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STUDY OF THE ADHESIVE ABILITY OF A. FERROOXIDANS BACTERIUM CELLS ON THE SURFACE OF ORE MINERALS OF THE SHOKPAR DEPOSIT

ШОҚПАР КЕН ОРНЫНЫҢ КЕН ДЕНЕЛЕРІНІҢ БЕТКЕЙІНЕ A. FERROOXIDANS БАКТЕРИЯ КЛЕТКАЛАРЫНЫҢ АДГЕЗИЯЛАНУ ҚАБІЛЕТІН ЗЕРТТЕУ

ИЗУЧЕНИЕ АДГЕЗИОННОЙ СПОСОБНОСТИ КЛЕТОК БАКТЕРИИ A. FERROOXIDANS НА ПОВЕРХНОСТИ РУДНЫХ ТЕЛ МЕСТОРОЖДЕНИЯ ШОКПАР

Annotation. *The behavior and the possibility of attachment of A. ferrooxidans strain cells to the sulfide-gold-bearing ore of the Shokpar deposit and the influence of ore particles on bacterial strains were investigated. The previous stages were carried out before the adsorption process and further for the bioleaching process, such as culturing of microorganisms Acid.ferrooxidans, granulometric analysis of ore composition from the Shokpar deposit. According to the amount of data from the study, the process of immobilization directly depends on the time that an experiment was set. Also, according to the quantitative indicators of cells from 3 strains of A. ferrooxidans, taken as an adsorbate, it was determined that 1 strain of Acid.ferrooxidans completely went into an inactive state during the 12-hour exposure time, and according to the data, this strain of bacteria turned out to be more suitable for ore particles with increases in biomass.*

Keywords: *bacterial-chemical leaching, Acidithiobacillus strain, Shokpar deposit, cell adsorption, immobilization.*

Андатпа. *Зерттеу жұмысында Шоқпар кен орнының алтынды-сульфидті кенімен биошаймалау жұмыстарын жүргізу негізінде алғаш рет зерттеу барысында қолданылатын темір тотықтырғыш бактерия дақылдарының аталған кен орнының кен бөліктеріне адсорбциялану қасиеті және бактерия штаммдарына кен бөлшектерінің тигізетін әсерін анықтау мақсатында зертханалық жағдайда зерттеу жұмысы жүргізілді. Шоқпар кен орнының сульфидті-алтынды кен бөлшектеріне A. ferrooxidans штамм жасушаларынының бекіну мүмкіндігі және процестің өту барысын зерттеуде адсорбция процесіне дейінгі алдыңғы кезеңдер және одан әрі биошаймалау технологиясы үшін Acid. ferrooxidans микроорганизмдерін қайталай егу әдісімен белсендіру; зерттеу объектісі, адсорбент ретінде «Шоқпар» кен орнынан алынған кен құрамына гранулометриялық талдау сынды зерттеу жұмыстары жүргізілді. Зерттеу жұмысының барысында иммобилизация процесі тәжірбие қойылған уақыт кезеңіне тікелей байланысты екендігі анықталды. Адсорбат ретінде алынған A. ferrooxidans 3 штаммының жасушаларының сандық*

көрсеткіштері бойынша Acid.ferrooxidans 1 штаммы экспозицияның 12 сағаттық уақытында толығымен белсенді емес күйге өтті және бактериялардың бұл штаммы биомассаның жоғарылауы көрсеткіштерімен кен бөлшектеріне анағұрлым сәйкес келетіндігі анықталды.

Түйін сөздер: *бактериялық-химиялық сілтісіздендіру, Acidithiobacillus штаммы, Шоқпар кен орны, жасушалардың адсорбциясы, иммобилизация.*

Аннотация. *Исследовано поведение и возможность прикрепления клеток штаммов A. ferrooxidans к сульфидно-золотоносной руде месторождения Шокпар и влияния рудных частиц на штаммы бактерий. Проведены предшествующие этапы до процесса адсорбции и далее для процесса биовыщелачивания, такие, как культирование микроорганизмов Acid.ferrooxidans, гранулометрический анализ состав руды с месторождения «Шокпар». В ходе исследования было установлено, что процесс иммобилизации напрямую зависит от периода времени, на который был поставлен эксперимент. По количественным показателям клеток из 3 штаммов A. ferrooxidans, взятое в качестве адсорбата, определено, что 1 штамм Acid.ferrooxidans за 12-часовое время экспозиции полностью перешел в инактивное состояние и по результатам данных, данный штамм бактерий оказалась более подходящим к рудным частицам с увеличением биомассы.*

Ключевые слова: *бактериально-химическое выщелачивание, штамм Acidithiobacillus, месторождение Шокпар, адсорбция клеток, иммобилизация.*

Introduction. One of the directions of scientific and technological progress in the field of processing and extraction of ore minerals is the use of integrated technologies that can significantly increase the totality of the use of this raw material and ensure environmental safety, as well as economic low costs. An alternative solution to such problems is bacterial leaching technology. In recent years, in the field of biogeotechnology of metals, chemolithotroph microorganisms have been widely used in leaching. Among iron-oxidizing bacteria, bacteria from the genus *A. ferrooxidans* occupy an exceptional position [1].

The rate of oxidation of iron and its oxide during bioleaching of metals from ore minerals is the main indicator of the effectiveness of this technology. The oxidative activity of iron-oxidizing bacteria is significantly affected by the amount of biomass or the concentration of cells, the oxidation conditions - the concentration of dissolved O2, CO2, the pH value, temperature, as well as the presence of inhibitory ions [2]. Therefore, the study of the physiological and vital characteristics of microorganisms and the ability to effectively use the results obtained, in turn, makes it possible to control the processes of leaching of metals. One of the features of microorganisms in direct leaching of metals is the adhesion capacity [3, 4]. Based on this, the use of immobilized microorganisms in bioleaching compared to free biomass in solution significantly increases the rate of bio-oxidation, in turn having several advantages such as: resistance to negative environmental factors, high biomass concentration and resistance of microorganisms, as well as ease of use [5,6].

The purpose of the work is to study the adhesive ability of *Acid. Ferrooxidans* strains on the surface of ore bodies of the Shokpar deposit.

Objects and methods of research. The object of the study is gold-bearing ore from the Shokpar deposit, which is geographically located on the territory of the Kordai district of the Zhambyl region (Fig. 1). According to the results of exploration work at this deposit, there are gold reserves of 12812 kg at a distance of 280 m to 450 m, including balance C1 + C2 and off-balance forms.

To accumulate biomass in solution, 3 strains of acidophilic thione bacteria from the genus *Acidithiobacillus ferrooxidans* were used as the initial seed inoculate. Considering the physiological properties, the initial culture of bacteria was grown in the environment of 9K Silverman and Lundgren of the following composition (g / l): $(NH_4)_2SO_4$-2.0; K_2NRA_4 - 1.0; $MgSO_4 \cdot 7H_2O$ –0,5; NaCl – 0,2; $FeSO_4 \cdot 7H_2O$ – 44.2; The acidity of the medium was brought to a pH of 2.5

with sulfuric acid [7.8]. On the development of the growth of *the Acid. ferrooxidans* were judged by the appearance of brown coloration of the medium caused by the formation of trivalent iron in a bacterial solution. The active cells of the strains were immobilized using the physical method of static adsorption on the surface of the substrate (ore bodies). According to the static method, the carrier (adsorbent) is placed in a suspension of the biological preparation and the mixture is left for inactivation for a certain period of time [9]. As a result, cells are deposited and subsequently adsorbed on the particles of the carrier.

Figure 1. Geographical location of the Shokpar field

To study the adsorption of microorganisms with particles of adsorbents, microscopy was performed. For this purpose, total preparations were prepared as follows: drops of bacterial suspension equal in volume were applied to a mesh with a film – a substrate and a 1% solution of phosphoric-tungstic acid (pH 2.0) was added for contrast. Exposure time – 30 seconds. Microscopy of objects was carried out on an electron microscope JEM-100 V.

For the quantitative accounting of cells of strains of *A. ferrooxidans* contained in 1 g, quantitative analysis was carried out by the method of marginal tenfold dilutions. In the study of the ore substrate, the ore mineral was crushed in a control sieve and ground in a mortar and the original suspension was prepared in a dilution of 1:10 [10,11].

The results of the study and their discussion.

One of the preceding stages of bioleaching and adsorption technology is the preparation of ore for upcoming work. Since the duration and stability of the adsorption process directly depend on the grinding of ores, the granulometric composition of the ore of the Shokpar deposit was determined by the method of sieve analysis on the control sieve U*1-ESL-K* in accordance with international standards ISO and DIN [12,13] (Fig. 2.).

According to the graph, it can be judged that the orebody, taken as an adsorbent by granulometric composition, mainly consists of small classes from 0.16 mm to 1 mm. For the adsorption process and further research, an ore from 0.5 mm honeycomb cells was selected. The initial ore was crushed with a mortar to 1.0-8.0 microns [14].

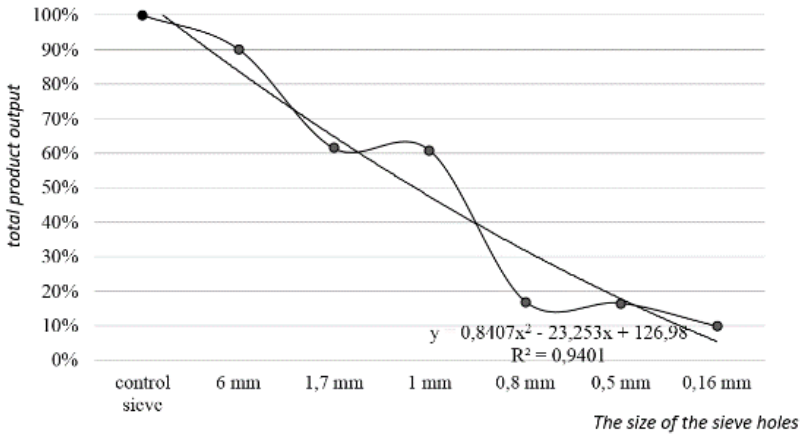

Figure 2. Characteristics of the size of the ore studied

Activated bacterial cells were separated from the culture fluid by centrifugation at 8000 rpm. And to separate the cells from iron ions, 2-3 times they were washed with distilled water acidified to a pH of 1.5-2.0 hydrochloric acid. For adsorption of cells on the surface of solids, the resulting suspension of cells of 20 ml was introduced into the crushed ore up to 1.08.0 mm in a mass of 15 g. The experiments were carried out in Erlenmeyer flasks with a volume of 250 cm^3. According to the static adsorption method, the carrier cell system was placed at rest for a certain period and the adhesion process was carried out (Fig. 3). Considering the properties of microorganisms, the experiments were carried out at an optimal pH level of 1.5-1.8. The temperature regime was maintained at 282 ° C±.

Figure 3. Adsorption of *A. Ferrooxidans* cells on the surface of ore minerals by static adsorption

For all strains of bacteria, the primary exposure time for inactivation of microorganisms was 60 minutes, 120 minutes, and 180 minutes. After the exposure time, the counting of the number of microorganisms in comparison with the original number was carried out by the method of tenfold dilutions (Fig. 4).

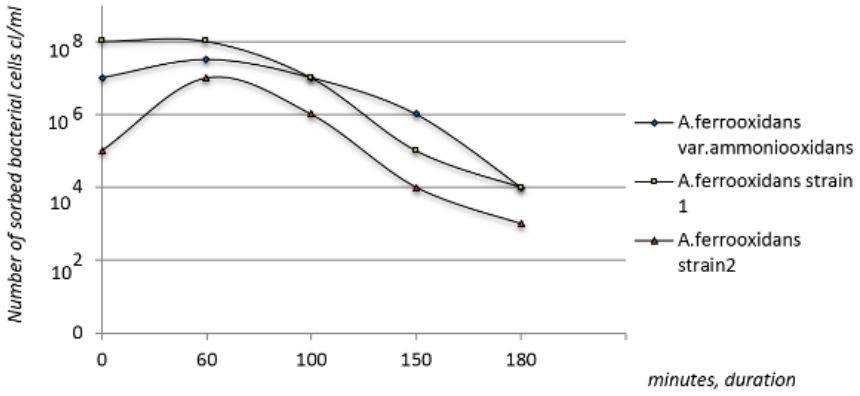

Figure 4. Sorption activity of *A. ferrooxidans* strains on the surface of ore-mineral particles (in different time ratios)

According to the graph, an increase in the number of cells *A. ferrooxidans var.Ammoniooxidans* was determined from the initial 10^7 cl/ml to 10^8 cl/ml and 1 strain of *A. ferrooxidans* from 10^5 cl/ml to 10^7 cl/ml in suspension after 60 minute exposure time. Quantitative indicators show that the ore body, as a solid substrate, positively affects the viability of bacteria and contributes to an increase in biomass. However, no changes were observed in 2 strains of *A. ferrooxidans*. As a result of 10-fold dilution after a 120-minute exposure time, the strain of *A. ferrooxidans var. Ammoniooxidans*, the content in the solution, which is 106 cl/ml, indicates the slow passage of the process of sorption by mineral particles of bacterial cells. In strain *A. ferrooxidans* 1, at the beginning of adsorption, the number of cells was 10^8 cl/ml as a result of this period of time, the number of bacteria in the suspension decreased to 10^7 cl/ml. After 180 minutes of exposure time, the active sorption of cells of *A. ferrooxidans* bacteria was shown by *A. ferrooxidans* strain 1 with a decrease in the cell content in the suspension to 10^4 cl/ml. Therefore, a microscopic study of this strain of bacteria was carried out (Fig. 5). Quantitative indicators of bacterial cells in suspension for the remaining two strains of *A. ferrooxidans* were 10^3 cl / ml. As a result of microscopy, we found that the cells of the bacterium attach to larger (5-7 mm) particles of ore bodies.

Thus, from the above interpretation of the electron microscopic image and the quantitative indicators of the 180-minute exposure time of inactivated cultures, it can be condemned that the process of complete adsorption of microorganisms still requires a significant period of time. Based on this, the exposure time for the adsorption of microorganisms was extended to 12 hours. The results of comparing the number of microorganisms before the study and after 12 hours of static adsorption are included in the diagram (Fig. 6).

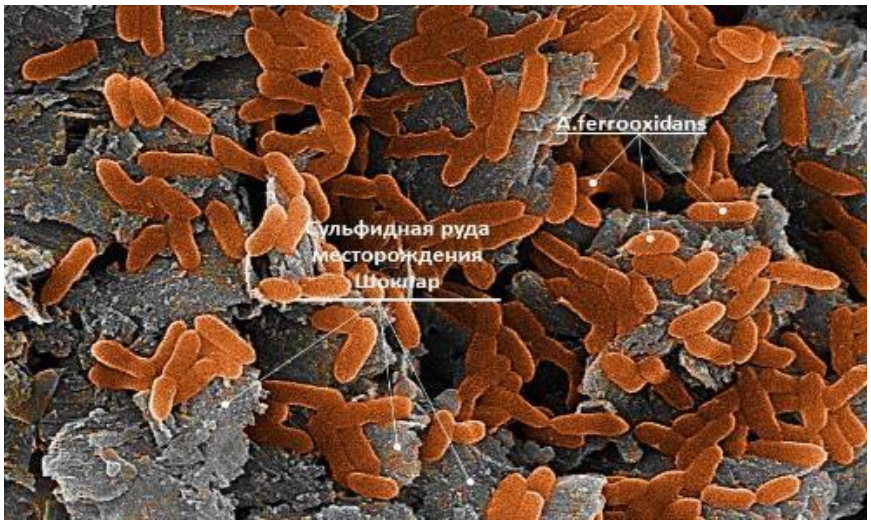

Figure 5. Adsorption of *A. ferrooxidans* bacteria cells on the surface of ore particles of the Shokpar deposit

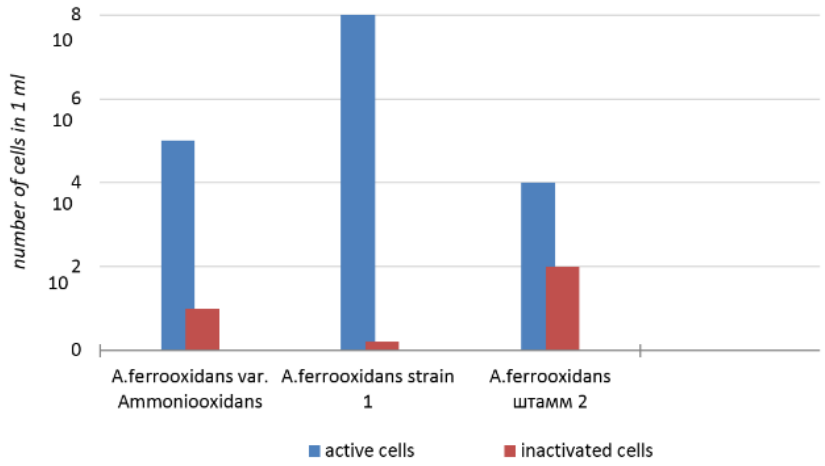

Figure 6. Quantitative indicators of cells *of A. ferrooxidans* strains in the active and adsorbed state

The experimental data obtained in the work indicate that in active cultures the highest rate was shown by *A. ferrooxidans* strain 1 (10^8 cl/ml). Next up is *A.ferrooxidans var. Ammoniooxidans*, where the number of cells is 10^5 cl/ml. The smallest number of cells has *A. ferrooxidans* strain 2 (10^4 cl / ml).

Whereas in variants with inactivated cultures the number of cells is in the range of $10^1 – 10^2$ cl/ ml, the highest rate also falls on the share of *A. ferrooxidans* strain 2 (10^2 cl /ml), and the smallest - in the culture of *A. ferrooxidans* strain 1 (0 cl / ml).

Conclusions. In the course of the work, it was shown that the process of immobilization of microorganisms on the surface of ore bodies by static adsorption directly depends on the duration of the time period for inactivation of bacterial cells. The most active sorption of cells was observed in the strain *A. ferrooxidans 1*. And, by a significant increase in the biomass in the solution, it can be observed that this strain of bacteria is the most suitable adsorbate for the gold-

bearing ore bodies of the Shokpar deposit. The results obtained will be used in the bioleaching of gold-bearing ores in comparison with the use of free biomass of bacterial cells.

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