and biological objects has been described theoretically and experimentally in studies such as [8, 17]. The use of multivortical MHD stirring to attach magnetic labels to biological objects is of interest because there appears a possibility to modify the bioagents by magnetic labels within minutes. Then, the solution and the magnetically labeled complexes can be separated through a magnetic separator in either a flow or a steady mode.

Biosorption of heavy metal cations occurs simultaneously through various mechanisms, which is evidenced by the differences in the sorption capacity of yeast in relation to, for example, the cations of Cu^{2+} , Cd^{2+} , and Pb^{2+} [18], as well as Fe²⁺, Pd²⁺, Co²⁺, Hg²⁺, and other heavy metal cations [1]. Magnetic labels are attached to yeast cells due to electrostatic interactions that can be described by the electrokinetic potential of the yeast surface and the magnetite. Such tests are described in [19]. There are also studies of determining the magnetic susceptibilities of magnetically operated, native and modified yeast cells [6, 20]. The results of the literature review have showed that the sorption capacity, the degree of removing heavy metal cations and the magnetic susceptibility of complexes may differ significantly, depending on the method of attaching magnetic labels.

The efficiency of attaching magnetic labels to yeast cells by using multivortical MHD stirring depends on the medium pH and the mass ratio of magnetic nanoparticles to yeast [21]. Multivortical MHD stirring in turn depends on the strength of the external magnetic field and the duration of stirring [22]. Therefore, it is necessary to compare the sorption characteristics of magnetically labeled yeast with the process parameters of attaching magnetic labels to yeast cells.

3. The purpose and objectives of the study

The aim of the study is to develop a magnetically operated biosorbent based on the yeast *S. cerevisiae* with optimal parameters of the production process for biosorption of copper cations. The determined optimal parameters of the production process in terms of the sorption characteristics can facilitate efficient use of the magnetically operated biosorbent and reduce the cost of removing heavy metal cations.

To achieve the aim, it is necessary to do the following tasks:

 to determine the parameters of multivortical MHD stirring to obtain an optimal magnetically operated biosorbent based on the yeast *Saccharomyces cerevisiae*;

 to specify the optimal parameters of producing a magnetically operated biosorbent;

 to obtain sorption isotherms of copper cations by magnetically operated biosorbents depending on the duration of the biosorbents' production;

to examine the magnetic susceptibility of the magnetically operated biosorbents.

4. Materials and methods of researching the characteristics of biosorbents

4. 1. Research materials and the experimental installation

The experiments were based on using the yeast *Saccharo-myces cerevisiae* produced by the CJSC Enzyme (Ukraine), crystalline copper $CuSO_4 \times 5H_2O$, a nanoscale magnetite solution based on ferric chloride $FeCl_3 \times 6H_2O$ and $FeCl_2$, nitric HNO₃, hydrochloric HCl and hypochloric HClO acids, and ammonia NH₄OH.

The process of attaching magnetic labels to yeast cells in the experiments is carried out inside an installation of multivortical MHD stirring that consists of a reactor and a system of electromagnets. A high gradient ferromagnetic matrix is placed in the reactor and is presented by a set of 20×20 parallel-aligned steel cylinders with a diameter of 0.5 mm each one. The distance between two cylinders in row l is 5 mm. The cylinders are fixed with plexiglass and are removable. Fig. 1, *a* shows the reactor with the matrix, whereas Fig. 1, *b* shows the direction of the vortices' rotation around a cylinder.

The reactor has the shape of a parallelepiped for an optimal use of the workspace of the magnetic system because the pole pieces are also made in the form of parallelepipeds. The pole pieces have a larger area than the base of the reactor to reduce the heterogeneity of the constant magnetic field H_0 on the borders of the system workspace.



Fig. 1. A reactor for multivortical MHD stirring: *a* shows the reactor with a matrix of steel cylinders:
1 – the reactor, 2 – a flanged basis, 3 – a removable basis

made of plexiglass for the matrix, 4 – steel cylinders;

b shows the rotation direction of vortices in the electrolyte around a ferromagnetic cylinder in a permanent magnet field

4.2. The magnetically operated biosorbent production method

Water solutions of nanoparticles of the magnetite Fe_3O_4 and yeast were prepared with a weight ratio ranging from 1:100 to 1:10, respectively. For this, yeast sample with a humidity of 74 % were weighted and diluted in water. The resulting suspension was supplied, first, with nitric acid to reduce the pH down to a range from 5.5 to 1 and then with a solution of magnetic nanoparticles with a concentration of 1 g/dm³.

The obtained mixtures were sent into the reactor for multivortical MHD stirring to attach magnetite nanoparticles to yeast cells. The reactor was situated in the workspace of the magnetic system. The strength of the permanent external magnetic field in the workspace ranged $H_0=0-320$ kA/m. The duration of stirring was varied from 0 to 10 min with an increment of 2 min.

The dry mass of the magnetically operated biosorbent was then determined by using the method described in [23]: 1 g of the yeast sample was dried to its constant weight in a drying cabinet at 105 °C to obtain 0.263 g of the dry weight.

4.3. The biosorption process method

The solutions of copper cations were prepared with concentrations ranging from 25 to 150 mg/dm^3 with an increment of 25 mg/dm^3 . Each solution separately was supplied with previously prepared 20 cm³ of a concentrated suspension of magnetically operated yeast cells and was mechanically mixed for 60 min at a frequency of 180 min⁻¹. The medium pH=5.5 during the biosorption process was controlled.

After the biosorption process, the solution was filtered through paper filters "white ribbon" or through a magnetic separator to remove the waste biosorbent and to determine the residual concentration of copper cations.

The concentration of copper ions Cu^{2+} was determined under normal conditions by measuring optical density of the blue color the complexes $[Cu(NH_3)_4]^{2+}$ in passing light with a wavelength of 590 nm on the spectrophotometer SP-46.

4. 4. The magnetic susceptibility determination method

The resulting suspensions of the yeast, nanoscale magnetite and magnetically operated biosorbent in distilled water with 0.4 g of the probe dry mass were sent for measuring by the method described in [24]. The measuring was controlled by determining the magnetic susceptibility of air in a tube under the same conditions as for the dried samples. The device for measuring the magnetic susceptibility consists of an electric circuit, an oscilloscope, an electronic frequency meter, and a merit device. The electric circuit includes a capacitor and a solenoid made of copper wire. In the middle of the solenoid, there is a cylindrical container with a sample for measuring. The measuring of magnetic susceptibilities was performed at frequencies of 6-12 MHz with an error not exceeding 1 %.

5. The results of studying the characteristics of the magnetically operated biosorbents

Fig. 2 shows the suspensions of yeast (100 mg/dm³) and magnetic nanoscale labels with a 1 % mass ratio of the magnetite to the magnetically labeled yeast biomass were mixed by MHD stirring for 10 minutes with $H_0=240$ kA/m and at various pH values; then, the received magnetically labeled biosorbents were used for removing copper cations from the model solutions in mechanical stirring for 60 minutes with the optimum pH 5.5 for the yeast. The initial copper cations concentration and the concentration of the yeast suspension in these experiments were 50 mg/dm³. The degree of extraction of cations R was calculated as the ratio of sorbed cations to the initial amount of copper cations.



Fig. 2. The degree of extraction of copper cations R by the magnetically operated biosorbent, depending on changes in the pH of the biosorbent production by MHD stirring:
1 - control (the native yeast suspension); 2 - the biosorbent with the relative weight of 1 % of the magnetite;
3 - the biosorbent with the relative weight 2 % of the magnetite; 4 - the biosorbent with the relative weight of 10 % of the magnetite

Fig. 2 shows that the most effective magnetically operated biosorbent with the degree of extraction of copper cations R=80 % is the magnetically labeled sample obtained by multivortical MHD stirring at pH=2.5.

In this study, MHD stirring was done through an installation described in [9]. Thus, with the known configuration of the flow in the working medium, the material of the matrix for MHD stirring, the electrolyte concentration and the temperature, it is only necessary to optimize the strength of the external magnetic field of the installation for the most efficient stirring effect.

Extraction degree of copper cations by magnetically labeled yeasts cells, depending on the H_0 during the multivortical MHD stirring of yeast cells and the magnetic labels before biosorption were studied. Fig. 3 shows yeast suspensions (100 mg/dm³) with magnetic labels in a mass ratio of 1 % were mixed using MHD stirring for 10 minutes at pH=2.5 with different values of the strength of the external magnetic field H_0 , and then they were sent for the sorption of copper cations.



Fig. 3. The degree of extraction of copper cations by magnetically labeled yeas cells, depending on the strength of the external magnetic field H₀ for the multivertical MHD stirring of yeast and magnetic labels:
1 - control; 2 - MHD stirring duration for 2 min; 3 - MHD stirring duration for 10 min

As shown in Fig. 3, an increase of the external magnetic field strength H₀ while producing each sample of magnetically labeled yeast cells increases the degree of copper cations extraction after biosorption. This happens up to H=240 kA/m and R=82 %, but a further increase in the strength of the external magnetic field does not significantly increase the extraction degree up to $H_0=280$ kA/m. In terms of optimizing the sorption characteristics depending on the preparation parameters for a magnetically labeled biosorbent, the optimal value of the magnetic field in multivortical MHD stirring of yeast with magnetic labels is $H_0^*=240$ kA/m. The cell wall disintegrates at higher values of $H_0>280$ kA/m when the other parameters remain unchanged, so sorption involves participation of cytosol components, so the degree of extraction increases. The destruction of the cell wall is an undesirable phenomenon, so the magnetic field $H_0 > 280 \text{ kA/m}$ is not considered.

Thus, the multivortical MHD stirring parameters were determined for preparing a magnetically operated biosorbent. The next step will be to optimize the parameters of preparing magnetically labeled yeasts – namely, the ratio of the mass of the magnetic labels to yeast and the duration of stirring.

Fig. 4 shows the maximum sorption capacity for copper cations of magnetically operated biosorbents depending on the ratio of the mass of magnetic labels to yeast in a range of $m_m/m_y=1-10$ % (the value 0% is control – the native yeast biomass *S. cerevisiae*).

As can be seen from Fig. 4, the magnetically labeled yeast suspension, with the ratio of the magnetite mass to the yeast

biomass being $m_m/m_y=10$ %, has the lowest sorption capacity and copper cations extraction degree among all observed ratios. When the mass ratios are 0 % (control) and 1 %, the maximum sorption capacity of the magnetically labeled yeast samples as to copper cations coincide, being 25.5 mg of Cu²⁺/g of dry mass, which is the largest of all represented. This can be explained by the fact that multivortical MHD stirring produces extensive cell surfaces with magnetite clusters with size of 60–350 nm, and the sorption capacity of such cells increases in comparison with native yeast cells [25], that leads to the same sorption capacity of native and magnetically labeled yeasts. In terms of the sorption capacity for copper cations, the best is the magnetically operated biosorbent with a ratio of $m_m/m_y=1$ %.



Fig. 4. The maximum sorption capacity Q_{max} of the magnetically labeled yeast as for copper cations, depending on the ratio of the magnetite mass to the yeast *S. cerevisiae* dry mass m_m/m_v

Fig. 5 shows the degree of extraction of copper cations by the magnetically operated biosorbent depending on the duration of MHD stirring of yeast and magnetic labels in case of the optimal ratio of $m_m/m_y=1$ %. We can conclude that with decreasing the duration of MHD stirring, the extraction degree and, therefore, the sorption capacity of the magnetically labeled yeast increases. For MHD stirring, the minimum duration is 2 min because during 1 min the working medium still contains unstirred elements – the so-called congestion zones.



Fig. 5. The degree of extraction R of copper cations by the magnetically labeled yeast, depending on the duration t of the biosorbent preparation

For further research on developing a magnetically labeled biosorbent, the optimal duration of MHD stirring was chosen to be 2 mins, with the ratio of 1 % of the magnetite mass to the yeast biomass. The obtained magnetically labeled biosorbent was used to study the degree of extracting copper cations, depending on the duration of the sorption process (Fig. 6). In all the experiments, the initial concentration of copper cations was 50 mg/dm^3 , and the biosorbent dry mass was 50 mg.



Fig. 6. The degree of extraction R of copper cations by sorbents based on the yeast *S. cerevisiae*, depending on the duration t of the sorption process: 1 - native yeast;
2 - the biosorbent prepared during 2 min of MHD stirring;
3 - the biosorbent prepared during 10 min of MHD stirring

As shown in Fig. 6, the degree of extraction of copper cations from the model solutions is 94% for native yeast and 89% for the optimal magnetically labeled yeast. These data correlate well with the results of similar studies of the extraction degree for iron cations - 82 % for native yeast and 63 % for magnetically labeled yeast. It should be noted that the degree of extraction from real samples of wasted water by magnetically labeled yeast in the case of iron concentration in the amount of 3.52 mg/dm^3 is about 50 %. A separate study was undertaken to determine the degree of copper cations extraction by a magnetic fraction of magnetically labeled yeast with a magnetic susceptibility of $\chi = 60 - 66 \cdot 10^{-4}$, i. e., after separation of magnetic fractions on a magnetic separator. The magnetic fraction of the biosorbent showed the same extraction efficiency as the magnetically operated biosorbent, with an error value in the results being less than 1 %: thus, the degree of copper cations extraction was 89 %.

Experiments were carried out to determine changes in the maximum sorption capacity of the magnetically operated biosorbent, depending on the duration of multivortical MHD stirring during attaching the nanoscale magnetite. Multivortical MHD stirring of the nanoscale magnetite and yeast with the weight ratio of 1 % was performed at pH=2.5 and H₀=240 kA/m, that is, at the most effective experimental parameters. Biosorption by the magnetically operated biosorbent of copper cations was carried out by mechanical stirring at the optimal pH.

The results of biosorption experiments at 18 °C were evaluated using the Langmuir model of monolayer sorption; they are presented in Fig. 7, where the empirical biosorption isotherms of copper cations are showed for different durations of the biosorbent preparation -2, 4, 6, 8, and 10 min - as well as control, without MHD stirring.

As shown in Fig. 7, with an increase in the duration of multivortical MHD stirring for preparing magnetically operated biosorbents, their sorption capacity decreases. So it should be assumed that the most effective biosorbent can be obtained by minimizing the duration of mixing yeast with nanoscale magnetic labels in the case of multivortical MHD stirring. The sorption capacity of magnetically operated biosorbents in the case of 2 min of multivortical MHD stirring remains equal to the sorption capacity of native yeast in the amount of 25.5-26 mg Cu²⁺/g of dry mass.



Fig. 7. The sorption isotherms Q=f(C) of copper cations by magnetically operated biosorbents under varying the duration of preparing the sorbent by the multivortical MHD stirring method: 1 - 0 min; 2 - 2 min; 3 - 4 min; 4 - 6 min; 5 - 8 min; 6 - 10 min

Tests were conducted to determine the magnetic susceptibilities of complexes of magnetic labels and yeast cells, depending on the duration of preparing and the mass ratio of the yeast and magnetic labels in the complexes. The experimental results are similar to those obtained in calculations according to the method of [25]. The experimental results are presented in Fig. 8, with the measurement error being less than 1 %.



Fig. 8. The magnetic susceptibility χ of biosorbent suspensions obtained under varied durations of preparing:
1 - the biosorbent with 1 % of the magnetite (equal to 1 mg/dm³ of the magnetite in the working medium),
2 - the biosorbent with 10 % of magnetite, 3 - control (a solution of the magnetite Fe₃O₄ of 100 mg/dm³)

The values of the magnetic susceptibility of the magnetically labeled cells indicate that they belong to paramagnetic substances. Apparently, the magnetic susceptibility of the magnetic labels Fe_3O_4 is $79.5\pm0.5\cdot10^{-4}$. It is known that the magnetic susceptibility of the yeast *S. cerevisae* is $-69.4\pm0.8\cdot10^{-6}$ according to [20]. The magnetic susceptibility of the magnetic susceptibility of

tical MHD mixing depends on the amount of the magnetite in the complexes, and it decreases with increasing the preparation duration. The magnetic susceptibility of the magnetically operated biosorbent with a weight of the nanoscale magnetite being 1 % made during 2 min is $58\pm0.5\cdot10^{-4}$, and in the case of a 10 min preparation the magnetic susceptibility is $56\pm0.5\cdot10^{-4}$. The reduced magnetic susceptibility, probably, indicates that multivortical MHD stirring makes the magnetic labels penetrate through the inner layers of the cell wall.

6. Discussion of studying the characteristics of the magnetically operated biosorbent

Multivortical MHD stirring significantly depends on the pH of the working medium. The optimal flow rates are observed when using a 7 % solution of nitric acid. In [26, 27], veast cell flows were studied depending on the duration of MHD stirring around a cylindrical element, and it was showed that an increase in the pH from 1 to 5 reduced the flow rate, depending on the duration of the process: at pH=1, V_1 =0.22 mm/s for MHD stirring for 0.5 min, V_2 =0.16 mm/s for MHD stirring for 10 min, and $V_3=0.06$ mm/s for MHD stirring for 15 min; otherwise, at pH=5, V_1 =0.07 mm/s for MHD stirring for 0.5 min, $V_2 = 0.06$ mm/s for MHD stirring for 10 min, and $V_3=0.05$ mm/s for MHD stirring for 15 min. Similar rates were observed in yeast samples under MHD stirring at low concentrations (0.01-4%) of the biomass, that is, when the solution viscosity was not a crucial factor for the flow resistance.

It should be noted that at pH=1 flow rates are higher, and they slow down when the pH is reduced; the optimal biosorbent is obtained at pH=2.5. The optimized strength of the external magnetic field for multivortical MHD stirring is 240 kA/m. The parameters of the magnetically operated biosorbent are: the ratio of the magnetite Fe₃O₄ to the yeast biomass should be 1 %, the duration of production should be 2 min, so the maximum sorption capacity is 25.5±0.5 mg Cu²⁺/g of the biosorbent dry mass.

The waste biosorbent with the mass ratio of the labels to the biosorbent being 1 %, it was removed from the solution using a magnetic filtration. Fig. 9 shows the effect of a magnetic field on the magnetically operated biosorbent with optimal parameters.

As shown in Fig. 9, *b*, the magnetically operated biosorbent with 1 % of the magnetite by the weight efficiently responds to the magnetic field. To remove the magnetically labeled yeast cells after the biosorption process, the matrix is a steel mesh with a complete set of one layer and two layers. For the single layer mesh, the extraction degree for the magnetically labeled yeas cells is at least 70 % if the flow of the solution is 5 mm/s, and it is up to 90 % at a solution flow of 1 mm/s; for the double layer matrix, the degrees are 90 % and 96 %, respectively. So it is worth noting that the capacity of the double layer matrix, and the two-layer matrix also has a higher number of local magnetic field heterogeneities. The steel mesh proved to be highly effective as a matrix for the magnetic filter in removing magnetically labeled yeast cells.

It should be noted that the maximum sorption capacity was observed at residual copper concentrations of about 100 mg/dm^3 when the concentration of the magnetically operated biosorbent was 50 mg/dm³. In real multicomponent solutions that entail further removal of copper, such high concentrations are very rare, so the maximum sorption capacity is not a decisive factor for magnetically labeled yeasts when it concerns cations of heavy metals.



Fig. 9. The influence of a magnetic field on the magnetically operated biosorbent: a - the suspension before the action of the magnetic field: 1 - the suspension of the magnetically operated biosorbent, 2 - a magnet NeFeB H=1.2 MA/m; b - the suspension after 2 min of the magnetic field action: 1 - the concentrated suspension of the magnetically operated biosorbent, 2 - a magnet

7. Conclusions

1. The parameters of multivortical MHD stirring to prepare an optimal magnetically operated biosorbent have been determined as follows: pH=2.5; the strength of the permanent magnetic field $H_0^*=240$ kA/m. The degree of extracting copper cations by a magnetically operated biosorbent that was prepared for 10 min of multivortical MHD stirring, with the mass ratio of the magnetic labels to the yeast being 1 %, is 80 %. 2. The parameters of preparing an optimal magnetically operated biosorbent are: the mass ratio of the nanosized magnetite to the yeast *S. cerevisae* – 1%; the duration of multivortical MHD stirring – 2 min; the degree of extraction of copper cations by the optimal magnetically operated biosorbent when the initial concentration of copper cations is 50 mg/dm³ – 89%. The maximum sorption capacity is $Q_{max}=25.5$ mg/g of the biosorbent dry mass, corresponding to the native yeast sorption capacity with respect to copper cations.

3. The magnetically operated biosorbent with the above preparation parameters shows a sorption isotherm of copper cations similar to native yeast, and the maximum sorption capacity is different from that of native yeast within the margin of error. There is a reduction of the maximum sorption capacity with respect to copper cations from 25.5 mg Cu²⁺/g of dry mass to 21.1 mg Cu²⁺/g of the sorbent dry mass with a corresponding increase in the duration of multivortical MHD stirring from 0 to 10 min when preparing magnetically labeled yeast.

4. The magnetic susceptibility of the magnetically operated biosorbent with 1 % of the magnetite weight produced in 2 min is $58\pm0.5\cdot10^{-4}$, and when it is produced for 10 min, the value is $56\pm0.5\cdot10^{-6}$. In general, magnetic susceptibility of suspensions of magnetically operated biosorbents is in the range of $55-70\cdot10^{-4}$, and it has the highest value for complexes with the ratio of 10 % by weight and the least duration of the preparation process. The removal of the spent magnetically operated biosorbent by a magnetic separator with a double layer steel mesh as a ferromagnetic matrix demonstrates effectiveness of at least 96 %.

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