

VARIANCE ANALYSIS FOR OPTIMIZATION OF THE GERMANIUM BIOLEACHING PROCESS FROM COAL BENEFICIATION DUMPS

I. A. Blayda
N. Yu. Vasylieva
T. V. Vasylieva
L. I. Sliusarenko

Odesa National Mechnykov University, Ukraine

E-mail: iblayda@ukr.net

Received 17.05.2017

The aim of the work was to optimize the process of germanium bioleaching from the dumps after coal beneficiation, namely, to determine the optimal composition of the new nutrient medium for acidophilic chemolithotrophic bacteria ensuring the maximum recovery of valuable metal in minimum time. We optimized the method of mathematical planning adapted to the plan in Greek-Latin squares. The calculations in this approach are based on the analysis of variance. The formal design of experiments has been carried out with four operating factors at four levels. The calculations were performed in Excel. The significance of the factor levels were analyzed using the Duncan's multiple range test, the uniformity of the variances was examined the Cochran test, and the significance of the factors was tested by the Fisher criterion for each day of the experiment. The obtained results were interpreted mathematically and biologically. The following combination of factors and their levels was recommended as optimum nutrient medium, g/dm³: KH₂PO₄ — 1.0; (NH₄)₂SO₄ — 2.0; KCl — 0.1; MgSO₄ — 0.5; NH₄Cl — 0.5; Na₂S₂O₃ — 5.0. The proposed composition allows the more than 90% quick extraction of germanium into the solution (in four days), which was previously impossible.

Key words: bioleaching, acidophilic chemolithotrophic bacteria, germanium, coal beneficiation, Greek-Latin squares, variance analysis.

Previously [1–7], rock waste of coal enrichment in Ukraine proved to be promising as a source of the rare metal germanium. The tested current biotechnological methods exploit the native microbiota activity. A singular community of mostly the heterotrophic and acidophilic chemolithotrophic bacteria is formed during the processes of formation, storage and storage in the studied man-made ecosystems [5]. There, the most active group of microorganisms in the native microbiota of coal waste substrates at coal dumps is a group of acidophilic chemolithotrophic microorganisms, both mesophilic and moderately thermophilic, of the genera *Acidithiobacillus* and *Sulfobacillus* [6]. In our work, the optimal parameters of the bioleaching process were selected and determined according to the recommendations on the use of nutrient media to activate a certain group of microorganisms, and conditions for their maximum growth and activity [8, 9]. Under constant conditions (ratio of solid (substrate)

to a liquid (nutrient medium) S:L = 1:10, pH ≤ 2.0, 30.0 ± 2 °C, 7 days, using a standard 9K nutrient medium with the addition of 44.5 g/dm³ FeSO₄·7H₂O as an energy source) the bioleaching process allows sufficiently high extraction of germanium and some of the heavy metals from the dumps into the solution [2]. The composition of nutrient medium is of utmost importance in the extraction of metals from the raw material. Specifically, phosphorus and nitrogen sources are necessary for the biomass growth of taxonomically different microorganisms, which also require energy sources to enhance their activity. Increasing the degree of germanium recovery into the solution in the shortest possible time is essential to intensify the developed biotechnological method. Mathematical methods of planning and analysis are widely used to optimize the studied biotechnological process with respect to this parameter, taking into account all possible factor effects with minimal number of experiments [10–15].

The aim of the work was to optimize the process of bioleaching germanium from the coal enrichment wastes, namely, to determine the optimal composition of the new nutrient medium, basing the experimental design on the variance analysis.

Materials and Methods

The study object was the rock waste of coal enrichment at the Chervonogradska coal preparation plant (CPP) of Lviv Coal Company by gravity separation and flotation methods. ROM coal was obtained in the mines of the Lviv-Volyn coal basin. The substrate is crystalline rock, comprised mostly of fairly large (5–7 mm) particles with a coal content up to 17.0%, sulfur up to 1.5% and organic weight up to 2.0%. Chemical composition, %: Fe — 4.46; Al — 1.39; Si — 15.90; Ti — 0.42; Ca — 1.72; Cu — $6.22 \cdot 10^{-3}$; Zn — $1.13 \cdot 10^{-2}$; Mn — $3.18 \cdot 10^{-2}$; Pb — $0.42 \cdot 10^{-2}$; Ni — $1.34 \cdot 10^{-2}$; Cd — $2.82 \cdot 10^{-4}$; Sn — $3.52 \cdot 10^{-2}$; Cr — $0.99 \cdot 10^{-2}$; V — $1.50 \cdot 10^{-2}$; Co — $1.16 \cdot 10^{-2}$; Sr — $2.11 \cdot 10^{-2}$; Ba — $5.19 \cdot 10^{-2}$; Zr — $1.73 \cdot 10^{-2}$; Rb — $1.41 \cdot 10^{-2}$; Nb — $1.40 \cdot 10^{-3}$; La — $4.80 \cdot 10^{-3}$; Ce — $6.90 \cdot 10^{-3}$; Ga — $1.21 \cdot 10^{-3}$; Ge — $2.60 \cdot 10^{-3}$. The substrate was stored in coal dumps for one year. The densities of aboriginal acidophilic chemolithotrophic microbiota are $6.4 \pm 0.6 \cdot 10^4$ cells/ml for mesophilic bacteria and $7.4 \pm 0.3 \cdot 10^8$ cells/ml for moderately thermophilic bacteria [5].

The fastest maximum extraction of germanium into the solution was selected as optimization parameter Y. The optimization was based on the method of mathematical experiment planning, adapted for the plan in Greco-Latin squares calculated using the variance analysis. The method itself consists in the determination and evaluation of individual factors and their combinations that cause the variability of the studied value [10–13]. The acting factors and their priority, the number of their levels and the size of the sample were

determined based on the requirements for planning the stages of the experiment and previous results. The formal planning of the experiment was carried out with four acting factors on four levels.

The value of the chosen variation interval should be greater than twice the quadratic error with which the level of this factor is fixed. The reason for this is that a small variation interval reduces the experimental area and hinders the search for the optimum. Thus the nutrient medium components serve as the factors of variation if there are sufficiently wide ranges of their concentration variations, known from the literature or established experimentally. The main requirement remains the importance of the factor for the growth of the microorganism.

Hence, four factors were chosen to determine the composition of the future nutrient medium. The factors were the nutrient medium components, standard sources of phosphorus and nitrogen for culturing the microorganisms of different taxonomic groups: A — KH_2PO_4 , B — K_2HPO_4 , C — $(\text{NH}_4)_2\text{SO}_4$, and D — energy sources ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Fe}_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$ or $\text{Na}_2\text{S}_2\text{O}_3$) [8, 9, 16]. The levels of factors are given in Table 1. As additional components, the following salts were used in concentration as standard additives in cultivation of various microorganisms [9, 16], g/dm³: KCl 1.0; MgSO_4 0.5; NH_4Cl 0.9.

Based on the matrix of the four-factor experiment for four levels according to Greek-Latin squares, Table 2 was compiled reflecting the factor combinations for carrying out the experiments.

The calculations of the variance analysis according to the plan in Greco-Latin squares are presented in [13, 14]. The calculations were performed in Excel. The importance of the factor level was analyzed based on the multiple rank Duncan test for each day of the experiment, the uniformity of variances was verified with the Cochran test, and the

Table 1. Factor levels (g/dm³), used in the variance analysis adapted for the plan in the Greco-Latin squares

Factors	Factor component concentration, g/dm ³			
	Level 1	Level 2	Level 3	Level 4
A — KH_2PO_4	0.0	1.0	2.0	3.0
B — K_2HPO_4	0.0	0.5	1.0	1.5
C — $(\text{NH}_4)_2\text{SO}_4$	0.0	2.0	3.0	5.0
D — energy sources	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 44.0	$\text{Fe}_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$ 15.0	$\text{Na}_2\text{S}_2\text{O}_3$ 5.0	$\text{Fe}_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$ 30.0

Table 2. Experimental conditions based at a four-factor four-level experiment matrix according to the Greco-Latin squares method

№.№ experiment	Level A	Level B	Level C	Level D	Optimization parameter
1	A1	B1	C1	D1	Y1
2	A1	B2	C2	D2	Y2
3	A1	B3	C3	D3	Y3
4	A1	B4	C4	D4	Y4
5	A2	B1	C2	D3	Y5
6	A2	B2	C1	D4	Y6
7	A2	B3	C4	D1	Y7
8	A2	B4	C3	D2	Y8
9	A3	B1	C3	D4	Y9
10	A3	B2	C4	D3	Y10
11	A3	B3	C1	D2	Y11
12	A3	B4	C2	D1	Y12
13	A4	B1	C4	D2	Y13
14	A4	B2	C3	D1	Y14
15	A4	B3	C2	D4	Y15
16	A4	B4	C1	D3	Y16

importance of factors was tested using the Fisher criterion (F). The value of the Fisher criterion was assumed to be significant at 95.0% ($P = 0.05$).

Bacterial leaching of metals was carried out in the previously determined optimal conditions: S: L = 1:10; pH ≤ 2.0; temperature 30.0 ± 2.0 °C, stationary cultivation for 7 days. The content germanium in solutions was analyzed using AAS-1 (Germany) and S-115PK Selmi (Ukraine) devices for atomic absorption spectroscopy. The reliability of the results was evaluated by the Student's test with a probability of $P < 0.05$. The "metal extraction ratio" is the ratio of the amount of metal passed into solution as a result of contact of the nutrient medium with the substrate in the presence of microorganisms (in%) to the original amount of this metal in the original solid substrate. The 100% corresponds to a complete transition of the metal from the substrate to the solution.

Results and Discussion

The results for determining the optimization parameter (the degree of germanium extraction in the solution for each day of the experiment) are given in Table 3 and in the Figure. The mean germanium recovery was determined in all of the 16 experiments in 1–7 days of

observations. The difference between the maximum and average indices of germanium extraction supports the advantage of a certain combination of factors with respect to the general variety: the greater the difference, the more effective the combination of factors. The obtained results were interpreted both from the mathematical and from the biological point of view [15].

The generalized table of calculated values was analyzed using the Fisher test (F_r) for all observation days (Table 4). The factor D is the most effective factor, and its maximum effect was recorded from the 4th to the 7th days of the study ($F_r \geq F_{st}$, where F_{st} is the table value of the Fisher test, equal to 3.49) [17]. Calculated with according to the Plokhinsky formula [18], the factor's influence on the optimization index (Table 5) was also the maximum for factor D. The tested indicator ranged from 58.9 to 73.2% for the 4th–6th days of the study. The maximum amount of germanium passed from the substrate to the leaching solution was also recorded on the 4th day of observation (Figure).

The daily calculation of the statistics according to the dispersion analysis adapted to the Greco-Latin square plan showed that in the 1st day of the experiment the Fisher criterion value was the maximum for almost all factors (Table 4). The only factor with the

Table 3. Extraction rate of germanium (%) during optimization planned in the Greco-Latin squares

№№ experiment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	0.52	15.63	18.34	25.05	17.88	31.68	36.95
2	8.54	12.44	17.61	23.78	14.34	29.68	29.14
3	4.54	7.72	6.23	39.94	16.88	17.43	16.34
4	7.51	2.72	9.35	1.15	0.40	8.17	7.26
5	5.08	4.81	7.26	82.60	62.18	86.87	76.34
6	8.26	5.54	10.35	2.36	9.99	0.36	2.25
7	3.36	12.70	11.98	0.00	10.44	7.26	1.45
8	11.80	13.25	15.79	48.38	42.94	60.46	68.45
9	9.98	6.98	11.35	4.36	8.26	8.62	1.49
10	13.40	11.44	10.35	93.50	93.50	80.25	70.00
11	9.26	14.34	11.98	45.66	68.89	60.55	47.75
12	4.54	11.17	7.89	0.00	5.08	6.35	3.00
13	6.54	8.71	12.53	42.39	43.94	39.40	37.85
14	9.98	7.81	14.43	4.36	9.35	9.08	7.00
15	5.81	12.35	10.44	0.00	4.18	1.45	0.90
16	10.89	9.80	16.52	36.58	52.11	57.37	65.05

Table 4. The values of the Fisher's exact test ($P = 0.05$)

Factor (dispersion source)	Fisher's exact test (F_p)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
A — KH_2PO_4	6.23	0.16	0.93	0.87	3.31	0.85	0.43
B — K_2HPO_4	9.80	0.61	0.51	1.02	0.35	0.80	1.21
C — $(\text{NH}_4)_2\text{SO}_4$	2.99	0.53	0.83	0.25	1.79	0.39	0.46
D — energy sources	7.59	2.24	1.67	12.4	11.66	8.95	8.47

Table 5. Indicators of factor influence (Plokhinsky's formula, %)

Factor (dispersion source)	Evaluation of the factor influence, %						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
A — KH_2PO_4	24.6	5.49	12.4	5.12	17.19	6.98	73.1
B — K_2HPO_4	35.5	2.5	9.5	6.23	1.84	6.47	4.74
C — $(\text{NH}_4)_2\text{SO}_4$	11.1	3.01	15.6	1.61	9.5	3.09	20.8
D — energy sources	29.18	7.21	22.1	73.2	58.9	64.2	4.71

Fisher criterion value below the tabulated one was factor C, $(\text{NH}_4)_2\text{SO}_4$. Testing the factor influence using the Plokhinsky's formula, it was shown that factors B (K_2HPO_4) and D (energy sources) exhibited the maximum influence on the optimization index on the first day of observation (Table 5). In this case, the optimization parameter (the degree of germanium extraction to the leaching solution) was minimal. The mean

indicator of germanium recovery from the general set of all 16 experiments for the first day was only 7.5%, and the maximum was 13.4% (Figure).

The most significant levels were determined using the multiple Duncan criterion, also taking into account all the information obtained in the experiment [12]. The significant levels of the active factors determined with the Duncan rank test are given in Table 6.

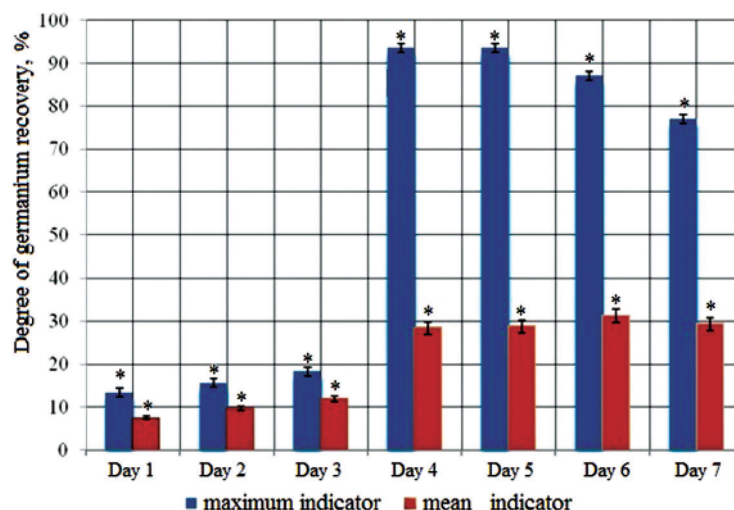


Fig. The mean and maximum indicators of germanium extraction into solution in the bioleaching process, optimized using dispersion analysis adapted to the Greek-Latin square planning
* — $P < 0.05$ compared to control (0)

Table 6. The generalized significant levels of active factors determined using the Duncan rank test

Time	Factor level			
	Factor A	Factor B	Factor C	Factor D
Day 1	A3	B2	C3	D2
Day 2	A1	B3	C1	D2
Day 3	A1	B2	C1	D2
Day 4	A2	B1	C2	D3
Day 5	A3	B1	C1	D3
Day 6	A3	B1	C1	D3
Day 7	A2	B1	C1	D3

Calculation of the Duncan rank test for the 1st day showed the significance of factor A (KH_2PO_4) at A3 level, factor B (K_2HPO_4) at B2 level, factor C ($(\text{NH}_4)_2\text{SO}_4$) at C3 level and factor D (energy sources) at D2 level (level values are given in Table 1). In addition, the calculated effect of the factor influence on the optimization parameter (Table 7) coincided with the data obtained using the multiple Duncan criterion.

The statistical data of the first experimental day also has a biological meaning. Bioleaching of metals is the result of the ore oxidation, the effectiveness of which depends on the amount and activity of chemolithotrophic bacteria of the aboriginal consortium of the studied "substrate-solution" system. The exponents of the germanium extraction into the solution indicate their leaching activity. All the factors used in the experiment are nutrient components of media for the cultivation of

chemolithotrophic bacteria, therefore their action is considered to stimulate the bacterial growth.

Thus, the first day seems to be a phase of bacterial adaptation. Accordingly, at this moment their metabolic activity is quite low. The extraction of metals in the solution can be the result of oxidative processes. This explains the low degree of germanium recovery into the solution at high Fisher criterion and the factor influence calculated by Plokhinsky's formula. In other words, we observe to a lesser extent the process of metal bioleaching due to the microbiotic activity rather than the process of chemical oxidation under the influence of medium factors.

A significant decrease in the Fisher criterion and the factor effect on the 2nd and 3rd days of the experiment (Tables 4 and 5) shows that the components of the leaching solution have a specific effect on the biomass growth

Table 7. The effects of the factor influences and levels in the biologically interpreted data on the process of germanium leaching from rock dumps

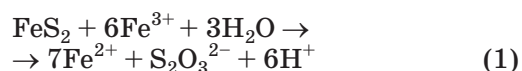
Time	Optimization factor							
	Factor A		Factor B		Factor C		Factor D	
	factor level	influence effect	factor level	influence effect	factor level	influence effect	factor level	influence effect
1	2	3	4	5	6	7	8	9
Day 1	A1	-0.025	B1	-0.0214	C1	-0.0037	D1	-0.0316
	A2	-0.0043	B2	0.0296	C2	-0.016	D2	0.016
	A3	0.0213	B3	-0.019	C3	0.017	D3	0.011
	A4	0.0079	B4	0.011	C4	0.003	D4	0.0039
Day 2	A1	-0.0025	B1	-0.0087	C1	0.0162	D1	0.021
	A2	-0.0082	B2	-0.006	C2	0.0038	D2	0.025
	A3	0.0125	B3	0.0215	C3	-0.01	D3	-0.015
	A4	-0.0018	B4	-0.0067	C4	-0.009	D4	-0.032
Day 3	A1	0.0157	B1	0.0034	C1	0.0249	D1	0.0123
	A2	-0.0077	B2	0.0124	C2	-0.0138	D2	0.0267
	A3	-0.0181	B3	-0.0197	C3	0.0	D3	-0.0205
	A4	0.0101	B4	0.0039	C4	-0.011	D4	-0.0185
Day 4	A1	-0.0617	B1	0.1158	C1	-0.0073	D1	-0.2285
	A2	0.0577	B2	0.0301	C2	-0.0166	D2	0.1320
	A3	0.0838	B3	-0.0737	C3	-0.0420	D3	0.3844
	A4	-0.0798	B4	-0.0722	C4	0.0660	D4	-0.2880
Day 5	A1	-0.18	B1	0.0475	C1	0.0928	D1	-0.199
	A2	0.0287	B2	0.033	C2	-0.0805	D2	0.1516
	A3	0.167	B3	-0.0405	C3	-0.1035	D3	0.3017
	A4	-0.015	B4	-0.0401	C4	0.0912	D4	-0.2543
Day 6	A1	-0.1077	B1	-3.9586	C1	0.0652	D1	-0.1989
	A2	0.0787	B2	0.1117	C2	-0.0048	D2	0.1769
	A3	0.0809	B3	-0.0183	C3	-0.0843	D3	0.3188
	A4	-0.0518	B4	-0.1094	C4	0.0239	D4	-0.2968
Day 7	A1	-0.078	B1	0.0958	C1	0.0941	D1	-0.1911
	A2	0.0846	B2	-0.0259	C2	-0.0228	D2	0.1801
	A3	0.00123	B3	-0.1412	C3	-0.0673	D3	0.3029
	A4	-0.0190	B4	0.0713	C4	-0.004	D4	-0.2918

and are less involved in chemical oxidation of the substrate. This time period corresponds to the initial stage of bacterial growth. The amount of germanium that passed into the solution is insignificant and corresponds to 15.63–18.34% (Figure). Analyzing the values of the Duncan test for the influence of the factors and their levels (Tables 6, 7), we can say that at the stage of bacterial growth, factor B is more important than factors A and C. The influence of the energy source factor at the D2 level does not change.

The maximum recovery of germanium (93.5%) was recorded on the 4th and 5th days of the experiment (Figure, Table 3). The obtained results correspond to the literature data. According to [19], the bacterial participants of the bioleaching process reach their maximum metabolic activity on the 4th–5th days. The factor D of all the factors, the source of energy, had the greatest influence on the process (Table 7), which is confirmed by a high Fisher test value significantly higher than F_{st} for the 4th and 5th days of observation (Table 4). Calculation of the Duncan rank test for the 4th and 5th days of the experiment showed a change in the levels of the acting factors (Table 6). Thus, the need for factor A then increased to the level of A2-A3 corresponding to 2.0–3.0 g/dm³. The need for factor B, on the contrary, decreased to the level of B1 (0.0 g/dm³); the need for factor C was at the minimum level of C1-C2, which corresponds to 0.0–2.0 g/dm³ (Table 2). Also in this time period, a change in the level of factor D was recorded. In the earlier stages of the experiment, trivalent iron was the most significant source of energy, and from the 4th day of observation the demand for thiosulphate prevailed. Such a change in the required energy source can be explained from a biological point of view considering the discovery of extracellular polymer compounds (EPC) released by microorganisms attached to the surface of a solid particle [20] and the hypothesis of an indirect bioleaching mechanism through the formation of thiosulphate [21].

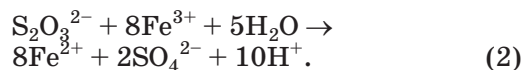
At the beginning of the process, contact bioleaching takes place, and oxidation is supported by the microorganisms attached to the solid surface of the substrate [22]. Here, an important role is played by the Fe³⁺ ion, which is part of both the extracellular exopolymer layer and the culture medium. As soon as the microorganism is attached to the surface of the metal sulfide insoluble in the acid (in the test substrate of the piles it is pyrite FeS₂), the Fe³⁺ ion begins an indirect

attack on the metal sulfide in the reaction [21]:

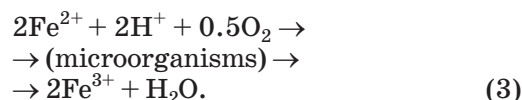


The attack mechanism lies in the fact that microorganisms oxidize the sulfur in pyrite, which leads to the formation of soluble compounds containing Fe²⁺, H⁺ and S₂O₃²⁻ ions. The EPC and the Fe³⁺ ion play an important role in the attachment of cells to the mineral and its further dissolution. EPC provide a strong contact between microbial cells and insoluble pyrite FeS₂, which is an electron donor in reaction (1). In addition, Fe³⁺ participates in the first stage of pyrite destruction, which requires the presence of a certain amount of Fe³⁺ in the nutrient medium at the beginning of the bioleaching process. This explains the significance of factor D at the level of D2 in the 1st–3rd days of the experiment (Table 6).

Starting from the 4th day, thiosulfate begins to play a more significant role as a source of energy. It is either formed by the reaction (1) or is introduced into the nutrient medium initially. Either the initial or intermediate product, thiosulfate eventually turns into sulfate under the influence of microorganisms and components of the medium through the formation of tetrathionate and trithionate in the reaction:



The resulting Fe²⁺ can be converted again to Fe³⁺ by such iron-oxidizing bacteria as *Acidithiobacillus ferrooxidans*:



Thus, the role of microorganisms at this stage is reduced to the formation of an oxidizer in the form of Fe³⁺ ions, which promotes a more complete leaching of the substrate components into the solution.

On the 6th and 7th days of observation, the germanium recovery into the solution decreased by 70%. At the same time, the current levels of factors remained unchanged (Table 6). The Fisher criterion (Table 4) unequivocally indicates the significance of the factor $D:F_r$ (8.95) $\geq F_{st}$ (3.49) and F_r (8.47) $\geq F_{st}$ (3.49).

From the obtained results it follows that:

1. The biotechnological process of leaching germanium from rock dumps of Chervonogradska CPP of the Lviv Coal Company was optimized using the method of Greco-Latin

squares mathematical planning based on the variance analysis.

2. These research, calculations and the analysis allowed to recommend a new composition of the optimal nutrient medium (ONM) for the fastest maximum extraction of germanium. The recommended combination of factors and their levels A2B1C2D3 corresponds

to the ONM composition, g/dm³: KH₂PO₄ — 1.0; (NH₄)₂SO₄ — 2.0; KCl — 0.1; MgSO₄ — 0.5; NH₄Cl — 0.5; Na₂S₂O₃ — 5.0.

3. The new ONM composition allows to achieve the 93.5% recovery of germanium from the coal dumps of Chervonogradska CPP in solution in for 4 days, which was impossible to obtain earlier.

REFERENCES

1. Blayda I., Vasyleva T., Slyusarenko L., Abisheva Z., Ivanytsia V. The germanium extraction from industrial wastes by microbiological methods. *XXVI International Mineral Processing Congress (IMPC 2012): New Delhi, India*. 2012, P. 550–558.
2. Blayda I. A., Vasileva T. V., Baranov V. I. The use of bio-hydrometallurgical technologies in solving problems utilization of manmade waste and receiving from them valuable metals. *Kompleksnoe ispolzovanie mineralnogo syrya*. 2015, V. 3, P. 75–82. (In Russian).
3. Blayda I. A., Vasileva T. V., Slyusarenko L. I., Khitrich V. F., Barba I. M., Ivanitcia V. O., Baranov V. I. The method of extracting rare metals from waste coal industry. *UA Patent 102926*. August 27, 2013. (In Ukrainian).
4. Blayda I. A., Vasyleva T. V., Baranov V. I., Slyusarenko L. I., Baklan V. Yu. Opportunities bacterial and chemical leaching of coal refuse with aim to germanium and gallium extraction. *Izvestiya Vuzov. Prikladnaya khimiya i biotekhnologiya*. 2013, 1 (4), 54–60. (In Russian).
5. Blayda I. A. The study of composition and activity of bacterial community of coal tailing. *Biotechnol. acta*. 2014, 7 (5), 94–100. doi: 10.15407/biotech7.05.094.
6. Blayda I. A., Vasileva T. V., Baranov V. I., Semenov K. I., Slyusarenko L. I., Barba I. N. Properties of chemolithotrophic bacteria new strains isolated from industrial substrates. *Biotechnol. acta*. 2015, 8 (6), 56–62. doi: 10.15407/biotech8.06.056.
7. Vasyliieva T. V., Blayda I. A., Slyusarenko L. I., Vasyliieva N. Yu., Chitrich V. F. Bacterial leaching of the metals from waste flotation of coals with the participation of thiosulfate, ferrous and ferric iron. *Microbiology & Biotechnology*. 2016, V. 3, P. 43–56. doi: [http://dx.doi.org/10.18524/2307-4663.2016.3\(35\).77956](http://dx.doi.org/10.18524/2307-4663.2016.3(35).77956). (In Russian).
8. Karavayko G. I. Practical Guide to biogeotechnology metals. *Moskva: AN SSSR*. 1989, 371 p. (In Russian).
9. Methods for General Bacteriology. V. 2. *Moskva: Mir*. 1984, 265 p. (In Russian).
10. Grachev Ju. P., Plaksin Ju. M. Mathematical methods of experiment planning. *Moskva: Nauka*. 2005, 296 p. (In Russian).
11. Adler Ju. P., Markova E. V., Granovskij Ju. V. Planning an experiment when searching for optimal conditions. *Moskva: Nauka*. 1976, 280 p. (In Russian).
12. Zedginidze I. G. Planning an experiment to study multicomponent systems. *Moskva: Nauka*. 1976, 390 p. (In Russian).
13. Radchenko S. G. Analysis of experimental data based on the use of multifactorial statistical mathematical models. *Matematichni mashini i sistemi*. 2005, V. 3, P. 102–115. (In Russian).
14. Ljubishhev A. A. Dispersion analysis in biology. *Moskva: Izd-vo Mosk. un-ta*. 1986, 200 p. (In Russian).
15. Tsarenko I. Yu., Roy A. O., Kurdish I. K. Optimization of nutrient medium for cultivation of *Bacillus subtilis* IMV V–7023. *Microbiol. J*. 2011, 73 (2), 13–19. (In Russian).
16. Lengeler J., Drevs G. I., Shlegel' G. (Eds). Modern microbiology. Prokaryotes. V. 2. *Moskva: Mir*. 2005, 496 p. (In Russian).
17. Likesh I., Ljaga J. Basic tables of mathematical statistics. *Moskva: Finansy i statistika*. 1985, 365 p. (In Russian).
18. Lakin G. F. Biometrics. *Moskva: Vysshaja shkola*. 1990, 352 p. (In Russian).
19. Ahmed Risikat Nike, Ahmed Risikat Nike, Alafara A. Baba, Sani Al-Hasan, Alamu Folake Bosede, Ajijolakewu Abiodun Kamoldeen. Bioleaching of Lead from Nigerian Anglesite Ore by *Acidithiobacillus ferrooxidans*. *J. Appl. Sci. Res*. 2012, 12 (8), 5591–5598.
20. Sand W., Gehrke T., Jozsa P.-G., Schippers A. (Bio) chemistry of bacterial leaching — direct vs. indirect bioleaching. *Hydrometallurgy*. 2001, V. 59, P. 159–175.
21. Schippers A., Sand W. Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. *Appl. Environm. Microbiol*. 1999, 65 (1), 319–321.
22. Rodriguez Y., Ballester A., Blazquez M. L. New information on the pyrite bioleaching mechanism at low and high temperature. *Hydrometallurgy*. 2003, V. 71, P. 37–46.

ДИСПЕРСІЙНИЙ АНАЛІЗ ДЛЯ ОПТИМІЗАЦІЇ ПРОЦЕСУ БІОВИЛУГОВУВАННЯ ГЕРМАНІЮ З ВІДВАЛІВ ВУГЛЕЗБАГАЧЕННЯ

*І. А. Блайда, Н. Ю. Васильєва,
Т. В. Васильєва, Л. І. Слюсаренко*

Одеський національний університет
імені І. І. Мечникова, Україна

E-mail: iblayda@ukr.net

Метою роботи була оптимізація процесу біовилуговування германію з відвалів вуглезабагачення, а саме визначення оптимального складу нового живильного середовища для ацидофільних хемолітотрофних бактерій, що забезпечує максимальне вилучення цінного металу за мінімальний термін. Для оптимізації використовували метод математичного планування експерименту, адаптований до плану греко-латинських квадратів, розрахунки в якому ґрунтуються на дисперсійному аналізі. Формальне планування експерименту проводили з чотирма діючими факторами (компонентами живильного середовища) на чотирьох рівнях (концентрацій). Розрахунки було виконано в програмі Excel. Проведено аналіз значущості рівня фактора на підставі множинного рангового критерію Дункана для кожного дня експерименту, перевірки однорідності дисперсій за допомогою критерію Кохрена, а також значущості факторів за критерієм Фішера. Інтерпретацію одержаних результатів проводили як із математичної, так і з біологічної точок зору. В результаті для оптимального живильного середовища рекомендували комбінацію чинників і їхніх рівнів, що відповідає складу цього середовища, г/дм³: KH_2PO_4 — 1,0; $(\text{NH}_4)_2\text{SO}_4$ — 2,0; KCl — 0,1; MgSO_4 — 0,5; NH_4Cl — 0,5; $\text{Na}_2\text{S}_2\text{O}_3$ — 5,0. Це дає змогу досягти вилучення германію в розчин більш ніж на 90% за короткий термін (чотири доби), що було неможливо раніше.

Ключові слова: біовилуговування, ацидофільні хемолітотрофні бактерії, германій, відвали вуглезабагачення, план греко-римських квадратів, дисперсійний аналіз.

ДИСПЕРСИОННИЙ АНАЛІЗ ДЛЯ ОПТИМІЗАЦІЇ ПРОЦЕСА БІОВИЩЕЛАЧИВАННЯ ГЕРМАНІЯ ІЗ ОТВАЛІВ УГЛЕБОГАЧЕННЯ

*І. А. Блайда, Н. Ю. Васильєва,
Т. В. Васильєва, Л. І. Слюсаренко*

Одесский национальный университет имени
И. И. Мечникова, Украина

E-mail: iblayda@ukr.net

Целью работы была оптимизация процесса биовыщелачивания германия из отвалов углеобогащения, а именно определение оптимального состава новой питательной среды для ацидофильных хемолитотрофных бактерий, обеспечивающей максимальное извлечение ценного металла за минимальный срок. Для оптимизации использовали метод математического планирования эксперимента, адаптированный к плану греко-латинских квадратов, расчеты в котором основываются на дисперсионном анализе. Формальное планирование эксперимента проводили с четырьмя действующими факторами (компонентами питательной среды) на четырех уровнях (концентраций). Расчеты были выполнены в программе Excel. Проведен анализ значимости уровня фактора на основании множественного рангового критерия Дункана для каждого дня эксперимента, проверки однородности дисперсий с помощью критерия Кохрена, а также значимости факторов по критерию Фишера. Интерпретацию полученных результатов проводили как с математической, так и с биологической точек зрения. В результате для оптимальной питательной среды рекомендовали комбинацию факторов и их уровней, что соответствует составу этой среды, г/дм³: KH_2PO_4 — 1,0; $(\text{NH}_4)_2\text{SO}_4$ — 2,0; KCl — 0,1; MgSO_4 — 0,5; NH_4Cl — 0,5; $\text{Na}_2\text{S}_2\text{O}_3$ — 5,0. Это позволяет достичь извлечения германия в раствор более чем на 90% за короткий срок (четверо суток), что было невозможно ранее.

Ключевые слова: биовыщелачивание, ацидофильные хемолитотрофные бактерии, германий, отвалы углеобогащения, план греко-римских квадратов, дисперсионный анализ.