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FACTORS AFFECTING BACTERIAL AND CHEMICAL PROCESSES OF SULPHIDE ORES PROCESSING

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Extraction of valuable components from sulphide ores using microorganisms is a recognized biotechnological method, combining several advantages over traditional methods of mineral processing. This paper presents the main factors affecting the bacterial-chemical leaching and methods of leaching with the participation of microorganisms. Some physical-chemical (temperature, pH, oxygen, carbon dioxide, nutrients, metals and other chemical elements) and microbial (cell count and microflora activity) properties are given, either directly or indirectly (suppressing or contributing to the growth and oxidative capacity of microorganisms) affecting the kinetics of the process. The paper discusses the characteristics of the mineral substrate, including galvanic interaction of sulfide minerals and the formation of passivating layers on the surface of the ore during oxidation, emphasizing the importance of the electrochemical interaction of the components of the leaching system. Bioleaching is a complex process, which is a combination of mainly chemical reactions mediated by the microbial component, therefore, to improve the kinetics, it is necessary to consider, monitor and regulate the listed range of factors.

Key words: bioleaching; sulphide ores; acidophilic chemolithotrophic microorganisms; physical-chemical properties; bioleaching methods

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Introduction. Bioleaching of ores, which is currently being actively researched, has received industrial application in some commercial processes, mainly abroad (South Africa, Mexico, USA, Brazil, Australia, Finland, China, Chile, etc.) [13, 18, 21, 28]. Extraction of 18 valuable components using microorganisms (*Acidithiobacillus* spp., *Leptospirillum* spp., *Sulfobacillus* spp. and others) is a recognized biotechnological method, combining a few advantages over traditional methods of mineral processing. With the existing shortcomings of bioleaching, manifested in some cases in the duration of the process and the difficulty of maintaining the activity of microbial cultures, the undeniable advantages of the method include simplicity of organization, self-sustainability, efficiency and environmental friendliness. The course and intensity of bioleaching is determined by its conditions.

The purpose of this work is to describe and analyze the main factors influencing the bacterial-chemical processes of sulphide ores processing.

According to [9], factors can be represented as follows:

– method and conditions of bioleaching (underground, heap, dump and tank methods, pulp mixing density and speed, type of reactor for tank bioleaching, geometry of ore layering for heap and dump methods);

– physical-chemical (temperature, pH, oxidation-reduction potential, availability and concentration of oxygen, carbon dioxide and nutrients, mass transfer, light, pressure, surface tension, the presence of inhibitors);

– microbiological (biodiversity, density and activity of population cells, resistance to metals and the adaptive ability of microorganisms);

– properties of the mineral component (type, mineral and chemical composition of the ore, particle size and surface area, porosity, hydrophobicity, galvanic interaction and the formation of secondary minerals).

Bioleaching methods. Over the past 65 years, various bioleaching technologies have been investigated, including those tested in laboratories and pilot projects, but not widely used in industry (for example, bioleaching in bioreactors such as «the flood-drain bioreactor», «the airlift bioreactor» and «the rotating-drum bioreactor») [28]. In general, the strategy of extracting valuable com-

ponents from ores has evolved from relatively inexpensive dumping and heap leaching, based on the principle of irrigation, to fully controlled processes in reactor installations that satisfy the principle of agitation.

Dump leaching is the oldest method of mineral processing. The nature of the structures and the size of the dump vary greatly – up to several hundred thousand tons of ore. The top of the dumps is constantly irrigated or for some time is flooded with a leaching solution, which is usually acidified water or an acidic solution of iron (III) sulfate. The latter is obtained by the oxidation of bivalent iron and regenerated with the help of bacteria [8, 26]. In some cases, to increase the degree of metal recovery, forced aeration and thermal insulation of the dumps are provided [1].

Heap leaching is mainly used in the processing of fine ore, which is not subjected to flotation. Crushed ore (up to 12 thousand tons) is put in layers in heaps on specially designated areas. The procedure is similar to dump leaching [1, 8].

Dump and heap leaching are traditionally used for low-grade and lean ores, tailings and wastes of metallurgical production, i.e. ore material not suitable for use in expensive reactor installations (extraction of copper and gold) [16]. With these methods, it is important to consider the microorganism growth and metabolism inhibition as a result of the lag phase in their development. Lag phase reduction can be achieved by continuously recirculating the solution with a new batch of microorganisms of the same species composition. This allows the formation of an actively oxidizing microbial population [17].

Underground leaching (in situ) is usually carried out in abandoned mines. The leaching solution with microorganisms is pumped into the ore body and passed through it. After the metals go into solution, the latter is collected and pumped out to the installation, where they are directly removed [8]. The procedure requires significant permeability of the ore body, but it prevents any leakage of the solution. Underground leaching is used mainly for extraction of uranium and copper.

Tank leaching is a process carried out in a cascade of reactors (tanks). This method has been actively used since the 1980s for the processing of rich ores and concentrates. It is more expensive in organization and operation but has several undeniable advantages. It provides careful control of important leaching parameters (temperature, pH, mixing rate, saturation level with nutrients and gases, etc.), as well as a homogeneous state of the pulp. The leaching lasts only a few hours or days, since the process is closed, harmful emissions and gases must be excluded. Efficiency is determined by the residence time of the pulp in the reactors [17]. In some cases, bioleaching acts in a constantly flowing (continuous) mode in non-sterile conditions, thereby contributing to the continuous selection of those species and strains of bacteria and archaea that can grow more efficiently. It is extremely important that microorganisms are always maintained in the phase of exponential growth, ensuring the intensification of the extraction of valuable components.

Physical-chemical properties. Temperature. Bioleaching is carried out in a different temperature range. This factor is selected based on the chosen technology and in accordance with the temperature optimum of development of the microorganisms to be used. Thus, in mesophilic conditions (30-42 °C), bacteria of the genus *Acidithiobacillus* are used more often, and at moderate temperatures (45-50 °C) – the genus *Sulfobacillus* and *Leptospirillum*. For a long time, the prospect of using extremophilic archaea (*Sulfolobus* spp., *Metallosphaera* spp.) was considered, the optimal growth temperature of which is 60 °C and higher [22]. However, due to the sensitivity of the membranes to aggressive technological conditions, their use may be limited.

Studies of the oxidation kinetics show that, as a rule, with a temperature increase of 10 °C, the rate of chemical reactions increases by 2 times. For some minerals, the decomposition rate of the sulfide matrix is much faster at 40 or 50 °C, for others (for example, chalcopyrite) the temperature must be increased up to 70 °C. Removal by increasing the temperature of sulfur or other oxidation products from the mineral surface can improve the kinetics of extraction of target metals from ore [24, 27].

The constancy of temperature is easy to achieve when conducting processes in a vapor process in reactors, in which this parameter is subjected to control. In the case of exothermic oxidation of sulphides, industrial reactors are cooled to prevent temperature fluctuations. For heap and dump leaching, the temperature gradient is more pronounced, as a result we can observe difficulty of controlling changes in the parameter. This is important at the initial stage of the process, when the content of sulphides is high. The reasons for such changes are the features of mineralogy and the reactivity of sulphides, the intensity of aeration and the composition of the leaching solution, as well as the characteristic self-heating of rocks. All these features determine the composition and distribution of microorganisms within heaps or dumps [23, 28].

Hydrogen value (pH). Since bioleaching microorganisms are acidophiles, maintaining a correct and constant pH value of the environment is an essential condition for their growth. Due to the possible changes in this parameter (upwards) during the oxidative dissolution of sulphide minerals, the effect of pH on the process of transition of valuable metals into solution can be very large. The pH range for the alkalization is very wide – from 1.5 to 8.0. Values in the range of 2.0-2.5 are optimal for the bacterial oxidation of bivalent iron and sulphide minerals. At pH < 2.0, growth inhibition of *Acidithiobacillus ferrooxidans* (a typical representative of bioleaching microflora) is observed, but the microorganism can adapt to even very low pH. At the same time, its optimum for growth and oxidation may vary among the same strains and depend on the conditions of the process carried out [1, 8].

Acidophilic microorganisms have a unique physiology. Even though they develop in acidic environmental conditions, their intracellular pH is neutral. The pH gradient of the internal and environmental microbial cells differs by 4-5 orders of magnitude. The maintenance of normal vital activity is partially provided by the positive membrane potential of the cell, which helps to limit the penetration of hydrogen protons inside. However, microorganisms are sensitive to lipophilic anions, such as thiocyanates and nitrates, which penetrate the cytoplasmic membrane and accumulate in response to the membrane potential inside the cell. Such accumulation of ions has a negative effect due to acidification of the internal environment, as well as due to the ability of anions to react with cellular components, such as enzymes. These phenomena require special attention in the organization of the technological process of bioleaching [23].

In the case of regulation of the synthesis of certain cellular components, *A. ferrooxidans* affects the change in the pH of the external environment [1, 4].

Oxygen and carbon dioxide. Maintenance of oxygen and carbon dioxide at the proper level is a prerequisite for good growth and high activity of microorganisms. Molecular oxygen is the final acceptor in metabolic oxidation reactions, and carbon dioxide is a source of carbon for constructive exchange [12].

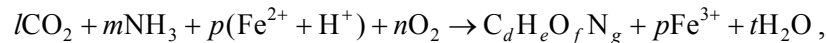
The microorganisms often used in bioleaching are obligate aerobes, so low oxygen concentrations have a negative effect on their metabolic activity and, consequently, the rate of oxidative processes. The solubility of O₂ in water at 35 °C is 8 g/m³ and decreases with increasing ionic concentration in solution [1, 13]. Low gas dissolution rates are associated with their overall ability to dissolve poorly in liquids. According to stoichiometry, the oxidation reaction of iron – one of the most important in bacterial and chemical processes – requires 0.07 g of oxygen per 1 g of oxidizable ferrous iron. Considering the solubility of O₂, this amount cannot be available from the solution. Therefore, its deficiency must be replenished. To maintain the metabolic activity of bacteria, the O₂ concentration must be at least 0.2 g·l⁻¹. In laboratory and industrial conditions, O₂ deficiency is eliminated by forced aeration, shaking and stirring [8]. It is extremely important to consider the fact that a high O₂ feed rate can lead to process limiting.

The rate of consumption of O₂ cells can be calculated by the formula

$$N_{AO} = \mu X / Y_O, \quad (1)$$

where N_{AO} – oxygen consumption, $g \cdot l^{-1} \cdot h^{-1}$; μ – cell specific growth rate, h^{-1} ; X – cell concentration, g (dry cell biomass) $\cdot l^{-1}$; Y_O – oxygen consumed by cells, g (of consumed O_2) $\cdot cells$.

To evaluate Y_O or any other product obtained, we present the microbial process as follows:



where l, m, p, n and t – stoichiometric coefficients; $C_dH_eO_fN_g$ – biomass.

To estimate the coefficients, the balance of C, H, O and N can be described. Using the expression $Y_{X/Fe} = M_C/55.8$, which allows to calculate the output of cells and ferrous iron, other equations can be solved, including $Y_{X/O} = M_C/32$. M_C is the relative molecular weight of the cells.

To constrain the limitation of the process, oxygen can be supplied, at least in the volumes in which it is consumed. Then the oxygen transfer rate

$$W_O = k_{LaO}(C_O^* - C_{LO}), \quad (2)$$

where W_O – oxygen transfer rate, $g \cdot l^{-1} \cdot h^{-1}$; k_{LaO} – volumetric oxygen transfer rate, h^{-1} ; C_O^* – oxygen saturation limit concentration, $g \cdot l^{-1}$; C_{LO} – dissolved oxygen concentration, $g \cdot l^{-1}$.

In large scale heap and dump leaching processes with natural aeration, k_{LaO} values can be $10 h^{-1}$ or less. The decrease in oxygen may be due to its consumption by microorganisms themselves. In the case of tank bioleaching (in reactors of various configurations), the oxygen transfer coefficient is $k_{LaO} = 40-70 h^{-1}$ and more. For example, under industrial conditions with a pulp density of 15-20 % solid, the concentration of dissolved oxygen is 15-25 % [12].

Bioleaching microflora is most often represented by autotrophic bacteria and archaea, i.e. microorganisms that metabolize carbon from air carbon dioxide to build their own cellular structures [25]. Its content in the air is low (0.03 % (v/v)). Even though the leaching microorganisms grow slowly, the need for carbon dioxide exceeds the actual rate of CO_2 transfer. This is mainly due to low equilibrium CO_2 concentrations:

$$N_{AC} = \mu X / Y_C, \quad (3)$$

$$W_O = k_{LaC}(C_C^* - C_{LC}), \quad (4)$$

where N_{AC} – carbon dioxide consumption, $g \cdot l^{-1} \cdot h^{-1}$; Y_C – carbon dioxide produced by cells, g (cells) $\cdot g$ (consumed CO_2) $^{-1}$ within the range of 0.50-0.55 $g \cdot g^{-1}$; W_C – carbon dioxide transfer rate, $g \cdot l^{-1} \cdot h^{-1}$; k_{LaC} – volumetric carbon dioxide transfer rate, h^{-1} ; C_C^* – equilibrium concentration of carbon dioxide, $g \cdot l^{-1}$, less than 1 $mg \cdot l^{-1}$, and C_{LC} – dissolved carbon dioxide concentration, $g \cdot l^{-1}$.

As in the case of oxygen, low or high concentrations of CO_2 can have an inhibitory effect on the microbial extraction of metals. The optimum concentration of CO_2 in the liquid phase of the pulp is the range of values of 0.003-0.007 $g \cdot l^{-1}$. It is known that carbon dioxide concentration of 4 % (v/v), introduced into the fermenter for cultivation of *A. ferrooxidans*, allows to reach the maximum growth rate of bacteria, and leads to the greatest oxidation of iron, copper and arsenic compounds [9].

Ensuring effective supply of oxygen and carbon dioxide is an important technological task. In tank bioleaching, gas exchange in the reactors is set and controlled automatically. It significantly depends on the design and volume of the tank used, the flow rate and the method of distribution of the gas component in it, the speed of mixing, and the type of mixer. Dump and heap leaching also require the air flow. The main driving force of air masses is the difference in their density, which depends on the temperature gradient inside the dump or heap. Air is supplied through pipe networks using low-pressure fans or blowers. Air masses are injected through the air distribution networks, which are up

to 50 mm in diameter and can be located at a distance of up to 2 m from each other. Throughout this pipe network, holes are drilled from the bottom. The frequency of their location depends on the amount of oxidized ore and the speed of the process. Similarly, aeration can significantly accelerate biooxidation reactions, shortening the leaching cycle [23].

Nutrient concentration. Microorganisms participating in bioleaching are chemolithotrophs, the sources of energy for which are compounds of iron and sulfur. All the necessary nutrients they can get from the environment. This is what happens with trace elements. However, for optimal growth and use of the main energy sources, they need the presence in the solution of ammonium, magnesium and phosphate salts, the deficiency of which can be observed in the natural environment [8, 14, 15]. In this regard, an excess of nitrogen, magnesium and phosphorus compounds is added to nutrient media, providing microflora with biogenic elements [2].

After carbon, nitrogen is the next important chemical element for the synthesis of cellular structures. The ammonium level of 0.2 mM is considered sufficient to meet the needs of *A. ferrooxidans* in nitrogen. Higher concentrations of inorganic and organic nitrogen can suppress iron oxidation. The required concentration of the element is determined by the amount of cell biomass participating in oxidative processes. In general, it is difficult to estimate the exact concentration of nitrogen, since bacteria can take it from the air [25].

Phosphorus and magnesium are equally important components in the constructive exchange of microorganisms. The first plays a role in the metabolism and growth of cells, the second is part of the enzymatic systems.

The production of phosphorus by microorganisms from the environment can be difficult. Its deficiency can lead to a negative effect on representatives of the bioleaching microflora, which is reflected in a change in the expression of genes encoding some proteins and, as a result, in restricting the growth of microorganisms [26].

The nutrient supply is best under reactor conditions, unlike heap and dump leaching, where the distribution of nutrients may be uneven [14].

Concentration of metals and other chemical elements. The chemical composition of the bioleaching solutions is very complex. Tolerance of microorganisms to high concentrations of metals and several other chemical elements is crucial in the processing of polymetallic raw materials [5]. It is known that chemolithotrophs can show resistance, and they can be adapted to high concentrations of components that are much higher than those observed in natural habitats.

The level of resistance may vary depending on the type and strain of the microorganism. It is known that *A. ferrooxidans* can show resistance to the following concentrations of metals in a nutrient medium, g/l: 30 Co^{2+} , 55 Cu^{2+} , 72 Ni^{2+} , 120 Zn^{2+} , 12 U_3O_8 and 160 Fe^{2+} . *A. ferrooxidans* and *Leptospirillum ferrooxidans* show approximately similar tolerance to Cu^{2+} , Zn^{2+} , Al^{3+} , Ni^{2+} and Mn^{2+} , but differ in resistance to Co^{2+} . *L. ferrooxidans* has sensitivity to metal already at a concentration of < 2 g/l. In comparison with these bacteria *A. thiooxidans* is the most sensitive because it is inhibited by the listed cations at a concentration of 5 g/l [25].

Of particular interest is iron, since in the trivalent form it serves as a powerful oxidizing agent for sulphides. In this regard, its concentration plays an important role in bioleaching. Increasing the concentration of ferrous iron to 5.6 kg/m³ increases the rate of oxygen consumption by acidophilic microorganisms and reduces the oxidation rate of Fe^{2+} . In turn, at high values of Fe^{3+} , inhibition of the oxidation of Fe^{2+} by *A. ferrooxidans* is possible [15, 19]. In the absence of other energy sources, ferric iron, as well as high concentrations of ferrous iron, also adversely affect the growth of bacteria. There is evidence that the concentration of Fe^{2+} above 0.108 M, added to sulfur compounds in the composition of salts of the nutrient medium, can significantly suppress the growth of *A. ferrooxidans*, using sulfur as an energy source [27].

When forming the chemical composition of leaching solutions, the following facts should be taken into account. Depending on the type of ore and the conditions of the processes in the reactors, the concentrations of chemical elements can amount to tens of grams per liter: 65 Zn, 60 Fe, 35 Cu, 25 Ni, 20 As, 5 Co, Mg < 1. In the leach solution, the heaps and the dump reach they are, g/l: 25 Fe, 25 Al, 23 Zn, 10 Mg, 6 Cu, 5 Ni, 8 As, Co < 1. In the latter case, the content of chemical elements within the heap or dump varies noticeably with depth. The reasons for their uneven distribution are the pH gradient, the presence of waste rock and several other factors that determine the different contact efficiency between mineral particles and the leach solution [28].

Microbiological signs. *The concentration of cells and the activity of the microbial component.*

The number of microorganisms is one of the main factors determining the success of the process and the stability of kinetic parameters. The concentration of microbial cells in the reactors with mechanical agitation, in comparison with dump and heap leaching, is high and can be of the order of 10³-10⁹ cells/ml. The increase in biomass can be carried out by accumulating cells from solution by centrifuging, using membrane filters or by immobilization in biofilm reactors [10]. In the latter case, the following carrier types are commonly used as immobilizer: glass beads, activated carbon, sand, polystyrene, polyurethane, ion exchange resin, nickel alloy threads, polyvinyl chloride, claydite, etc. Thereby, iron oxidation rate depends on reactor design and air efficiency use can reach 5-6 g/(l·h). Biofilms can be used in different types of bioreactors: rotating biological contactors, fermenters with a packed carrier layer, and fluidized-bed reactors [4].

The activity of microorganisms is determined by the ability to oxidize iron, reduced sulfur compounds and metal sulfides [3]. It is known that it is different for various strains. Highly active strains can be isolated directly from mines. Moreover, their activity can be enhanced by UV irradiation or by manipulation of the genome [1]. Another way is to adapt microorganisms to individual technological indicators of the process or their combination. Adaptation helps microorganisms to survive and grow successfully in unusual conditions and shortens the lag phase in the development of isolates [7].

Mineral substrate. The mineral composition of the substrate, its degree of grinding, and the presence of impurities strongly influence the kinetics and dominance of certain species in the microorganism populations [20, 27]. With a high content of carbonates, the level of acidity of the medium is significantly reduced and inhibits bacterial activity. Increasing the pH in this case is compensated by the addition of acid to the solution, thereby maintaining optimal conditions for the vital activity of microorganisms. However, this leads to additional costs.

The leaching rate also depends on the total surface area of the mineral substrate available for dissolution. A decrease in particle size leads to an increase in the contact area of the leach solution with mineral grains and acceleration of the extraction of the target components. The optimal particle size is 42-44 μm [1]. An increase in the total mineral surface can also be ensured by an increase in the density of the pulp, which leads, on the one hand, to a better yield of metals in the solution, on the other, to an excess of inhibiting components and a toxic effect on microorganisms [8].

During the bioleaching process, precipitated iron compounds and elemental sulfur are formed on the mineral substrate, creating a diffusion layer that adversely affects the kinetics of the process. However, if the layer is porous or does not create an additional diffusion barrier, then the reaction rate does not depend on its thickness [11].

Galvanic interaction of sulfide minerals. The galvanic interaction of minerals determines the rate and sequence of their dissolution. Each sulphide mineral in the mixture, with its own resting potential, will act as a cathode or as an anode. Accordingly, between them there is an electric current. So, in the presence of two sulphides in the system, a galvanic vapor is formed, and, the cathode always acts as a sulfide with a higher electrode potential, and as the anode with a lower potential. With the passage of electric current between the sulfides at the anode, oxygen is released, which accelerates the oxidation of

the anode sulfide. Hydrogen released at the cathode will, conversely, protect cathode sulfide from oxidation. Thus, galvanic interaction not only increases the dissolution reaction, but also preferably leaches a certain mineral. The galvanic effect depends on several factors: the resting potential of sulfide minerals, the nature and duration of contact, the presence of oxygen, the nature of the electrolyte, pH, conductivity and the presence of other compounds, and the grain size of the reacting sulfide minerals [11].

Conclusions

1. Bioleaching is a complex process, which is a combination of chemical reactions mediated by the biological component, that are influenced by a number of factors such as leaching method, temperature, pH, gas content and gas exchange rate, concentration of nutrients, metals and other chemical elements, amount and activity of the microbial component, features of the mineral substrate and galvanic interaction of sulphides.

2. To organize the process, it is important to consider the mineral features of the ore, which determine the choice of the method of bacterial and chemical processing. Hence, for lean and low-grade ores, heap and dump bioleaching are used, for rich ores and concentrates – the tank one. At the same time, the mineral composition of the substrate, its degree of grinding, and the presence of impurities strongly influence the kinetics and dominance of certain species in the populations of microorganisms.

3. High temperature ($> 40\text{ }^{\circ}\text{C}$) is one of the key factors that determine not only an increase in the dissolution rate of metals, but also the species composition of microbial cultures.

4. The peculiarity of the physiology of bacteria and archaea used in bioleaching and the need to maintain the target components in a dissolved state, in order to avoid the formation of secondary precipitates, require a constant pH correction to no more than 2.0-2.5.

5. Since molecular oxygen is the ultimate acceptor for microorganisms in metabolic oxidation reactions, and carbon dioxide is a carbon source for constructive exchange, ensuring the gas composition is at the proper level ($0.2\text{ g}\cdot\text{l}^{-1}\text{ O}_2$ and $0.003\text{-}0.007\text{ g}\cdot\text{l}^{-1}\text{ CO}_2$) serves as a prerequisite for high microbial activity and leaching kinetics.

6. The chemical composition of bioleaching solutions is very complex and, despite the adaptation mechanisms of microorganisms, can critically affect their activity. Often, solutions are characterized by high concentrations of metals (up to 200 g/l) and low concentrations of nutrients (nitrogen, phosphorus and magnesium), the deficiency of which must be compensated by the addition of ammonium, magnesium salts and phosphorus compounds.

7. Galvanic interaction of metal sulfides can have an independent effect on the kinetics of the process. The features of the electrochemical interaction of sulphide composing ore can both increase and inhibit the rate of their dissolution, determining the sequence of bioleaching of each.

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