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INTERACTION BETWEEN HEAVY METALS AND MICROORGANISMS DURING WASTEWATER TREATMENT BY ACTIVATED SLUDGE

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ABSTRACT

In order to study the main regularities of the interaction of metals with microorganisms, a range of concentrations of heavy metals is determined. The kinetics of extraction of heavy metals by the example of Cu (II), Co (II), Cr (II) from the aquatic environment by microorganisms of the genera Pseudomonas and Micrococcus was studied. It is shown that adsorption is the main mechanism of metal absorption by microorganisms. The dependence of the number of adsorbed metal ions is described by the Freundlich equation for both Pseudomonas and Micrococcus, the coefficients of the equation are determined. It is established that the optimum temperature of adsorption of metals by microorganisms is 293 K. The regularities of natural processes in systems of microorganisms - heavy metals - substituting elementsthat allow finding methods of their control by human are studied. It has been established that the amount of microorganisms in the intracellular space or on the surface of the cell wall of microorganisms of the same or another metal influences the amount of extracted metal from the aquatic environment. Microorganisms containing calcium in their composition accumulate heavy metals 1.3-1.5 times less.

Keywords: microorganisms, pseudomonas, micrococcus genera, active sludge, heavy metals, resistance of microorganisms, biosorption.

INTRODUCTION

In the biological treatment of industrial wastewater containing heavy metal impurities, the latter are extracted from the aqueous medium by the active sludge components.

Active sludge of biological treatment facilities includes three components: biological (microorganisms, protozoa, algae, fungi); organic (nucleic and amino acids, proteins, polysaccharides, humic acids, etc.); Inorganic (hydroxides, phosphates, carbonates, silicates, etc.).

The composition of the microflora of activated sludges is formed depending on the composition of the wastewater being treated, the concentration of dissolved oxygen, temperature, pH, BODratio: N: P, flow hydrodynamics.

The main species composition of the microorganisms of the active sludge is represented by such morphological groups of bacteria as Pseudomonas, Bacterium, Sarcina, Micrococcus. Normally working sludge contains a small amount of filamentous bacteria (Sphacrotilusnatans, Cladothrixdichotoma).

In systems with living cells, the question of the resistance of the microbial organism to the effect of increased (in comparison with physiological) concentrations of heavy metals becomes fundamental.

Resistance of microorganisms to heavy metals is due to four possible mechanisms:

- biosorption - due to binding metals by the functional groups of the cell surface;
- bioaccumulation - due to interaction with the components of the cytoplasm and active groups of enzymes;
- formation of a less toxic product due to oxidation-reduction reactions in the cytoplasm;
- a decrease in the accumulation of metals in the cytoplasm through the efflux system.

The process of metal adsorption on the surface of microorganisms involves its binding by cell wall and / or cytoplasmic membrane, as well as substances of the capsule and extracellular secretions. It occurs due to the interaction of metal ions with negatively charged groups on the surface of microorganisms and their secretions and does not depend on temperature, the presence of an energy substrate or metabolism inhibitors.

Having penetrated into the cell (2nd mechanism) cations that have a large relative atomic mass, such as Hg (II), Cd (II), Ag (I), can interact with SH-groups of enzymes and inhibit their activity. Cations of other heavy metals can enter into interactions as "counterparts" of physiological ions, for example, Cd (II) instead of Zn (II) or Ca (II), Ni (II) and Co (II) instead of Fe (II), Zn (II) instead of Mn (II), thereby inhibiting the function of the corresponding physiological ion.

Due to oxidation-reduction potentials (ORP), Hg (II) (+430 mV), chromate (+929 mV), arsenate (+ 139

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mV) and Cu (II) (-268 mV) can be reduced by the third mechanism. By this mechanism Co (II) (-701 mV), Ni (II) (-678 mV), Zn (II) (-1180 mV) can not be restored

There are limitations for the energy metabolism of microorganisms according to the OBR mechanisms, which are determined by the oxidation-reduction potential range for most aerobic cells (-421 mV ÷ +808 mV) at 30 ° C and pH = 7. Certainly, the theoretical values of standard ORPs do not correspond to the potentials that are necessary for the restoration of metals by microorganisms in nutrient media, since both the activity and the concentration of metals under real conditions are much lower than the standard ones.

Studies of the main regularities of the interaction of heavy metals contained in cleaned effluents, especially industrial ones with microorganisms, are extremely insufficient, despite numerous publications devoted to the study of biological wastewater treatment [1-3]. This led to the formulation of a study which involves finding means (methods) for regulating the process of biological treatment of industrial wastewater and the reverse process - extraction of heavy metals from excess active sludge with subsequent utilization in agriculture or other industries.

MATERIALS AND METHODS

The microorganisms of the Pseudomonas and Micrococcusgenera, which predominate in the biocenosis of active sludge atbiological treatment plants (BTP), were chosen as the object of the study.

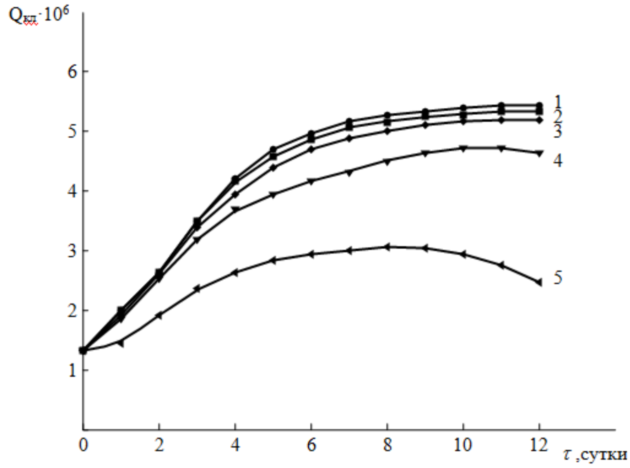
Bacteria Pseudomonas and Micrococcus were cultured on a dense nutrient medium and then separated from the nutrient agar by flushing the culture with a small amount of saline.

After treatment of microorganisms with solutions of heavy metals it is necessary to separate them from the solution. To separate the microorganisms, centrifugation was performed at a speed of 8000 rpm for 10 minutes and then the centrifuge was passed through a microbial filter.

Determination of heavy metal ions in solutions was carried out using a Perkin-Elmer 460 atomic absorption spectrophotometer.

RESULTS AND DISCUSSIONS

All paragraphs must be indented. All paragraphs must be the example of Cu (II) (Figure-1) shows the effect of the concentration of heavy metals in the aquatic environment on the growth dynamics of microorganisms. Performed researches have allowed defining a range of concentrations of heavy metals for studying of the basic patterns of interaction of metals with microorganisms.

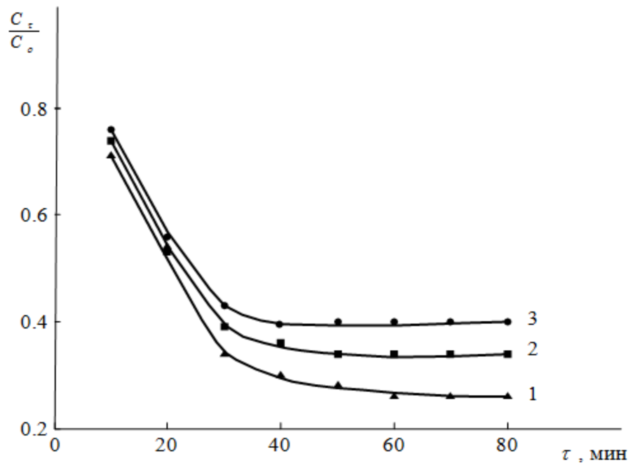


Cu (II) concentration: 1 - without Cu (II); 2 - 10 mg/dm3; 3 - 50 mg/dm3; 4 - 100 mg/dm3; 5 - 500 mg/dm3

Figure-1. Growth rates of Pseudomonas bacteria.

The conducted studies showed that the toxic effect is negligible at metal concentrations up to 100 mg / dm3 for resistant microorganisms. As a consequence, it was this range that was chosen for further research.

Saturation of microorganisms with metals is achieved in 40 minutes (Figure-2), and the amount of absorbed metal depends on the concentration in the initial solution. The higher the initial concentration of the metal, the more its quantity is absorbed by the microorganisms in the same time interval (Figure-3), whereby the specific absorbed masses of metals for Micrococcus are approximately 20% higher than for Pseudomonas.



C_0 = 100 mg/dm3; T = 295 K; metal ion: 1 - Co (II); 2- Cu (II); 3 - Cr (III)

Figure-2. Kinetics of heavy metal ion extraction from water by Pseudomonas.

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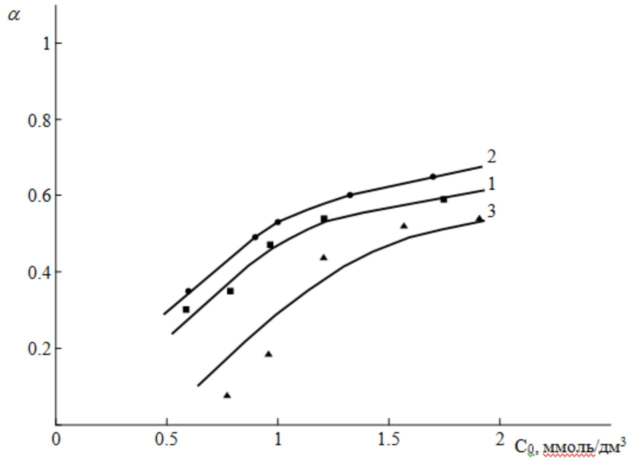


α - rate of ion extraction; metal ion: 1 - Co (II); 2 - Cu (II); 3 - Cr (III)

Figure-3. Rate extraction of metal.

The example of microorganisms of the genus Pseudomonas shows the absorption of metals by microorganisms from aqueous media in the studied concentration range. Absorption occurs to a certain residual concentration, which practically does not increase with an increase in their initial concentration. Similar patterns were obtained when working with microorganisms of the genus Micrococcus.

The calculation of the average rate of metal absorption by microorganisms shows that it is the highest in the first minutes of the process. The longer the phase contact is, the lower the absorption rate. The rate of metal uptake increases with an increase of the initial concentration to 60 mg/dm3, after which an increase in the metal concentration in the aqueous phase does not lead to a significant increase in the rate of extraction by microorganisms.

The nature of the adsorption isotherms (Table-1, Figure-4) makes it possible to describe the dependence of the number of adsorbed metal ions by the Freundlich equation for both Pseudomonas and Micrococcus:

$$a = K \cdot C_p^{1/n},$$

where Cp is the equilibrium concentration of the metal ion; n and K are constants) [4]. According to [5], K is a constant, in vivo conditions, determining the degree of affinity of the metal for microorganisms.

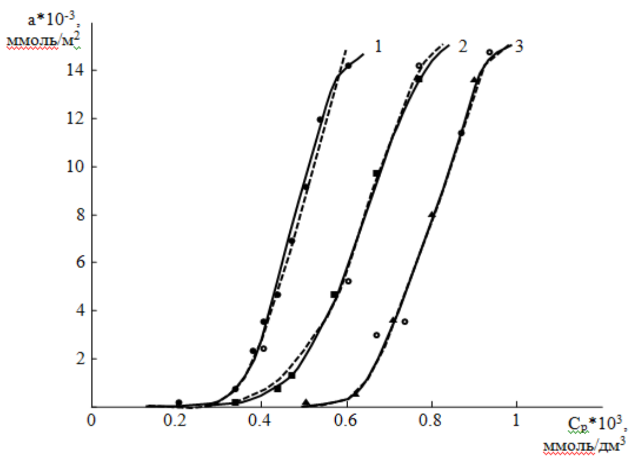
Table-1. Adsorption metal ions by micrococcus bacteria at T=293 K.

Co (II)		Cu (II)		Cr (III)	
$C_p \cdot 10^3$, mol/dm^3	$a \cdot 10^{-3}$, mmol/m^2	$C_p \cdot 10^3$, mol/dm^3	$a \cdot 10^{-3}$, mmol/m^2	$C_p \cdot 10^3$, mol/dm^3	$a \cdot 10^{-3}$, mmol/m^2
0,400	2,41	0,403	1,96	0,677	0,80
0,450	3,46	0,456	2,86	0,750	1,83
0,428	5,12	0,463	4,17	0,715	3,80
0,502	7,41	0,554	6,11	0,831	6,13
0,623	11,47	0,598	8,46	0,908	9,67

Table-2. The Freundlich equations constants*.

Constant	Cu (II)	Co (II)	Cr (III)
K*	$\frac{1,5 \cdot 10^5}{5,62 \cdot 10^4}$	$\frac{1,4 \cdot 10^5}{1,45 \cdot 10^5}$	$\frac{3,7 \cdot 10^4}{5,0 \cdot 10^4}$
n*	$\frac{0,188}{0,246}$	$\frac{0,227}{0,231}$	$\frac{0,147}{0,141}$
* in numerator– data for Pseudomonas, in denominator - for Micrococcus			

Table-2 shows the values of the coefficients n and K at T = 293 K; pH = 6.8.



a (mmol/m2) - adsorption; Cp - equilibrium concentration (mmol/dm3); pH = 6,0; T = 293 K; metal ion: 1 - Co (II); 2 - Cu (II); 3 - Cr (III)

Figure-4. Adsorption isotherm of metal ions by Pseudomonas bacteria.

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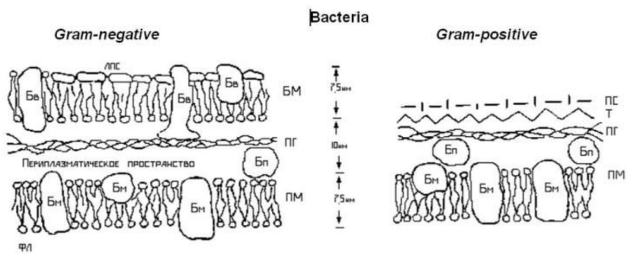
Theoretical isotherms of sorption have been constructed according to certain coefficients, which agree well with the experimental ones for both Pseudomonas and Micrococcus at the initial metal concentrations to 100 mg / dm3 (the dashed lines in Figure-4). It was not possible to describe the dependence at high metal concentrations for Pseudomonas by the above-mentioned equation.

Slightly different sorption properties of Micrococcus cells can be a manifestation of physiological adaptation to increased metal concentrations, which is due to the specific structure of the cell membrane, in particular, the presence of glycoproteins, capable of absorbing significant amounts of metals [6].

Pseudomonas bacterial cells possess a plasmid resistance mechanism against heavy metals (including Co (II), Ni (II), Cu (II), Cd (II), etc.) [7-10], at the basis of which lies the chemosmotic system of "ejection" of metal ions in exchange for protons. The functioning of this system leads to the creation of a zone with a high pH around the cells and increased concentration of heavy metal ions, which can contribute to the formation of hydroxocomplexes in the extracellular space. Comparison of these data suggests that Pseudomonas and Micrococcus have different mechanisms of resistance to heavy metals.

Data that characterize the sorption properties of the strains studied suggested that the two-dimensional structure (Figure-5) of the Pseudomonas cell wall limits to some extent the sorption capacity of the strain. According to [5], the metal-precipitating capacity of the cell wall of gram-negative bacteria is inferior to that of gram-positive bacteria.



PhL - phospholipids; LPS - lipopolysaccharides; OM - the outer membrane; PG - peptidoglycan layer; PM - plasma membrane; PS - polysaccharide; T - teichoic acid; Pou - proteins of the outer membrane; Pps - proteins of the periplasmic space; Ppm - proteins of the plasma membrane

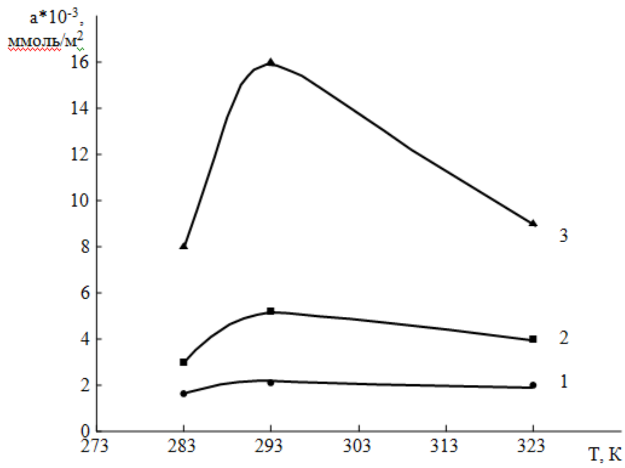
Figure-5. Structure of bacterial cell wall.

On the example of Cu (II) and microorganisms of Pseudomonas, the effect of temperature in the range of 283 - 323 K on the absorption process was studied. From the experimental data, the constants of the Freundlich equation are determined (Table-3).

Table-3. The Freundlich equations constants meanings for Cu (II) absorption.

T, K	283	293	323
K	$3{,}7 \cdot 10^4$	$1{,}5 \cdot 10^5$	$7{,}3 \cdot 10^4$
n	0,262	0,188	0,184

The effect of temperature on the adsorption of metals by microorganisms is of extreme nature (Figure-6), which is associated with a change in the living conditions of mesophylls Pseudomonas and Micrococcus, especially at high concentrations of metals.



a (mmol/m^2) - adsorption; Cp - equilibrium concentration (mmol/dm^3):
1 - 0,3; 2 – 0,5; 3 - 0,7; T (K) - temperature

Figure-6. Adsorption of metals by Pseudomonas bacteria versus temperature characteristics.

Since any changes in the environment are a reflection of the change in the direction of the chemical and chemical-biological processes that take place in it, the knowledge of the laws of natural processes in systems of microorganisms - heavy metals - substituting elements can allow us to find methods of controlling them by human.

Based on the analysis of the mechanisms of metal extraction from wastewater by microorganisms, we made the assumption that it is possible to reverse the process by creating certain conditions for the concentration of nontoxic metals in the system.

According to experimental data, the amount of extracted metal from sewage water by microorganisms depends on the concentration of metal in the aqueous phase.

The comparative data (Table-4) show that the amount of microorganisms in the intracellular space or on the surface of the cell wall of microorganisms of the same or another metal affects the amount of extracted metal from the aquatic environment.

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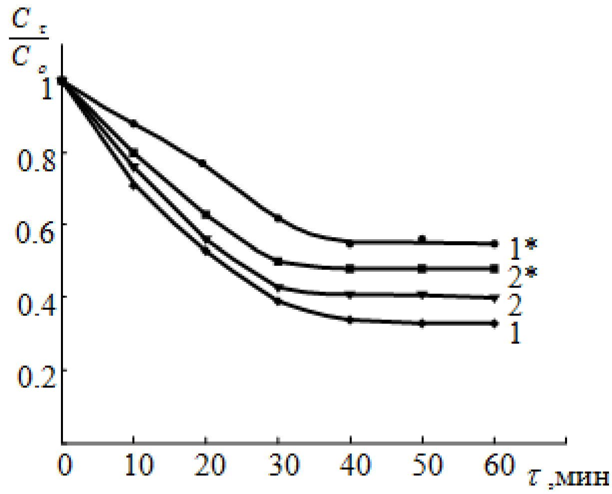
Table-4. Metal mass fraction absorbed by bacteria, cultivated on different media (concentration of metal in medium 25 mg/dm^3, T = 295 K, pH=6, 8).

Metal	Metal mass fraction absorbed by bacteria					
	Pseudomonas			Micrococcus		
	Nutriculture medium	Nutriculture medium with calcium	Nutriculture medium with specific metal	Nutriculture medium	Nutriculture medium with calcium	Nutriculture medium with specific metal
Cu	1,0	0,68	0,36	1,0	0,68	0,38
Co	1,0	0,76	0,63	1,0	0,80	0,63
Cr	1,0	0,87	0,65	1,0	0,89	0,70

The decrease in the absorption of heavy metals grown on media containing heavy metal can be explained by changes in the properties of the membrane, or by regulating the intake of metals at the level of membrane transport processes [11]. The mechanism of resistance in bacteria is based on a change in the cell permeability, either in changing the orientation of membrane proteins or in the composition and structure of phospholipids, which leads to the protection of SH-groups with which Cu (II) and Co (II) probably bind. Thus, resistance to Cu (II), Co (II) in microorganisms occurs at the molecular and membrane transport levels.

Reduction of the ability to absorb heavy metals in resistant cells can be achieved due to the loss a less specific transport system of a cell. It is established [12] that, analogous to phosphates, the entry of Mg (II) into the bacterial walls occurs through two systems, one of which is less specific and constitutive, the other is specific and synthesized inducibly. Loss of the cell of the first system leads to the fact that it acquires resistance to other bivalent cations Co (II), Ni (II), Mn (II), which enter through the transport system Mg (II) [13].

When using microorganisms grown on Ca-containing media, the effect of calcium is manifested. In this case, the kinetics of extraction of heavy metals from aqueous media by microorganisms is similar to the kinetics of extraction of heavy metals by cultures of microorganisms. Microorganisms containing calcium in their composition accumulate heavy metals 1, 3-1, 5 times less, the degree of saturation with heavy metals is also smaller (Figure-7).



Initial concentration of metal (C_0) – 100 mg/dm^3; T = 295 K; pH = 6,8; 1, 2 - nutriculture medium; 1*,2* - Nutriculture medium with calcium (C_{Ca} = 25 mg/dm^3); metal: 1 - copper (Cu), 2 - chromium (Cr)

Figure-7. Kinetics of heavy metal ion extraction from water by Pseudomonas.

Microorganisms grown on Ca-containing media, extract divalent cations (Cu (II), Co (II)) in lesser degree and the presence of calcium in the microorganism slightly affects the extraction of trivalent chromium. For a particular metal, the change in the character of saturation does not depend on the type of microorganisms. Depending on the charge, the metal ion interacts with certain areas on the surface of the microorganism or inside it.

If we compare the amount of the secondarily absorbed metal, the presence of calcium in the initial microorganisms in a certain concentration range exerts a less significant influence than the presence of the absorbed metal at the same concentration of the calcium cation in the nutrient medium for the growth of microorganisms. The relative amount of cations of divalent metals re-absorbed by microorganisms grown on a modified medium is less than that of chromium. It is likely that copper and cobalt occupy areas analogous to calcium on the surface, that is, they compete, while chromium occupies other parts or forms more stable compounds. Calcium influences the extraction of heavy metals from aquatic environments by microorganisms, which indicates the principal possibility of seeking the replacement of toxic heavy metal ions by calcium or magnesium.

CONCLUSIONS

The example of microorganisms of the Pseudomonas genera and Micrococcus of the active sludge prevailing in the biocenosis shows that a complex complex of microorganisms of activated sludge accumulates metals from sewage by adsorption or by mechanisms that depend on the processes of the metabolism of microorganisms, for example, by transport of ions into cells. These processes, called "biosorption" lead to the accumulation (accumulation) of metals in microbial biomass and a decrease in their concentration in the environment surrounding microorganisms. Based on the analysis of the mechanisms of metal extraction from wastewater by microorganisms, we made the assumption that it is possible to reverse the process by creating certain conditions for the concentration of nontoxic metals in the system. The studies of the regularities of extraction of heavy metals from aqueous media by microorganisms of activated sludge (Pseudomonas and Micrococcus) grown on media containing calcium ions have shown the possibility of changing the direction of the process by creating appropriate conditions.

a) It has been established that the toxic effect for the resistant microorganisms of the genera Pseudomonas and Micrococcus is insignificant at metal concentrations up to 100 mg / dm3.

b) It has been established that adsorption is the main mechanism of metal absorption by microorganisms.

c) The dependence of the number of adsorbed metal ions can be described by the Freundlich equation for both Pseudomonas and Micrococcus, and the coefficients of the equation have been determined.

d) The effect of temperature has been studied using the example of Cu (II) and microorganisms of the genus Pseudomonas. It has been established that the optimum temperature for the adsorption of metals by microorganisms is 293 K.

e) It has been assumed that the reverse process can be performed when certain conditions are established for the concentration of non-toxic metals in the system.

f) It has been established that the amount of microorganisms in the intracellular space or on the surface of the cell wall of microorganisms of the same or another metal influences the amount of extracted metal from the aquatic environment. Microorganisms containing calcium in their composition accumulate heavy metals 1, 3-1, 5 times less.

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