BIOTECHNOLOGIES APPLICATION AT ARSENIC-BEARING MATERIALS PROCESSING

V.A. Luganov[1], T.A. Chepushtanova[1], G.D. Guseynova[1], B. Mishra[2]

[1] Satbayev University, Kazakhstan, Almaty, 050013, str. Satpayev 22; T.chepushtanova@satbayev.university, tanya2305@list.ru
[2]Worcester polytechnic institute. USA, 100 Institute Road, Worcester, MA 01609; bmishra@wpi.edu

ABSTRACT. The arsenic problem is a global problem. Results obtained in this research allow us to formulate directions for development of environmentally friendly technologies for processing arsenic-bearing ore and technogenic materials. Behavior of autotrophic thionic bacterias was studied (*Thiobacillus thrioparis, Thiobacillus thiooxidans, Thiobacillus ferrooxidans*), as well as arsenite-oxidizing and arsenate-reducing bacteria. Their influence on soil, different calcium arsenate cakes and cinders were studied. Model tests in laboratory conditions for determination of possibilities for development of microbiological processes with formation of versatile arsenic forms at the presence of arsenate cakes, sulfide sublimates and soils at various temperatures were conducted. It was established that the development of arsenate-reducing bacterias is associated with the presence of higher arsenic oxides in sulfide sublimates, which play the role of electron acceptors in inactive chains of bacteria and reduce As_2O_5 until trivalent form. Experiments were carried out with pure cultures of bacteria potentially capable of transforming arsenic in arsenic-containing products. In variants using arsenate-reducing bacteria, arsenic content decreased from 11.6-17.0 mg / l to 3.0-11.6 mg / l, which indicates that calcium arsenate-cake does not affect the viability of arsenate-reducing bacteria, which can precipitate soluble arsenic into insoluble arsenic compounds.

Keywords: micro-bioleaching, arsenic cake, cinders, sulfide sublimates, thionic bacteria, solubility

ПРИМЕНЕНИЕ БИОТЕХНОЛОГИЙ ПРИ ПЕРЕРАБОТКЕ МЫШЬЯКСОДЕРЖАЩИХ МАТЕРИАЛОВ

В.А. Луганов[1], Т.А. Чепуштанова[1], G.D. Guseynova[1], B. Mishra[2]

[1] Satbayev University, Казахстан, Алматы, 050013, Ул. Сатпаева 22; T.chepushtanova@satbayev.university, tanya2305@list.ru
[2] Worcester polytechnic institute. США, 100 Institute Road, Вочестер, MA 01609; bmishra@wpi.edu

ABSTRACT. Проблема мышьяка является общемировой проблемой. Полученные результаты позволяют сформировать направления развития экологически безопасных технологий переработки мышьяксодержащих рудных и техногенных материалов. В работе изучалось поведение автотрофных тионовых (*Thiobacillus thrioparis, Thiobacillus thiooxidans Thiobacillus ferrooxidans)*, а также мышьякокисляющих и мышьяквосстанавливающих бактерий. Проведены модельные испытания для определения возможности развития микробиологических процессов с образованием подвижных форм мышьяка в лабораторных условиях в присутствии арсенатных кеков, сульфидных возгонов и почв при различных температурах. Установлено, что развитие арсенатвосстанавливающих бактерий связано с наличием в сульфидных возгонах высших оксидов мышьяка, которые играют роль акцептора электронов в нательной цепи бактерий и восстанавливают As_2O_5 до 3-х валентной формы. Выполнены эксперименты с чистыми культурами бактерий, потенциально способными к трансформации мышьяка в мышьяксодержащих продуктах. В вариантах с использованием арсенатвосстанавливающих бактерий содержание мышьяка снижалось от 11.6-17.0 мг/л до 3.0-11.6 мг/л, что свидетельствует о том, что арсенат-кальциевый кек не влияет на жизнеспособность арсенатвосстанавливающих бактерий, которые способны осаждать растворимый мышьяк в нерастворимые соединения мышьяка. Установлено, что результаты микробиологических исследований должны учитываться при выборе способа и условий захоронения техногенных мышьяковых продуктов.

Ключевые слова: микробиологическое выщелачивание, арсенатный кек, огарки, сульфидные возгоны, тионовые бактерии, растворимость

Introduction

The current practice of arsenic-containing wastes (cakes) recycling in special facilities does not prevent the possibility of soil, air, etc. getting into them, which can cause or inhibit the transformation of some compounds into others.

Long-term storage of arsenate cakes in landfills can lead to their transformation under the influence of external conditions and microorganisms capable of oxidizing and / or reducing arsenic. This will cause a change in the solubility of arsenic compounds and will affect the possibility of arsenic transfer to the environment.

A similar transformation of arsenic can also occur in sulfide sublimates obtained during sulfidizing roasting of arsenic raw materials. For example, arsenic sulfide As_2S_3 (orpiment) is very slightly soluble in water, while As_2S_5 is practically insoluble in water, (Xian-Chun, 2018). At the same time, during long-term storage in air under the influence of moisture and microorganisms, even such poorly soluble arsenic compounds as arsenic sulfides can transform into soluble forms, Nguyen (2015). The effectiveness of the use of biotechnology is also described in the works about arsenic tolerance and bioleaching from realgar based on response surface methodology, published by Lei Yan (2017) and about column bioleaching of arsenic and heavy metals from gold mine tailings by *Aspergillus fumigates,* published by Bahi Jalili (2012).

The purpose of this work was to conduct model tests to determine the possibility of the development of microbiological processes with the formation of versatile forms of arsenic in laboratory conditions in the presence of arsenate cakes, sulfide sublimates, and soils at different temperatures, as well as to conduct laboratory model experiments with pure cultures of bacteria, potentially capable of transferring hardly soluble arsenic compounds into a versatile form.

The studying of autotrophic thionic bacteria, as well as arsenic-oxidizing and arsenic-reducing bacteria, which changing the valence of arsenic can form arsenic compounds with different solubility are objects of interest.

Methods and materials

For the studies, we used soil that did not contain arsenic, washed out and not washed from oxides, sublimates of sulfidizing roasting of arsenic concentrate and arsenate-calcium cakes, as well as initial technological cakes. Mineralogical composition of unwashed fumes (%): As_2S_3 - 45, As_4S_4 - 32, As_2S_5 - 10, As_2O_3 - 13, their chemical composition (%): As - 62.4, S - 32.5, O - 5.1. The washed sublimates contained traces of oxides.

The number of microorganisms was determined by plating samples on elective media. The experiment was carried out in duplicate. Experimental vessels were filled with 100 g of soil, 1 g of sublimate or cake, 500 ml of distilled water with pH 7.0-7.2. Half of the test vessels were incubated at room temperature (+ 18-20 ºC), and the other half at +4 ºC.

The work studied the behavior of autotrophic thionic bacteria (*Thiobacillus thrioparis, Thiobacillus thiooxidans Thiobacillus ferrooxidans*), as well as arsenic-oxidizing and arsenic-reducing bacteria.

Thionic bacteria differ in their ability to grow at different pH of the environment. In the presence of sulfur-containing compounds, thionic bacteria gain advantage, for which environmental conditions (pH, temperature, and humidity) will be optimal. A decreasing of pH as a result of the activity of one group of bacteria can suppress the activity of this group of bacteria, and this niche will be occupied by another group for which the formed conditions will be optimal.

The influence of the composition of the medium on the behavior of arsenite-oxidizing and arsenate-reducing bacteria in systems with sublimates was studied using media of various compositions (Table 1).

Table 1. *Composition of media in vessels*

№	Environment	Additive amount, g/l
1	Soil without additives	-
2	Soil + Calcium Lactate	3.5 g/l
3	Soil + Sodium Acetate	3 g/l
4	Soil + yeast extract	1 g/l
5	Soil + glucose	5 г/л
6	Soil + sulfur	1 g per 100 g soil
7	Soil+ $Ca(OH)_2$	1 g/l

In all experiments, the content: $(NH_4)_2SO_4$ - 1g/L, KH_2PO_4 - 1g/l. In all solutions, the pH was 7.5. In experiments with the addition of $Ca(OH)_2$, the initial pH value was 9-10.

Results and discussion

Influence of microorganisms on the arsenic behavior at materials storage in soil

Arsenate calcium cake. The experiment was carried out in duplicate for 7 months. In each of the four experimental vessels, 100 g of soil was introduced, then 10 g of arsenate-calcium cake. Each vessel was added to 500 ml of sterile water with a pH of 7.0-7.2. Half of the test vessels were incubated at room temperature (+ 18-20 ºC), and the other at +4 ºC. The room temperature was recorded and the experimental vessels were mixed daily.

Thionic bacteria were not detected throughout the experiment, while the number of arsenite-oxidizing bacteria increased during the first 4 months, and by the end of the experiment (after 7 months), a decrease in their number was observed (Fig.1). The development of arsenite-oxidizing bacteria confirms the presence of a certain amount of arsenite in the arsenate-calcium cake. In the ionic form, the ongoing reaction is described by the equation: $As^{3+} - 2\bar{e} = As^{5+}$.

As can be seen from fig. 1, during the first four months, the amount of arsenic that passed into the solution increased.

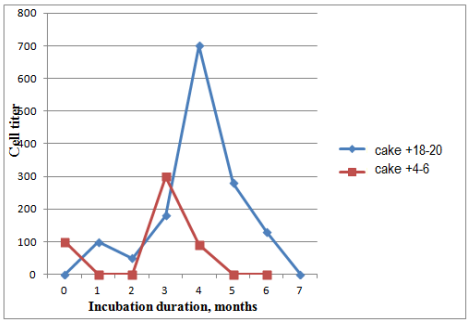

Fig. 1. Content of arsenite-oxidizing bacterias in vessels with arsenate-calcium cake (cells/ml) - at + 18-20 ºC, - at + 4-6 ºC

The solubility of $3Ca_3(AsO_4)\cdot 2Ca(OH)_2$ is higher than that of $3Ca(AsO_2)_2$, which led to an increase in the content of soluble arsenic in the solution. Determination of the number of arsenate-reducing bacteria is shown in Fig. 2. The arsenic content in the model solution at a temperature of +4 ºC slowly increased throughout the experiment.

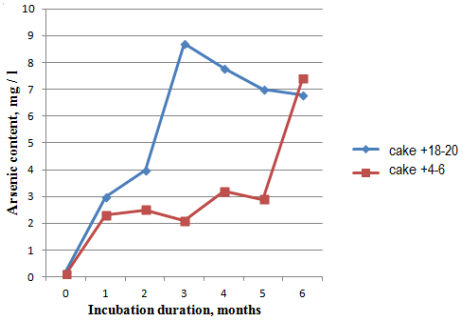

Fig. 2. The content of soluble arsenic in model solutions with arsenate calcium cake(- at + 18-20 ºC, - at + 4-6 ºC)

Arsenate-reducing bacteria are anaerobic bacteria capable of using arsenate as the final electron acceptor and reducing it to trivalent arsenite. In the presence of sulfate ions in the medium, which can also be reduced by bacteria to sulfide ion, the formation of practically insoluble arsenic sulfide As_2S_3 is possible, Fig.3.

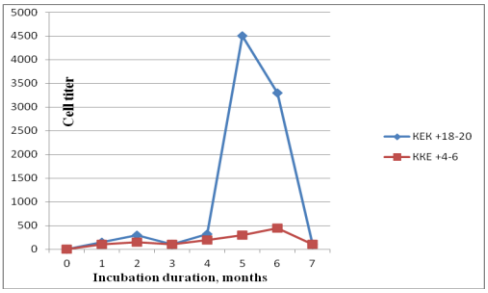

Fig. 3. The content of arsenate-reducing bacteria in the vessels with arsenate calcium cake (- at + 18-20 ºC, - at + 4-6 ºC)

The reaction can proceed in accordance with the equations:

$$As^{5+} + 2e = As^{3+} \quad (1)$$
$$S^{6+} - 8e = S^{2-} \quad (2)$$
$$2As^{3+} + 3S^{2-} = As_2S_3 \quad (3)$$

Table 2. *pH of media and the content of microorganisms in samples after a month of incubation*

Arsenite oxidizing bacteria		Arsenate-reducing bacteria	
pH	cells / ml	pH	cells / ml
Soil without additives (Sample 1)			
7.45/6.95*	50/9.0*	6,95/6.95*	0/0*
Soil + calcium lactate (Sample 2)			
7,42/7,48*	140/25,5*	7,08/7,0*	1,1/0*
Soil + sodium acetate (Sample 3)			
7,53/7,65*	140/0*	7,2/6.9*	0/0*
Soil + yeast extract (Sample 4)			
7,57/7.56*	0,5/0*	7,24/7.0*	0/0*
Soil + glucose (Sample 5)			
7,53/7.46*	5.0/1.2*	7,7/6.8*	0/0*
Soil + sulfur (Sample 6)			
7,5/7.66*	0/0*	7,2/7.0*	0/0*
Soil + Ca (OH) 2 (Sample 7)			
7.0/6.25*	0,4/2*	7,2/7.15*	0/0*

* Numerator - at room temperature. Denominator - at 4 °C.

Thus, in the presence of arsenate-calcium cake, arsenite-oxidizing bacteria first begin to develop, which oxidize trivalent arsenic to a soluble pentavalent form, and then anaerobic arsenate-reducing bacteria develop, which reduce arsenate and convert it into insoluble arsenic sulfide. Due to this, the observed decrease in the arsenic content in the test vessels occurs by the end of the experiment at a temperature of + 18-20 ° C. At the same time, during the incubation of the vessels at + 4 °C, the amount of arsenate-reducing bacteria is very low. Therefore, the formation of arsenic sulfide does not occur, and by the end of the experiment the content of soluble arsenic increases to 6.3 mg/l.

The results obtained allow us to say that in experiments simulating the storage of arsenate-calcium cake in soil under high humidity conditions, autotrophic thionic bacteria are unable to affect the mobility of arsenic. At the same time, the active growth of arsenite-oxidizing bacteria and the subsequent development of arsenate-reducing bacteria indicate that these bacteria affect the mobility of arsenic. The subsequent development of arsenate-reducing bacteria is associated with the presence of arsenates in the cakes, which play the role of an electron acceptor in the respiratory chain of bacteria and reduce arsenate to the trivalent form with the formation of arsenic sulfides. A decrease in the concentration of soluble arsenic by the end of the experiment suggests that the formation of a sparingly soluble arsenic compound is possible under the influence of arsenate-reducing bacteria.

Sulfide sublimates

The results of studies of the behavior of bacteria in the presence of washed sulphide sublimates showed that thionic bacteria were not detected during the experiment, i.e. sulfide forms of arsenic are inert with respect to the studied microorganisms. Next, we checked the survival of arsenite-oxidizing and arsenate-reducing bacteria under conditions of prolonged contact with washed sulfide arsenic sublimates in various environments (Table 2). As follows from the results obtained, arsenate-reducing bacteria are not detected in samples as early as 1 month after the start of the experiment, i.e. they do not participate in the processes.

In the second series of experiments, the behavior of arsenous fumes in the presence of arsenite-oxidizing bacteria was studied. As a result of the experiments performed, it was found that various additives in the form of calcium lactate, sodium acetate, yeast extract, glucose do not have a noticeable effect on the transition of arsenic into solution.

The transition to the solution of arsenic is mainly associated with the oxides contained in the sulfide fume. Oxidation from As^{3+} oxides to As^{5+} promotes the transfer of arsenic into solution. As is known, As_2O_5 has a higher solubility than As_2O_3 - the solubility of As_2O_3 at 25 °C in 100 g of water is 2.1 g and As_2O_5 is 65.8 g.

The transition of arsenic into solution is more noticeably influenced by the presence of elemental sulfur. The results of studies on the behavior of arsenite-oxidizing and arsenate-reducing bacteria under conditions of long-term contact (within 7 months) with washed and unwashed sulfide arsenous sublimates in the presence of soils are presented in Fig.4-6.

As can be seen, at 20 °C the number of arsenite-oxidizing bacteria remained almost unchanged during the first three months (Fig 4).

After four months, the number of arsenite-oxidizing bacteria reached a maximum and gradually decreased to zero by the end of the experiment.

The development of arsenite-oxidizing bacteria is apparently associated with their participation in the oxidation of arsenic oxide (As_2O_3) in accordance with the equation $As_3 + -2\bar{e} = As_{5+}$. This is also indicated by a sharp increase in the content of arsenic in the solution in the first three months of the experiment (Fig 5).

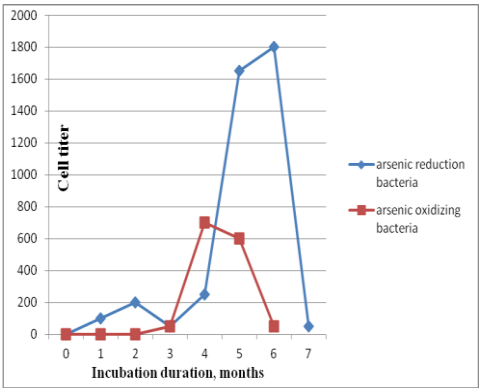

Fig. 4. Change in the number of bacteria depending on the duration

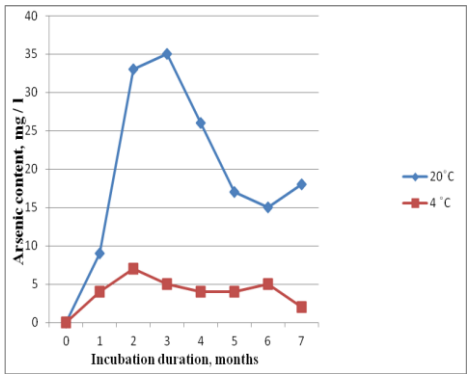

Fig. 5. Change in the content of arsenic in the solution depending on the duration (sample of unwashed fumes)

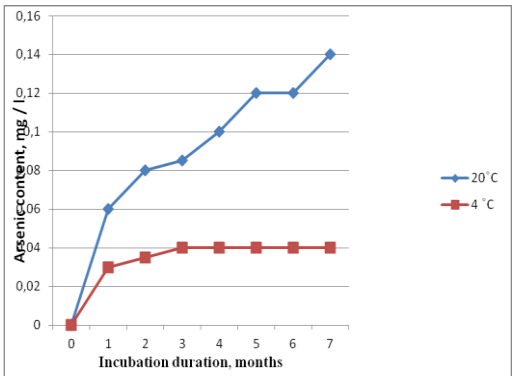

Fig. 6. Change in the content of arsenic in the solution depending on the duration (sample of the washed sublimate)

At room temperature, arsenate-reducing bacteria develop intensively starting from the fourth month (Fig. 4) and reach their maximum numbers by the sixth month of the experiment. By the end of the experiment, the number of arsenate-reducing bacteria gradually decreased.

In model vessels with the introduction of sulfide fumes, the content of arsenic passing into the solution increased during the first three months: starting from the fourth month, a tendency to decrease in its amount was observed (Fig.5).

Thus, in the presence of unwashed sulfide sublimate, arsenite-oxidizing bacteria first begin to develop, which oxidize trivalent arsenic oxide to a soluble 5-valent form, and then anaerobic arsenate-reducing bacteria develop, which restore pentavalent arsenic compounds and convert it into sparingly soluble arsenic forms 3-valent. Due to this, the observed decrease in the arsenic content in the test vessels by the end of the experiment occurs. The decrease in the concentration of bacteria by the end of the seventh month is due to the fact that the nutrient medium introduced into the samples at the beginning of the process was exhausted. No additional soil was introduced into the system.

In order to exclude the effect of arsenic oxide forms, a study was carried out of the possibility of the development of microbiological processes under similar conditions in the presence of sulfide fumes washed from arsenic oxides. The change in the content of arsenic in solutions depending on the duration (Fig.6) indicates that in the absence of oxide forms of arsenic, the solubility of fumes decreases sharply and the content of arsenic does not exceed 0.15 mg/l. And this amount of arsenic went into solution due to the dissolution of arsenic oxides present in the sublimates). The development of microbiological processes is not observed, i.e. the sulfide forms of arsenic are inert with respect to the microorganisms under study.

The results obtained allow us to say that in experiments simulating the storage of sulfide fumes in soil at high humidity, autotrophic thionic bacteria are unable to affect the mobility of arsenic. At the same time, the active growth of arsenite-oxidizing bacteria and the subsequent development of arsenate-reducing bacteria indicate that these bacteria affect the mobility of arsenic.

The fact that arsenite-oxidizing bacteria develop first indicates the possibility of the 3-valent oxide forms of arsenic present in arsenic products to be oxidized by bacteria to the highest forms. It can be assumed that the partial dissolution of sulphide sublimate is associated with the properties of the soil used, because humic and fulvic acids present in the soil can affect the solubility of metal ions. This is also confirmed by the fact that the maximum increase in the arsenic content in the solution is noted after two months of experiments, when the active growth of arsenite-oxidizing bacteria has not yet begun. The subsequent development of arsenate-reducing bacteria is associated with the presence of higher arsenic oxides in sulfide sublimates, which play the role of an electron acceptor in the underwear chain of bacteria and reduce As_2O_5 to the 3-valent form. The results show that the studied microorganisms under the studied conditions practically do not affect the sulfide forms of arsenic, participating in the transformation of only oxide compounds contained in sulfide sublimates.

To obtain stable sulfide fumes, it is advisable to carry out additional sulfidization before disposal.

The effect of pure cultures of bacteria on the transformation of arsenic in arsenic-containing products

Arsenate calcium cake

This study included experiments with arsenate-calcium cake and fume after sulfidizing roasting of the concentrate to determine the possibility of the effect of pure cultures of bacteria on the solubility of arsenic from these products. For this purpose, the vessels containing the media optimal for the development of the corresponding group of bacteria were inoculated with pure cultures of bacteria. Every 1.5 months, an analysis was carried out for the content of thionic bacteria, arsenate-reducing and arsenite-oxidizing bacteria in these vessels, changes in pH and the content of total arsenic in the solution. The studies were carried out with non-sterile arsenic-containing products. 1 g of arsenate-calcium cake or sublimate after sulfidizing roasting of the concentrate was added to the vessels with the appropriate medium. 400 ml of the appropriate medium and pure cultures of bacteria were added to each vessel. The experiment was carried out in 2 replicates.

Analysis of the results obtained indicates that bacteria T.thiooxidans were not detected after 1.5 months and until the end of the experiment. The abundance of T.ferrooxidans decreases to 10 cells / ml after 4.5 months, and by the sixth month they are also not detected. T.thioparus bacteria with an initial content of 109 cells / ml were not detected after 1.5 months and until the end of the experiment. The content of As_3 + oxidizing bacteria gradually decreases, but even after six months it is 103-4 cells / ml. The number of As_5 + reducing bacteria at their initial content of 106 cells / ml decreases and by the end of the experiment is 102-3 cells / ml.

After 1.5 months of the experiment, practically in all variants, including the control variants without the introduction of bacteria, an increase in the pH of the medium is observed, which, apparently, is associated with the transition of hydroxyl groups present in the arsenate-calcium cake into the solution. Therefore, every 1.5 months after sampling, the pH of the media in all variants was brought to the level corresponding to each medium.

The data on the content of soluble arsenic showed that in the variant with T.thiooxidans, an increase in its amount from 20 mg/L at the beginning of the experiment to 150 mg/L after 4.5 months and a slight decrease by the sixth month is observed.

The arsenic content in the variant using T.ferrooxidans is approximately the same both in the control and in the experiment. The increase in the arsenic content during the experiment is apparently associated with the low pH of the medium (2-2.5), in which the culture was grown, which contributed to the dissolution of a part of the arsenate-calcium cake.

In experiments with As_3^+ oxidizing bacteria, the concentration of arsenic in the solution slightly increases during the experiment, and this is noted both in the variants without bacteria, and with bacteria.

When T.thioparus is grown, the content of soluble arsenic increases from 0.2 mg / l at the beginning of the experiment to 10-90 mg/l after 6 months. A similar trend was observed in the variants without bacteria, although in this case the arsenic content was lower. When growing As_5 + reducing bacteria, a decrease in the content of soluble arsenic was noted throughout the experiment in the variant with bacteria and an increase in its content in the control.

Thus, the results showed that the thionic bacteria *Thiobacillus thiooxidans, Thiobacillus thioparus, and Thiobacillus ferrooxidans*, despite the creation of optimal conditions for their growth, are unable to grow in the presence of calcium arsenate cake. Therefore, these bacteria cannot influence the mobility of arsenic in arsenate calcium cake. At the same time, the growth of arsenite-oxidizing and arsenate-reducing bacteria under such conditions indicates that they can influence the transformation of arsenic in arsenate-calcium cake.

With the aim of a more detailed study of the effect of arsenite-oxidizing and arsenate-reducing bacteria on the mobility of arsenic in arsenate-calcium cake, additional studies were performed with these microorganisms.

The objective of this stage of research was to study the effect of arsenite-oxidizing and arsenate-reducing bacteria on the mobility of arsenic in arsenate-calcium cake under optimal conditions for the growth of the studied bacteria.

The results of the work showed that after three months of the experiment, the number of arsenite-oxidizing bacteria decreased slightly from 108-9 cells / ml in the initial sample to 106-7 cells / ml. The number of arsenate-reducing bacteria did not change and amounted to 103-4 cells / ml.

The content of arsenic in vessels with arsenite-oxidizing bacteria increased from 6.4-7.4 in variants with bacteria to 20-26.6 mg / L, while in the control variants this value increased from 8.0-9.4 to 38-44.4 mg / L after three months.

In variants with the use of arsenate-reducing bacteria, the arsenic content decreased from 11.6-17.0 mg / L to 3.0-11.6 mg / L after three months of the experiment, which indicates that arsenate-calcium cake does not affect the viability of arsenate-reducing bacteria, which are able to precipitate soluble arsenic. into insoluble arsenic compounds.

The results of the X-ray phase analysis - Table 4.

The analysis of the obtained results showed that arsenite-oxidizing bacteria practically do not change the cake composition in comparison with the original untreated cake.

In all variants, both in the control without bacteria and in the variants with bacteria, the disappearance of arsenic acid $H_3AsO_4 \cdot 3H_2O$ is noted, which, apparently, is associated with its dissolution.

It was found that when arsenic acid goes into solution, then pentavalent arsenic is reduced by bacteria with the subsequent formation of arsenic-calcium complexes. In variants with the use of arsenic-reducing bacteria, all sodium sulfur-containing salts ($Na_2S_5O_{16}$, Na_2SO_4) and β-$NaCaAsO_4$ disappear, although they are present in the original untreated cake and in controls without bacteria. It is known that arsenate-reducing bacteria are able to use sulfur from these compounds in the process of metabolism with its subsequent reduction.

Thus, arsenate-reducing bacteria influence the composition of the arsenate-calcium cake. They are able to reduce all water-soluble arsenic salts contained in the cake, with the formation of insoluble arsenic complexes with calcium, and can reduce sulfates and polysulfates, using them as the final electron acceptor.

Sulfide sublimates

Studies have shown that throughout the experiment, the number of the studied groups of bacteria changed in different ways. Thus, bacteria with *T.thiooxidans* were not detected after 1.5 months of the experiment.

Analysis of the data on changes in pH showed that during incubation of vessels with *T.thiooxidans*, no change in the pH of the medium was observed in comparison with the control variants without bacteria, which also confirms the absence of viable cells in the medium. There were also no visual differences between the experimental and control variants. It should be noted that after 1.5 months the content of soluble arsenic increased in the variant with the *T.thiooxidans* culture to 400-600 mg/L in the test vessels and 800 mg/L in the control vessels without bacteria, and then gradually decreased.

In experiments with *T.thioparus*, the arsenic content increased from 9-20 mg/L at the beginning of the experiment to 1000 mg/L after 1.5 months in the variant with bacteria and 1200 mg/L in the control. Perhaps this is due to the solubility of sublimate polysulfides under slightly alkaline conditions.

The number of *T. ferrooxidans* bacteria began to decrease from 103 cells/ml in the initial sample to 0-102 cells/ml by the

third month of the experiment, and after 4.5 months of the experiment, bacteria were not detected.

Table 4 - *Results of X-ray phase analysis of microbiological samples of arsenate-calcium cake* *

Culture under study	Nutritional supplement	Compound	Approximate content,%
Arsenite oxidizing bacteria	Glucose	$Ca_5(AsO_4)_3OH \cdot H_2O$ $Ca_5(AsO_4)_3OH$ $CaSO_4$	30 10 20
Control without bacteria	Glucose	$Ca_5(AsO_4)_3OH \cdot H_2O$ $Ca_5(AsO_4)_3OH$ $CaSO_4$	30 30 10
Arsenate-reducing bacteria	Calcium lactate	$Ca_5(AsO_4)_3OH \cdot H_2O$ $Ca_5(AsO_4)_3OH$ $CaSO_4$	25 25 10
Control without bacteria	Calcium lactate	$Ca_5(AsO_4)_3OH \cdot H_2O$ $Ca_5(AsO_4)_3OH$ $CaSO_4$	30 20 10

* Calcium arsenate cake (original, untreated) contains, %: $Ca_5(AsO_4)_3OH \cdot H_2O$ – 10; $Ca_5(AsO_4)_3OH$ – 10; $CaSO_4$ – 5; $CaCO_3$ – 40; $Ca(OH)_2$ – 20; β-Na-$CaAsO_4$ – 5; $H_3AsO_4 \cdot 3H_2O$ – 5; $Na_2S_5O_{16}$ – 5.

It should be noted that in the variants with the culture of T.*ferrooxidans*, after 4.5 months, upon visual examination, the sublimate, which was a light suspension on the surface of the medium, dissolved, and only precipitates of iron hydroxides formed in the oxidation of iron by the culture of *T.ferrooxidans* were visible in the vessels. In the control variants without bacteria, the sublimation practically did not change and was on the surface of the medium. Apparently, *T.ferrooxidans*, under favorable conditions, is able to use sulfur from the sublimate polysulfides, thereby dissolving the sublimate. This is confirmed by the results of chemical analysis, according to which, after 1.5 months of the experiment, the arsenic content in the variant with *T.ferrooxidans* bacteria was 600-800 mg/l, while in the control variants this figure was 200 mg/l.

Arsenite-oxidizing bacteria grew well in the presence of sublimate and their number increased from 103-4 cells/ml in the initial sample to 107-8 cells/ml throughout the experiment. The content of soluble arsenic in the experimental variant after 1.5 months was 200 mg/l, while in the control it was 900-1000 mg/l. It is difficult to explain why the experimental variant after 1.5 months found 5 times less arsenic than the control one. As a result, it was established that arsenite-oxidizing bacteria practically do not affect the solubility of fumes.

Of greatest interest are the results obtained in experiments with arsenate-reducing bacteria. The content of arsenate-reducing bacteria was approximately at the same level throughout the experiment and amounted to 103-6 cells / ml. If the content of soluble arsenic forms at the beginning of the experiment in the variant with bacteria was 40-50 mg/l, and after 1.5 months it increased to 200 mg/l, then in the control variant this value increased from 10 to 300 mg/l. Three months later, in the variant with bacteria, the content of soluble arsenic decreased to 20 mg/l, while in the control variant this value increased to 400-500 mg/l. The formation of a finely dispersed yellow-orange sediment, presumably arsenic sulfide, was visually observed, which settled to the bottom and walls of the experimental vessels. In the experimental vessels, an increase in the pH of the medium was noted, which also indicated a good development of bacteria and their reduction of arsenate to arsenite.

Conclusion

The study of the influence of microorganisms on the transformation and solubility of arsenic in arsenate-calcium cake and sublimates of sulphiding roasting shows that thionic bacteria have no significant effect on the stability of these products during long-term storage. Arsenite-oxidizing and arsenate-reducing bacteria affect the stability of arsenic compounds in the studied materials: in sulfide sublimates, oxygen-containing arsenic compounds undergo the main transformation, in arsenate cakes, successive oxidation processes occur - reduction of the corresponding arsenite-arsenate forms of arsenic. To regulate the activity of these bacteria in order to reduce the solubility of arsenic, certain nutrient additives are required in the soil. The stabilization of sulfide fumes is also facilitated by a decrease in the content of arsenic oxide forms in their composition, which are most susceptible to microbiological dissolution.

The results of microbiological studies can be useful in choosing the method and conditions for the disposal of technogenic arsenic products.

References

Xian-Chun Zeng, Ye Yang, Wanxia Shi, Zhaofeng Peng, Xiaoming Chen, Xianbin Zhu,Yanxin Wang, 2018, Microbially Mediated Methylation of Arsenic in the Arsenic-Rich Soils and Sediments of Jianghan Plain. Front. Microbiol.

Nguyen, Van Lee, Jong-Un, 2015, A comparison of microbial leaching and chemical leaching of arsenic and heavy metals from mine tailings. Biotechnology & Bioprocess Engineering., Vol. 20 Issue 1, 91-99.

Lei Yan, Huixin Hu, Shuang Zhang, 2017, Arsenic tolerance and bioleaching from realgar based on response surface methodology by *Acidithiobacillus ferrooxidans* isolated from Wudalianchi volcanic lake, northeast China. Electronic Journal of Biotechnology. Volume 25, 50-57.

Bahi Jalili Seh-Bardan, Radziah Othman, Samsuri Ab Wahid, 2012, Column Bioleaching of Arsenic and Heavy Metals from Gold Mine Tailings by *Aspergillus fumigatus.* CLEAN. Soil. Air. Water.Volume 40, Issue 6, 565-666.