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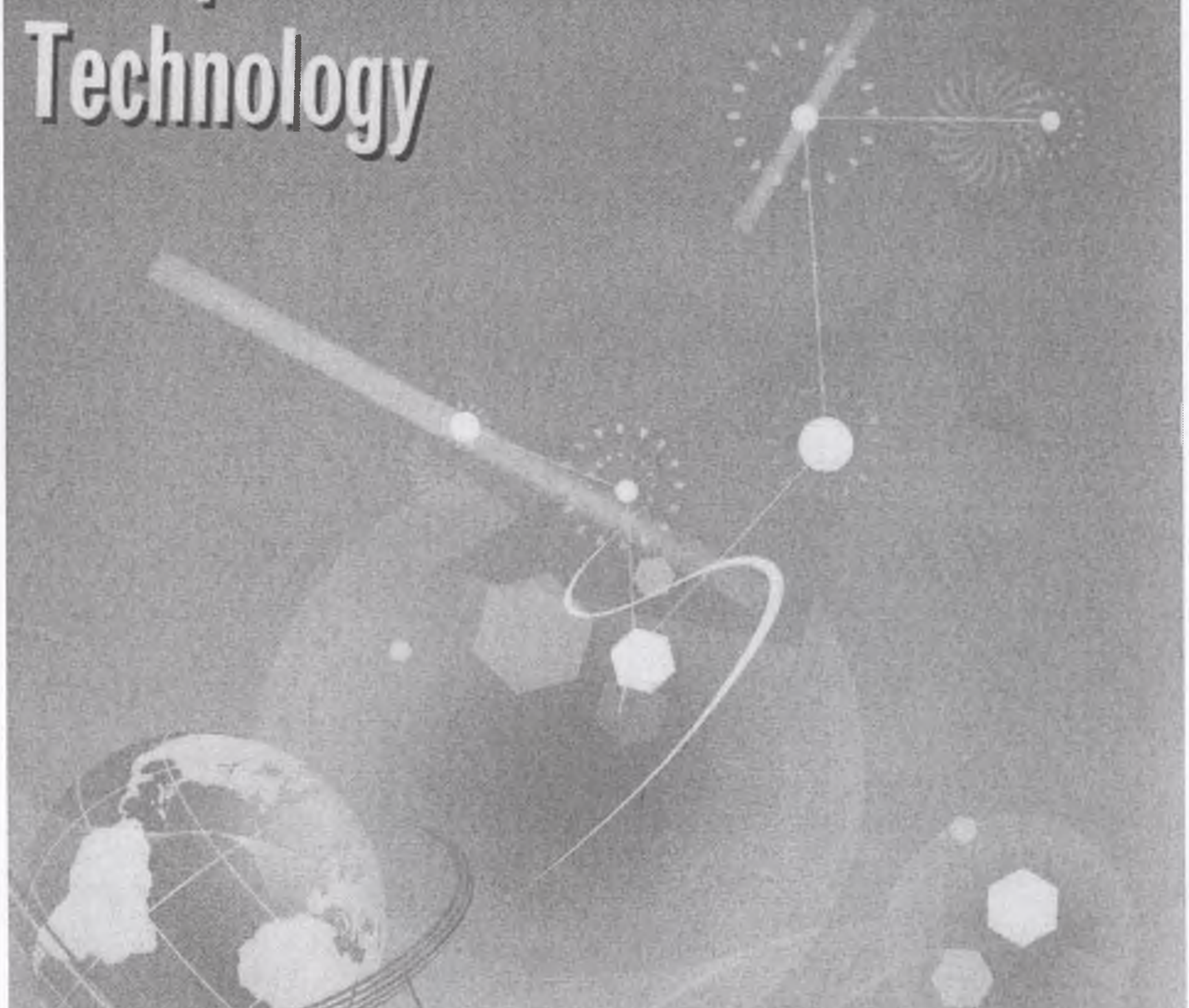

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IMMOBILIZATION OF MICROORGANISM CELLS ON THE DIATOMITE SURFACE

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Abstract

The immobilization of *Torulopsis kefir var kumis, Rhodotorulla glutinis, Pseudomonas mendocina H3* microorganisms cells on a diatomite surface is studied. For increase of degree of an immobilization it is offered to use in quality spacer cationic polyelectrolyte – polyethyleneimine (PEI). Possibility of use received biosorbents for removal of Cu^{2+} and Pb^{2+} ions from solutions is shown.

Keywords: immobilization, adsorption, diatomite, microorganism, cell.

Introduction

Now preservation of the environment is one of essential problems of mankind. Various ways of water treating, air, protection of the infected soils against aeration etc. In the field of water treating, despite variety of methods are developed, the most widespread and effective are sorption methods [1-4]. And last years scientists and technologists gravitate to use of natural adsorbents. Such sorbents are microorganisms cells which, thanks to presence on their surface of various functional groups, possess high sorption ability [5-7]. However their wide use at water treating is interfered by difficulty of branch from solutions.

Effective way of simplification of process of branch of cells from solutions is the attachment to firm surfaces. In this connection a research objective was optimization of conditions of an immobilization of *Torulopsis kefir var kumis, Rhodotorulla glutinis, Pseudomonas mendocina H3* cells on a diatomite.

Experimental

As the carrier of microorganisms cells used a diatomite of the Mugodzhar region (Kazakhstan).

As adsorbents of metals ions of microorganisms cells (tab. 1) from a collection of department of biotechnology of Al-Faraby Kazakh National University served.

Table 1

The characteristic of microorganisms cells

Microorganisms cells	Kind	The size, a micron
Torulopsis kefir var kumis	Yeast	(2,2-4,7) x (2,6-5,2)
Rhodotorulla glutinis	Yeast	(2,5-3,0) x (6,0-6,5)
Pseudomonas mendocina H3	Bacteria	(0,6-0,8) x (1,8-2,0)

ζ – potential of a cells surface defined by microelectrophoresis method under the formula Gelmgolts-Smoluhovsky.

For an immobilization of microorganisms cells their suspension with the fixed concentration mixed with diatomite suspension, then a mix maintained within 2,5 hours. After that a diatomite with immobilizing cells separated from supernatant in which defined quantity of cells.

For an immobilization of microorganisms cells on a diatomite in the presence of PEI at first a diatomite surface modified with PEI by mineral keeping in PEI solution corresponding concentration within 2,5 hours, then diatomite particles separated from a solution of polymer and mixed with suspension of cells.

After 2.5 hours defined concentration of the cells which have been not connected with a surface of a diatomite.

Results and discussion

The diatomite is natural high-porous material which represents a perspective material for use as a sorbent possessing high mechanical durability and considerable of sorption capacity [1, 2]. The important factor is also that this material is rather cheap mineral raw materials. The experiments made earlier by us on adsorption of Cu^{2+} and Pb^{2+} ions on a diatomite surface have shown that at concentration of $CuSO_4$ and $Pb(NO_3)_2$ $1\cdot10^{-3}$ mole/l metals ions almost completely contact a diatomite surface. Thus degree of extraction of metals ions makes 91,6 % for Cu^{2+} and 89,8 % for Pb^{2+} [8]. However such degree of extraction doesn't solve a water treating problem as residual concentration of metals ions exceeds maximum permissible concentration which for Cu^{2+} and Pb^{2+} ions is equal 0,1-4,0 and 0,2-1,0 mg/l accordingly [9].

For increase of degree of clearing it is possible to use microorganisms cells which also can take metals ions from solutions [10, 11]. For an attachment of microorganisms cells to a diatomite surface at first spent their immobilization on a diatomite mixture of cells suspensions and a diatomite with the subsequent keeping of a mix. In table 2 results of experiences on an immobilization of cells on a diatomite are resulted.

Table 2

Results of experiences on an immobilization of cells on a diatomite

$C_0\cdot10^{-5}$, cell/ml	$C_{eq}\cdot10^{-5}$, cell/ml	Immobilization degree, %	$A\cdot10^{-5}$, cell/g
	Torulopsis kefir var kumis		
2.19	0,09	91,32	21,0
4.38	0,43	90,88	38,5
8.76	0,86	90,18	79,0
10.95	1,45	86,75	95,0
13,14	1,74	86,83	114,0
17,52	2,30	86,87	152,2
21,90	2,83	87,09	190,7
	Rhodotorulla glutinis		
2.04	0,23	88,81	18,1
4.08	0,48	88,31	36,0
8,16	1,05	87,05	31,1
10.20	1,35	86,82	88,5
12,24	1,71	86,10	105,3
16.32	2,29	86,01	140,3
20,40	2,91	85,78	174,9
	Pseudomonas mendocina H3		
2.40	0.89	63,00	15,1
4.81	1,92	60,21	28,8
9.60	4,33	54,90	52,7
12.00	5,75	52,11	62,5
14.41	7,2	50,02	72,0
19.22	9,69	49,54	95,1
24.03	12,45	48,15	115,5

Basically, the immobilization of cells in this case represents their adsorption on a diatomite surface. Therefore results of experiences can be presented as well in the form of isotherms adsorption (fig. 1). Apparently from table 1, cells *Torulopsis kefir var kumis* and *Rhodotorula glutinis* on 85,78-91,32% are attached to a diatomite surface, and *Pseudomonas mendocina H3* – only on 48,15-63%. It, apparently, is connected with features of cells: in their sizes and a variety of the functional groups responsible for interaction with a diatomite.

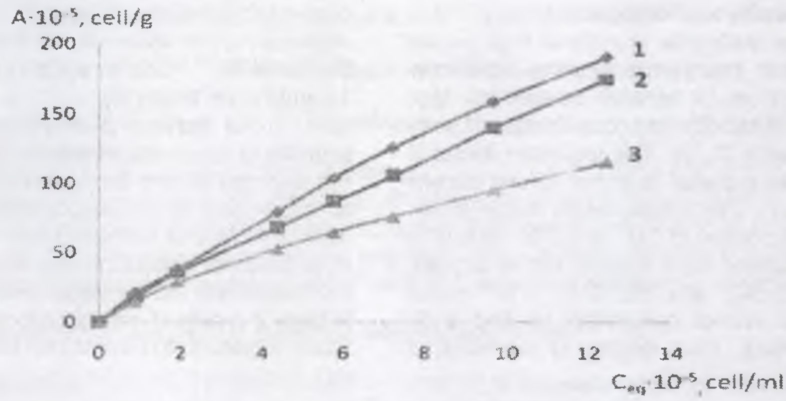

Fig. 1 Adsorption of cells on a diatomite surface:
1 – Torulopsis kefir var kumis; 2 – Rhodotorulla glutinis;
3 – Pseudomonas mendocina H3

According to the data [6-7], on a surface of cells of yeast are carboxyl, phosphatic, hydroxilic and amino groups. The main component of a diatomite is SiO_2 (table 3).

Table 3

The Chemical contents of a diatomite, %

Contents of a diatomite	SiO_2	Al_2O_3	Fe_2O_3	K_2O	MgO	Na_2O	TiO_2	CaO	Cl
Quality, %	65,83	8,66	2,98	1,16	1,01	0,75	0,49	0,47	0,56

As it is known [12], silanolic surface groups of silicacontaining dispersions depending on pH mediums can be in dissociated, not dissociated and protonated conditions:

$\equiv SiOH + H^+ \rightarrow \equiv SiOH_2^+$ (the acidic medium)

$\equiv SiOH + OH^- \rightarrow \equiv SiO^- + H_2O$ (the alkaline medium)

$\equiv SiOH$ – (the neutral medium)

As initial cells suspensions had pH, close to 5, and diatomite suspensions – pH=6 it is possible to assume that H-bonds will be defining type of interactions of surfaces of cells and a diatomite.

The size of a time of a diatomite it agree [13] makes 60 nanometers – 1 micron. The size of cells is in limits of several microns. It follows from this that not all microorganisms cells can enter into a time of a diatomite and be fixed in them. Therefore for increase of effect of an attachment of microorganisms cells to a diatomite surface in quality of spacer used PEI.

Choice PEI is caused by that it represents cationic polyelectrolyte which is well adsorbed on negatively charged surfaces. It is known also [14] that it posses of complex forming properties at the expense of formation donor-acceptors to bond between atoms of nitrogen of imines groups and ions of transition metals.

Microorganisms cells of have mainly negative charge [15-16], to it testifies also curve dependences ζ – potential of barmy cells from pH mediums (fig. 2).

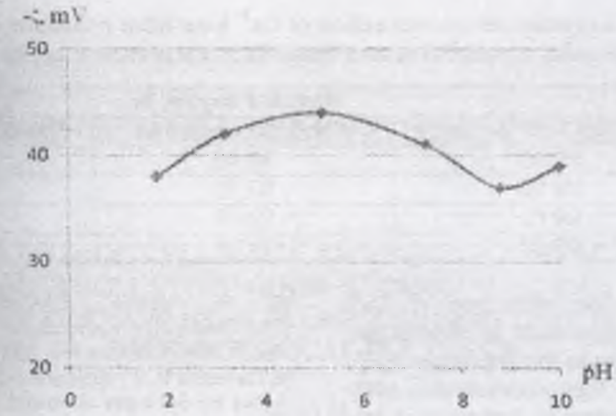

Fig.2 Effect of medium pH on the electrokinetical potential of *Torulopsis kefir var kumis* cells

The negative charge of a surface of a diatomite is caused of dissociation of SiOH-groups. Therefore amino groups of PEI can electrostatic be drawn both to a diatomite surface, and to a cells surface. However results of experiences on an immobilization of cells on a surface of a diatomite in the presence of PEI have shown that polymer use not always favorably for an immobilization of cells.

Curve dependences of degree of an immobilization of cells on PEI concentration (fig.3) pass through maxima. The maximum attachment of *Torulopsis kefir var kumis* and *Rhodotorulla glutinis* cells is observed at PEI concentration 0,01 base-mole/l, and *Pseudomonas mendocina H3* – at 0,03 base-mole/l. Obviously, these concentration of polymer it is enough for a covering of a surface of a diatomite and creation on it positively charged sites for linkage of microorganisms cells. The further increase in PEI concentration, possibly, leads to closing of a time and roughness's on a mineral surface that, accordingly, will promote decrease in its specific surface. Surplus PEI also is undesirable in view of that it can flocculating particles of a diatomite and microorganisms cells.

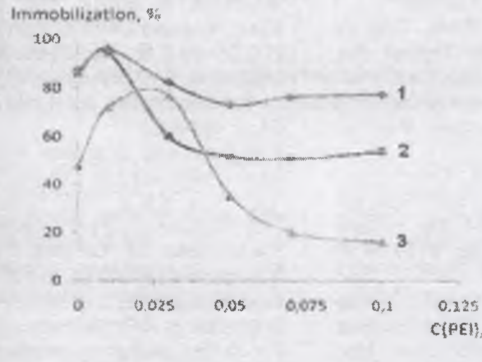

Fig. 3 Dependence of degree of an immobilization of cells from PEI concentration:
1 – *Torulopsis kefir var kumis*; 2 – *Rhodotorulla glutinis*;
3 – *Pseudomonas mendocina H3*

It is necessary to notice that increase of degree of an immobilization of cells in the presence of PEI has appeared the most considerable in case of *Pseudomonas mendocina H3* cells, having the least size among the used cells.

Table 4

Results of experiences on extraction of Cu^{2+} ions from solutions by means of microorganisms cells, immobilizing on a diatomite in the presence of PEI. $C_0(Cu)$ =64 mg/l.

Time, min.	Removal degree, %		
	Torulopsis kefir var kumis	*Rhodotorula glutinis*	*Pseudomonas mendocina H3*
30	99,90	99,46	99,85
60	99,90	99,96	99,92
90	99,62	99,95	99,87
120	99,95	99,96	99,85

Experiences on extraction of Cu^{2+} ions from solutions by means of microorganisms cells, immobilizing in the presence of PEI (tab. 4), have shown their considerable sorption ability. In 30 minutes of contact of a biosorbent with a solution degree of extraction of Cu^{2+} ions for all systems exceeds 99%. It it is possible to explain that all components of the given system: microorganisms cells, a diatomite and PEI can take separately from a solution Cu^{2+} ions. It is natural that their joint action provides the clearing of a solution corresponding to norms of ecological safety [9].

Thus, use as a biosorbent of metals ions microorganisms cells, immobilizing on a diatomite, creates conditions for the maximum water treating.

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