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Cyanide Detoxification by Microbial Consortia of Natural-Industrial Complexes of Gold Heap Leaching

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Abstract—Microorganisms that have adapted not only to high concentrations of pollutants but also to environmental conditions develop in autochthonous microbial communities of natural-industrial complexes of gold heap leaching. The biotechnological potential and diversity of autochthonous microbial communities involved in cyanide detoxification was evaluated by the example of a deposit situated in the Sakha (Yakutia) Republic. Under the zoning conditions of the ore heap, the biological component had a greater impact on cyanide destruction than chemical transformation. Metabolically active representatives of a microbial consortium are capable of surviving developed under these conditions. Phylotypes of the genus *Serratia* and family Alcaligenaceae that are capable of cyanide destruction and are potentially promising for the detoxification of wastes of gold heap leaching were revealed.

Keywords: cyanides, gold heap leaching, destruction, autochthonous microbial communities
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INTRODUCTION

Potentially hazardous cyanide-containing waste, spent ore heaps, and technological solution are produced during gold heap leaching (HL) conducted in outdoor areas of industrial facilities (Fig. 1) [1]. This waste has a negative impact in natural-industrial complexes on the air, soil, and surface and ground waters [2]. Waste decontamination and remediation are based mainly on chemical approaches. Spent technological solution is detoxified and discharged into the environment according to allowable discharge standards (ADS), whereas spent ore heap is recultivated [1, 2]. Cyanide destruction can occur due to the effect of natural factors, but this process in outdoor areas of natural-industrial complexes proceeds relatively slowly [3]. Ecofriendly and cost-effective bioremediation technologies based on the use of degrading bacteria are being developed around the world to improve the detoxification of cyanide-containing compounds [4–7].

Microorganisms that have adapted not only to high concentrations of pollutants but also to environmental conditions develop in autochthonous microbial communities of natural-industrial complexes of gold HL. The conditions in different zones of ore heap during the storage should be taken into consideration during the study of bacterial consortia that detoxify cyanide. The structure of the ore heap was characterized by heterogeneity of the temperature, humidity, and free oxygen content; four layers were distinguished [3]. First, the upper layer, 0.2 to 0.5 m in thickness, is in direct contact with the atmosphere and is subjected to seasonal temperature fluctuations, since the average temperatures vary from 20°C (in summer) to −18°C (in winter). Its humidity depends on the precipitation quantity and evaporation and varies from 10 to 15% within a year. The first layer does not affect the behavior of toxic compounds during detoxification [3]. The second layer lies beneath the first layer up to the depth of frost penetration (1.3–3.0 m) and is saturated with oxygen due to the draining of technological solutions. Its humidity is almost constant due to the prevention of evaporation by the upper layer. However, as in the first layer, its temperature regime is subject to seasonal fluctuations. In the third layer, which is situated beneath the frost penetration, temperature fluctuations are insignificant (of 0 to 4°C). If anoxic zones are absent in this layer, ore material can stay in aerobic conditions down to the base of the heap. It is typical for final stages of toxic compound destruction. The fourth layer is the deepest horizon and differs from the third by its oxygen deficit; the oxygen is largely consumed by the ore material due to biogeochemical reactions [3].

The goal of the present work was to study cyanide biodetoxification and reveal the main representatives

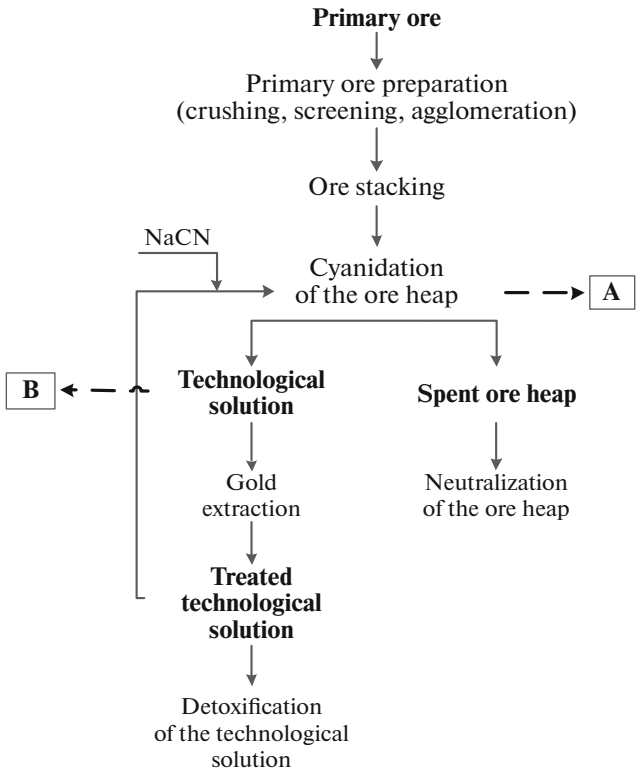

Fig. 1. Flowsheet of gold HL. Arrows indicate sampling sites: A—ore mass of the operating ore heap; B—technological solution.

of the microbial consortia of natural-industrial complexes of gold HL performing cyanide destruction during model experiments.

MATERIALS AND METHODS

The samples were collected from a deposit in Sakha (Yakutia) Republic (Russia) in 2014. Samples of the technological solution and ore from the operating ore of the gold HL heap were collected in sterile containers and then transported and stored at 0–4°C.

Evaluation of the biotechnological potential of autochthonous microbial communities of natural-industrial complexes of gold HL. The biotechnological potential of the microbial consortia was evaluated in experiments modeling the zoning conditions of a HL ore heap. The experiments were performed with sterilized and nonsterilized ore samples for 262 days. Samples were collected bimonthly for complex analysis and sterility control. To estimate sterility, aqueous extracts from preliminary sterilized samples were inoculated on NSY solid nutrient medium containing the following (g/L): nutrient broth—1.0, soy peptone—1.0, yeast extract—1.0, and agar—15.0. They were incubated at 20–25°C. The growth (development of colony forming units (CFUs)) was monitored after 3 and 7 days of incubation. Samples that did not demonstrate growth after 7 days of cultivation were considered to be sterile.

Representative ore samples were collected from the general sample for complex analysis. They were obtained by the quartering method. To equalize the chemical composition of the moisture of the ore mass, the ore sample was washed with technological solution and divided into two equal parts. One of these parts was used as a control during biodegradation. The control was subjected to fractional sterilization (autoclaved three times for 1 h at 121°C with an interval of 24 h) [8]. After cooling, the sample was washed with technological solution sterilized by filtration through 0.22 μm nitrocellulose membrane filter (Millipore, United States). To maintain sterility, the sample was incubated in a sterile container equipped with a nitrocellulose filter to avoid aeration with nonsterile air. Sampling was performed in a sterile box.

The cultivation conditions were selected with respect to the heterogeneity of the ore heap. The experiments were performed at a given temperature mode (20°C, 4°C, –18°C) and with the presence or absence of aeration. The designations of the ore samples were the following: **S0**—primary ore mass; **S1**—ore mass incubated with aeration at 20°C; **S2**—ore mass incubated with aeration at 4°C; **S3**—ore mass incubated without aeration at 4°C; **S4**—ore mass incubated at –18°C. Oxygen removal (degassing) of the primary sample was not conducted. These conditions corresponded to the characteristics of the layers of stored HL ore heap described above, with the exception of the first layer, which was not studied due to its insignificant thickness and impact on cyanide destruction [3].

Water and alkaline extraction methods were used to determine the content of toxic compounds in the moisture of the ore mass [3]. The contents of cyanide and thiocyanate were determined in alkaline extracts to prevent cyanide release into the gaseous phase in the form of hydrocyanic acid. The concentrations of nonvolatile toxic substances were determined in aqueous extract prepared with sterile distilled water. Chemical analysis was performed by the following standard methods: the contents of calcium, sodium, and chloride ions were determined by titrimetric methods (PND F 14.1:2.95–97, PND F 14.1:2.98–97, and PND F 14.1:2.96–97, respectively), the sulfate content was determined by the turbidimetric method (PND F 14.1:2.159–2000), cyanides and thiocyanates were determined by photometric methods with pyridine and barbituric acid (PND F 14.1:2.56–96 and PND F 14.1:2:4.156–99, respectively), and the total salt content was determined by the gravimetric method (PND F 14.1:2:4.114–97). The elemental composition was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) according to the protocol (PND F 14.1:2:4.135–98). The content of mobile toxic compounds in the mois-

Table 1. Chemical composition of the technological solution used in the experiments

Component*	Concentration, mg/L	TLV, mg/L***	Measurement range, mg/L
Total salt content	960.0		10.0–1000.0
Calcium	160.0	180.0	1.0–500.0
Magnesium	<3.0	40.0	1.0–50.0
Chloride	18.0	300.0	10.0–250.0
Sulfates	37.0	100.0	2.0–1400.0
Cyanides	44.1	0.05	0.005–500.0
	307.0**		
Thiocyanates	7.8	0.1	0.02–200.0
	9.1**		
Aluminum	1.4	0.04	0.010–50.0
Arsenic	0.013	0.05	0.0010–50.0
Bismuth	<0.01	0.1	0.010–10.0
Cadmium	<0.005	0.005	0.00010–10.0
Cobalt	0.064	0.01	0.0010–10.0
Copper	17.2	0.001	0.0010–50.0
Iron	<0.005	0.1	0.050–50.0
Manganese	<0.005	0.01	0.0010–10.0
Nickel	0.35	0.001	0.0010–10.0
Lead	<0.010	0.1	0.0010–10.0
Antimony	<0.005	0.05	0.0050–50.0
Zinc	0.010	0.01	0.0050–50.0

* All components, excluding cyanides and thiocyanates, were analyzed before the addition of concentrated solutions of NaCN and KSCN.
** The content of cyanides and thiocyanates after the addition of concentrated solutions of NaCN and KSCN.
*** TLV correspond to the values accepted in the fish industry.

ture of the ore mass was calculated as described previously [3].

Study of the diversity of microbial consortia. To determine microbial diversity, the total bacterial DNA was extracted with an AxyPrep Bacterial Genomic DNA kit (Axygen, United States). Amplification of the V4–V8 fragments of 16S rDNA was performed with the following primers: 5'-CCATCTCATCCCTG-CGTGTCTCCGAC-TCAG-*X*CCAGCAGCYGCG-GTAAN-3' and 5'-CCTATCCCCTGTGTGCCTTG-GCAGTC-TCAG-GACGGGCGGTGTGTACAAG-3', where *X* is a 10-nucleotide barcode unique for each sample.

Metagenomic sequencing of 16S rDNA fragments was carried out with GS Junior+ reagents on a 454 GS Junior System pyrosequencer (Roche, United States). Bioinformatic processing was performed with the RDP Pipeline service (https://pyro.cme.msu.edu) [9, 10]. A total of 5807 to 6685 sequences with an average read length of 498 bp were analyzed. Chimeric sequences, as well as sequences less than 300 bp in length, were removed from the analysis. Phylotypes (operational taxonomic unit (OTUs)) were separated when the cluster distance comprised 0.03.

RESULTS AND DISCUSSION

In the studied area of the natural-industrial complex, gold HL is used. At the industrial sites of the complex, prepared ore heap is irrigated with technological solution containing a high concentration of cyanide-containing compounds. To evaluate the biotechnological potential and diversity of autochthonous microbial consortia, the ore mass of the operating ore heap (Fig. 1a) and technological solution (Fig. 1b) were used.

Technological solution was supplemented with concentrated NaCN and KSCN solutions to increase the content of cyanides and thiocyanates from 44.1 to 307.0 mg/L and from 7.8 to 9.1 mg/L, respectively (Table 1). The ore mass was washed with the obtained technological solution (pH 12.1). The whole nonsterile and sterile mass ore mass had pH levels of 10.8 and 10.9, respectively (Table 2). Its moisture contained

Table 2. Concentrations of the components (mg/L) in the moisture of the ore mass of nonsterile and sterilized samples

Component	Nonsterile sample	Sterilized sample	TLV*, mg/L	Measurement range
Calcium	173.3	488.2	180.0	1.0–500.0
Magnesium	7.4	16.7	40.0	1.0–50.0
Chloride	<10.0	<10.0	300.0	10.0–250.0
Sulfates	932.9	1308.3	100.0	2.0–1400.0
Cyanides	81.6	83.0	0.05	0.005–500.0
Thiocyanates	13.3	15.6	0.1	0.02–200.0
Aluminum	1.42	0.21	0.04	0.010–50.0
Arsenic	1.33	1.07	0.05	0.0010–50.0
Bismuth	<0.010	<0.010	0.1	0.010–10.0
Cadmium	<0.005	<0.005	0.005	0.00010–10.0
Cobalt	0.20	0.27	0.01	0.0010–10.0
Copper	20.3	16.9	0.001	0.0010–50.0
Iron	0.50	0.67	0.1	0.050–50.0
Manganese	<0.005	<0.005	0.01	0.0010–10.0
Nickel	0.50	0.35	0.001	0.0010–10.0
Lead	<0.005	<0.005	0.1	0.0010–10.0
Antimony	<0.005	<0.005	0.05	0.0050–50.0
Zinc	0.08	<0.005	0.01	0.0050–50.0

* TLV correspond to the values accepted in the fish industry.

Table 3. Main parameters of the biodiversity of microbial consortia developing under natural conditions of natural-industrial complexes of HL and during the model experiment

Sample	Cultivation conditions	Sequences number	OTU	Diversity indexes	
				Chao 1	Shannon
S0	Primary ore mass	6685	51	79.9	1.5
S1	20°C; $+O_2$	5935	32	41.0	0.8
S2	4°C; $+O_2$	6352	41	56.0	1.3
S3	4°C; $-O_2$	5807	29	45.5	1.0
S4	−18°C; $+O_2$	6366	40	55.2	1.1

considerable concentrations of cyanide (1632.0 and 1660.0 TLV, respectively) and thiocyanate (133.0 and 156.0 TLV, respectively), as well as heavy metals, including aluminum, arsenic, cobalt, copper, iron, nickel, and zinc.

Autochthonous bacterial communities developing under the natural conditions of natural-industrial complexes of gold HL were characterized by low species diversity.

The OTU number was 51, whereas the species diversity according to the Chao 1 and Shannon indexes comprised 79.9 and 1.5, respectively (Table 3). Analysis of the α-diversity of the ore heap microbiome revealed that the species accumulation curves sharply raised and then reached a plateau (Fig. 2). These results confirmed that the number of sequences obtained in the present work was sufficient to characterize the biodiversity of the communities.

The phylotypes detected in the microbial consortium were assigned to eight phyla. Representatives of four phyla were predominant (*Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes*), whereas four phyla were minor (*Cyanobacteria*, *Acidobacteria*, *Verrucomicrobia*, and *Planctomycetes*). The share of the phylum *Proteobacteria* was the largest (99.22%) and *Beta-* and *Gammaproteobacteria* were the main classes (Table 4). Phylotypes identified at the genus level as representatives of *Serratia* (42.42% of total number of sequences), *Malikia* (1.84%), *Bordetella* (1.66%), and *Silanimonas* (1.39%), as well as representatives of *Comamonadaceae* (26.03%) and *Alcaligenaceae* (24.86%) at the family level, were predominant.

To evaluate the biotechnological potential of the autochthonous microbial communities of natural-industrial complexes, comparative analysis of the dynamics of cyanide destruction in nonsterile and sterilized ore mass was performed. Since the conditions in the ore heap were inhomogeneous due to zoning, the effects of positive and negative temperatures, as well as presence and absence of aeration, were studied.

Prolonged incubation of the ore mass resulted in a considerable decrease in the cyanide content, especially in nonsterile samples S1–S3. At negative temperatures, the cyanide content in both nonsterile and sterilized ore mass (S4) decreased insignificantly (from 82.0 and 83.0 to 70.0 mg/L, respectively) (Fig. 3a and 3b).

In nonsterile ore mass (S1–S2), the CN^- content decreased below the TLV after 65 and 140 days of incubation at positive temperatures (Fig. 3 a). In sterilized ore mass, the cyanide concentrations after 262 days of incubation under the same conditions were 40.0 and 70.0 mg/L (Fig. 3b). These results confirmed that the biological component had a more considerable effect on the destruction of cyanide-containing compounds than chemical transformations. It should be noted that absence of aeration during incubation also affected cyanide destruction. In nonsterile ore mass (S3) under anaerobic conditions, the cyanide concentration decreased 1.5 times more slowly than in presence of oxygen (Fig. 3a).

Since under the cyanide concentrations decreased below TLV after 65 days of incubation anaerobic in anaerobic conditions, it can be assumed that the autochthonous microbial community possessed a high biotechnological potential and played a significant role in cyanide detoxification under natural conditions. The main taxonomic groups of bacteria of the community potentially capable of cyanide destruction were revealed by metagenomic sequencing of the amplicons of V4–V8 variable regions of 16S rDNA.

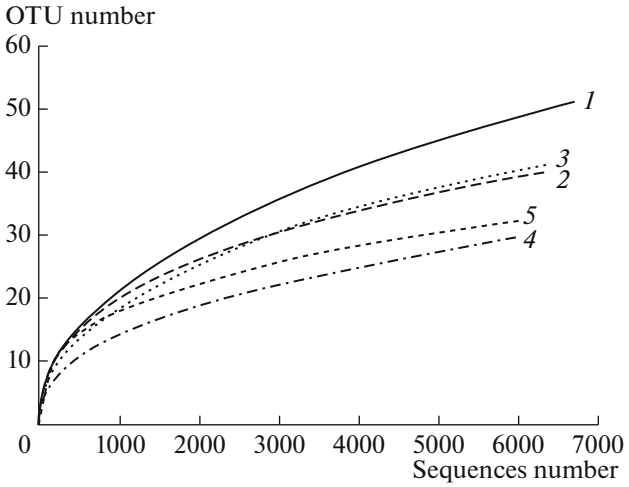

Fig. 2. Diversity of the microbiomes in nonsterile ore mass (*1*), after incubation under model conditions at 4°C with aeration (*2*), 18°C with aeration (*3*), 20°C with aeration (*4*), and 4°C without aeration (*5*).

The species accumulation curve and diversity indexes of microbial community are shown on Fig. 2 and in Table 3. Under model conditions of zoning of the ore heap of gold HL, indicators of species abundance and diversity were lower than those in the primary ore mass. When the ore mass was incubated with aeration at 4 and –18°C, the OTU numbers decreased to 41 and 40 and the Chao 1 index values decreased to 56.0 and 55.2, whereas Shannon index values were 1.3 and 1.1 (Table 3). In the ore mass, where intensive cyanide destruction occurred (20°C, $+O_2$), the phylotypic diversity was significantly lower than that in the primary ore mass (32 OTU) (Table 3). The lowest values of the parameters of species abundance and diversity were revealed for the microbial community cultivated in absence of aeration ($-O_2$) at 4°C. The OTU

Table 4. Share of predominant phylogenetic groups of bacterial consortia developing under natural conditions of natural-industrial complexes of HL and during the model experiment

Phylum/Class	Share, %				
	S0	S1	S2	S3	S4
Proteobacteria	99.22	98.63	99.56	99.68	99.31
Betaproteobacteria	53.38	89.20	53.01	37.55	40.82
Gammaproteobacteria	45.44	7.96	46.35	61.79	57.79
Alphaproteobacteria	0.36	1.33	0.16	0.30	0.46
Deltaproteobacteria	n.d.	n.d.	n.d.	n.d.	0.02
Actinobacteria	0.36	1.34	0.16	0.08	0.26
Firmicutes	0.25	n.d.	0.17	0.08	0.18
Bacteroidetes	0.10	n.d.	0.03	0.02	0.09
Unclassified sequences and minor phyla	0.07	0.03	0.08	0.14	0.15

n.d.—not detected.

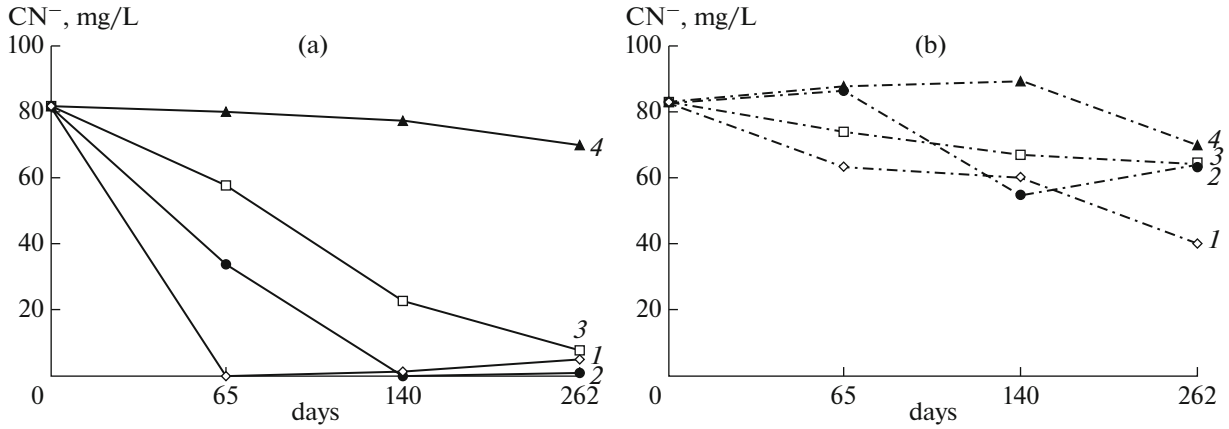

Fig. 3. Dynamics of cyanide destruction in nonsterile (a) and sterilized (b) ore mass after incubation under conditions of zoning at 20°C with aeration (*1*), 4°C with aeration (*2*), and without aeration (*3*), and at −18°C with aeration (*4*).

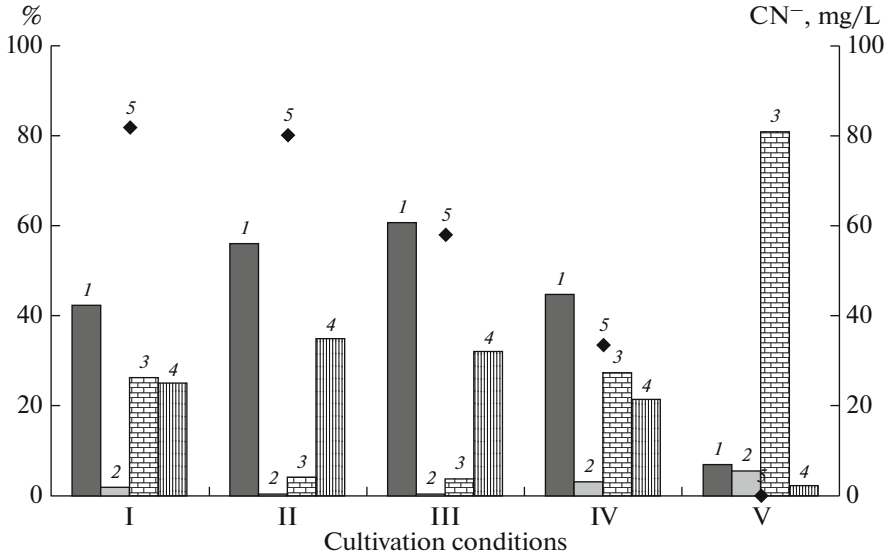

Fig. 4. Dynamics of cyanide destruction (*5*) after 65 days of incubation under model conditions of the primary ore mass (I), at −18°C with aeration (II), 4°C without aeration (III) and with aeration (IV), and at 20°C with aeration (V), as well as the share of the phylotypes of (%) *Serratia* (*1*), *Malikia* (*2*), *Comamonadaceae* (*3*), and *Alcaligenaceae* (*4*) predominant in microbial communities developing under the natural conditions and in the model medium.

number was 29, and the Chao 1 and Shannon indices comprised 45.5 and 1.0, respectively (Table 3). In this case, oxygen deficit caused the death of obligately aerobic bacteria and resulted in a considerable decrease in the species diversity of the autochthonous consortium and the efficiency of cyanide destruction.

The results demonstrated that only those representatives of a microbial community that were able to survive and demonstrate metabolic activity under certain environmental conditions developed under conditions of zoning of the ore heap.

The microbial consortium composition did not change in comparison to the initial composition under model zoning conditions of the HL ore heap after 65 days of incubation. Representatives of the same phyla were detected. *Proteobacteria* (Table. 4), as well as phylotypes of *Serratia*, *Malikia*, *Comamonadaceae,* and *Alcaligenaceae,* were predominant. The structure of the microbial consortia depended on the cultivation conditions. The proportions of *Alcaligenaceae* and *Serratia* decreased, whereas the shares of *Comamonadaceae* and *Malikia* increased during cultivation at 20°C (Fig. 4, V). Cultivation at negative temperatures (−18°C) or without aeration (4°C, $-O_2$) caused a change of the proportions of the main phylotypes in microbial communities. The bacterial consortium composition under aerobic conditions at 4°C was similar to that in the primary ore mass (Fig. 4, IV).

Comparison of the predominant phylotypes and dynamics of cyanide destruction demonstrated that, when the CN^- content decreased to TLV, the share of the phylotypes *Comamonadaceae* and *Malikia*

increased (Fig. 4). These results suggested that these bacteria were resistant to cyanide-containing compounds but did not confirm their considerable role in cyanide degradation. It is known that the absence of aeration resulted in inhibited growth of strict aerobes, including representatives of the genus *Malikia* [11], and a decrease in its share in the consortium. Cultivation under aerobic conditions at positive temperatures caused a decrease both in the cyanide concentration and the phylotypes *Serratia* and *Alcaligenaceae* (Fig. 4). It can be assumed that these representatives of autochthonous bacterial consortia were involved in the destruction of cyanide-containing compounds, which resulted in detoxification of the stored ore heap of HL.

At the present time, data on the diversity, composition, and structure of microbial consortia of HL waste are presented in a limited number of works (conducted mainly by foreign researchers) [6]. In our previous works [12, 13], we demonstrated the wide distribution of the certain representatives of the autochthonous microbial community in HL waste, regardless of geographic location, regional specificity, and the state of the ore heap.

CONCLUSIONS

Thus, it was shown that autochthonous microbial consortia of natural-industrial complexes of gold HL possessed high biotechnological potential and played a key role in the detoxification of cyanide-containing compounds. Representatives of the genus *Malikia* of the family *Comamonadaceae,* which are resistant to cyanide, as well as representatives of the genus *Serratia* and family *Alcaligenaceae*, which may be promising for the treatment of cyanide-containing HL waste, were detected in the communities.

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