Electrophoretic mobility of magnetically labeled yeast cells *S. cerevisiae*

Y.V.Karpenko, S.V.Gorobets

National Technical University of Ukraine "KPI", College of Biotechnology and Bioengineering, Department of Bioinformatics, 37 Peremogy Ave., 03056 Kyiv, Ukraine

Research of ζ-potential of cell wall of magnetically labeled yeast cells was proposed by measuring electrophoretic mobility to determine the nature and mechanisms of cell wall interaction with environment. The results showed that biosorption of copper cations (II) and attaching the magnetic beads clearly reduce the electrophoretic mobility of cells and thus the interaction with functional groups distributed on the cell surface takes place in these processes.

Для определения характера и механизмов взаимодействия клеточной стенки с внешней средой предложено исследовать ζ-потенциал клеточной стенки магнитоуправляемых клеток дрожжей путем измерения электрофоретической подвижности. Полученные результаты показали, что биосорбция катионов меди (II) и прикрепление магнитных меток однозначно уменьшают электрофоретическую подвижность клеток, это значит в этих процессах взаимодействия происходят с функциональными группами, распределенными на поверхности клетки.

1. Introduction

Yeasts *Saccharomyces cerevisiae* are capable to unlimited growth in both diploid and haploid phases. Diploid cells are slightly larger than haploid cells. Yeasts are fungi that have adapted to exist in an environment with a high content of saccharides [1]. Usually *S. cerevisiae* are unicellular and reproduce by budding. Cell wall completely covers yeast cells and limits the internal contents of cells. Uniformity of the wall structure is broken only in budding areas where daughter cells form and separate. Cell wall is separated from the cytoplasm membrane by plasmatic membrane. Plasma membrane is the outer membrane surface. It regulates the flow of substances into the cell and from it, and it takes part in chemical interactions with other cells. Vesicles formed by plasma membrane disconnect and merge with lysosomes in some cases. In this way the cell can adsorb dense particles (phagocytosis) or droplets (pinocytosis) from the environment [2]. Surface phenomena such as adhesion and adsorption occurring on the edge of the distribution of cell wall-external environment play an important role in biotechnology. For example, there is a problem of microorganism's cells immobilization in various industrial biotechnological processes and wastewater treatment technologies.

The surface layer of cell is a dynamic structure [3], which responds to environmental change through ions and macromolecular components adsorption. The microorganism's cells reaction depends on the presence of charged groups which are the different components on the cell wall surface. The yeast cells surface has a negative charge [1] under normal conditions of their existence. Dense particle's charged groups can connect or disconnect from the surface functional groups of cell depending on many factors, especially, changes in the

value of pH, and depending on the distance between the surfaces: two cells or cell and substrate, or cells and magnetic particles. DLVO (Derjaguin, Landau, Verwey, Overbeek) theory describes the cellular adhesion in a wide range of phenomena [3], including creation of new biosorbents and biosorption of heavy metals. In this theory cell wall is a surface with distributed electric charge. Surface is described by its electric potential. Electric potential on the surface of yeast cells *S. cerevisiae* is formed by functional groups and other surface formations, and can be calculated from the electrophoretic mobility [4].

Process of metal biosorption by living cells is two-stage process [5]. First, metal ions are adsorbed on the surface of cells by interacting with functional groups such as carboxyl, phosphate, hydroxyl and amino groups. The first step, also called passive biosorption, is not dependent on metabolism and takes few minutes with each of these metal binding mechanisms or combinations: coordination, complexation, ion exchange, physical adsorption (e.g. electrostatic interactions) or inorganic micro-sedimentation. Passive biosorption represents reversible dynamic equilibrium, so adsorption/desorption process takes place. Metal ions bounded to the surface can be washed by other ions or acid chelate supplement. The second step is metal ions penetration through the cell membrane and penetration into the cell, it is called an active biosorption. Metal adsorption by lifeless cells is mainly a passive way, besides, in case of dead cells certain restrictions can be overridden that arises in the case of alive cells using such as: need for nutrient medium, sensitivity to extreme values of pH or higher metal ions concentration, etc. [6, 7].

The presence of the surface charge on yeast cells makes them possible to bind cations and in addition the ability to interact with the magnetic beads in magnetic fluid. The magnetic fluid used in this research is a suspension of nanosized magnetic particles stabilized by hypochlorous acid in aqueous solution. It is typically for magnetic fluids that individual nanosized particles (magnetic beads) have distributed electric charge on the surface that changes its value depending on the environment: it is negative in alkaline and positive in acid [8]. Complexes yeast cell-magnetic beads generated by stirring biomass with magnetic fluid can be effectively removed by high-gradient magnetic separation after biosorp-

tion. However, probably joining magnetic beads to cell wall takes place by the same functional groups that participate in copper biosorption. Therefore it is necessary to determine the ζ-potential of yeast cell wall and to identify the impact of associated magnetic beads on sorption capacity of magnetically labeled complexes by measuring the electrophoretic mobility.

The aim of the work was to determine electrophoretic mobility of yeast cells *S. Cerevisiae* native and modified by magnetic beads attachment with different mixing methods of yeast suspension with magnetic fluid, and after the copper cations (II) biosorption process. Quantity of magnetic beads that was added to the yeast suspension was limited by the value for which the cells with mechanically attached magnetic beads lost mobility in an electric field, i.e. the cells do not move in electric field. The beads attaching process was conducted by different mixing methods of magnetic fluid with yeasts solution: 1) mechanical stirring using a mechanical device and 2) multivertex magnetohydrodynamic mixing with a high-gradient ferromagnetic matrix in cuvet placed in the working gap of an electromagnet. Methods of mixing are identical to those described in [9].

2. Materials and methods

We used yeasts *Saccharomyces cerevisiae* produced by CSC "ENZYM", crystalline copper $CuSO_4 \cdot 5H_2O$, ferric chloride $FeCl_3 \cdot 6H_2O$ and $FeCl_2$, nitric HNO_3, HCl and hypochlorous HClO acids and ammonia NH_4OH, potassium buffer solution, sodium phosphate pH = 6.86, glycerol.

Preparation of magnetic beads. Method of magnetic fluid preparation is similar to [8, 10]. 2 M solution of ferric chloride (II) was prepared in 2 M solution of HCl and 1 M solution of ferric chloride (III). 1 M ferric chloride (III) and 10 ml of 2 M ferric chloride (II) were mixed in 40 ml aqueous solution and added 500 ml 0.7 M solution of ammonia. Sediment was separated from the supernatant by permanent magnets. The resulting residue was stirred with 200 ml hypochlorous acid for 10 min on a magnetic mixer. Then again the sediment was separated by permanent magnet and mixed with hypochlorous acid. Procedure was repeated twice. The resulting gel was mixed with 200 ml of water for 5 min, 200 ml of 2 M hypochlorous acid residue was added and separated by the permanent magnet. Thus we repeated three times mixing with water

Table 1. Samples with their treatments for electrophoretic mobility measurement

Sample number	Heat treatment of *S. cerevisiae* cells		Method of attachment of magnetic beads		
	Untreated	Treated with 60°C, 30 min	Mechanical mixing with different ratio of biomass to mass of beads		Multivertex magnetohydrodynamic mixing with magnetic fluid with mass ratio 100:1
			100:1	50:1	
Sample 1	×				
Sample 2		×			
Sample 3	×		×		
Sample 4		×	×		
Sample 5		×		×	
Sample 6	×		×		
Sample 7		×			×

and then added 200 ml of 2 M hypochlorous acid and precipitated by permanent magnet. Gel was centrifuged with frequency 2000 min^{-1} for 1 h and supernatant was removed. 3 g of gel was dissolved in 7 ml of water. The effective particle size varies from 5 nm to 20 nm [10].

Preparation of Saccharomyces cerevisiae solution. Weight of yeasts was hold at 4°C during 3 weeks. Then 2 g of yeasts initial biomass was taken on analytical scales. Biomass was mixed with water in the 500 ml flask by mixing in mechanical stirrer with frequency speed 180 min^{-1} for 15 min. That way the uniform cell distribution was reached with an average size 5–15 μm in volume with concentration of $8 \cdot 10^9$ cells/l^3. The resulting solution was used in further methods of sample preparation. Yeasts aged 2 days and prepared by the same method were used as controls — sample 1.

Preparation of heat-treated S. cerevisiae cells. Yeasts solution with a known concentration was closed hermetically and heated to a temperature of 60°C and kept at constant temperature during 30 min. After processing the solution was cooled to room temperature — sample 2.

Preparation of complexes Saccharomyces cerevisiae cell — magnetic beads. Untreated and heat-treated yeasts solutions were mixed separately with a magnetic beads solution with the ratio of yeast cells totally dry mass to the beads mass was 100:1 in accordance to further measurements of electrophoretic mobility. 1 ml of magnetic fluid with a concentration 0.1 g/l^3 was added to

99 ml of solution with yeast concentration 100 mg totally dry substance/l^3 for this reason. Also the sample of untreated magnetically labeled yeast cells was prepared with mass ratio 50:1, i.e. adding magnetic beads in the amount of 2 % of totally dry mass of yeast cells.

The resulting solution was stirred by different methods including mechanical mixing during 30 min and multivertex magnetohydrodynamic one during 10 min. Multivertex magnetohydrodynamic mixing with ferromagnetic high-gradient matrix was conducted in a homogeneous permanent magnetic field using the method described in [11].

The methods of preparation of the samples are represented in Table 1. Treatment type of biomass and way of magnetic beads attachment are marked in Table 1 by "×" for each sample, prepared for the study of electrophoretic mobility.

Preparation of yeast cells in sorption of Cu^{2+} ions. Solvents of sample 3, sample 5 and sample 1 for comparison were used for this process. Initial copper ions concentration in solution was 100 mg/l^3. Biosorption was intensified by mechanical mixing with frequency 180 min^{-1}. Copper Cu^{2+} ions concentration measurement conducted after 120 min [11] because after this time in the system metal ions-biosorbents the sorption equilibrium was set. For the concentration measurement the separate probe was taken in which the magnetically labeled biosorbent was filtrated. Determination of copper Cu^{2+} ions concentration was carried out at room temperature by measuring the intensity of blue color of complexes $[Cu(NH_3)_4]^{2+}$ ob-

tained by adding ammonia NH_4OH to filtrate, in light-filter with wavelength 590 nm using a spectrophotometer SF-46 by the procedure described in [9].

Preparation of yeast cells in desorption of Cu^{2+} ions. Nitric acid was added to samples of biosorption systems to reduce to value pH = 2 after biosorption, since it is known that after passive biosorption cations of heavy metals can disconnect from the cell wall in the processing by relevant agent [12]. The system was left on 2 h.

Preparation of samples for measurement of electrophoretic mobility. For each study to measure electrophoretic mobility the untreated samples and heat-treated samples were prepared in order to Table 1, and magnetically labeled yeast cells by diluting model solutions 20 times, in order to be able to observe the movement of individual cells. pH value in measuring electrophoretic mobility of the cell samples adjusted in the range from 1 to 8 with increments of approximately 1 by adding nitric acid or alkali $NaHCO_3$ with different molar concentrations in the range 0.05–0.15 mol/l. Low molar concentration is due to follow laminar regime of electroosmosis flow.

Conducting experiments with electrophoretic mobility measurements. From each sample it was taken 2 ml and deposited onto a piece of glass on which gold electrodes were installed. System closed by covering glass and tightening mechanisms. The resulting sample mounted on narrow table of microscope, which worked on the enlightenment model (increase in the eyepiece — ×5, increased lens — ×20, an overall increase — ×100). After setting the image sharpness in glasses voltage 9 V conveyed to the electrode. The distance between the electrodes R = 30 mm. When visually selected cell approached to the beginning of the scale, stopwatch turned on. There was 20 cells to account for standard error of measurement — 2 %. To determine the overall electrophoretic mobility was used the following formula [12]:

$$\mu_{ek} = V/E = (L/T)/(U/R), \qquad (1)$$

where μ_{ek} — electrokinetic mobility, m^2/Vs; V — average velocity of yeast cells in an electric field, m/s; E — electric field between electrodes, V/m; L — path length of cell, m; T — time of observation, s; U — potential difference applied to the electrodes, V; R — distance between electrodes, m.
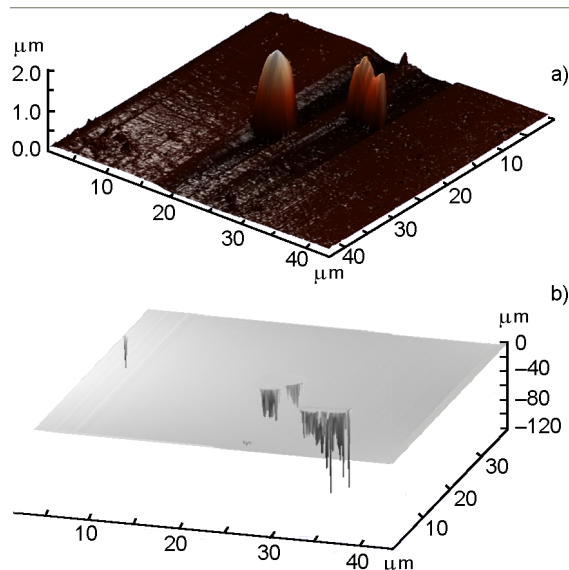

Fig. 1. Images of magnetically labeled yeast cells obtained by a) method of atomic force microscopy, b) method of magnetic force microscopy.

Thus, in experiments simultaneously measured two quantities — length of the path traversed cells, and time, which was passed for this way.

Since formula (1) determines apparent electrokinetic mobility, it is necessary to consider mobility electroosmosis flow to determine the actual electrophoretic mobility of cells. Let's use formula [13]:

$$U_{ek} = \varepsilon Ed/(\eta + \varepsilon^2 d/\kappa a), \qquad (2)$$

where U_{ek} — velocity of cells in the fluid, m/s; ε — charge of the mobile part of the electrical double layer, C; d — thickness of the electrical double layer, m; η — viscosity of solution, Pa·s; κ — electrical conductivity of the solution, Cm·m; a — radius of particle, m.

Formula (2) can be rewritten as follows:

$$U_{ek} = \varepsilon Ed(1 - \eta\kappa a/\varepsilon^2 d)/\eta.$$

If you divide and multiply equation (2) to $\varepsilon^2 d/\eta\kappa a$, then the formula (2) can be written as two limiting cases:

$$U = \varepsilon Ed/\eta, \qquad (3)$$

at $\varepsilon^2 d/\eta\kappa a <<|1$, and

$$U = a\kappa E/\varepsilon \qquad (4)$$

at $\varepsilon^2 d/\eta\kappa a >> 1$.

In our case, the formula will work (3) with direct proportionality between the charge and electrophoretic mobility, since the inequality (5) due to the low molarity of the electrolyte:

$$\varepsilon^2 d / \eta \kappa a \ll 1. \quad (5)$$

In the case of ideal electrokinetic flow in which electrokinetic field intensity is proportional to the speed u_{zah} local electric field [14]:

$$U_{ek} = \mu_{ek}E = u_{cell} \pm u_{eo} = (\mu_{cell} \pm \mu_{eo})E. \quad (6)$$

To take into account mobility of electroosmosis flow on electrophoretic mobility of cells was measured separately for all conductivity of electrolytes. According to the measurement using the difference between the total mobility and electrokinetic mobility of electroosmosis flow actual the electrophoretic mobility of cells was calculated.

$$\mu_{cell} = \mu_{ek} - \mu_{eo}, \quad (7)$$

where μ_{cell} — electrophoretic mobility of cell, m^2/Vs; μ_{eo} — mobility of electroosmosis flow, m^2/Vs.

Electroosmosis flow mobility can be determined from the equation of the specific electrical conductivity of electrolytes [12]:

$$\kappa = (\mu_+ + \mu_-)FCa, \quad (8)$$

where κ — electrical conductivity of the electrolyte, $Cm \cdot m^{-1}$; μ_+ — mobility of electrolyte cations, m^2/Vs; μ_- — mobility of electrolyte anions, m^2/Vs; F — Faraday number (96.500 cells/mol); C — molar concentration, mol/m^3; and a — the degree of dissociation.

According the fact that the polarity of the electrolytes used in the experiments is very low, you can apply Kolraush law:

$$\lambda^\infty = (\mu_+^\infty + \mu_-^\infty)F = \lambda_+^\infty + \lambda_-^\infty, \quad (9)$$

where λ^∞ — molar conductivity of electrolyte at infinite dilution, $Cm \cdot m^2/mol$; λ_+^∞ — molar conductivity of electrolyte cations at infinite dilution, $Cm \cdot m^2/mol$; λ_-^∞ — molar conductivity of electrolyte anions at infinite dilution, $Cm \cdot m^2/mol$.

So the sum $\mu_+^\infty + \mu_-^\infty$ is electroosmosis mobility at infinite dilution μ_{eo} of electrolyte and it is equal to the ratio of λ^∞/F. In the real solution it is necessary to measure
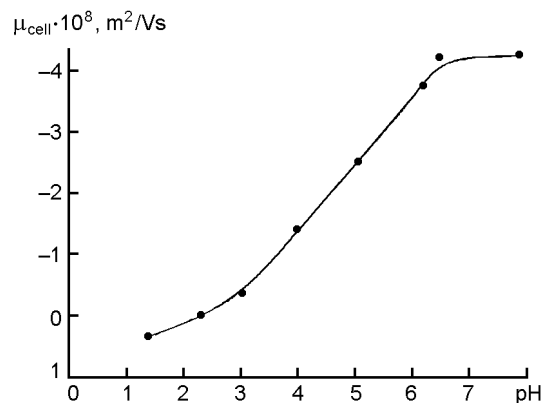

Fig. 2. pH-dependent curve of electrophoretic mobility of yeast cells (control sample 1).

the molar conductivity of electrolytes. Using the conductometry method based on determination of conductor resistance by measuring the current strength, electrical conductivity of the electrolyte can be determined and as a consequence — the ratio λ/F, which is a electroosmosis mobility μ_{eo} for each electrolyte, which was used in the research.

For all experiments it was measured the value of current strength, which passes through the electrolyte solution before nitric acid or alkali $NaHCO_3$ in the absence of a biological agent. On the basis of measurements it was found that the value of electrophoretic mobility of the flow was in the range of 0.005–0.009 m^2/Vs and was taken into account in calculations of the measurement results.

3. Results and discussion

Charged groups can connect or disconnect depending on the changing of pH value or ionic strength of solution, and depending on approaching of charged surface or another cell. Therefore, an important step is the selection of electrolytes that will not make a significant error in the experiment. These electrolytes are solutions or buffer solutions with a high coefficient of dilution. Fig. 2 shows dependence of electrophoretic mobility of baking yeast cells measured after 2 days of exposure.

As shown in Fig. 2, untreated yeast cells have a maximum value of electrophoretic mobility $-4.25 \cdot 10^{-8}$ m^2/Vs at values pH > 7 and zero mobility between pH = 2–3. It should be noted that the surface functional groups of yeast cell wall create distributed charge and consequently form the wide dif-
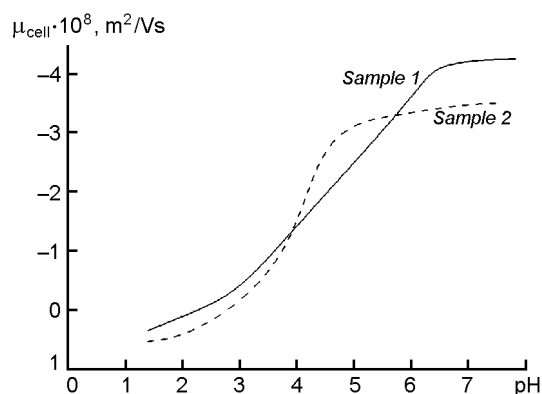
Fig. 3. pH-dependent curves of electrophoretic mobility of heat-treated and untreated yeast cells.
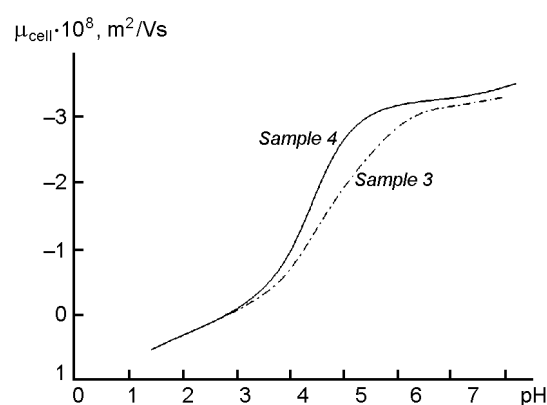

Fig. 4. pH-dependent curves of electrophoretic mobility of magnetically labeled yeast cells produced by mechanical mixing.
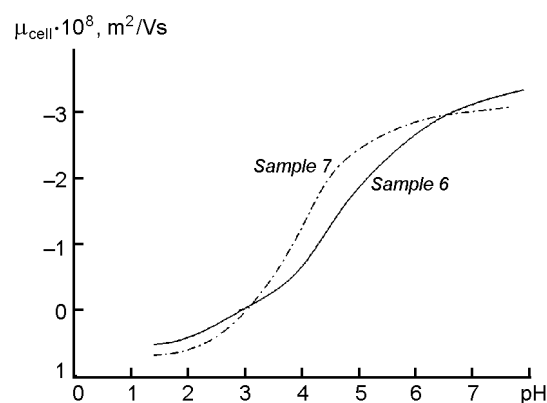

Fig. 5. pH-dependent curves of electrophoretic mobility of magnetically labeled yeast cells produced by different methods of mixing.

fusion layer in the normal direction to the cell surface and possess most electrokinetic potential. It should be noted that the extracellular activity of protein components of living organisms could theoretically improve the expected results because there is a need to explore the dead yeast cells. From this perspective, the next Fig. 3 shows the dependence of electrophoretic mobility of heat-treated yeast cells on pH.

Results of the samples comparison according to types of treatments are presented in Fig. 3, 4 and 5. As shown in Fig. 3 the maximum of electrophoretic mobility is $-3.5 \cdot 10^{-8}$ m^2/Vs for dead cells, and it is less than for the living cells. However, this may indicate a degradation of biopolymers as a result of high temperature, but on the other hand it is known that proteins are able to regenerate itself after exposure to critical temperatures. Similar to the previous experiment electrophoretic mobility of the cells of the second sample is zero at pH between 2–3.

As shown in Fig. 3, in the case of thermal processing of biomass the cells electrophoretic mobility varies: it becomes lower in comparison to raw cells in the range of pH less than 4 and more than 6 at all other similar terms and it becomes higher in the range pH = 4–6. Thus in the range pH = 4–6 heat-treated cells have more distributed electric charge and therefore able to better passive biosorption, confirming the fact that heating of yeast before biosorption increases their sorption capacity [7].

Fig. 4 shows the electrophoretic mobility of untreated (sample 3) and heat-treated (sample 4) magnetically yeast cells produced by mechanical mixing of biomass with a magnetic fluid with a ratio of yeast to the

magnetic labels 100:1, depending on pH. As shown in Fig. 5, in the case of heat treatment before attachment of magnetic labels the electrophoretic mobility of yeast cells greater in magnitude than for untreated cells. If you compare sample 3 and sample 4 with a control sample 1 in the range pH 4–6 you can see that electrophoretic mobility of cells is higher in sample 4 when compared with the control and lower in sample 3 compared with control. As in the previous comparison samples in Fig. 3 and Fig. 4 shows that in case of heat treatment cell electrophoretic mobility greater than that of untreated cells.

Fig. 5 shows the electrophoretic mobility of magnetically labeled yeast cells produced by mechanical mixing (sample 6) and by multivertex magnetohydrodynamic mixing (sample 7). In the case of mechanical mixing electrophoretic mobility of the cells is greater than in the case of multivertex

Table 2. Electrophoretic mobility of yeasts cells in sorption studies

Relative weight of beads	0 % (sample 1)	1 % (sample 3)	2 % (sample 5)
Cell state	μ_{cell}, m^2/Vs·10^8		
After beads attachment	−2.293	−1.595	−1.095
After biosorption of copper ions	−0.099	−0.297	−0.292
After desorption of copper ions	0.698	0.695	0.691

magnetohydrodynamic mixing. This is a consequence of acid adding for multivertex magnetohydrodynamic mixing, and using of high intensity values of the magnetic field of electromagnet.

The values of electrophoretic mobility of cells at pH = 8 decrease in the following order: sample 3 — $-3.5 \cdot 10^{-8}$ m^2/Vs, sample 6 — $-3.34 \cdot 10^{-8}$ m^2/Vs and sample 7 — $-3.08 \cdot 10^{-8}$ m^2/Vs.

If we compare electrophoretic mobility of samples presented in Fig. 5 in pH = 4.5, i.e. the optimal pH for biosorption, we can see that sample 7 has the largest modulo value. So heat-treated magnetically labeled biosorbents has the largest number of functional groups, and therefore has the best adsorption characteristics comparing with other samples. These facts confirm the results of [9]. In terms of processes that occur in the studies: in the case of magnetic beads attachment primarily we deal with magnetic beads adhesion on the cell wall surface and in the case of removal of heavy metals — copper ions adsorption on the cell wall surface. Both processes are related to surface phenomena, and therefore in the study of cell wall of yeast *S. cerevisiae* it should be noted that it is necessary to consider the possibility of penetration of the magnetic beads and heavy metal ions through the cell wall into the cell. From this point of view copper ions sorption and desorption by magnetically labeled yeast cells is presented in Table 2.

For easy interpretation of the research results Table 2 shows the data of electrophoretic mobility measurements after appropriate types of treatment as described in Materials and Methods. The research results showed that the electrophoretic mobility of magnetically labeled cells decreases with increasing of relative amount of magnetic beads on the cell surface.

The values presented in Table 2 change their sign after desorption at pH below 2.

4. Conclusions

Electrophoretic mobility of magnetically labeled yeasts under the same pH is lower than mobility of untreated and heat-treated yeast cells. As known from the literary sources the magnetic beads have distributed positive charge in acidic environments, and their presence on the cell surface takes a certain number of binding sites that can be used in heavy metals biosorption. Thus, the number of attached magnetic beads is essential in creation of magnetically labeled biosorbents. For example, if we compare the maximum values of electrophoretic mobility of untreated yeast cells (sample 1) and magnetically labeled cells with a relative weight of beads 1 % (sample 3), then at pH = 7 ratio takes place $(\mu_1 - \mu_3) \cdot 100$ % / $\mu_1 =$ 20 %. For this reason, it was investigated electrophoretic mobility of magnetically labeled cells in the case of copper ions sorption / desorption.

In [9] the best sorbent is complex of magnetic beads and yeast cells, obtained by multivertex magnetohydrodynamic mixing. In the studies of electrophoretic mobility sample 7 has the greatest electrophoretic mobility before biosorption at pH = 4.5 and is similar to the one described in [9], and thus confirms the result of better passive biosorption by higher potential distributed on the cell surface.

After copper ions biosorption the electrophoretic mobility is significantly reduced, indicating the binding sites filling on the cell surface by cations. After quantitative analysis it was found that the residual concentration of copper ions is about 10 mg/l^3 that indicating a 90 % efficiency removal of cations from the model solution. Also worth noting that after biosorption the electrophoretic mobility of magnetically labeled samples is greater than mobility of untreated cells. This concludes existing of competition between the magnetic beads and copper cations for free sites. After desorption the electrophoretic mobility reduced on 30 % in untreated cells and on 40−60 % in magnetically labeled cells. So there is

correlation between the residual concentration of copper cations and electrophoretic mobility of the cells. And therefore it is possible to quantify the effectiveness of biosorbents.

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Електрофоретична рухливість магнітокерованих клітин дріжджів *S. cerevisiae*

Ю.В.Карпенко, С.В.Горобець

Для визначення характеру і механізмів взаємодії клітинної стінки з навколишнім середовищем запропоновано дослідити ζ-потенціал клітинної стінки магнітокерованих клітин дріжджів шляхом вимірювання електрофоретичної рухливості. Отримані результати показали, що біосорбція катіонів міді (II) і прикріплення магнітних міток однозначно зменшують електрофоретичну рухливість клітин, отже в цих процесах взаємодія відбувається з функціональними групами, розподіленими на поверхні клітини.