Original Research

Bioleaching of Metals from Wastes of Phosphoric Fertilizers Production

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Abstract

Phosphorus-containing wastes formed after the production of phosphorus fertilizers in Shymkent, due to intensive urbanization were within the city limits and for reason of processes of water-wind erosion began to pose a serious threat to the environment and the population of the city. It was found that the formation of the fractional composition of wastes was influenced by the conditions of processing of raw materials, weather and climatic parameters and time characteristics of storage. The greater part of the waste are silicate and calcium-containing compounds. One of the options for waste disposal is considered the possibility of bio-leaching of valuable components from it. The choice of phase 1 nitrifies and micromycetes is justified by neutral or slightly acidic pH values of their cultivation, which will allow to abandon the use of acidophilic bacteria or sulfuric acid leaching. A variant using a strain of acidophilic bacteria *Alicyclobacillus tolerans ST* confirmed the effectiveness of their use in the bioleaching of magnesium, aluminum, potassium, calcium, phosphorus, REE such as lanthanum, cerium and neodymium. The use of micromycete *Aspergillus niger AsIA* proves its effectiveness in the extraction of zirconium and silver. The use of nitrifying bacteria *Methyloversatilis thermotolerans* MSO increases the extraction of barium and iron.

Keywords: bioleaching, phosphorus containing waste, *Alicyclobacillus tolerans*, *Aspergillus niger*, *Methyloversatilis thermotolerans*

Introduction

Mining and processing of mineral metal-containing raw materials is aimed, mainly, on the extraction of one component. As a result, most of the valuable metals go to wastes. At the same time, intensive urbanization processes lead to the fact that the boundaries of residential places are gradually beginning to absorb the territory of industrial wastes storage. Gradually the accumulated and stored in the open-air wastes begin to represent a serious threat to the environment and, in particular, to public health. At the same time, wastes are the complex of valuable components suitable for further use.

It is known a number of studies devoted to the behavior of rare earth elements in phosphorite ores [1, 2] and natural water [3-5]. It was investigated the possibility of rare earth elements composition obtaining from deposits in the Southwestern Sinai, the characterization of the residue shows the presence of

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uranium, rare-earth elements (REE), aluminum, and zinc that can be valorized [6].

From the known phosphate raw materials that are processed for fertilizers, apatite concentrates containing about 0.9% of rare earth elements [7, 8] is of the greatest practical value as a source of rare earth elements. In phosphorites, the content of rare earth elements is significantly lower and usually does not exceed 0.1% and their extraction is associated with certain technological difficulties [9, 10].

According to [11] one of the cost-effective and environmentally friendly methods of processing low-grade raw materials, which can include waste processing of ores, is bioleaching. However, it is known that the biological leaching allows extracting of REE from raw materials by using of microorganisms composition that destroy the metal-containing minerals by oxidizing of ferrous iron to ferric iron, and sulfur of mineral sulfides to sulfuric acid and converting the metals from the minerals into solution. Under mesophilic conditions, acidophilic thionic bacteria, which are used in hydrometallurgy, play a leading role in oxidative processes [12-14]. Most commonly used for bioleaching are the bacteria *Acidithiobacillus ferrooxidans* [11, 15, 16]. The range of used in biohydrometallurgy microorganisms involves the application of *Chromobacterium violaceum* [17], *Sulfobacillust hermosulfidooxidans* [18, 19], *Acidiphilium multivorum, Leptospirillum ferrooxidans* [19, 20], *Aspergillus niger* [21]. At using of bacterial and micromycete cultures for the bioleaching of REE from phosphorus-containing wastes it was established the direct relationship between the volume of citric acid produced by the strain of *Gluconobacteroxydans FCC* and the degree of

extraction of lanthanum [22]. There are studies related to the development of technology for the extraction of rare earth elements from phosphogypsum *in situ* [23]. On the other hand, at cultivation of microorganisms for bioleaching different nutrient media are used, which are certain solutions of salts with different pH values and which can also affect the metals, behavior.

More than 50.0 million tons of phosphorus-containing wastes are stored on the territory of the South Kazakhstan region near Shymkent city, which, due to erosion processes, represent a serious threat to the environment and public health. The solution of this problem is important not only for Kazakhstan, but also for a number of countries with phosphorus-containing waste in their territories. An especially acute environmental problem arises in the presence of concomitant radioactive elements, which complicates the implementation of waste recycling processes. In studies by Mäkinen et al. [2] the possibility of preliminary selective separation of uranium from phosphorus-containing apatite ores with uranium impurities has been shown. It was found that the mixture used is fluorapatite and standard uranium ore and requires 24-hour sulfuric acid leaching at pH = 1 to obtain a 100% phosphorus yield. At the same time, 89% of uranium is dissolved in 3 days at pH>2, and leaching is improved by the use of Fe^{3+} oxidizer. The maximum 95% yield of uranium was observed with prolonged 28-day leaching. These studies show one of the ways to solve the problem of utilization of phosphorus-containing slags. The possibility of reducing the processing time of metal-containing waste using an environmentally friendly method is shown in [12]. The authors argue that direct biological leaching of waste light-emitting

Fig. 1. Place of storage of phosphorus-containing waste in Shymkent: the markers shows the location of the sampling sites A, B, C, D (Google Earth, The view from the height: 2.24 km).

diodes (WLED) provides low yields of copper, nickel, and gallium, while detection of all metals is improved by phased indirect biological leaching. At a pulp density of 2g/mL, the recovery efficiency of copper, Nickel and gallium is 83%, 97% and 84%, respectively. The leaching time was reduced from 30 days to 15 days. From a technological point of view, the study showed that step-by-step indirect bioleaching with biogenic iron results in maximum recovery of valuable metals from WLED at low cost and through a short, simple and environmentally friendly process.

Each study shows the role of microorganisms in metal bioleaching processes, where microbial-free nutrient media variants or chemical analogues of microbial metabolites are used as controls. In our work the aim was to study the role of different ecological groups of microorganisms on the processes of extraction of metals from phosphorus-containing slags in Shymkent.

Material and Methods

The object of the study is phosphorus-containing wastes, the dumps of which in the amount of 50.0 million tons are located on the territory of Shymkent city (Fig. 1). Coordinates of the waste storage site: e.l. 42°15'42.92'', n.l. 69°43'15.57''. Height above sea level: 619 m. Visually, phosphorus-containing slags can be divided into 4 conditional groups: A-light loose, B-dark loose, C-dark dense, D-light dense (Fig. 2).

The studies used strains of sulfur-oxidizing bacteria – *Alicyclobacillus tolerans* ST (sin. *Sulfobacillus thermosulfidooxidans*), micromycete *Aspergillus niger AsIA*, bacterium *Methyloversatilis thermotolerans MSO* in the order *Nitrosomadales*.

Determination of Fe^{+2} and Fe^{+3} was carried out by a volumetric trilonometric method in technical solutions, which was distributed to measure the iron content in solutions in the range from 0.1-10 g/l [24].

Inductively coupled plasma spectrometer with mass spectrometric detection of Varian–820 MS [25,26].

The elemental composition was determined by the method of atomic adsorption analysis on the spectrometer AAnalyst 800 (Perkin-Elmer) [27].

X-ray diffractometric analysis was carried out on the automated diffractometer DRON-4 with Cu Kα – radiation, β-filter. Conditions for recording of diffraction patterns are: U = 35 kV; I = 20 mA; scale: 2000 impulse; time constant 2s; recording of theta-2theta; detector 2 deg/min [28].

Video fixation of the material was carried out with using of video card of electron-raster microscope JSM 649LV produced by the company JEOL (Japan) with the system of energy dispersive microanalysis INCA Energy 350 of the firm Oxford instruments (UK), associated with the system of structural analysis of polycrystalline objects HKL Basis.

Microbiological Examination

Sampling of water and ore was carried out by aseptic special samplers in accordance with the guidelines from the depths of 20-40 cm.

For the cultivation of sulfur-oxidizing bacteria used Vaxman's medium (g\l water): $(NH_4)_2SO_4$ – 0.2; $MgSO_4 \times 7H_2O$ – 0.5; KH_2PO_4 – 3.0; $CaCl_2$ $6H_2O$ – 0.25; $FeSO_4 \times 7H_2O$ – 0.,0001, $S°$ – 10. pH 3,0. For the cultivation of micromycetes Chapek's medium was used, g/l of water: sucrose – 30.0 or glucose – 20.0; $NaNO_3$ – 2.0; K_2HPO_4 – 1.0; $MgSO_4 \times 7H_2O$ – 0.5; KCl – 0.5; $FeSO_4 \times 7H_2O$ – 0.1. For cultivation of 1 phase nitrifying bacteria the Vinogradsky`s medium was used, g/l of water: glucose – 20.0; K_2HPO_4. 1.0; $MgSO_4 \times 7H_2O$ – 0.5; $CaCO_3$ – 20.0; yeast extract –10.0; the solution of microelements – 1.0 ml.

The bacteria required for biological leaching were inoculated on the selective nutrient medium in the thermostat of the brand №TS-1/80 SPU TU 9452-002-00141798-97. The process of cultivation was continuously aerated. At the nutrient medium preparation the scale of the brand "Scout–Pro" was used, and for sterilization the autoclave of the brand SPGA–100–I–HHwas used. Cultivation of microorganisms was carried out at 25ºC for 7 days.

Molecular Genetic Identification of Bacteria Strains

Taxonomic analysis of isolated microorganisms was carried out by PCR analysis. Genomic DNA was isolated from diurnal bacterial cultures using the PureLink Genomic DNA Kit according to the manufacturer's protocol (Invitrogen, Carlsbad, USA). The concentration of DNA and the PCR product in the samples was determined on a Qubit® 2.0 fluorimeter
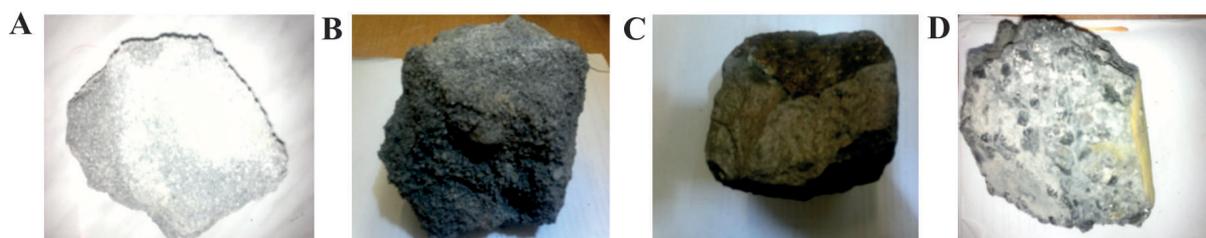

Fig. 2. Types of phosphorus containing wastes.

using the Qubit TMds DNA HS Assay Kit (Life Technologies, Oregon, USA).

PCR was performed on an Eppendorf ProS amplifier (Hamburg, Germany) with an amplification mode: 94ºC – 30 sec; 55ºC – 1 min; 72ºC – 40 sec. – only 30 cycles; 72ºC – 10 min. Amplification results were viewed on a 1.2% agarose gel. PCR products were purified by CleanSweep ™ PCR Purification reagent (ApplideBiosystems, USA).

The Experiment Scheme

Under model conditions, the influence of the composition of elective medium and microorganism's monocultures on the processes of metals leaching from phosphorus-containing wastes in percolation mode was studied. The experiment was carried out according to the scheme:

1. Nutrient medium of Vaxman
2. Nutrient medium of Vaxman +*Alicyclobacillustolerans ST*;
3. H_2SO_4 -3,0 g/l.
4. Chapek's medium;
5. Chapek's medium + *Asp. niger AsJA*
6. Vinogradsky medium;
7. Vinogradsky medium + *Methyloversatilis thermotolerans MSO+Pseudomonas stutzeri NJA*.

Exposure period – 30 days, the ratio S: L = 1:3. Temperature: 22-24ºC. The titer of microorganisms- 10^7-10^9cell/ml. The experiment was carried out in glass percolators.

Statistical Analysis of the Results

Experiments were carried out five times in repetition, calculate the standard 0.95>P>0.80. Statistical processing was performed using the statistical software package Microsoft Excel. By the number of measurements and in general diagnostic group determined the arithmetic mean [29].

Results and Discussion

The problem of recycling of metal-containing waste remains one of the acute environmental problems in many countries of the world. Of particular relevance is the issue of waste processing located near settlements. Such a picture has developed in the South of Kazakhstan, where due to intensive urbanization, the former territory of the plant for processing phosphorus-containing raw materials into fertilizer was in the border of the city of Shymkent. Currently, an open landfill of phosphorus-containing waste with a volume of more than 50.0 million tons and occupying an area of more than 16.0 hectares poses a serious threat to the environment due to the processes of water and wind erosion, as well as the close location of underground and surface water sources. One of the first steps of studying
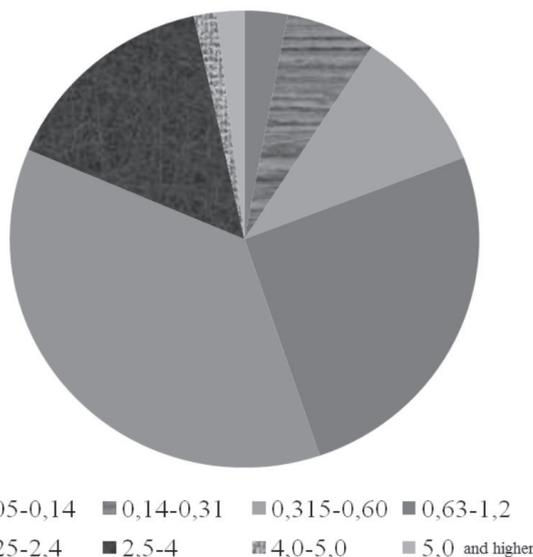

Fig. 3. Fractional composition of granulated phosphorus-containing wastes [%].

this problem was the study of physical and chemical characteristics of phosphorus-containing wastes.

The fractional composition of phosphorus-containing wastes at the storage sites was studied for research work. It is established that the granulated wastes differ by the dominant fractions (Fig. 3), while their formation is influenced not only by the technological parameters of the raw materials processing, but also by weather and climatic conditions and time characteristics of storage. At the storage sites, the dumps of phosphorus-containing wastes are represented by the exhausted ores and pelletized slag, visually dominated by samples of size 10.0-18.0 cm, occasionally samples of size up to 30.0 cm are marked. The mineralogical structure of the wastes is presented in Table 1.

The results show that the dominant part of the wastes consists of silicate and calcium-containing minerals. These minerals are represented by pseudowollastonite (a-CaOxSiO$_2$), cuspidine (3CaOxCaF$_2$x2SiO$_2$), ferrophosphorus Fe$_2$P, melilite – Ca$_2$(Al, MgSi) Si$_2$O$_7$, akermanite – Ca$_2$MgSi$_2$O$_7$, rankinite 3CaOx2SiO$_2$, fluorapatite – Ca$_5$(PO$_4$)3F, whitlockite (NaF), fluorite (CaF$_2$) and silicocarnotite (5CaOxP$_2$O$_5$xSiO$_2$).

The elemental composition of the wastes varies in a wide range and depends on various factors such as the depth of wastes position in the dumps, the origin and type of wastes, the susceptibility to erosion processes, etc. The results of REM showed that the studied wastes differ not only visually, but also in the internal structure and content of individual elements (Fig. 4). So the content of such prevalent elements as phosphorus is found in the range of 0.50 to 21.95 weight %, silicon content ranges from 1.16 to 13.49 weight %; calcium content ranges from 0.42 to 25.37 weight %, iron content is from 0.26 to 59.71 weight %. Aluminum is presented more evenly in different types of wastes

Table 1. Mineralogical structure of phosphoric wastes [wt %].

Compact wastes:								
SiO_2	CaO	Al_2O_3	MgO	FeO	P_2O_5	SO_3	F	S
40.20±2.81	48.46±4.22	0.8±0.05	4.07±0.41	From trace till 0.30	1.28±0.12	No trace	2.85±0.22	No trace
Granulated wastes								
42.68±4.05	45.17±3.9	2.88±0.21	3.56±0.29	from 0.00 till 0.58	0.82±0.01	from 0.00 till 1.42	from 0.00 till 1.95	from 0.00 till 0.51

Table 2. Elemental composition of phosphorus-containing wastes [ppb].

Elements	A	B	C	D
Te 125	1.23	1.28	0.42	1.20
La 139	282.85	462.72	8.03	14.83
Ce 140	283.49	480.68	17.13	18.60
Nd 146	222.60	379.02	12.94	12.87
Tm 169	2.74	4.78	0.05	0.15
Ta 181	0.72	1.61	3.20	1.48
Tl 205	0.61	0.96	0.10	0.30
Th 232	10.61	20.09	0.23	1.33
U 238	42.44	68.07	259.39	8.42

from 0.31 to 1.2 weight %, potassium is presented from 0.02 to 0.46 weight %. Titanium, vanadium and chromium were observed only in one sample in the amount of 0.99; 0.25 and 0.37 weight % respectively. In all samples of phosphorus-containing wastes some rare earth elements were discovered, the amount of which ranges: lanthanum – from 8.03 to 462.71 ppb; cerium – from 17.13 to 480.68 ppb; neodymium – from 12.87 to 379.02 ppb; thulium – from 0.05 to 4.78 ppb. In addition, such actinoids as thorium, the content of which

in samples varies from 0.23 to 20.09 ppb and uranium, the content of which in samples varies from 8.42-259.39 ppb, were noted. The contents of the individual elements are presented in Table 2.

The strains of microorganisms used in the studies are characterized by the following features: *Alicyclobacillus tolerans ST* (*Sulfobacillus thermosulfidooxidans*): is gram-positive, strictly aerobic bacterium. The bacteria are acidophilic and produced endospores. The optimum growth temperature for *A. tolerans ST* is 37-42ºC, and can grow in the 20-50ºC range. The optimum pH is 2.5-2.7, and can grow in pH 1.5-5.0. It was first isolated from oxidizable lead-zinc ores in Uzbekistan [30, 31].

Methyloversatilis thermotolerans MSO – species from Nitrosomonale family, cells are Gram-stain-negative, non-motile rods (0.3–0.4 × 1.2–2.6 μm), that multiply by binary fission.

Aspergillus niger AsIA: the colonies color is from pure white to slightly yellowish, appearance is from woolly to velvety. The growth rate is moderate. The hyphae are septate and colorless. Conidial heads are spherical, with radial arrangement of conidia chains.

The main idea of the research was to study the role of isolated strains of microorganisms in the bioleaching of valuable components from phosphorus-containing slags of Shymkent, as was written above. The role of sulfobacteria in the bioleaching of a number of metals from both ores and man-made waste has, in fact, been studied comprehensively [32], therefore, the variant
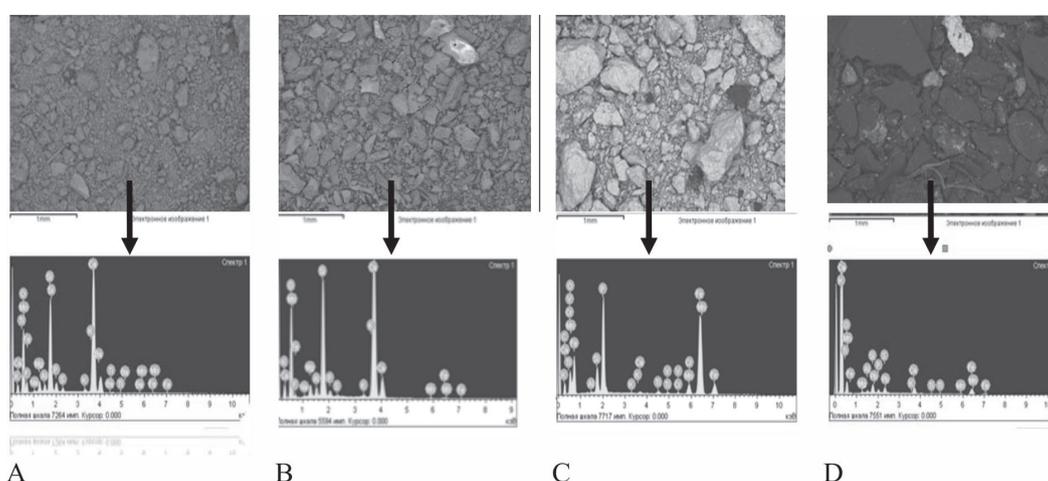

A   B   C   D
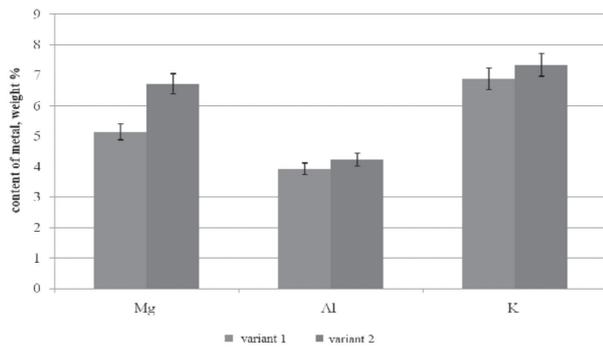
Fig. 4. The results of REM analysis.

Fig. 5. The influence of medium 9K (variant 1) and *Alicyclobacillus tolerans ST* (variant 2) on the extraction of magnesium, aluminum and potassium from phosphorus-containing solutions.

Fig. 7. Extraction of barium and iron with the use of the elective Chapek's medium and nitrifying bacteria *Methyloversatilis thermotolerans MSO*.

using the *Alicyclobacillus tolerans ST* strain in our studies is used only to confirm the indisputable role of this microorganism in the processes of bioleaching of phosphorus-containing waste in acidophilic conditions. Really, the use of acidophilic bacteria *Alicyclobacillus tolerans ST* allow to increase the yield of magnesium, aluminum and potassium ions into the productive solution on 30.64%; 7.58% and 6.45% respectively, in comparison with variant where only the elective medium is used (Fig. 5). In addition, at using of sulfobacteria the increase in the yield of calcium and phosphorus into the solution on 6.00% and 17.10%, respectively, is noted.

On the other hand, it is known that acidophilic conditions with a pH of 1.5-3.5 are necessary for bioleaching of metal-containing substrates with the help of thio and sulfobacteria [33]. The acidity of the medium is maintained with the help of sulfuric acid added or formed as a result of the metabolism of sulfobacteria. This is probably one of the downsides of using thio and sulfobacteria in biohydrometallurgy. This issue may be particularly acute in the case of possible bioleaching of phosphorus-containing slags in Shymkent due to the close location of settlements with surface and underground sources of water resources.

The analysis of the literature data shows versatile studies of the possibility of using microscopic *Aspergillus* fungi for the bio-recovery of metals from
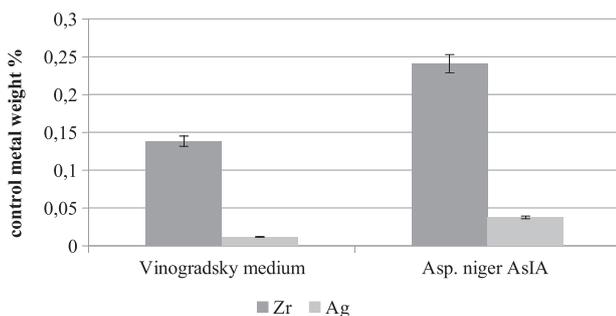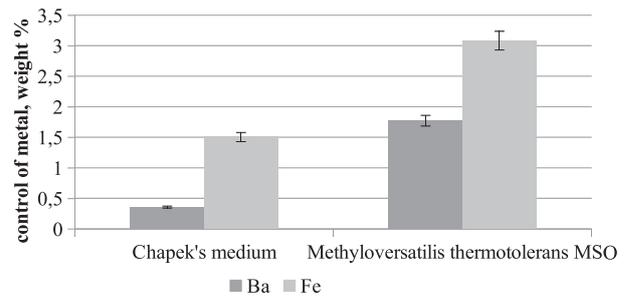
metal-containing waste [34]. The advantages of using this group of microorganisms include the availability and cheapness of cultivation, rapid adaptation to changing environmental conditions, optimum pH of 4.0 and above. In addition, the isolation of *Aspergillus niger AsIA* strain with confirmed characteristics of non-pathogenicity and non-allergenicity for humans and warm-blooded animals allowed us to consider this micromycete as a promising microorganism for biogeotechnological purposes. The use of micromycetes *Asp. niger AsIA* is effective at the extraction of metals of value such as zirconium and silver, at that the increase of these metals yield into solution in the variant with use of micromycetes is 74.11% and 18.76%, respectively (Fig. 6).

In addition, the role of a group of bacteria from the *Nitrosomonales* family, which is still little known in biohydrometallurgy, was studied in this study. Nitrifying bacteria of phase 1 nitrification participate in the biogeochemical nitrogen cycle in Nature, where they participate in the conversion of ammonium ions into nitrite ions [35,36]. It is known that nitrite ions are active oxidants, which suggested their possible participation in the oxidation of sulfide minerals. Along with this, the optimum growth conditions of nitrifies takes place at neutral pH values, which, if positive results are obtained, in the future will allow to create

Fig. 6. Extraction of zirconium and silver in variants with use of elective medium and micromycetes *Asp. niger AsIA*.
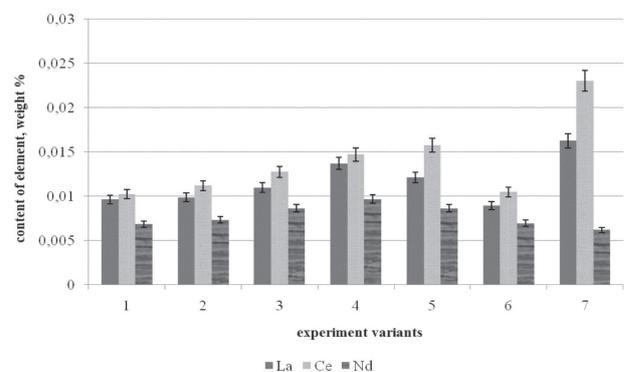

Fig. 8. Influence of elective media and microorganisms on the extraction of lanthanum, cerium and neodymium from phosphorus-containing wastes.

an environmentally safe technology for processing man-made waste. The use of nitrifying bacteria *Methyloversatilis thermotolerans MSO* contributes to the increase of the extraction of barium and iron ions into the solution almost twice or on 96.67% and 104.78%, respectively (Fig. 7).

It is found that the use of elective media together with microorganisms increase the yield of rare earth metals into the productive solution in comparison with the control version, where only elective media are used. So, at using of the strain *Alicyclobacillus tolerans ST* the increase of lanthanum extraction is 0.00023 wt. % or 2.38% from total yield; the increase of cerium extraction is 0.00097 wt. % or 9.48% from total yield, the increase of neodymium extraction is 0.00051 wt. % or 7.45% from total yield (Fig. 8). At the extraction of lanthanum and neodymium with the help of *Asp. niger AsIA* in the liquid Czapek's medium it is found the reduce of elements extraction in to the productive solution on 11.58% and 10.64%, respectively. The use of nitrifying bacteria *Methyloversatilis thermotolerans MSO* promotes the increase of the lanthanum yield into the solution on 81.87%; cerium – 19.84%.

Conclusions

On the formation of the phosphorus-containing waste's fractional composition of the South of Kazakhstan not only the conditions of raw materials processing, but also the weather and climatic parameters and time characteristics of storage are influenced.

The dominant part of wastes are silicate and calcium-containing compounds represented by pseudowollastonite, cuspidine, ferrophosphorus, melilite, akermanite, rankinite, fluorapatite, whitlockite, fluorite and silicocarnotite.

The role of different groups of microorganisms in bioleaching of valuable metals from phosphorus-containing wastes was studied in the research. The choice of phase 1 nitrifies and micromycetes for biogydrometallurgical purposes was justified by the conditions of their cultivation in neutral or weakly acidic environments, which can create an alternative to traditional methods of sulfuric acid leaching or bio-leaching by acidophilic bacteria.

The used strains of microorganisms selectively leach metals. The variant with the use of acidophilic bacteria *Alicyclobacillus tolerans ST* have demonstrated the effectiveness of their use in biohydrometallurgy, because it showed an increase in the extraction of such metals as magnesium, aluminum, potassium, calcium phosphorus, from rare earth elements such as lanthanum, cerium and neodymium. The use of micromycetes *Asp. Niger AsIA* proves to be effective in the extraction of metal of value such as zirconium and silver. Application of nitrifying bacteria *Methyloversatilis thermotolerans MSO* helps to increase the extraction of barium and iron ions into the solution.

Conflict of Interest

The authors declare no conflict of interest.

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