



Studying some characteristics of cyanide destructors for the purpose of decontamination of cyanide-containing waste

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ABSTRACT

The subject of article is about isolation and study of endemic cyanide-resistant strain on the Madneuli mine, for further microbiological decontamination of cyanide-containing waste of the gold mining company. In the result of performed studies, for the first time in Georgia, two active cyanide-destructor stains were isolated from the liquid and solid objects contaminated with industrial cyanides: The strain #5 *Bac.cyanooxidans* and #12 *Bac.subtilis*. In the result of performed works, in laboratory conditions, in the mode of serial passage, on model solutions, some characteristics of development of cyanide-destructor microorganisms in the process of cyanide-destruction were determined. Nutrient is an impact of content, impact of the bacterial mass on the process of destruction, cyanide destruction characteristics of specific strains and their association.

Keywords: Cyanide-resistance, Microorganism, Strain, Waste, Cyanide-destruction, Performed works.

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Introduction

Development of mining and processing industry is dependent on increase of anthropogenic impact on the environment. These phenomena are especially visible during operation of gold mines of various formations, where cyanides are used for extracting gold from mostly gold-containing ores. During a heap leaching of these ores, which is performed on open pits, potentially dangerous cyanide-containing waste is produced. They are negative factors of environment impact, because there might be their leakage into soil, water and atmosphere after evaporation. The gold-copper-polymetallic mine of Madneuli is located in the ore-containing zone of Bolnisi, which is the forming part of the Artvin-Bolnisi belt and is situated in the central part of the Alpine-Himalayan belt. The mine is the northern-western part of the Armenia-Karabagh structural metallogenic zone.

Secondary quartzites are widespread in the mine.

The secondary quartzites containing gold and silver are located in the superficial part of the mine,

above the gold and copper. Mineralization is associated with the late blueish-gray quartzes, which are in the form of stockworks, separate rich veins and vein zones [1]. During mining of chalcopyrite, gold-containing quartzites were stored on dumps, with the perspective of their further processing. Later the technology of gold extraction from these quartzites were implemented. This is the heap leaching method using cyanides, which is carried out in open pits. A thick polyethylene film covers the bottom of the pit. The ore is arranged in the form of 5-meter layers. Extracted gold is collected in the heap cover and is pumped to the collecting tank, from where it is transferred to adsorption columns. Depending on ore type and company's capacity, an annual consumption of cyanides reaches tens of even hundreds of tons. Therefore, there is a problem of cyanide contamination, and it is necessary to neutralize the cyanides in the waste. Destruction of cyanides may be carried in three ways: chemical, biological and complex chemical-biological methods.

Among the biological methods, most promising, economical and environment-friendly is the micro-

biological method of cyanide destruction. Therefore, researchers focus on obtaining cyanide-destructor bacterial strains and their use in bio-decontamination of cyanide-containing waste.

Currently, various types of heterotroph microorganisms are obtained, which carry out destruction of cyanide compounds. These are the representatives of the following genus: *Pseudomonas*, *Bacillus*, *Achromobacter*, *Rhodococcus*, *Serratia* and other [2- 5].

Study objects and methods

The objects of isolation of cyanide-destructor microorganisms were solid and liquid waste of heap leaching of gold-containing secondary quartzites carried out by cyanidation technique. Isolation of strains and their further study was conducted by means of techniques approved in microbiology, which are given in the textbook of practical works [6].

For the purpose of microflora revival and activation, the solid probes were moisturized by sterile water, and all probes were placed into a thermostat at the temperature of 28 °C for 24 hours. After incubation, probe of 10 g was diluted with sterile water (1:10), the mix was placed on shaker for 10 minutes, after which the probes, according to the approved technique, by tenfold dilution, were inoculated 3 times repeatedly in Petri dishes on beef peptone agar, to which 10 mg/l of sodium cyanide was added to suppress other associated microflora. Cultivation of microorganisms were conducted in stationary conditions in the thermostat at 28 °C for 4 days. Microorganisms were recorded by visual examination of the inoculations and also by their microscopic study. The number of microorganisms were calculated for 1 g of absolutely dry ore or 1 ml of water.

After cultivation for 4 days, isolated colonies were produced on the nutrient medium. In the result of conducted study, 18 cyanide-resistant strains were isolated and obtained. Those strains are considered cyanide-resistant, which grow well or weakly on cyanide-containing nutrient medium.

From 18 strains, isolated for obtaining working strains, were selected the strains with clearly expressed ability for destruction of cyanides.

The selection was conducted on Petri dishes on Podolskaya nutrient agar #1 [7] with the following content: $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ – 3,0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ –

0,5; FeCl_3 – 0,01; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ – 0,01; saccharose – 5,0; agar – 2%. The prepared source of nitrogen was not added to the nutrient medium, to give a possibility to microorganisms to use nitrogen from NaCN as a source of nitrogen. Cyanide was added to the nutrient medium after sterilization. Cyanide concentration intervals of 10-20 mg/l; 30-40 mg/l and 50-60 mg/l were taken. Cultivation was conducted in stationary conditions in the thermostat at temperature of 28 °C. Resistance of the strains against cyanides were assessed according to growth on nutrient medium.

It should be noted that all strains from 18 expressed resistance to cyanide to some extent, but two active strains were distinguished among them: The strains #5 and #12.

These strains were examined on purity in accordance with inoculation technique on various nutrient media. In parallel, they were microscopically studied using a digital microscope Omax.

For strengthening and improving cyanide-destruction features of the isolated strains, they were adapted to the increased concentrations of cyanides by means of their serial high passage.

Adaptation of strains was conducted on Petri dishes in #1 solid synthetic nutrient medium, to which sodium cyanide were gradually added with increased concentrations (50-135 mg/l) according to the results of tests. 3-5 passages were needed for adaptation to each increased concentration of cyanide (I passage – 50 mg/l, II passage – 70 mg/l, III passage – 100 mg/l, IV passage – 115 mg/l, V passage – 135 mg/l). Cultivation was conducted in stationary conditions in the thermostat at temperature of 28 °C.

After obtaining active working strains, their morphological-cultural and physiological-biochemical characteristics were studied. Based on the obtained data, the strain # 5 was identified as *Bacillus cyanooxidans*, and the strain #12 – *Bacillus subtilis*. Identification of bacteria was conducted according to the Bergeys bacteria determinant technique.

As it is known, for growth and development of live organisms, including microorganisms, availability of two elements – nitrogen and carbon is critically important. Synthetic nutrient medium used by us, was providing the microorganisms with critically important elements. For clarifying the issue – whether the isolated cyanide-resistant microorganisms had the ability to absorb these elements or not, we conducted tests, in which the solid and liquid nutrient media of similar content were used.

For determining nitrogen absorption ability of microorganisms from cyanide compound, the tests were conducted in Petri dishes, agar synthetic medium #1, which contained NaCN (20 mg/l) as a source of nitrogen in one variant, and NH₄Cl (0,5 g/l) in another.

For determining the source of carbon, the same cyanide-containing synthetic medium #1 in one variant contained carbohydrates (saccharose - 5 g/l, Na lactate - 1.5 g/l), and in the second variant it did

not contain an organic compound. The 24 hours cultures of strain #5 *Bac.cyanooxidans* and the strain #12 *Bac.subtilis* were used as inoculants. The tests were conducted at the temperature of 28 0C in the thermostat in stationary conditions for 5 days. We assessed the results of tests according to growth of bacteria on the solid nutrient medium, and also by analyzing cyanide in the solutions. The results of the test are given in the Table 1.

Table 1. *The nutrient is an impact of growth of cyanide-destruction microorganisms*

Strain #	Source of nitrogen		Source of carbon		
	Zone # 1 NaCN	Zone # 1 NH ₄ Cl	Zone # 1 NaCN		
			Saccharose	Na lactate	Without carbohydrate
5, <i>Bac.cyanooxidans</i>	+	+	+	+	-
12, <i>Bac.subtilis</i>	+	+	+	+	-

+ - Growth of bacterium
- - absence of growth

The amount of bacterial mass is very important for studying the cyanide destruction process.

The tests for studying this issue were conducted in Erlenmeyer flasks of 250 ml volume, in which 100 ml of synthetic nutrient #1 were introduced as well as increased concentrations of cyanide (10-50 mg/l). Cyanide-destruction microorganisms - 24 h cultures of the strain #5 *Bac.cyanooxidans* and the

strain #12 *Bac.subtilis* were used as inoculants. The tests were conducted in stationary conditions in the thermostat at temperature of 28 0C. We assessed the results of the tests according to the number of microorganisms using the direct counting method in microscope, and also by determining a dry bacterial mass and analyzing cyanide compounds in the solution. The results of the test are given in the Table 2.

Table 2. *Impact of the bacterial mass on the cyanide destruction*

Value, №	1	2	3	4	5
Cyanide concentration, mg/l	10	20	30	40	50
Bacterial titer	10 ⁹	10 ⁹	10 ⁷	10 ⁶	10 ³

The issue was studied concerning the impact of certain strains (types) of isolated heterotroph bacteria and their association on the activity of cyanide-destruction.

The tests for studying this issue were conducted in Erlenmeyer flasks of 250 ml volume, in which 100 ml of synthetic nutrient #1 with saccharose were introduced and CN was added in the amount

of 20 mg/l and 30 mg/l. The 24 h cultures of strain #5 *Bac.cyanooxidans* and the strain #12 *Bac.subtilis* and their united bacterial suspension were taken as inoculants. The amount of inoculant was 10% of the initial liquid (each strain in the association in equal amount of 5%). Tests were conducted in condition of shaking, by thermostating, at 28 0C. The results of the test are given in the Table 3.

Table 3. *Cyanide compounds destruction by isolated heterotroph bacteria and their association*

Strain #	Concentration of CN, mg/l		Incubation time, h
	Initial	Final	
5,	20	15	24
Bac.cyanooxidans	30	20	48
12,	20	4	24
Bac.subtilis	30	08	48
5, 12	20	0	24
Bac.cyanooxidans; Bac. subtilis	30	0	48

Results and discussion

For the first time in Georgia, works were conducted on the Madneuli mine for isolation and study of endemic cyanide-destructor microorganisms, for using them later for microbiological decontamination of gold mining company's waste.

During the study process, 18 strains of cyanide-resistant microorganisms were screened from solid and liquid waste of the mine, contaminated with cyanides, from which two active strains #5 and #12 were selected. Based on the study of morphological-cultural and physiological-biochemical characteristics of the strains, the strain #5 was identified as *Bac.cyanooxidans*, and the strain #12 as *Bac.subtilis*.

The results of conducted adaptation showed that after each passage the resistance ability of microorganisms against cyanide was increasing (which we assessed according to the developed bacterial mass). The strain #5 was adapted to the concentration of cyanide of 100 mg/l, and the strain #12 - to 135 mg/l concentration. Further increase of cyanide was not performed because further increase of NaCN concentration significantly suppressed the growth of bacteria.

In the study process of ability of isolated strains to use cyanide as a nitrogen and carbon source, good growth of bacteria was detected in the nutrient medium when NH_4Cl , and also NaCN were present. During visual examination of microorganisms on the solid medium, larger growth of strains was detected in the variant with NH_4Cl compared to

the variant with NaCN, however, the latter variant also produced a good growth. Therefore, it became evident that the strain #5 and the strain #12 *Bac. cyanooxidans* have the ability to absorb nitrogen from cyanide, but, at the same time, they do not use cyanide as a carbon source, because, without organic compounds, autotrophic growth of strains on NaCN-containing medium was not detected. While in case of use of saccharose and lactate as carbon and energy source, the strains produced a good growth.

These data match the characteristics of cyanide-destruction. In stationary conditions, after incubation for 5 days, the content of CN was determined in the final solutions (initial solution CN - 20 m). In case of source of nitrogen being NaCN and NH_4Cl , the destruction of CN was 99.2% and 99.5%, respectively. In case of source of carbon being saccharose and lactate, the destruction of CN was 99.2% and 99.5%, respectively, and the obtained results are close to each other, and in case of carbohydrate presence in the nutrient medium, CN destruction did not occur. It became clear that the presence of organic compound in the medium is necessary for development of cyanide-destructor microorganisms and the process of cyanide destruction.

Limitation occurs during the serial passage of microorganisms (gradual decrease of nutrient components in the medium and accumulation of metabolism substances), as well as inhibition (toxic impact of cyanide compounds' concentration). After introducing in the bacteria nutrient medium, they

continue reproduction until a content of some component needed for them reaches a minimum value after which the reproduction stops.

The test results showed that concentration of CN impacts the number of microorganisms (mass). Total destruction of cyanides depends on the initial number of microorganisms in the solution. As the test results showed, 10-20 mg/l cyanide concentration in the medium had weak impact on the growth of microorganisms, no limitation and cyanide inhibition effect on the microbic mass occurred.

With cyanide concentrations above 30 mg/l, the inhibition effect appears and the specific growth rate of bacteria decreases. With cyanide concentrations of 50 mg/l, the inhibition effect even more increased and biochemical activity of the microorganisms decreased.

The test results show that in the serial passage, the more is CN concentration, the less is bacterial mass. Highest value of cyanide destruction is obtained in case of bacterial titer of 109 CFU/ml.

In case of combination of isolated strains in an association, they complement each other, because of which the destruction ability of the association increases.

During conducting the test, the change of ration between various bacteria was detected in the population. At the same time, the continuity of type content was retained, which was proven microscopically, and by inoculation on an agar synthetic nutrient medium. Also, the strains in the pure culture showed different ability of CN destruction. The strain #12 *Bac.subtilis* showed more active ability of CN destruction compared to the strain #5 *Bac.cyanooxidans*. The strain #5 *Bac.cyanooxidans* destructs 10 mg/l cyanide for 48 hours (initial - 30 mg/l), and the strain #12 *Bac.subtilis* in the same time destructs 22 mg/l cyanide. Their combined association carries out the total destruction of cyanide within the same time period.

Conclusion

For the first time in Georgia, works were conducted on the Madneuli mine for isolation and study of endemic cyanide-destructor microorganisms, for using them later for microbiological decontamination of gold mining company's waste.

18 strains of cyanide-resistant microorganisms were screened from solid and liquid waste of the mine, contaminated with cyanides, from which two active strains #5 and #12 were selected. Based on

the study of morphological-cultural and physiological-biochemical characteristics of the strains, the strain #5 was identified as *Bac.cyanooxidans*, and the strain #12 as *Bac.subtilis*.

For strengthening and improving cyanide-destruction features of the isolated strains, they were adapted to the increased concentrations of cyanides, after which the strain #5 *Bac.cyanooxidans* was adapted to the cyanide concentration of 100 mg/l in the nutrient, which the strain #12 *Bac.subtilis* - 135 mg/l of cyanide concentration.

The isolated strains can use cyanide as a source of nitrogen, but they cannot use it as a source of carbon. For the source of carbon, they need existence of organic compound in the medium. In these conditions, the strains may carry out 99% destruction of cyanide with its initial concentration of 20 mg/l.

The cyanide concentration impacts the number of microorganisms (mass); the more is cyanide concentration, the less is bacterial mass. Highest value of cyanide destruction is obtained in case of bacterial titer of 109 CFU/ml. In case of combination of isolated strains in an association, they complement each other, because of which the destruction ability of the association increases. The strain #5 *Bac.cyanooxidans* destructs 10 mg/l cyanide for 48 hours (initial - 30 mg/l), and the strain #12 *Bac.subtilis* in the same time destructs 22 mg/l cyanide. Their combined association carries out the total destruction of cyanide within the same time period.

The concentration of cyanide in the medium has an impact on the cyanide destruction process. Bacterial titer, nutrient is content.

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