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COMPOSITION AND ACTIVITY OF BACTERIAL COMMUNITY OF COAL TAILING

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The aim of this research was to study the composition of aboriginal bacterial community of coal tailing and to evaluate lixiviation activity of different groups of microorganisms belonging to this community. Using standard microbiological techniques we obtained and quantified the saving cultures of microorganisms from different physiological groups — filamentous fungi, heterotrophic microorganisms, mesophilic and thermophilic moderately acidophilic sulfur-oxidizing chemolithotrophic bacteria. Their oxidative activity was also established. The optimal results were achieved for collective leaching of rare and heavy metals into the solution under thermophilic conditions, which are favorable for the growth and activity of *Sulfobacillus* and under mesophilic conditions with the usage of ferrous iron as an energy substrate. This confirms the leading role of *A. ferrooxidans* in the processes of bacterial leaching of metals. Comparing our results with the available literature data we made a conclusion that the qualitative composition of acidophilic chemolithotrophic bacteria living in technogenic waste did not differ from the microbiocenose structure of natural sulfide ores.

Key words: coal tailing, the aboriginal bacterial community, chemolithotrophic acidophilic bacteria, leaching activity.

Coal tailing and piles of coal are dangerous for the environment but, on the other hand, they could be considered as significant industrial raw materials to obtain rare metals. There are more than 1,100 slagheaps in our country which contain more than 160 million tons of dangerous but significant raw materials [1, 2]. Biotechnological methods are the most promising to extract valuable components from coal tailing and to decrease its toxicity. Using these methods it is possible to process unprofitable depleted raw materials with the minimal resource costs and environment damage [3–5]. In this case it is necessary to carry out a complex chemico-biological evaluation of the initial substrate i.e. to study quantitative and qualitative composition, leaching activity of aboriginal bacterial community and possibility of selective isolation of active cultures of acidophilic chemolithotrophic bacteria.

The aim of this work was to study the composition of the aboriginal bacterial community living in coal tailing and to evaluate what contribution in leaching activity is made by different groups of microorganisms which are present there. This investigation is continuation of the work started earlier concerning the evaluation of quantitative and qualitative composition of microbiocenoses from energetic waste [9].

Materials and Methods

The object of investigation was the coal tailing forming as a result of coal processing which is used in mines of Lviv-Volyn coal basin by gravity and flotation methods at Central processing factory (CPF) "Chervonogradskaya" LTD "Lvivska coal company".

We investigated the aboriginal bacterial community living in technogenic raw materials. In particular, we developed conditions to detect a wide range of different microbiota representatives and to evaluate their participance in the process of metal leaching. To detect metals in solid substrates the quantitative analysis was carried out using an atomic emission spectrometer EMAC-200 CCD (Russia).

To detect different groups of microorganisms by the method of enrichment cultures we carried out the incubation of probes on corresponding nutrient media (Table 1).

Table 1. **Composition of media used to detect acidophilic chemolithotrophic bacteria**

Mineral components	Concentration of mineral components in nutrient media, g/dm^3	
	9K for *Acidithiobacillus*	Modified 9K* for *Sulfobacillus*
$NH_4(SO_4)_2$	3,0	0,45
KCl	0,1	0,05
K_2HPO_4	0,5	–
KH_2PO_4	–	0,05
$MgSO_4$	0,5	0,5
$Ca(NO_3)_2$	0,01	0,014
Na_2SO_4	–	0,15
Yeast extract	–	0,20
	Source of energy	
$Na_2S_2O_3$	5,0	–
S^0	2,0	–
$FeSO_4.7H_2O$	44,5	30,0

Note. Here and after: 9K* is 9K modified nutrient medium 9K for *Sulfobacillus*.

We used media 9K* and 9K for acidophilic chemolithotrophic bacteria, Gorbenko medium for heterotrophic microorganisms and Czapek medium for filamentous fungi [10–13].

Biomass accumulation of microbiocenosis representatives from the investigated coal tailing and definition of their leaching activity were carried out maintaining the ratio of the solid and liquid phases 1:10. To provide it we put into each flask 2.0g of the investigated substrate and add 20.0 ml of corresponding nutrient medium, which is preferable for the certain group of microorganisms. In the control experiments we carried out metal leaching from preliminarily autoclaved substrate by steril leaching solutions of the corresponding nutrient medium. Bacterial inoculations were incubated at 30.0±2.0 °C or 45.0±2.0 °C, pH ≤ 2.0 for 5 days. Microorganism development was tested by the changing of appearance of the savings culture (appearance of the light turbidity or layer on the surface) and by the change of both: colour and pH of the cultural liquid. The amount of acidophilic chemolithotrophic bacteria and heterotrophic bacteria after 5 days of cultivating was determined by the inoculation of bacterial suspensions (tenfold serial dilution) on the agar medium of the same composition. The amount of spore-forming bacteria was determined after preliminary thermal treatment at 80 °C for 15 min [10, 11].

Biogeochemical activity of bacterial community of heterotrophic and chemolithotrophic (mesophilic and moderately thermophilic acidophilic) bacteria living in technogenic waste was detected by the measurement of concentration of metals which transferred from the solid phase into cultivation medium. "The degree of metal extraction" was considered as the ratio (%) of the metal amount, which transferred into solution as a result of the contact of nutrient medium with the substrate in the presence of microorganisms, to the initial amount of this metal in the origin solid substrate. The full metal transition from the substrate into solution was taken as 100%.

The content of metals in solutions was detected by atomic absorption spectroscopy using AAS-1 (Germany) and C-11 PK Selmi (Ukraine) [14]. Micrographs of substrate samples were obtained by the scanning electron microscope Superprobe 733 JEOL (Japan) for X-ray microanalysis.

Statistical treatment of the data was performed using Student t-test. When the content of elements in the samples was determined the standard deviation for three repeated measurements did not exceed 0.024–0.078.

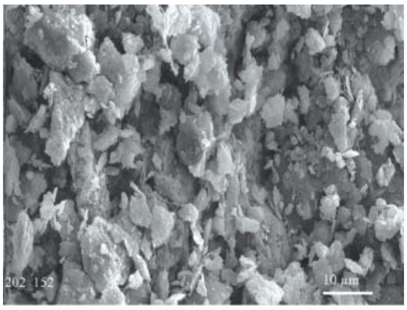
Results and Discussion

Chemical composition of the product is shown at Table 2 and Fig. 1.The initial substrate is a sufficiently crystallized solid layered argillaceous rock of silty mudstone (with a predominance of montmorillonite), kaolinite, mineral quartz sandstone type, pyrite with the content of carbon up to17.0%, sulfur up to 1.5% and organic mass up to 2.0%. Particle size distribution of the product is varied and represented in the vast majority of sufficiently big particles with sizes 5mm (34.3%) and 7 mm (18.6%). There are also smaller particles with sizes1mm (8.2%), 2 mm (11.7%) and 3 mm (15.6%). Quantitative analysis of the initial substrate showed that the main components of the coal tailing were rare and non-ferrous metals (germanium, gallium, plumbum, zinc, molybdenum, tungsten, stannum, beryllium, zirconium, bismuth, selenium, cadmium, mercury etc), their amount in some cases exceeded industrially significant concentration.

As a result of the work carried out using the standard microbiological methods we obtained savings cultures of microorganisms from different taxonomic groups-filamentous fungi, heterotrophic microorganisms, mesophilic and moderately thermophilic acidophilic chemolithotrophic sulfur-oxidizing bacteria. The results of determination of microbiocenosis composition in coal tailing are presented in table 3.

Despite the fact, that technogenic waste of power industry is considered as bioinert i.e. depleted by organic compounds we found a great number of heterotrophic microorganisms, spore-forming and nonspore forming in the coal tailings. The number of microscopic fungi is insignificant.

a



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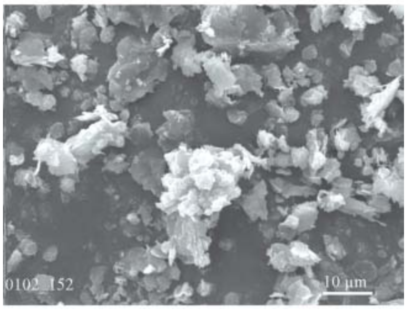

Fig. 1. **Micrographs of coal tailing:**
a — initial substrate;
b — the substrate after microbiological processing

Under acidophilic conditions the aboriginal bacterial community is presented by chemolithotrophic bacteria from *Acidithiobacillus*, *Sulfobacillus*, *Leptospirillum* and *Acidimicrobium* as well as by archaebacteria which belong to *Acidianus*, *Sulfolobus* and *Ferroplasma* [6, 7, 15–17]. The obtained results let us suggest that in the investigated substrate from coal tailing there are representatives of *Acidithiobacillus*, mesophilic and moderately thermophilic. The wide range of the oxidizable sources of energy confirms the presence of A. *ferrooxidans* bacteria which are able to use as the substrate elemental sulfur, all its reduced compounds (S^0, SO_3^{2-} and other), ferrous oxide and natural sulfide mineral [5, 8, 18–20]. The number of aboriginal representatives from this group of microorganisms, isolated on the medium 9K with the different sources of energy under mesophilic conditions, was insignificant and did not exceed 10^4 CFU /ml. As opposed to *A. ferrooxidans*, *A. thiooxidans* bacteria which are able to oxidize only reduced sulfur compounds (thiosulfate and sulfur) [5, 8, 21] from the investigated mineral raw material under mesophilic conditions also were isolated with the titre not exceeding 10^4 CFU/ml. The largest number of microorganisms (10^8 CFU/ml) was isolated under thermophilic conditions on the media 9K with thiosulfate and 9K* with ferrous, that indicates the presence of *A. caldus* and *Sulfobacillus* in the aboriginal bacterial community. The obtained results are in accordance with the literature data which confirm the presence of sulfobacilli in the same ecological niche as thiobacteria i.e. in the piles of sulfide ores, acidic hydrothermal and mine water (where the temperature can reach 60 °C) which are capable to oxidize sulfur compounds, Fe^{2+} and sulfide minerals [5, 8, 13, 22].

At the next stage of our investigation it was studied leaching activity of some representatives of microbiocenosis which was formed in the coal tailing. It has to be noted that in all control experiments the leaching metals into the solution was not observed, the degree of metal recovery did not exceed 0.5–1.0%. When the degree of metal recovery was determined in case of heterotrophic spore-forming and nonspore forming microorganisms living in the coal tailings (Fig. 2) the steril Gorbenko medium was served as a control (control 1 and control 2); in case of filamentous fungi we used the steril Czapek medium (control 3). In spite of active

Table 2. **Metal concentration in the coal tailing**

Element	Industrial concentration [1, 2], mg/kg	Detected concentration, mg/kg
Copper	45,0–60,0	62,18
Zinc	65,0–70,0	112,52
Manganese	850–10^3	317,72
Plumbum	18,0–22,0	42,20
Nickel	80,0–120,0	134,20
Cadmium	45,0–55,0	2,82
Iron	$(1{,}5–2{,}0)\cdot10^3$	$44{,}57\cdot10^3$
Gallium	10,0–15,0	12,10
Germanium	5,0–7,0	26,00
Stannum	90,0–120,0	351,9
Chromium	190,0–210,0	99,1
Vanadium	140,0–160,0	150,0
Cobalt	37,0–42,0	116,1
Aluminium	$(2{,}5–5{,}0)\cdot10^3$	$13{,}9\cdot10^3$
Silicon	–	$159{,}0\cdot10^3$
Zirconium	160,0–220,0	173,0
Niobium	19,0–22,0	14,0
Lanthanum	25,0–29,0	48,0
Cerium	25,0	69,0
Rubidium	90,0	141,0
Strontium	80,0	211,0
Barium	250,0–400,0	519,0
Titanium	$4{,}0\cdot10^3$	$4{,}2\cdot10^3$
Calcium	–	$17{,}2\cdot10^3$

Table 3. **Quantitative and qualitative composition of microbiocenosis in coal tailing (colony forming units-CFU/ml of savings cultures)**

Heterotrophic microorganisms			Acidophilic chemolithotrophic bacteria				
			Mesophilic bacteria oxidazing different sources of energy — *Acidithiobacillus*			Moderately thermophilic bacteria	
						Sulfobacillus	*Acidithiobacillus*
Gorbenko medium		Czapek medium	Medium 9K			Medium 9K*	Medium 9K
			Source of energy				
Spore-forming bacteria	Nonspore forming bacteria	Filamentous fungi	$Na_2S_2O_3$	S^0	Fe^{+2}	Fe^{+2}	$Na_2S_2O_3$
$1{,}9 \pm 0{,}24\times10^6$	$1{,}2 \pm 0{,}28\times10^7$	$5{,}0 \pm 0{,}32\times10^3$	$5{,}3 \pm 0{,}43\times10^4$	$1{,}6 \pm 0{,}24\times10^4$	$6{,}4 \pm 0{,}64\times10^4$	$7{,}4 \pm 0{,}37\times10^8$	$8{,}9 \pm 0{,}59\times10^8$

growth of the heterotrophic microorganisms from the aborginal community, their contribution into destruction of crystalline structure of investigated coal tailing and following metal leaching is negligible and hardly reaches 15.0% (Fig. 2). The highest relative activity was shown in case of spore-forming microorganisms. The highest degree of metal recovery was the following: 22% of Germanium and 28% of Cadmium.

The aboriginal representatives from *Acidithiobacillus* (mesophilic and moderately thermophilic) and *Sulfobacillus* microorganisms possess the high oxidizing activity towards the majority of registered metals in the investigated coal tailing (Fig. 3, 4). When the degree of metal recovery was determined in case of mesophilic acidophilic chemolithotrophic bacteria living in the coal tailings (Fig. 3) the steril 9K medium was served as a control : in

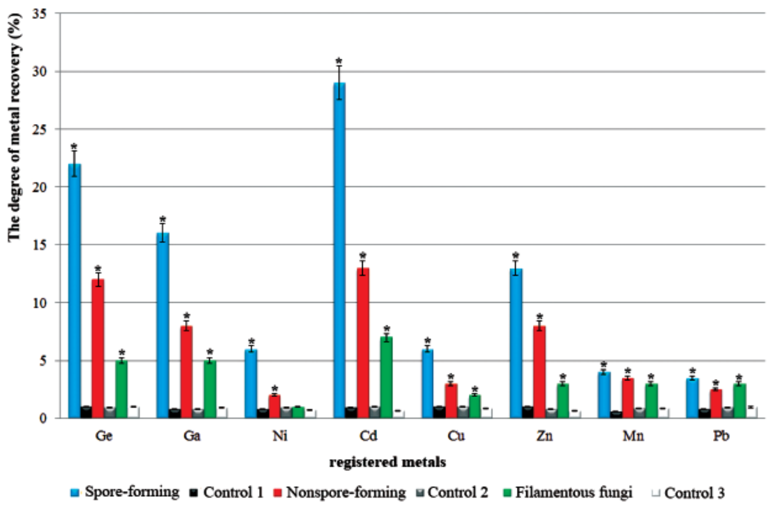

Fig. 2. **The degree of metal recovery (%) by the association of heterotrophic bacteria living in coal tailing.**
Here and after: * — $P < 0.05$ compared to the control; in all control experiments the leaching of metals into the solution was not observed, the degree of metal recovery did not exceed 0.5–1.0%.

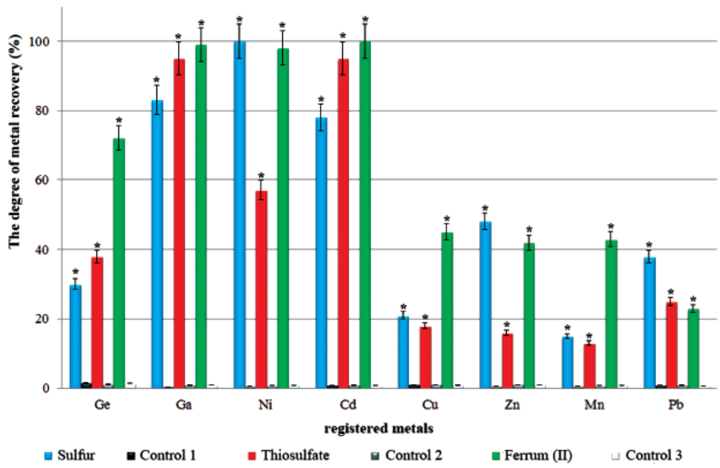

Fig. 3. **The degree of metal recovery (%) by the association of mesophilic acidophilic chemolithotrophic bacteria living in the coal tailings**

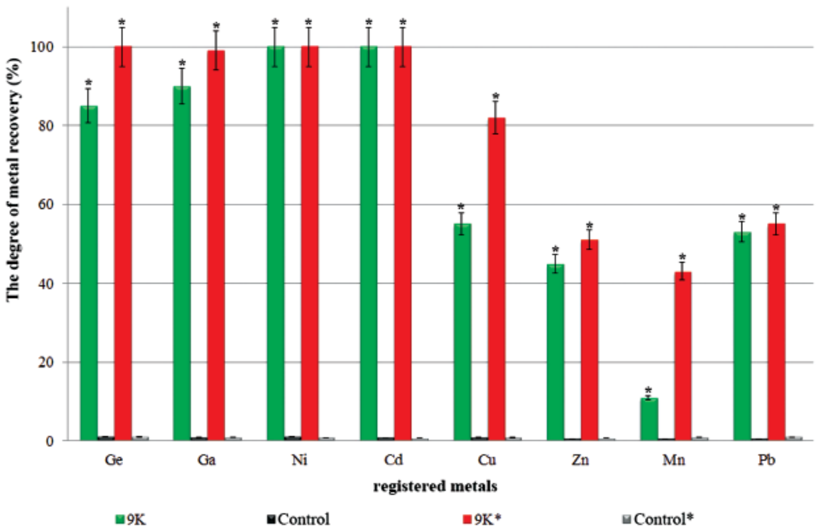

Fig. 4. **The degree of metal recovery (%) by the association of moderately thermophilic acidophilic chemolithotrophic bacteria living in the coal tailings**

case of S^0 as a source of energy-control 1,in case of $Na_2S_2O_3$-control 2, in case of $FeSO_4 \cdot 7H_2O$-control 3. When the degree of metal recovery was determined in case of moderately thermophilic acidophilic chemolithotrophic bacteria living in the coal tailings (Fig. 4) the steril 9K medium with $Na_2S_2O_3$ was served as the control and the steril 9K* medium with $FeSO_4 \cdot 7H_2O$ as the control*.The optimal results of collective metal leaching into solution of rare and heavy metals were achieved under mesophilic conditions with the usage of ferrous as a source of energy. It confirms the leading role of A.*ferrooxidans* in the processes of bacterial metal leaching [5, 6, 18–21].

The optimal results were achieved under thermophilic conditions with the usage of 9K* with ferrous as a nutrient medium and a source of energy. It confirms the high oxidizing activity of *Sulfobacillus* microorganisms. The obtained data are in accordance with the previous literature data concerning sulfobacillus ability as well as thiobacteria to participate in the degradation of natural ores and coal tailing resulting in leaching metals in the solution [5, 8, 13].

So, it was established that in the process of formation of coal tailings, their warehousing and keeping under influence of certain technogenic and natural factors the highly efficient aboriginal bacterial community is formed. It was mainly presented by heterotrophic and acidophilic chemolithotrophic bacteria. Their high oxidizing activity allows us to leach metals from the coal tailing with high efficiency. This is accompanied by destruction of sufficiently stable structures, the void formation and the increase of substrate amorphous after microbiological treatment (Fig. 1, *b*). These results complement our earlier assessment data concerning qualitative and quantitative composition of microbiocenoses from energy waste [9]. Comparing the obtained results with the literature data we made a conclusion that qualitative composition of acidophilic chemolithotrophic bacteria living in technogenic waste does not differ from the structure of microbiocenoses from the natural sulfic ores [3,6-8, 15, 16, 20, 21].

The obtained results concerning dominating microorganisms in aboriginal bacterial community and their leaching activity are highly important to develop and optimize the highly efficient biotechnological methods of metal recovery from the mineral materials of technogenic origin.

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СКЛАД Й АКТИВНІСТЬ БАКТЕРІАЛЬНОГО УГРУПОВАННЯ ВІДХОДІВ ЗБАГАЧЕННЯ ВУГІЛЛЯ

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Метою роботи було вивчення складу аборигенного бактеріального угруповання, що населяє відходи збагачення вугілля, а також оцінювання впливу на активність вилуговування різних груп мікроорганізмів, що входять до його складу. Мікробіологічними методами одержано й кількісно оцінено накопичувальні культури мікроорганізмів різних фізіологічних груп — міцеліальних грибів, гетеротрофних мікроорганізмів, мезофільних і помірно термофільних ацидофільних хемолітотрофних сіркоокиснювальних бактерій, встановлено їхню окиснювальну активність. Оптимальні результати колективного вилуговування в розчин як рідкісних, так і важких металів отримано за термофільних умов, сприятливих для росту й активності представників роду *Sulfobacillus*, а також мезофільних — з використанням як енергетичного субстрату двовалентного заліза, що підтверджує провідну роль *A. ferrooxidans* у процесах бактеріального вилуговування металів. Результати власних досліджень і дані літератури свідчить про те, що якісний склад ацидофільних хемолітотрофних бактерій, що мешкають у техногенних відходах енергетики, практично не відрізняється від структури мікробіоценозів природних сульфідних руд.

Ключові слова: відходи збагачення вугілля, ацидофільні хемолітотрофні бактерії, бактеріальне вилуговування металів.

СОСТАВ И АКТИВНОСТЬ БАКТЕРИАЛЬНОГО СООБЩЕСТВА ОТХОДОВ УГЛЕОБОГАЩЕНИЯ

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Целью работы было изучение состава аборигенного бактериального сообщества, населяющего отвалы углеобогащения, а также влияния на выщелачивающую активность различных групп микроорганизмов, входящих в его состав. Микробиологическими методами получены и количественно оценены накопительные культуры микроорганизмов различных физиологических групп — мицелиальных грибов, гетеротрофных микроорганизмов, мезофильных и умеренно термофильных ацидофильных хемолитотрофных сероокисляющих бактерий, установлена их окислительная активность. Оптимальные результаты совместного выщелачивания в раствор как редких, так и тяжелых металлов были получены в термофильных условиях, благоприятных для роста и активности представителей рода *Sulfobacillus*, а также в мезофильных — при использовании в качестве энергетического субстрата двухвалентного железа, что подтверждает ведущую роль *A. ferrooxidans* в процессах бактериального выщелачивания металлов. Результаты собственных исследований и данные литературы свидетельствует о том, что качественный состав ацидофильных хемолитотрофных бактерий, обитающих в техногенных отходах энергетики, практически не отличается от структуры микробиоценозов природных сульфидных руд.

Ключевые слова: отвалы углеобогащения, ацидофильные хемолитотрофные бактерии, бактериальное выщелачивание металлов.