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ANALYSIS OF INFLUENCE OF MICROORGANISMS ON HEAVY METALS ADSORPTION BY ALUMINOSILICATE SORBENTS

The article is devoted to the problems of sorption purifying of the water environments polluted by heavy metals ions. The influence of microorganisms on sorption properties of aluminosilicate sorbents is examined. It was studied sorption of Fe^{2+}, Cu^{2+} ions with bacterial cell in the free state immobilized on a surface of aluminosilicate sorbents and also at formation of biofilms. It is shown that the presence of the immobilized microorganisms increases the limiting capacity of heavy metals sorption by aluminosilicate sorbent. The formation of biofilms on the surfaces of sorbent increases heavy metals resistance of microorganisms and their sorption efficiency in comparison with the aluminosilicate sorbent.

Introduction. Environmental pollution by heavy metals is one of actual environmental problems. Ion-exchange pitches, carbon and alumosilicate sorbents are most widely used for cleaning of wastewater from heavy metals. Sorbents with well developed internal surface of micropores reaching 1000 m^2/g, possess the greatest efficiency and sorption capacity for binding of ions of heavy metals (HM) in the range of 100–580 mg/g [1, 2].

One of shortcomings of microporous sorbents is pollution of their surface by organic, inorganic pollutants, and also microorganisms. Fast development on a surface of sorbents of bacteria and other microorganisms leads to change of sorption properties of such sorbents.

Main part. The purpose of the real work – the analysis of binding of bacteria on a surface of aluminosilicate sorbents and their influence on sorption of heavy metals.

As an object of the research we used aluminosilicate sorbent on TU 2163–001–01115840–94 with a specific surface of 30 m^2/g. Sorbent was fractioned with the help of sieves selecting granules with the size of (0.9 ± 0.1) mm.

Pure cultures of bacteria from the collection of the department of biotechnology and bioecology: *Bacillus sp*., *E. coli*, of *Pseudomonas sp.* and also the strains allocated from sewage were used.

Collection cultures of bacteria were sowed in a loop on the slanted nutritious agar (NA) and cultivated during 3 days. Then washouts of cultures in the nutritious broth (NB) cultivated during 2 h for transferring cells to the exponential growth phase. After it cells have been sediment by centrifugation at 8000 rev./min. within 10 min., washed three times in physiological solution from the nutrient medium under the same conditions of centrifugation.

Assessment of concentration of microorganisms in water was carried out by the turbidimetric method [3]. For this purpose calibration curve of light intensity changing at 600 nm (D_{600}) from concentration of cells in suspension were built.

A total number of microorganisms in water media were determined by their cultivation in nutritious agar as

$$N = a \cdot 10^f / V, \quad (1)$$

where a – average number of the grown colonies on the plates; f – dilution degree of suspension of cells; V – suspension volume placed on agar surface. Concentration of microorganisms in water media varied in the range of 10^6–10^8 CFU/ml.

The maintenance of Fe^{2+}, Cu^{2+} ions in water media was determined by a chelatometric method based on optical density testing of heavy metals complexes with EDTA (EthyleneDiamineTetraacetic Acid) at 370 nm and 750 nm, respectively. For this purpose calibration curves of optical density changes from concentration of HM previously were built.

The process of HM sorption was studied in a static mode. Stationary sorption of HM was carried out in flasks V = 50 cm^3 containing 2 g of a sorbent and 20 cm^3 of HM solution of the corresponding concentration. Flasks were stirred up in a shaker during 1 h selecting on 2 cm^3 of the sample within 10 min., 2 cm^3 of a complexon of EDTA were added (1:1) and then optical density of the received complexes HM–EDTA was measured.

A value of HM sorption by a sorbent was determined by expression

$$A = \frac{(C_0 - C_p) \cdot V}{m}, \quad (2)$$

where C_o, C_p – initial and equilibrium HM concentrations; V – solution volume; m – dry mass of sorbent.

Correspondence of HM sorption to the equation I. Langmuir [4] was checked:

$$A = \frac{A_\infty \cdot K \cdot C}{(K \cdot C + 1)}, \quad (3)$$

where A_∞ – the limiting capacity of HM sorption; K – constant of Langmuir; C – equilibrium concentration of HM.

Value A_∞ was found by transforming the expression (3) in the return coordinates $1/A$ from $1/C$.

Sorption of microorganisms at the surface of aluminosilicate sorbent was registered by turbidimetric method as the change of concentration of cells in water after sorbents particles sedimentation.

Dry biomass was determined by its weighting on analytical scales after centrifugation of cells and their drying up to the constant weight at 105°C.

For studying of influence of microorganisms on sorption properties of a sorbent daily culture of cells washed by centrifugation in physiological solution were passed through a column with aluminosilicate granules up to the full saturation of a sorbent. Exit of microorganisms from a column was registered by the changes of optical densities at 280 nm and 600 nm. Columns with the cells of bacteria immobilized on a sorbent were used further for building of biofilms. For this purpose glucose solution (10^{-4} M) was passed through the columns during 3 days.

After adsorption of microorganisms on the aluminosilicate granules and formations of biofilms solutions of HM were passed through the received samples under the same conditions, as well as in the case of an initial aluminosilicate sorbent.

Assessment of viability of microorganisms in presence of HM was carried out by reductase method in accordance with State standard 23454–79 by means of redox-dye methylene blue (MB) [5], and also by the method of diffusion of substances into agar.

Concentration of MB in solution before and after connection with sorbent was measured at 680 nm. Knowing the surface area occupied by a molecule of MB, and the concentration of the connected dye, the size of a specific surface of a sorbent was accounted as it is described in [6].

The obtained data were processed statistically, using Microsoft Excel software.

Comparative study of sorption properties of an aluminosilicate sorbent based on analysis of isotherms of HM sorption was made before and after microorganism's immobilization and after formation of a biofilm.

Time of sorption of HM was chosen equal 2 h as a record of kinetic curves of sorption showed that the equilibrium state in the analyzed case was established during 1–2 h and further didn't change.

At first A_∞ value of HM sorption by an aluminosilicate sorbent was estimated. The results of the analysis of the received isotherms of HM sorption are given in fig. 1.

Apparently from Fig. 1, Fe^{2+} binding is well described by the equation of an isotherm of monomolecular sorption of I. Langmuir. Similar dependence is observed and in the case of Cu^{2+}. The calculated values A_∞ are given in the table.

At the second stage sorption of bacteria on a surface of an aluminosilicate sorbent was studied (Fig. 2).

As seen from the table a specific capacity of binding of cells on a surface of a sorbent varies in the range of 0.45–0.75 mg/g for the studied microorganisms.

Unlike an aluminosilicate sorbent the isotherm of HM sorption on the immobilized microorganisms has more complicated character (Fig. 3).

The area of low concentration of HM in which sorption submits to Langmuir's equation, and the area of high concentration described by dependence of G. Freundlich is allocated [3]. It is probably connected with penetration of HM at high concentration inside the cells and their death.

The calculated values of Fe^{2+}, Cu^{2+} sorption on aluminosilicate sorbent and biosorbents in the free and immobilized states are given in the table at 20°C.

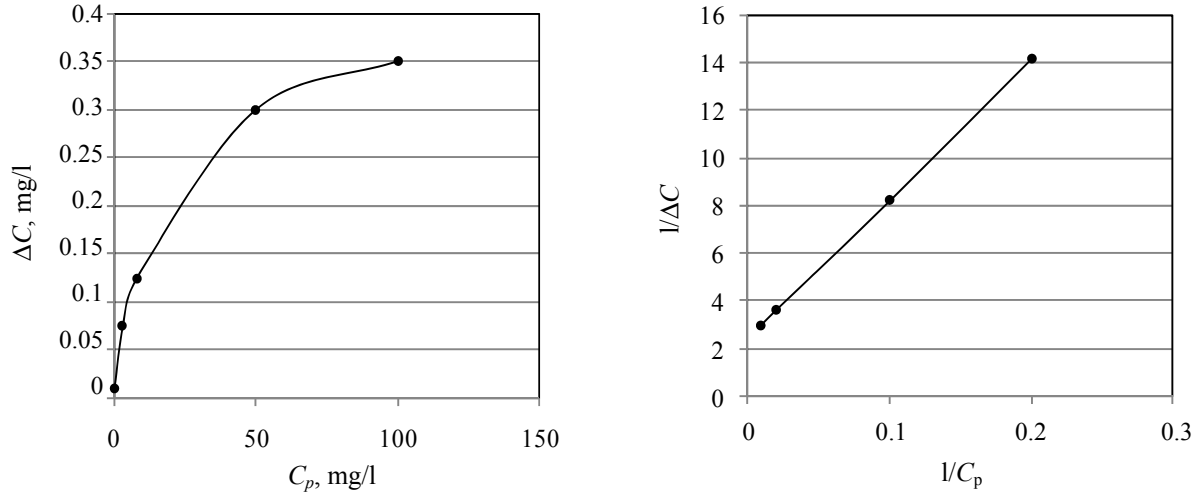

Fig. 1. Isotherm of Fe^{2+} sorption on aluminosilicate sorbent in straight and return coordinates

Table

Sorption capacity of sorbents and biosorbents

Sorbents	Sorbated object	A_∞, mg/g
Aluminosilicate granules	Fe^{2+}	2.2 ± 0.1
Aluminosilicate granules	Cu^{2+}	2.9 ± 0.4
Aluminosilicate granules	*E. coli*	0.45 ± 0.05
Aluminosilicate granules	*Pseudomonas sp.*	0.75 ± 0.10
E. coli	Fe^{2+}	3.5 ± 0.3
Pseudomonas sp	Fe^{2+}	27.6 ± 0.5
E. coli on aluminosilicate sorbent	Cu^{2+}	6.5 ± 0.4
Pseudomonas sp biofilm on aluminosilicate sorbent	Fe^{2+}	120.1 ± 0.8

As it is seen from the table, a sorption capacity of aluminosilicate sorbent in the presence of the immobilized microorganisms of *E. coli* increases by 2–3 times in comparison with the initial sorbent.

In actual practice the majority of microorganisms immobilized on a surface of sorbents, can form biofilms. In that case their stability to HM can be changed.

Fig. 1 shows the reductase activity of *Bacillus sp*. in suspension as well as in biofilm (Fig. 4). According to the received data cells of microorganisms are 3–4 times steadier against copper ions being a part of a biofilm, than in a free state.

It was checked also how the formation of a biofilm influences the sorption properties of an aluminosilicate sorbent (Table).

In the case of sorption of *Pseudomonas sp*. cells, steady against HM and formed a biofilm on a surface of aluminosilicate sorbent, a value of A_∞ for Fe^{2+} increased at 50–60 times in comparison with an aluminosilicate granules.

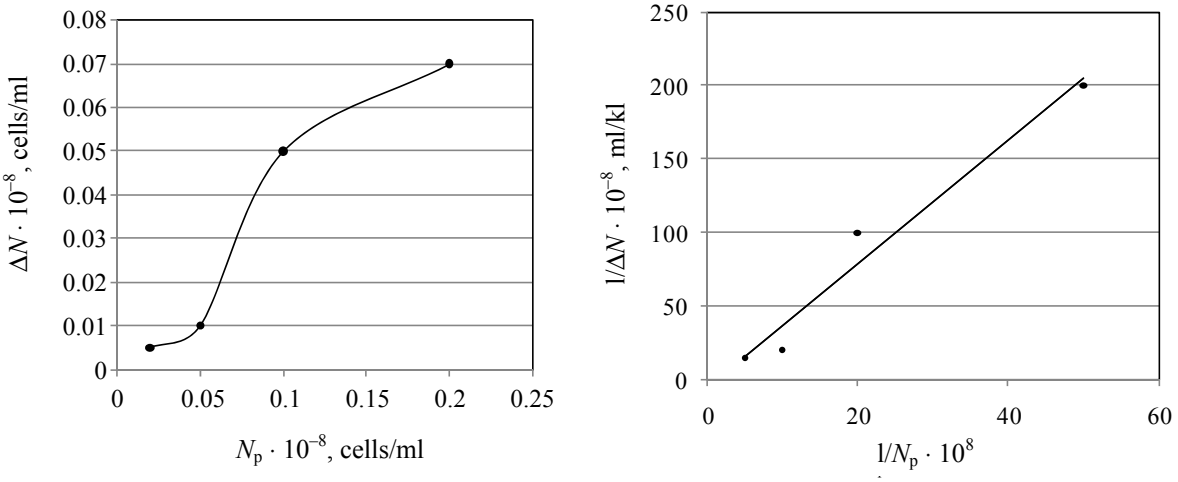

Fig. 2. Isotherm of *E. coli* sorption on aluminosilicate sorbent in straight and return coordinates

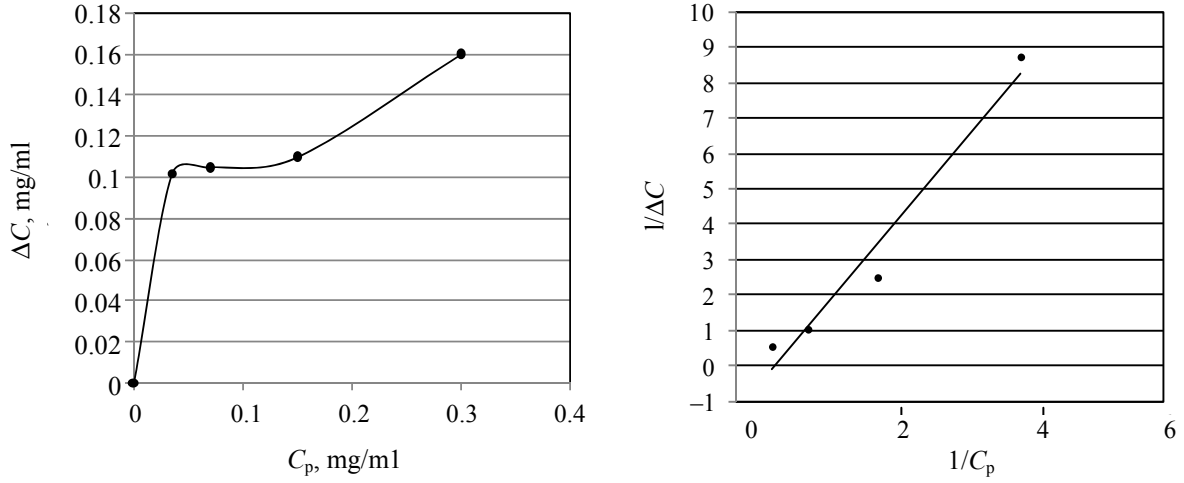

Fig. 3. Isotherm of Cu^{2+} sorption on *E. coli* in straight and return coordinates

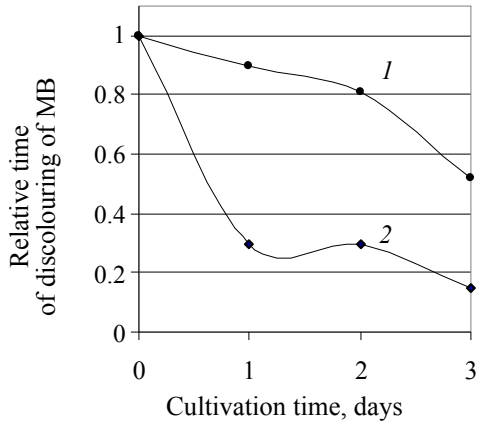

Fig. 4. Reductase activity of *Bacillus sp.* in the presence of Cu^{2+} (10^{-3} M): *1* – cells in NB; *2* – cells in a biofilm

Conclusion. The obtained data show that microorganisms are well attached to a surface of an aluminosilicate sorbent and form biofilms.

Steady to HM cultures of cells poses a higher sorption capacity of HM than usual cells.

Bacteria cells show the increased resistance to HM in the immobilized state and keep the viability at higher concentration of HM in comparison with free microorganisms.

Formation of a biofilm by the steady cultures of cells increases their sorption capacity to HM on 1–2 orders in comparison with the initial aluminosilicate sorbent and also increases stability of cells to HM in comparison with cells in a free state.

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