очищення сфлокульованого шламу до 99 % і зменшує винесення твердої фази з фугатом.

Ключові слова: модуль очищення шламів, полідисперсні шлами, деструкція флокул, міцність флокул, швидкість осідання, удосконалення центрифуги.

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STUDY OF MODIFICATION OF MAGNETICALLY LABELED YEASTS *Saccharomyces cerevisiae* FOR COPPER CATIONS Cu^{2+} REMOVAL

Досліджено сорбційну ємність магнітомічених дріжджів S. cerevisiae, отриманих за допомогою багатовихрового магнітогідродинамічного перемішування суспензії з нанорозмірним магнетитом Fe_3O_4, в залежності від модифікації клітинної стінки. Також досліджено метилювання аміногруп, етерифікацію карбоксильних груп, обробку лугом і екстракцію ліпідів магнітомічених дріжджів S. cerevisiae. Отриманні результати показали вклад компонентів клітинної стінки в максимальну сорбційну ємність біосорбенту по відношенню до катіонів міді Cu^{2+}.

Ключові слова: *магнітомічені дріжджі S. cerevisiae, сорбційна ємність біосорбенту, функціональні групи.*

1. Introduction

The development and perfection of methods of waste water treatment from different origin pollutants, including heavy metals cations such as a copper, iron, etc, remain relevant today. The yeasts *S. cerevisiae* are not the most effective biosorbent of heavy metals, but their availability largely overlaps competitors. Besides this type of yeasts it is well studied that simplifies the search of ways of modification or activation of biomass for a biosorption.

Sorption capacity towards copper cations Cu^{2+} of magnetically labeled biosorbent based on the yeast *S. cerevisiae*, obtained by multi-vortical magnetohydrodynamic (MHD) stirring depends on the number of attached nanosized magnetite Fe_3O_4 [1]. A passive biosorption by magnetically labeled yeasts takes place apparently due to the stoichiometric ratios of functional groups of the cellular wall components to the metal ions and physical adsorption by electrical interactions [2], and also due to a sorption by magnetite. The magnetite has significant electrokinetic potential [3] and is able to absorb copper cations. Carboxyl and amino groups presented in mannan–protein layer and lipoproteins can be regarded as major groups involved in passive biosorption. There is a necessity to educe, what amount of functional groups and also components of cellular wall has been blocked by magnetite during multi-vortical MHD stirring.

2. The object of research and its technological audit

The object of research is magnetically labeled biosorbent. Magnetically labeled biosorbent is a suspension of cells modified by attaching magnetic labels to the cellular wall using multi-vortical MHD stirring. The technological characteristics of magnetically labeled yeast are the following:

– mass ratio of magnetic labels towards yeasts – 1 %;
– sorption capacity towards copper cations Cu^{2+} – 25 mg/g dry mass of sorbent;
– magnetic susceptibility – $5{,}5 \cdot 10^{-3}$–$7 \cdot 10^{-3}$;
– biosorption optimal pH – 5,0–5,5;
– duration of storage – 2 days.

Manufacturing process settings of magnetically labeled yeast (multi-vortical MHD stirring):

– strength of the external magnetic field – 240–280 kA/m;
– working environment pH – 2,5–3;
– process duration (cycle) – 2 min.

One of the main drawbacks of magnetically labeled biosorbent based on yeast *S. cerevisiae* is low sorption capacity comparing to other biosorbents, such as brown algae. To increase the sorption capacity of yeast it is necessary to clarify the contribution of cellular wall components in passive sorption of copper cations. Therefore, the main focus of the study is to identify the contribution

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of components of cellular walls in magnetically labeled yeast sorption capacity.

3. The aim and objectives of research

The aim of research – to prospect influence of chemical modification of biomass on a sorption capacity of the magnetically labeled biosorbent based on the yeasts *S. cerevisiae* and on magnetite of Fe_3O_4.

To achieve this aim it is necessary to solve the following objectives:

– to study the sorption capacity of the magnetically labeled yeasts, modified by methods that allow to extract the components of cellular wall or block functional groups in terms of biosorption;

– to compare results to similar researches for native yeasts;

– to study the sorption capacity of the extracted components of cellular wall of magnetically labeled yeasts *S. cerevisiae* in terms of the functional groups attracted in a biosorption, and nanosized magnetite.

4. Research of existing solutions of the problem

The studies on the removal of heavy metals by means of biosorption proceed in particular in the search of new biosorbents [4]. Researchers pay attention to possibility of modification of yeasts cellular wall to recover it potential in removal of heavy metals cations [5]. So, to understand mechanisms of biosorption the different methods of biomass treatment are used, for example, thermal [6], chemical [7], and also methods to lay-up necessary components [8]. Also in [8] viability of cells is showed after covering by the layer of polyelectrolyte. Attaching of magnetic labels to the yeast cells takes place very effectively due to the method of multi-vortical MHD stirring [9]. A resulted magnetically labeled biosorbent found its implementation in water treatment technology of galvanic production from a copper and chrome, and also iron [3].

The functional groups represented on the surface of yeast cell, such as amino, carboxyl, sulfhydryl, phosphoryl absorb cations of heavy metals [10] with different efficiency. Nanosized magnetite [11], which is used to create magnetically labeled cells [12], also absorbs cations of metals on the surface. For example, in [13] showed that the magnetite is an effective sorbent, but the downside is coagulation of particles that impairs its industrial use. Industrial application of yeasts *S. cerevisiae* is already seen as a real technology for wastewater treatment [14].

5. Methods of research

Yeast S. cerevisiae. For each study was used 25 g of pressed yeasts production of PJSC «Company enzyme» (Ukraine) with humidity 74 %, which were dissolved in water. The suspension was stirred for 60 min at 120 rpm to separate from the surface of the cell wall excreta. After stirring the yeast slurry is centrifuged at 2000 rpm for 10 min swift cell and precipitate was washed with distilled water to a pH of 5,5 – sample 1.

Magnetically labeled biosorbent. To attach magnetic labels to yeast cells was used magnetic fluid, obtained by [11]. The process of manufacturing of magnetically labeled biosorbent made by the method described in [3] with multi-vortical MHD stirring. For this yeast slurry with volume of 100 cm^3 was mixed with magnetic liquid so that the ratio of the mass of yeast to the mass of magnetic labels was 100 : 1. The resulting solution was transferred to an electrochemical camera with ferromagnetic matrix and adjusted 56 % nitric acid HNO_3 to pH 2,5. The camera installed in the workspace of magnetic system, which generates a permanent magnetic field with strength of 240 kA/m. Stirring was performed 2 min. The suspension of magnetically labeled biosorbent directed to magnetic separation after stirring. Magnetic fraction was centrifuged at 2000 rpm for 10 min, the precipitate was washed with distilled water to pH 5,5. No magnetic fraction re-sent to the previous stages. Magnetically labeled biosorbent was combined in one capacity – sample 2.

Modification of biosorbents surface. Half the volume of suspensions of samples 1 and 2 were sent to treatment with alkali (samples 3 and 4, respectively), and half for processing acetone (samples 5 and 6, respectively).

Alkali processing was performed as follows [15]: argued suspension of biosorbent by 0,1 N NaOH to pH 10 and stirred 2 hours heating at the same time to 40 °C. The resulting solution was left for 1 day at 4 °C, and then centrifuged at 6000 rpm for 10 min. The supernatant was poured separately and aligned pH to 5,5. Decantate was washed to pH = 7 and sent to biosorption of copper cations Cu^{2+}, or for further modification of functional groups.

Extraction of lipids was determined as described [16] with the rate of 75 cm^3 acetone per 1 g dry weight yeast was stirred 4 h heating at the same time to 40 °C. The resulting solution was left for 1 day at 4 °C, and then centrifuged at 6000 rpm for 10 min. As in the previous case, the supernatant and the filtrate were investigated separately.

The second half by volume of suspensions of samples 1, 2 and extracts of samples 3–6 were again split into 2 parts and sent for the chemical processing. The first part was modified with formaldehyde, formic acid (examples 7, 8 and 11, 12), and the second – by adding methanol (samples 9, 10 and 13–16). As for lipids from samples 5 and 6 methylation of amino groups was not done.

Methylation of amino groups. The processing by formalin (formaldehyde) aims denaturation of proteins and amino blocking. The treatment was carried out by [17]. For this purpose 5 g of yeast diluted in 100 cm^3 of formaldehyde (HCHO), and then 200 cm^3 of formic acid (HCOOH). The mixture was stirred for 6 hours at 150 rpm with a temperature of 40 °C. The resulting biomass was centrifuged at 2000 rpm for 10 min, filtrate was washed with distilled water with 0,2 M sodium carbonate and was used for biosorption.

Esterification of carboxyl groups. The treatment was carried out by [18]. Native or magnetically labeled yeasts were diluted in 300 cm^3 of methanol. To the suspension was added 5 cm^3 of hydrochloric acid as a catalyst. The solution was stirred 6 hours at 150 rpm. The resulting mixture was centrifuged at 2000 rpm for 10 min, decantate was washed with distilled water of 0,2 M sodium carbonate.

Processing of biomass of magnetically labeled yeast and removed from the cell wall components are presented in Table 1.

Biosorption of copper cations. The process of extracting copper cations by modified sample was carried out with mechanical stirring at 180 rpm for 60 min at pH 5,5.

To remove the spent biosorbent used centrifugation at 10,000 rpm for 5 min. Magnetically labeled samples were previously removed by magnetic separation because magnetite effectively absorbs ammonium ions, which are used to determine the concentration of copper in solution.

Table 1

The scheme of the experimental samples

Sample No.	Type of modification					
	Attachment of magnetic labels	Processing of suspension		Extract from biomass	Blocking of functional groups	
		Alkali	Acetone		$-NH_2$	–COOH
1	×	×	×	–	×	×
2	*	×	×	–	×	×
3	×	*	×	×	×	×
4	*	*	×	×	×	×
5	×	×	*	×	×	×
6	*	×	*	×	×	×
7	×	×	×	–	*	×
8	*	×	×	–	*	×
9	×	×	×	–	×	*
10	*	×	×	–	×	*
11	×	*	×	*	*	×
12	*	*	×	*	*	×
13	×	*	×	*	×	*
14	*	*	×	*	×	*
15	×	×	*	*	×	*
16	*	×	*	*	×	*

Note: «*» – marked used modification, «×» – processing is not carried out, «–» – not possible to obtain.

High-gradient magnetic separation of biosorbent. To remove the spent magnetically labeled biosorbent solution is passed through a magnetic separator, which is a container of high-gradient ferromagnetic steel matrix in the form of a grid with cell of 0,5 mm. The separator is in flow mode, with a productivity 10 dm^3/h, the strength of the external magnetic field of 300 kA/m. Removed mass of magnetically labeled biosorbent washed from matrix of separator after the separation process by passing distilled water. Biosorbent solution is concentrated by means of 2–3 consecutive centrifugations at 2000 rpm for 10 min if necessary.

Determination of optical density of solutions. The concentrations of yeast, magnetically labeled biosorbent and magnetite were examined by spectrophotometry at a wavelength of 590 nm for all three types of samples. Cation concentrations of copper (II) – initial and final were determined by adding ammonia solution and determining the optical density of the blue color complex $[Cu(NH_3)_4]^{2+}$ at a wavelength of 540 nm.

Calculation of sorption capacity of extracts. To determine the contribution of extracted substances to the sorption capacity of yeasts was used in the calculation of the difference between weight of dry matter of yeast before and after extraction. To calculate the contribution of blocked functional groups in extracts from total sorption capacity of extract was subtracted sorption capacity of extract with specific blocked functional group.

6. Research results

The maximum sorption capacity biosorbents derived from native and magnetically labeled yeast by modifying cell wall, is shown in Fig. 1. The magnetically labeled yeast obtained by multi-vortical MHD stirring process with the following parameters:

– strength of the external magnetic field installation;
– 240 kA/m;
– working environment pH – 2,5;
– the duration of the process – 2 min.

The ratio of weight of magnetic labels to mass yeast – 1 %, magnetic susceptibility of complexes magnetic labels-yeast cell – $5{,}6 \pm 0{,}1 \cdot 10^{-3}$. As shown in Fig. 1, magnetically labeled and native yeasts (samples 1 and 2) have the same within the error sorption capacity with respect to copper cations – $25{,}5 \pm 0{,}3$ mg Cu^{2+}/g dry matter sorbent. This similarity is explained by optimized manufacturing method of magnetically labeled biosorbent. Processing suspensions of native and magnetically labeled yeasts by alkali NaOH leads to a decrease sorption capacity of both samples 3 and 4 in equal amounts – down to $21{,}6 \pm 0{,}2$ mg/g, indicating that the structure of the cell wall components remain the same.

A similar situation is observed in the extraction of lipids from the cell wall of both biosorbents using acetone – reducing the capacity of samples 5 and 6 to $22{,}1 \pm 0{,}2$ mg/g. In other words, nanoscale magnetic labels are not localized on lipids in the cell wall of magnetically labeled yeast.

From the literature, for example [2], is known that from all functional groups represented by cell wall components for biosorption of metal cations the most important are amino carboxylic –COOH and $-NH_2$. Fig. 1 shows the comparison of sorption capacity native and magnetically labeled yeast in case of blocking these functional groups.

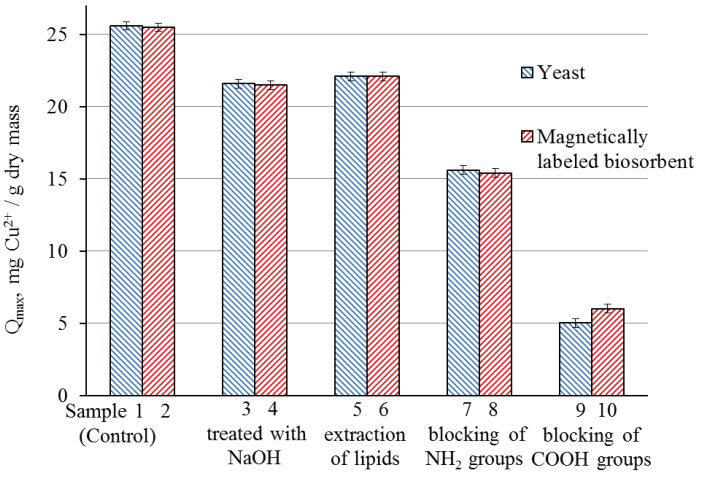

Fig. 1. The maximum sorption capacity Q_{max} of modified native and magnetically labeled yeasts relative to copper cations Cu^{2+}, depending on the method of modification

Since methylation of amino groups by formaldehyde and formic acid goes by reaction of Eschweiler-Clark:

$$RNH_2 \xrightarrow{CH_2O,HCOOH} RN(CH_3)_2 + CO_2 + H_2O, \quad (1)$$

which reduces the sorption capacity relative to copper cations for native and magnetically labeled yeasts to

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15,6 ± 0,3 and 15,4 ± 0,3 mg/g, respectively. In other words, nanosized magnetite does not react with amino groups of the cell wall of yeast and does not block them in the biosorption of copper cations.

In the case of esterification of the carboxyl groups by reaction:

$$RCOOH+CH_3OH \xleftrightarrow{HCl} RCOOCH_3+H_2O, \qquad (2)$$

sorption capacity of the cell walls of yeast significantly reduces – to 5 ± 0,3 and 6 ± 0,3 mg/d for the native yeast and magnetically labeled respectively. The lowest sorption capacity in case of modification of the cell wall by methanol confirms the fact that the carboxyl group involved in copper cations biosorption most. It is interesting that the sorption capacity of magnetically labeled yeast in this modification differs significantly from the native yeast. If we assume that the residual sorption capacity after esterification of carboxyl groups represented by all other components of the cell wall, we can suppose that the difference in sorption capacity belongs to nanoscale magnetic labels capacity and, besides, assume that magnetic labels block carboxyl groups by electrostatic interactions with them.

To find out insertion and impact on copper cations binding by nanoscale magnetite labels were conducted studies on sorption of copper cations by extracted components of the magnetically labeled yeast cell wall and the results were compared with native yeast. The results are shown in Fig. 2.

As shown in Fig. 2, sorption capacity due to amino groups of components of the cell wall removed using alkaline NaOH (samples 11 and 12) is the same for native and magnetically labeled yeasts. On the contrary, in the same extracts carboxyl groups of amgnetically labeled yeast are blocked by magnetite at 15 % (sample 13 for native and sample 14 for magnetically labeled yeasts). In the lipid extracts from magnetically labeled yeast (sample 16), a decrease of deposit carboxyl groups only within the error, so make the finding of a sorption of copper cations by magnetite in lipids is failed.

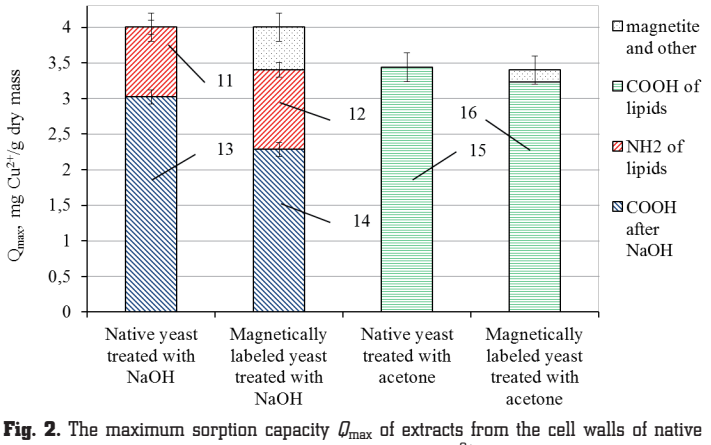

Fig. 2. The maximum sorption capacity Q_{max} of extracts from the cell walls of native and magnetically labeled yeasts relative to copper Cu^{2+} cations in terms of mass of sorbent

In terms of technical and economic feasibility of using of magnetically labeled biosorbent can conclude that if the ratio of the mass of magnetic labels to yeast of 1 % a maximum sorption capacity of magnetically labeled yeast is equal to sorption capacity of native yeast. In other words, nanosized magnetite attached to yeast cells blocks the equivalent number of binding sites for copper cations to the number of sites that it provides.

7. SWOT-analysis of studies

Strengths. The study, presented in the experimental part, provide information about the contribution of the major functional groups (carboxyl and amino) on the sorption of metal cations by magnetically labeled yeast and contribution to this sorption capacity of nanosized magnetite. Among the strengths of this study is analysis of the contribution of functional groups and selected components of the cell wall in sorption capacity of complexes magnetic labels-yeast cells obtained by multi-vortical MHD stirring. Use of the data gives partial information about the location of nanosized magnetite in the cell wall and its electrostatic interaction with charged functional groups and quantitative analysis of these interactions. The criterion for assessing the amount of functional groups and electrostatic interactions in such a case is the sorption capacity relative to copper cations.

The main advantage of magnetically labeled yeast with 1 % by weight of magnetite is that their sorption capacity is equal to sorption capacity of native yeast S. cerevisiae, and magnetically labeled biosorbent can be removed from the work environment quickly and efficiently through magnetic separation.

Weaknesses. Among the weaknesses of this work is that it is not possible to allocate separately each component of the cell wall and explore the quantitative contribution to the sorption capacity. This is due to the fact that the dynamic structure of the cell wall has constant variety of processes including sorption, chemical transformations, complex and so on. Thus, not all functional groups involved in the recovery of copper cations. On the other hand, research of selected components, such as lipids, is excessive in relation to their sorption capacity, as they interact with other components in the cell wall, in particular through carboxyl groups.

Among the negative factors of the study include the lack of information about other functional groups, such as sulfide, phosphoryl, etc.

Opportunities. Understanding the mechanism of attachment of magnetite to the yeast cells and biosorption is limited. Opportunities disclosure formation of magnetically labeled cells obtained by MHD stirring underlying theoretical justification and quantitative analysis of the mechanisms that take place in the cell wall. In particular, results of electron paramagnetic resonance spectrometry of magnetite localizations are necessary for further researches.

Implementation of magnetically labeled biosorbents for wastewater treatment from heavy metal cations enables fine purification of water to concentrations less than 1 mg/dm^3. Comparing with existing biosorbent, yeast or waste yeast production are cheaper. The economic effect of the order of 10^4–10^5 UAH/year and higher, depending on production volumes.

Threats. The complexity of the implementation of the results is that the obtained results are in optimal parameters for a process of attaching magnetic labels to yeast and for process of biosorption of copper cations. In the

real case there is biosorption process from multicomponent mixtures, and therefore may be involved in various mechanisms of sorption by cell wall.

Additional costs at treatment plants are primarily associated with the need of technological stages of production of magnetically labeled biosorbent and magnetic separation of the spent sorbent.

On the basis of SWOT-analysis of results can be characterized by the following main research areas: comprehensive analysis of the factors influencing the sorption capacity and stability of the magnetic characteristics of magnetically labeled yeast, research of biosorption from multicomponent mixtures.

8. Conclusions

1. Sorption capacity of magnetically labeled yeast obtained by multi-vortical MHD stirring of yeast with 1 % of magnetite by weight is investigated. It is established that the sorption capacity of magnetically labeled yeast reduces after treatment with alkali NaOH, acetone, formaldehyde and formic acid from methanol from 25,5 mg/g of dry sorbent to 21,6, 22,1, 15,6 and 5 mg/g, respectively.

2. Compared sorption capacity of native and magnetically labeled yeast *S. cerevisiae* is compared. It is established that the maximum sorption capacity of magnetically labeled and native yeast changes same after treatment with alkali, acetone or formaldehyde with formic acid. And in the case of processing with methanol magnetically labeled yeast sorption capacity is more than for native and is 6 mg/g of the dry matter.

3. Sorption capacity of lipids and proteins of the cell wall of magnetically labeled yeast *S. cerevisiae* is investigated. It is found that nanosized magnetite does not affect the sorption of cations copper by lipids of the cell wall and amino groups represented on the surface of yeast cells, and affects the sorption capacity of proteins, including covers 15 % of the carboxyl groups of components extracted from cell wall using NaOH that are involved in biosorption of copper cations.

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ИССЛЕДОВАНИЯ ВЛИЯНИЯ МОДИФИКАЦИИ МАГНИТОМЕЧЕНЫХ ДРОЖЖЕЙ Saccharomyces cerevisiae НА ИЗВЛЕЧЕНИЕ КАТИОНОВ МЕДИ Cu^{2+}

Исследована сорбционная емкость магнитомеченых дрожжей *S. cerevisiae*, полученных с помощью многовихревого магнитогидродинамического перемешивания суспензии с наноразмерным магнетитом Fe_3O_4, в зависимости от модификации клеточной стенки. Также исследованы метилирование аминогрупп, этерификация карбоксильных групп, обработка щелочью и экстракция липидов магнитомеченых дрожжей *S. cerevisiae*. Полученные результаты показали вклад компонентов клеточной стенки в максимальную сорбционную емкость биосорбента по отношению к катионам меди Cu^{2+}.

Ключевые слова: магнитомеченые дрожжи *S. cerevisiae*, сорбционная емкость биосорбента, функциональные группы.

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