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Golodova I.V., Rube V.A., Lezhneva M.Yu., Dolzhnikov V.S.
ESTIMATION OF AUTHENTIC EXISTENCE OF COMPONENT IN COMPLEX MIXTURE OF THE PHYTOGENESIS ON THE BASIS OF PROBABILISTIC CRITERIA ................................................. 68

Hitrin S.V., Fuks S.L., Suhanova E.N., Filatov V.Yu.
LOW-WASTE PRODUCTION DEVELOPMENT OF WASTE UTILIZATION OF POLYTETRAFLUORETHYLENE .............................................................................................. 73

Mamedov Kh.F.
RADIOLYTIC DESTRUCTION OF MYCOTOXINS IN WATER SOLUTIONS, CEREALS AND FRUIT ................................................................. 76

Nasyrova Z.H., Ernazarova A.E.
PROBLEMS OF SCHOOL CHEMICAL EDUCATION IMPROVEMENT .................................................................................................................. 80

Niyazbekova A.B., Kuanysheva G.S., Rakhova A.S., Akatev N.V., Mukaeva M.M.
QUANTUM-CHEMICAL CALCULATIONS OF ELECTRONIC STRUCTURE OF POLYPHOSPHATE COMPLEXES OF MANGANESE, COBALT, COPPER AND ZINC ............................................................................... 82

Pogosyan A.S.
GETTING OF SMART MATERIALS: NANOSTRUCTURED ALUMINUM OXIDE AND CARBON NANOMATERIALS ON ITS BASIS ........................................................................ 85

Tazhibayeva S.M., Korzhymbayeva K.B., Orazymbetova A.B., Musabekov K.B., Zhubanov A.A., Burkitbaev M.M.
IMMOBILIZATION OF MICROORGANISM CELLS ON THE DIATOMITE SURFACE ....... 90

BIOLOGICAL SCIENCES

Agaeva M.A.
THE STUDY OF CELLULOSE AND PECTOLYTIC ACTIVITY OF FUNGI COLLETOTRISHUM GLOEOSPORIOIDES PENZ AND EPICOCUM NIGRUM LINK CAUSING LEAF BLIGHT OF SUBTROPICAL CULTURES .............................................................. 96

RADIATION SAFETY OF FOOD PRODUCTS OF AGRICULTURAL PRODUCTION IN INDUSTRIAL REGIONS OF KAZAKHSTAN ........................................................................ 98

Egerev E.S., Syvatova N.V., Yarultina L.L.
THE BALANCE OF MICROELEMENTS IN ORGANISM OF CHILDREN LIVING NEAR ROAD... 102

Gubina A.V.
THE USE OF GROWTH FACTORS IN THE PROCESS OF LIANAS' CULTIVATION FOR VERTICAL GARDEN ............................................................................................... 108

Kayumova G.G., Shaikhislamova M.V., S ltdikova A.A., S ltdikov F.G.
MECHANISMS OF HORMONAL REGULATION OF CHILDREN AND TEENAGERS MUSCULAR ACTIVITY ....................................................................................................... 112
IMMOBILIZATION OF MICROORGANISM CELLS ON THE DIATOMITE SURFACE

Tazhibayeva S.M., Korzhynbayeva K.B., Orazymbetova A.B., Musabekov K.B., Zhubanova A.A., Burkitbaev M.M.

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Kazakhstan

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.

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.

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>
<thead>
<tr>
<th>Microorganisms cells</th>
<th>Kind</th>
<th>The size, a micron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torulopsis kefir var kumis</td>
<td>Yeast</td>
<td>(2.2-4.7) x (2.6-5.2)</td>
</tr>
<tr>
<td>Rhodotorulla glutinis</td>
<td>Yeast</td>
<td>(2.5-3.0) x (6.0-6.5)</td>
</tr>
<tr>
<td>Pseudomonas mendocina H3</td>
<td>Bacteria</td>
<td>(0.6-0.8) x (1.8-2.0)</td>
</tr>
</tbody>
</table>

Table 1

\(\zeta\) – potential of a cells surface defined by microelectrophoresis method under the formula Geimgolts-Smoluhovsky.

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.

Acknowledgements

2.5 hours defined concentration of the 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 dealing 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 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.

### 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 that this material is rather cheap mineral raw materials. The experiments made earlier on adsorption of Cu$^{2+}$ and Pb$^{2+}$ ions on a diatomite surface have shown that at concentration of CuSO$_4$ and Pb(NO$_3$)$_2$ 1·10$^{-3}$ 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.

### Results of experiences on an immobilization of cells on a diatomite

<table>
<thead>
<tr>
<th>Cu$^{2+}$ cell/ml</th>
<th>Cu$^{2+}$ 10$^{-3}$ cell/ml</th>
<th>Immobilization degree, %</th>
<th>A·10$^{-6}$ cell/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torulopsis kefir var kumis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.10</td>
<td>0.09</td>
<td>91.32</td>
<td>21.0</td>
</tr>
<tr>
<td>4.33</td>
<td>0.43</td>
<td>90.88</td>
<td>38.5</td>
</tr>
<tr>
<td>8.76</td>
<td>0.86</td>
<td>90.18</td>
<td>79.0</td>
</tr>
<tr>
<td>16.95</td>
<td>1.45</td>
<td>86.75</td>
<td>95.0</td>
</tr>
<tr>
<td>13.14</td>
<td>1.74</td>
<td>86.83</td>
<td>114.0</td>
</tr>
<tr>
<td>17.52</td>
<td>2.30</td>
<td>86.87</td>
<td>152.2</td>
</tr>
<tr>
<td>21.90</td>
<td>2.83</td>
<td>87.09</td>
<td>190.7</td>
</tr>
<tr>
<td>Rhodotorulla glutinis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.04</td>
<td>0.23</td>
<td>88.81</td>
<td>18.1</td>
</tr>
<tr>
<td>4.08</td>
<td>0.48</td>
<td>88.31</td>
<td>36.0</td>
</tr>
<tr>
<td>8.16</td>
<td>1.05</td>
<td>87.05</td>
<td>31.1</td>
</tr>
<tr>
<td>16.20</td>
<td>1.35</td>
<td>86.82</td>
<td>88.5</td>
</tr>
<tr>
<td>12.24</td>
<td>1.71</td>
<td>86.10</td>
<td>105.3</td>
</tr>
<tr>
<td>16.32</td>
<td>2.29</td>
<td>86.01</td>
<td>140.3</td>
</tr>
<tr>
<td>20.40</td>
<td>2.91</td>
<td>85.78</td>
<td>174.9</td>
</tr>
<tr>
<td>Pseudomonas mendocina H3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.40</td>
<td>0.89</td>
<td>63.00</td>
<td>15.1</td>
</tr>
<tr>
<td>4.81</td>
<td>1.92</td>
<td>60.21</td>
<td>28.8</td>
</tr>
<tr>
<td>9.60</td>
<td>3.33</td>
<td>54.00</td>
<td>52.7</td>
</tr>
<tr>
<td>12.00</td>
<td>5.75</td>
<td>52.11</td>
<td>62.5</td>
</tr>
<tr>
<td>14.41</td>
<td>7.2</td>
<td>50.02</td>
<td>72.0</td>
</tr>
<tr>
<td>19.22</td>
<td>8.89</td>
<td>49.54</td>
<td>95.1</td>
</tr>
<tr>
<td>24.03</td>
<td>12.45</td>
<td>48.15</td>
<td>115.5</td>
</tr>
</tbody>
</table>

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 Rhodotorulla 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.

It is connected with features of cells: in their sizes and a variety of the functional groups responsible for interaction with a diatomite.

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 possesses 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 $\zeta$ - potential of barmy cells from pH mediums (fig. 2).
The negative charge of a surface of a diatomite is caused by dissociation of SiOH-groups. Therefore amino groups of PEI can electrostatically be drawn both to a diatomite surface and to a cells surface. However results of experiments 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 often pass through maxima. The maximum attachment of *Torulopsis kefir var kumis* and *Rhodotorula 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.

It is necessary to notice that 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.
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. \( \text{Cu}(\text{Cu}) = 64 \text{ mg/l.} \)

<table>
<thead>
<tr>
<th>Time, min.</th>
<th>Torulopsis kefir var kumis</th>
<th>Rhodotorula glutinis</th>
<th>Pseudomonas mendocina H3</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>99.90</td>
<td>99.46</td>
<td>99.85</td>
</tr>
<tr>
<td>60</td>
<td>99.90</td>
<td>99.96</td>
<td>99.92</td>
</tr>
<tr>
<td>90</td>
<td>99.62</td>
<td>99.95</td>
<td>99.87</td>
</tr>
<tr>
<td>120</td>
<td>99.95</td>
<td>99.96</td>
<td>99.85</td>
</tr>
</tbody>
</table>

Experiences on extraction of Cu^{2+} ions from solutions by means of microorganisms cells, immobilizing on a diatomite in the presence of PEI (tab. 4), have shown their considerable sorption ability. In 30 minutes of contact with a biosorbent, a solution degree of extraction of Cu^{2+} ions for all systems exceeds 99%. 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.

References


