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OPTIMIZATION OF THE COAL BACTERIAL DESULFURIZATION USING MATHEMATICAL METHODS

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The aim of the work was to optimize the process of bacterial desulfurization of energy coal, namely, to determine the influence of the component composition of the nutrient medium and the conditions of the process, which ensure the maximal development and activity of the aboriginal association of acidophilic chemolithotrophic bacteria and, as a consequence, the maximal index of sulfur decrease in coal in minimal time. We used the method of mathematical planning of the experiment adapted to the plan in Greek-Latin squares. The calculations in this approach are based on the analysis of variance (ANOVA). The formal planning of experiments has been carried out with four operating factors (nutrient medium components) at four levels (concentrations). The calculations were performed in Excel. The selection of operating factors and their combinations was made with the usage of unifactor ANOVA, correlation analysis and the method of principal components PCA. Researches were carried out in R 3.4.0 program and were founded on data of the preliminary evaluating experiments. *Acidithiobacillus ferrooxidans* Coal 17 aboriginal strain was used to obtain the most significant desulfurization effect. This strain was isolated from the investigated coal, studied and identified. The significance of the factor level for each nutrient medium component was analyzed using the Duncan's multiple range test, the uniformity of the variances was examined with the Cochran's test, and the significance of the factors was tested by the Fisher's criterion. As a result, for the optimal nutrient medium the next combination of factors and their levels, which corresponds to the composition, g/dm^3, was recommended: $(NH_4)_2SO_4$ — 0.15; K_2HPO_4 — 0.50; $FeSO_4.7H_2O$ — 44.50; KCl — 0.10; $MgSO_4 \cdot 7H_2O$ — 0.10; $Ca(NO_3)_2$ — 0.10; yeast extract — 0.025% (vol.); strain *A. ferrooxidans* Coal 17 (titre $1 \cdot 10^8$ CFU/ml) — 1.60% (vol.). This makes it possible to reduce the sulfur content in coal by 66.31% in a short period (seven days). This result could not be got before.

Key words: desulfurization, aboriginal association, acidophilic chemolithotrophic bacteria, plan in Greek-Latin squares, variance analysis.

The extracted coal in most cases does not meet the requirements of consumers by the main qualitative indicators: the content of sulfur, ash content, humidity, calorific value and sintering properties. Thus, coal from the mines of the Lviv-Volyn coal basin belongs to high-sulfur, low-calorie raw materials, since it contains not less than 4.5% of total sulfur (including pyrite — up to 2.0%). Decrease the sulfur content at the stage of its enrichment can increase the quality, thermal characteristics of coal and, consequently, its cost, as well as reduce emissions of toxic compounds of sulfur in the atmosphere during its combustion. The improvement of the quality of coal raw materials for high-quality coking and energy coal, which is in demand on the market, is achieved by enriching with various physico-chemical methods: gravity, magnetic separation, electric separation, flotation, and others [1]. With the development of biotechnology, more and more attention is being paid to environmentally safe and resource-saving microbiological methods of coal desulfurization, namely biodesulfurization [2–4]. The analysis of literature data suggests that pure cultures of ACB are mainly used for desulfurization, in particular *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* — type and collection strains, as well as isolated from specific sources — various samples of coal, drainage acidic mine waters [3–5]. There is practically no data on the association of mixed cultures and consortia of own microbiots of coal substrates usage for this purpose, although the activity of such communities is widely used and well-proven for the bioleaching of metals from sulphide ores and man-made waste [2, 4].

The aim of the work was to optimize the process of bacterial desulfurization of energy coal, namely, to determine the influence of the component composition of the nutrient medium and the conditions of the process, which ensure the maximal development and activity of the aboriginal association of acidophilic chemolithotrophic bacteria and, as a consequence, the maximal index of sulfur decrease in the coal in minimal time.

Materials and Methods

Studies on desulfurization were carried out with a batch of natural coal, which enters the Central Enrichment Plant (CEP) “Chervonogradska” directly from the coal mines of the Lviv-Volyn coal basin. Coal has the following initial characteristics: Sgeneral — 2.82%; ash content — 18,10%; specific heat of combustion — 16.60 MJ/kg.

For preliminary evaluation studies and determination of the most favorable conditions for the process of microbiological coal desulfurization using the activity of acidophilic chemolithotrophic aboriginal bacteria, the nutrient media 9K, 9K* and Letain recommended for ACB growth were used [2, 6, 7]. To these media, divalent iron was added as a source of energy in the form of salt $FeSO_4 \cdot 7H_2O$ at various concentrations. The composition of the media is given in Table 1. Experiments were performed in a thermostat by stationary cultivation at pH ≤ 2.0; temperature 30.0 ± 2.0 °C; duration — 28 days. The ratio of solid (coal) to liquid (nutrient medium) phases (S:L) as 1: 5 or 1:10 was provided. Weekly, the control of the process was performed, based on the value of the residual sulfur in the treated coal (%) and the titre of ACB (CFU/ml) of the aboriginal association grown in the appropriate nutrient medium. The titre of cells was determined by the classical microbiological method of serial dilutions. Sterile coal and nutrient media were the control.

Acidithiobacillus ferrooxidans Coal 17 strain, which was isolated from the aboriginal association of natural coal of the Lviv-Volyn coal basin, was used to intensify the process of desulfurization. The strain is studied by basic biological properties. It has been found that strain cells are thin, short gram-negative mobile cells (Fig. 1). It, as a typical representative of ACB, is capable of growing in mixotrophic conditions in the presence of 0.02 % of yeast extract or glucose, but is not able to grow in complete media (MPA, Gorbenko). The temperature and pH range for growth are within (4.0–35.0) ± 2.0 °C and 1.0–4.5, respectively. Bivalent iron, thiosulfate, sulfur were used as a source of energy. By all indications and on the basis of polymerase chain reaction (PCR) method, it is referred to as *Acidithiobacillus ferrooxidans*. The *A. ferrooxidans* Coal 17 strain was pre-cultivated in a liquid medium 9K with 12.0 g/dm^3 of $FeSO_4 \cdot 7H_2O$ to a titre index of $1 \cdot 10^8$ CFU/ml (corresponding to an optical density of 0.9).

Based on the results of preliminary evaluative studies, an experiment was modeled to optimize the composition of the medium

Table 1. **Composition of nutrient media, g/dm^3**

Components	**Nutrient medium**		
	Letain	9K	9K*
$(NH_4)_2SO_4$	0.15	3.00	0.20
KCl	–	0.10	0.10
KH_2PO_4	0.10	–	–
K_2HPO_4	–	0.50	0.10
$MgSO_4 \cdot 7H_2O$	0.50	0.50	0.40
$Ca(NO_3)_2$	0.01	0.10	–
Yeast extract (YE)	–	–	0.02

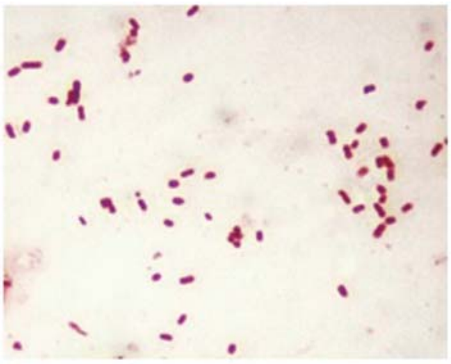

Fig. 1. **Microphotography of *Acidithiobacillus ferrooxidans* Coal 17 bacteria strain** (magnification ×1000)

for microbial desulfurization of coal. As an experiment plan, a Greek-Latin square was used with four factors at four levels of variation [8–12]. The choice was based on the fact that the Greek-Latin squares help to discover the most effective factors combination, without taking into account the interfacial interactions, and to minimize the number of calculations and laboratory manipulations. According to the constructed matrix of four-factor experiment for four levels, based on the principle of Grec-Latin squares, a table was formed in which the combinations of factors for experiments performing are represented (Table 2).

The optimization parameter Y was a vector containing the percentage of residual sulfur content in the coal after the substrate treatment with appropriate nutrient medium (according to the matrix of the experiment, Table 2).

The choice of operating factors and their combinations was made using unifactor dispersion analysis, correlation analysis and principal component analysis (PCA) in program R 3.4.0, based on the determination of the influence of the component composition of nutrient media, the variation of the $FeSO_4 \cdot 7H_2O$ concentration and the S:L ratio on the growth indices of the aboriginal mesophilic association of acidophilic chemolithotrophic bacteria and the efficiency of the desulfurization process [13].

When choosing the interval of variation, it was taken into account that its value should be more than double the quadratic error, from which the level of this factor is fixed. This is due to the fact that a small variation interval decreases the area of the experiment and slows down the search of the optimum. Therefore, the components of the nutrient medium, for which there are quite wide ranges of concentration variations known from literature or experimentally established, act as factors of variation. The main requirement is the significance of the factor for the growth

Table 2. **Conditions of the experiment according to the matrix of four-factor experiment for four levels, based on the principle of Greek-Latin squares**

Experiment number	Level A	Level B	Level C	Level D	Parameter of optimization
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

of a microorganism as a source of phosphorus, nitrogen or energy.

Calculations to find out the most effective combination of factors based on the results of a four-factor experiment on optimizing the composition of the medium for desulfurization were based on a dispersion analysis, which allows us to estimate the influence of operating factors and separate their influence from the variability given by the random variable [8, 9, 14, 15]. The significance of linear effects was checked by Fischer's criterion (F) — Fisher's criterion value was taken as significant at 95.0% ($P = 0.05$) [4, 10]. The determination of significant ranks was carried out using the Duncan's multiple criterion [10], which allows us to determine the significance of the difference between the effects of levels of factors introduced into the plan with more than two levels with greater reliability, since at the same time all information obtained in the experiment is used [6, 10]. The Cochran's criterion (G) was used to evaluate the equality of dispersions. The calculations were performed in Excel and R 3.4.0.

The analysis for sulfur was carried out by Eshka method [16].

The titre of acidophilic chemolithotrophic bacteria representatives was determined by seeding of 10-fold sequential dilutions of the bacterial suspension on dense media of the same composition. The stained microscopic specimen was studied using a Primo Star PC (Germany) light microscope.

Results and Discussion

The previous studies performing showed the possibility of microbiological desulfurization of coal using an aboriginal association of acidophilic chemolithotrophic bacteria, and also detected the most influential factors and their combinations. The conditions of the experiments that led to the most significant results are given in Table 3. The content of sulfur was determined on the 28th day of cultivation.

The obtained results indicate that the maximal abundance of an aboriginal mesophilic association of acidophilic chemolithotrophic bacteria in all variants of experiments was recorded already in the first 7 days of experiments. Absolute maximal titre of microorganisms ($(7.3 \pm 0.7) \cdot 10^5$ and $(6.8 \pm 0.6) \cdot 10^5$ CFU/ml) was recorded after 7 days of experiment using 9K nutrient medium with 44.50 g/dm^3 of $FeSO_4 \cdot 7H_2O$ and the ratio S:L = 1: 10 (Var5) and 9K* medium with 25.00 g/dm^3 of $FeSO_4 \cdot 7H_2O$ at S:L = 1: 5 (Var6), respectively. However, after 21–28 days of cultivation, the abundance indices of the aboriginal mesophilic association of acidophilic chemolithotrophic bacteria significantly decreased in all cases. Thus, for variants of experiments with previously highest parameters (Var5 and Var6), the decrease in the number was more than two orders of magnitude — up to $(1.6 \pm 0.1) \cdot 10^3$ and $(5.4 \pm 0.5) \cdot 10^2$ CFU/ml, respectively. However, using a 9K* nutrient medium with a yeast extract (Var6), a slight repeated increase in the aboriginal ACB biomass amount was observed after 21 days of cultivation (Fig. 2, Table 3). The mixotrophic conditions of this experiment result in the formation of a more complex microbial association and, consequently, in the formation of residual biomass due to the loss of part of the population. In such conditions, short-term secondary growth of the amount of ACB bacteria is possible, for which this biomass is an additional source of nutrition. It should be noted that this variant of the experiment provided the maximal decrease in sulfur content in coal — up to 1.40%, which corresponded to 50.35% (Table 3). This indicates a rather high activity of the aboriginal association of ACB, aimed at destroying the stable crystalline structures of the initial coal in order to use sulfur in its composition as a source of energy for its life. This is accompanied by the extraction in the solution from the initial substrate of trace elements, which can act as an additional source of nutrition for the growth and activity of the aboriginal microbial community of acidophilic chemolithotrophic bacteria, which was observed from the 21st to the 28th day of the experiment.

Analysis of the influence of the component composition of media recommended for the ACB cultivation, the variation of $FeSO_4 \cdot 7H_2O$ concentration and the ratio of S:L on the growth indices of aboriginal mesophilic association of acidophilic chemolithotrophic bacteria and the effectiveness of desulfurization process, performed with the usage of unifactor dispersion (Fig. 3, 4) and correlation (Fig. 5, 6) analysis, as well as the PCA method, confirmed the existence of differences between the numbers of bacteria depending on the factors mentioned and the intensity of desulfurization process and allowed us to determine finally the factors that need to be included in optimization matrix.

The analysis of the obtained results shows that the same factors contribute to

Table 3. **Indicators of coal desulfurization efficiency at the variation of nutrient media composition and conditions (S:L)**

Research index	Combination: medium — $FeSO_4{\cdot}7H_2O$, g/dm^3 — S:L	Indicators measured				
		CFU/ml				$S_{general}$, %
		7 days	14 days	21 days	28 days	
Var1	Letain $FeSO_4{\cdot}7H_2O$ — 15,00 г/дм^3 S:L =1:5	$(1.5\pm0.1)\cdot10^5$	$(2.7\pm0.2)\cdot10^2$	$(2.8\pm0.3)\cdot10^2$	$(2.3\pm0.2)\cdot10^2$	1.85±0.05
Var2	Letain $FeSO_4{\cdot}7H_2O$ — 44.50 г/дм^3 T:P=1:5	$(1.9\pm0.2)\cdot10^5$	$(3.5\pm0.3)\cdot10^2$	$(9.4\pm0.8)\cdot10^2$	$(4.9\pm0.4)\cdot10^2$	1.90±0.05
Var3	Letain $FeSO_4{\cdot}7H_2O$ — 44.50 г/дм^3 S:L =1:10	$(5.3\pm0.5)\cdot10^5$	$(5.3\pm0.5)\cdot10^3$	$(7.8\pm0.7)\cdot10^2$	$(3.5\pm0.3)\cdot10^2$	1.70±0.03
Var4	9K $FeSO_4{\cdot}7H_2O$ — 44.50 г/дм^3 S:L =1:5	$(5.3\pm0.5)\cdot10^5$	$(5.0\pm0.5)\cdot10^3$	$(6.7\pm0.6)\cdot10^2$	$(6.8\pm0.7)\cdot10^2$	1.99±0.05
Var5	9K $FeSO_4{\cdot}7H_2O$ — 44.50 г/дм^3 S:L =1:10	$(7.3\pm0.7)\cdot10^5$	$(4.8\pm0.5)\cdot10^4$	$(3.7\pm0.3)\cdot10^3$	$(1.6\pm0.1)\cdot10^3$	1.95±0.05
Var6	9K* $FeSO_4{\cdot}7H_2O$ — 25.00 г/дм^3 S:L =1:5	$(6.8\pm0.6)\cdot10^5$	$(3.2\pm0.3)\cdot10^3$	$(5.4\pm0.5)\cdot10^2$	$(9.7\pm0.8)\cdot10^2$	1.40±0.02

the achievement the maximal titre of the aboriginal association of ACB (shown in Fig. 3 as a decimal log of the CFU/ml value in 7 days of the experiment) and desulfurization (in Fig. 4 in the form of the final sulfur content in the processed coal Sgeneral,%). This is the evidence of the interrelation between these indicators.

On the development of ACB aboriginal association, the presence of KCl (Pearson correlation coefficient r = 0.77), K_2HPO_4 (r = 0.64), $(NH_4)_2SO_4$ (r = 0.57) and $Ca(NO_3)_2$ (r = 0.51) have a significant effect, which is characterized as a positive linear correlation with a high level of coupling. The presence of KH_2PO_4, by contrast, negatively affects

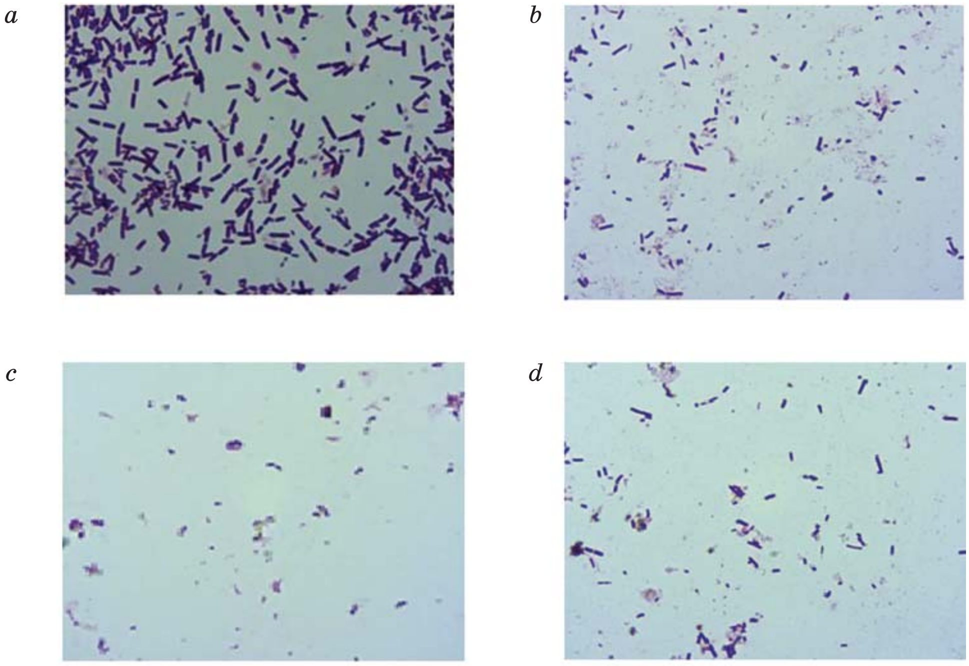

Fig. 2. **Microphotographs of the microbial landscape of the aboriginal mesophilic association of acidophilic chemolithotrophic bacteria in Var6 of the experiment during the cultivation period, days:**
a — 7; *b* — 14; *c* — 21; *d* — 28 (magnification ×1000)

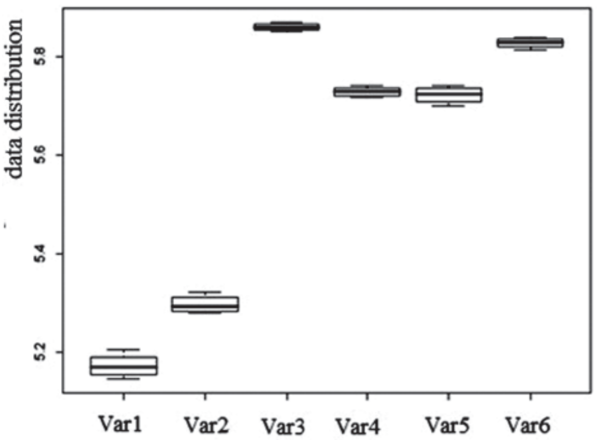

Fig. 3. **Unifactor dispersion analysis of the influence of nutrient medium composition, quantity of $FeSO_4·7H_2O$ and S:L on the titre of AHB aboriginal association**
($F_{fact} = 827.39$; $F_{tabl} = 3.098$, at a given $P = 0.05$)

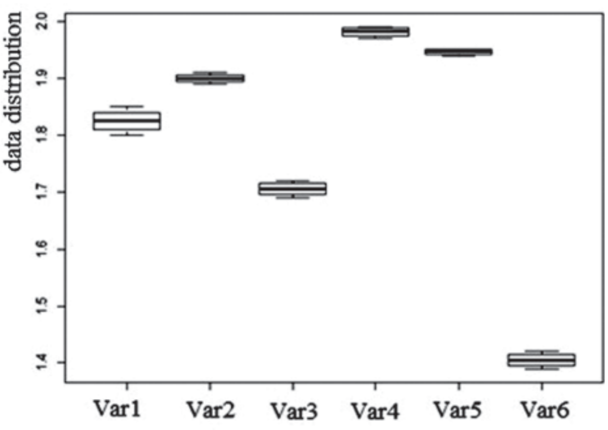

Fig. 4. **Unifactor dispersion analysis of the influence of nutrient medium composition, quantity of $FeSO_4·7H_2O$ and S:L on desulfurization indicator**
($F_{fact} = 1116,8$; $F_{tabl} = 3,098$, at a given $P = 0.05$)

the growth of the aboriginal association of ACB, as evidenced by the negative Pearson's correlation coefficient ($r = -0.56$) (Fig. 5).

In examining the dependence of the desulfurization index on the components of nutrient media, it was noted that the maximal effect on the decrease of sulfur amount in the coal is given by the presence of $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ ($r = -0.84$). Also important is the presence of K_2HPO_4, as it is shown by the Pearson's correlation coefficient at the level of −0.82. The effect of KCl is slightly smaller: $r = -0.57$. However, in this case, the presence of KH_2PO_4, on the contrary, contributes to the increase of sulfur in the coal: $r = 0.84$ (Fig. 6).

It is interesting that the influence of the energy source ($FeSO_4·7H_2O$), according to the results of the correlation analysis, gives a multidirectional effect, depending on the investigated indicator. Thus, its presence positively affects the growth of the aboriginal association of ACB, as evidenced by the Pearson's correlation coefficient ($r = 0.47$) (Fig. 5), however, the presence of $FeSO_4·7H_2O$ has an adverse effect on the desulfurization process ($r = -0.3$) (Fig. 6).

The effect of $MgSO_4·7H_2O$ on the studied parameters is characterized by a weak negative correlation level, as evidenced by the Pearson's correlation $r = -0.34$ for biomass growth (Fig. 5) and $r = -0.32$ for the degree of desulfurization (Fig. 6).

The use of the PCA method allowed us to form the certain factor groups based on their distribution by main components (Table 4). Thus, according to the data obtained by us, it can be seen that the first main component (PCA1) is $(NH_4)_2SO_4$, K_2HPO_4, KH_2PO_4 and $Ca(NO_3)_2$. These factors have the greatest impact on the growth of the aboriginal association of ACB. Combining the obtained data by the PCA results with the results of the correlation analysis, potassium dihydrogen phosphate (KH_2PO_4) should be removed from this group, the presence of which inhibits the growth of ACB association and contributes to the maintenance of sulfur in the coal. The second main component (PCA2) is $MgSO_4·7H_2O$, KCl and yeast extract. Together, the first two components account for 92.93% of the dispersion (Table 4).

A practically identical combination of factors was noted for the desulfurization parameter (Table 5).

The energy source ($FeSO_4·7H_2O$) for all significant parameters (change in bacteria titre of ACB association and the intensity of desulfurization process) were classified in the third main component (PCA3), which explained from 6.0 to 7.0% of dispersion.

Having obtained all the necessary indicators for the reasoned formation of an optimization planning matrix based on the variance analysis adapted for the plans in Greek-Latin squares (Table 2), we have identified the factors and their levels (Table 6). Thus, to determine the optimal composition of the solution for desulfurization, four factors of the nutrient medium components were used: A — the ratio of salts $(NH_4)_2SO_4$ and K_2HPO_4 with concentrations from 0.1 to 0.5 g/dm^3; B — strain *A. ferrooxidans* Coal 17,% (volume); C — YE,% (volume); D — $FeSO_4·7H_2O$, g/dm^3 [11, 14]. Levels of factors are given in Table 6.

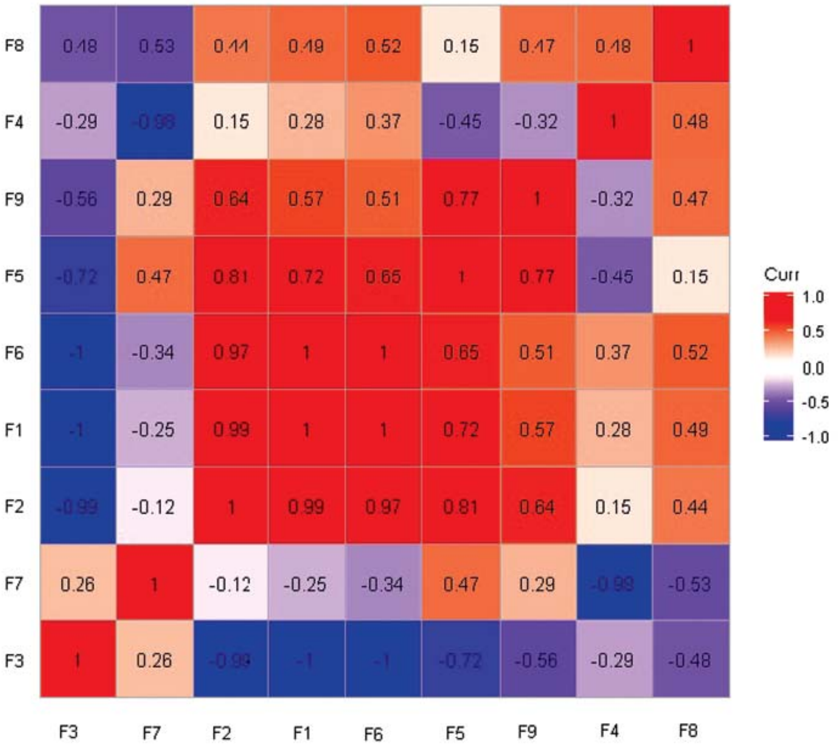

Fig. 5. **Pearson's correlation coefficients (*r*) between the titres (CFU/ml in the form of a decimal logarithm) and the concentrations of nutrient medium components, where F1 – $(NH_4)_2SO_4$; F2 – K_2HPO_4; F3 – KH_2PO_4; F4 — $MgSO_4.7H_2O$; F5 — KCl; F6 – $Ca(NO_3)_2$; F7 – YE; F8 – $FeSO_4.7H_2O$**

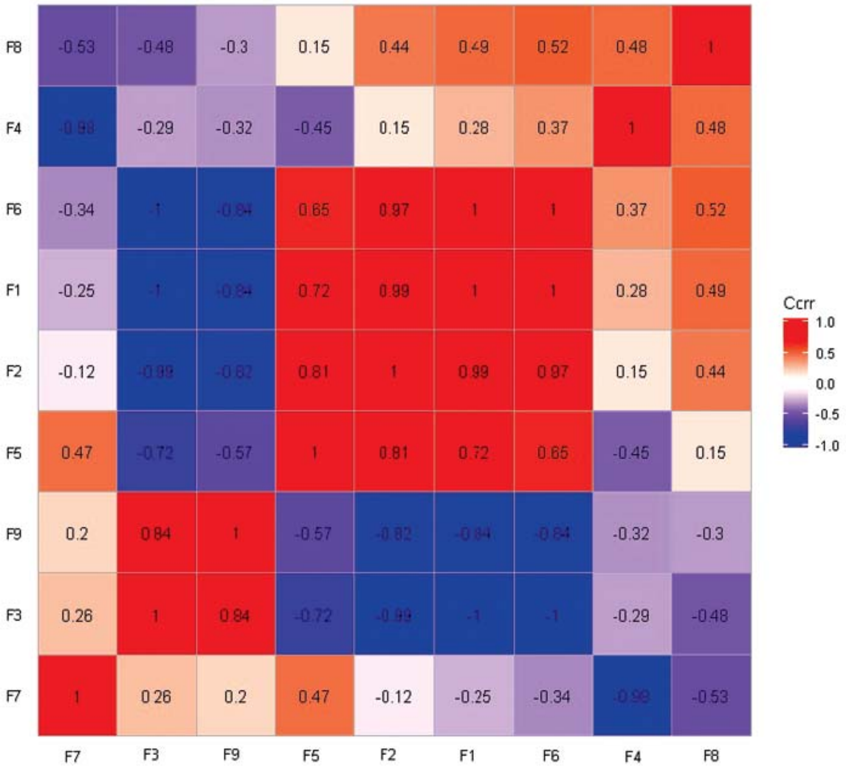

Fig. 6. **Pearson's correlation coefficients (*r*) between the desulfurization indicators and the concentrations of nutrient media components, where F1-$(NH_4)_2SO_4$; F2–K_2HPO_4; F3–KH_2PO_4; F4-$MgSO_4{\cdot}7H_2O$; F5-KCl; F6–$Ca(NO_3)_2$; F7-YE; F8–$FeSO_4{\cdot}7H_2O$**

Table 4. **Characteristics of the main components and their explicit dispersion for the distribution of titre index of ACB aboriginal association**

Components of nutrient media	Main components	
	PCA1	PCA2
$(NH_4)_2SO_4$	–0.44*	–**
K_2HPO_4	–0.43	–
KH_2PO_4	0.44	–
$MgSO_4{\cdot}7H_2O$	–	0.57
KCl	–	–0.46
$Ca(NO_3)_2$	–0.44	–
YE	–	–0.59
Explicit dispersion,%	61.72	31.21
Cumulative dispersion,%	61.72	92.93

Note. * The negative values of the component indicate the position of the point in the coordinate system of the main components (PCA); ** "–" means that the load of this factor is insignificant and is less than 0.3.

Table 5. **Characteristics of the main components and their explicit dispersion for the distribution of desulfurization degree indicator**

Components of nutrient media	Main components	
	PCA1	PCA2
$(NH_4)_2SO_4$	–0.44	–
K_2HPO_4	–0.43	–
KH_2PO_4	0.44	
$MgSO_4{\cdot}7H_2O$	–	0.57
KCl	–	–0.46
$Ca(NO_3)_2$	–0.44	–
YE	–	–0.59
Explicit dispersion.%	61.72	31.21
Cumulative dispersion,%	61.72	92.77

Table 6. **Levels of factors used in the dispersion analysis adapted for the plan in Greek-Latin squares**

Factors	Level 1	Level 2	Level 3	Level 4
A — $(NH_4)_2SO_4$; K_2HPO_4, g/dm^3	0.10:0.10	0.15:0.50	0.30:0.10	0.30:0.50
B — *A. ferrooxidans* Coal 17,% (volume)	0.0	0.80	1.60	3.50
C — YE,% (volume)	0.0	0.025	0.050	0.075
D — $FeSO_4{\cdot}7H_2O$, g/dm^3	0.0	15.00	30.00	44.50

As additional components, the following salts were used in concentration, g/dm^3: KCl — 0,10; $MgSO_4 \cdot 7H_2O$ — 0.10; $Ca(NO_3)_2$ — 0.10; since they are the standard components of nutrient media for chemilototrophic microorganisms cultivating [2, 17]. The process of microbial desulfurization was performed in a thermostat by stationary cultivation at pH≤2.0; temperature 30.0 ± 2.0 °C; within 7 days; the ratio S:L = 1: 10.

According to the results of the experiments, the optimization parameter the vector Y (Table 7) was obtained, which included quantitative measurements of residual sulfur in the coal after desulfurization process with appropriate nutrient medium usage (with the addition of the corresponding amount of *A. ferrooxidans* Coal 17 strain with a titre of $1 \cdot 10^8$ CFU/ml to achieve the maximal effect).

The indicators of the statistical criteria that were guided during optimization process are given in Table 8.

Fisher's calculated criterion clearly indicates the importance of the factors A, B and D, since the inequality $Fr \geq Fst$ (where *Fst* is the tabular value of Fisher's criterion, equal to 3.49) is observed, and their statistical significance is recognized at $\boldsymbol{P}$ = 0.05 (Table 8). The force of factor influence was estimated using the Pohinsky formula, which allows determining what percentage of the optimization index (Y) variation is determined by the operating factor. The calculated factors of the influence force of the factor were almost equal for the factors A, B and D (respectively 26.30, 26.13 and 26.70%). The total cumulative percent, which explains the variation of the indicator for all factors, was 93.63%. The obtained value suggests that unrecognized factors that could affect the change in the desulfurization index were not detected, i.e., the influence of extraneous factors, or the existence of processes that would occur in parallel with the desulfurization process, are not observed, and the result is likely depends on the activity of *Acidithiobacillus ferrooxidans* Coal 17. The maximal indexes of influence force of the factors coincided with those factors for which significant values of Fisher's criterion were determined (Table 8). The calculated Cochran's criterion (*Gr*) in comparison with the table value (*Gst* = 0.68) unequivocally confirms the homogeneity of dispersions for all factors and at all levels.

Analyzing the obtained factors and calculated criteria, one can conclude that the presence of the combination $(NH_4)_2SO_4$ and K_2HPO_4, the $FeSO_4 \cdot 7H_2O$ salt as an energy source and the addition of the strain *A. ferrooxidans* Coal 17 significantly and practically equally influence on the desulfurization process intensity. The effect of the yeast extract was insignificant.

Ranking of factor levels after mathematical interpretation of the obtained results using the multiple Duncan's criterion allowed us to determine that the factor A (ratio $(NH_4)_2SO_4$: K_2HPO_4) at A2 level, factor B (*A. ferrooxidans* Coal 17) at B3 level, factor C (yeast extract) at C2 level and factor D ($FeSO_4 \cdot 7H_2O$ as a source of energy) at D4 level have the greatest influence on desulfurization process of coal from mines of the Lviv-Volyn coal basin (Table 9).

Thus, based on the calculated indicators, an optimal combination of operating factors was determined, which should be used to intensify the process of coal from the mines of the Lviv-Volyn coal basin desulfurization: A2B3C2D4. This

Table 7. **Desulfurization degree (%) during optimization according to the plan in Greek-Latin squares**

Experiment number	Y	Experiment number	Y
1	2.30±0.03	9	1.90±0.03
2	1.82±0.02	10	2.20±0.03
3	1.70±0.02	11	1.90±0.03
4	1.65±0.02	12	1.92±0.03
5	1.50±0.02	13	1.82±0.02
6	1.20±0.02	14	2.05±0.03
7	1.95±0.03	15	1.70±0.02
8	2.20±0.03	16	2.00±0.03

Table 8. **Indicator of criteria that were calculated at optimization the desulfurization process of coal**

Factors	**Fischer's calculated criterion (F_r)**	**Cochran's calculated criterion (G_r)**	**Assessment of influence force of the factor (according to Pohinsky formula)**
A — $(NH_4)_2SO_4$:K_2HPO_4, g/dm^3	5.69	0.47	26.30
B — *A. ferrooxidans* Coal 17, % (volume)	3.53	0.43	26.13
C — YE,% (volume)	1.62	0.46	14.50
D — $FeSO_4 \cdot 7H_2O$, g/dm^3	13.07	0.52	26.70

Table 9. **Analysis scheme using Duncan's multiple rank criterion**

Factors	**Factor levels**				**Ranges on demand * (r Sy)**
	A1(1.70)	A4(1.75)	A3(1.88)	A2(1.98)	
A1(1.70)	–	0.05	0.18	0.28	0.527
A4(1.75)	–	–	0.13	0.23	0.550
A3(1.88)	–	–	–	0.1	0.561
	B2(1.70)	B1(1.80)	B4(1.87)	B3(1.93)	
B2(1.70)	–	0.10	0.17	0.23	0.527
B1(1.80)	–	–	0.07	0.13	0.550
B4(1.87)	–	–	–	0.06	0.561
	C1(1.72)	C3(1.85)	C4(1.86)	C2(1.88)	
C1(1.72)	–	0.13	0.135	0.15	0.527
C3(1.85)	–	–	0.01	0.02	0.550
C4(1.86)	–	–	–	0.01	0.561
	D1(1.60)	D2(1.75)	D3(1.93)	D4(2.03)	
D1(1.60)	–	0.15	0.33	0.43	0.527
D2(1.75)	–	–	0.18	0.28	0.550
D3(1.93)	–	–	–	0.1	0.561

Note: * r [3.20; 3.34; 3.41] Sy = 0.16.

corresponds to the final composition of the nutrient medium, g/dm^3: $(NH_4)_2SO_4$ — 0.15; K_2HPO_4 — 0.50; $FeSO_4 \cdot 7H_2O$ — 44.50; KCl = 0.10; $MgSO_4 \cdot 7H_2O$ — 0.10; $Ca(NO_3)_2$ — 0.10; yeast extract — 0.025 % (volume); *A. ferrooxidans* Coal 17 strain (titre $1 \cdot 10^8$ CFU/ml) — 1.60 % (volume).

When verifying the results, it has been shown that the final indicator of sulfur content in the investigated coal entering the Chervonogradska CEP from the mines of the Lviv-Volyn coal basin (with stationary cultivation at pH≤2.0; temperature 30,0 ± 2,0 °C; within 7 days; the ratio S:L = 1:10, the nutrient medium of the established optimal composition) is 0.95%, which corresponds to a degree of desulphurisation of 66.31%.

Then, applying the mathematical method of experiment planning, the number of experiments has been minimized to study the influence of many factors (in particular, the composition of the nutrient medium, the treatment time, the S: L ratio) on the desulfurization process of natural coal from the mines of the Lviv-Volyn coal basin.

According to the results of the work, it has been established that the combined effect of certain unrelated factors affects on the total abundance of aboriginal mesophilic association of acidophilic chemolithotrophic bacteria, which, in turn, is decisive for the efficiency of desulfurization process.

The composition of the optimized nutrient medium (ONM), g/dm^3 has been specified: $(NH_4)_2SO_4$ — 0.15; K_2HPO_4 — 0.50; $FeSO_4 \cdot 7H_2O$ — 44.50; KCl = 0.10; $MgSO_4 \cdot 7H_2O$ — 0.10; $Ca(NO_3)_2$ — 0.10; yeast extract — 0.025% (volume); *A. ferrooxidans* Coal 17 (titre $1 \cdot 10^8$ CFU/ml) — 1.60% (volume). The new ONM in quantitative composition was different from those that are generally accepted and recommended for the cultivation of acidophilic chemolithotrophic bacteria.

The degree of desulfurization when using a new nutrient medium can decrease the content of sulfur in the coal to 0.95%, which corresponds to a loss of sulfur content by 66.31% for 7 days, which could not be obtained before. This has a positive effect on the general characteristics of the processed coal: the ash content is decreases to 12.88 % and the specific heat of combustion increases to 19.07 MJ/kg.

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ОПТИМІЗАЦІЯ БАКТЕРІЙНОЇ ДЕСУЛЬФУРИЗАЦІЇ ВУГІЛЛЯ З ВИКОРИСТАННЯМ МАТЕМАТИЧНИХ МЕТОДІВ

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Метою роботи була оптимізація процесу бактерійної десульфуризації енергетичного вугілля, зокрема визначення впливу компонентного складу живильного середовища та умов здійснення процесу, що забезпечують максимальний розвиток і активність аборигенної асоціації ацидофільних хемолітотрофних бактерій і, як наслідок, максимальний показник зниження сірки у вугіллі за мінімальний термін. Для оптимізації використовували метод математичного планування експерименту, адаптований до плану греко-латинських квадратів, розрахунки в якому ґрунтуються на дисперсійному аналізі (ANOVA). Формальне планування експерименту проводили з чотирма діючими факторами (компонентами живильного середовища) на чотирьох рівнях (концентрацій). Розрахунки виконували в програмі Excel. Вибір діючих факторів і їх комбінацій було зроблено з використанням однофакторного дисперсійного аналізу, кореляційного аналізу та методу головних компонент PCA у програмі R 3.4.0 на підставі результатів попередньо проведених оцінювальних експериментів. Для отримання найбільш значущого ефекту з десульфуризації використовували аборигенний штам *Acidithiobacillus ferrooxidans* Coal 17, виділений із досліджуваного вугілля. Для кожного компонента живильного середовища проведено аналіз значущості рівня фактора на підставі множинного рангового критерію Дункана, перевірки однорідності дисперсій за допомогою критерію Кохрена, а також значущості факторів за критерієм Фішера. В результаті для оптимального живильного середовища рекомендували комбінацію чинників і їхніх рівнів, що відповідає складу, г/дм^3: $(NH_4)_2SO_4$ — 0,15; K_2HPO_4 — 0,50; $FeSO_4{\cdot}7H_2O$ — 44,50; KCl — 0,10; $MgSO_4{\cdot}7H_2O$ — 0,10; $Ca(NO_3)_2$ — 0,10; дріжджовий екстракт — 0,025% (об.); штам *A. ferrooxidans* Coal 17 (титр $1{\cdot}10^8$ КУО/мл) — 1,60% (об.). Це уможливлює зниження вмісту сірки у вугіллі на 66,31% за короткий термін (сім діб), чого не можна було досягти раніше.

Ключові слова: десульфуризація, аборигенна асоціація, ацидофільні хемолітотрофні бактерії, план греко-римських квадратів, дисперсійний аналіз.

ОПТИМИЗАЦИЯ БАКТЕРИАЛЬНОЙ ДЕСУЛЬФУРИЗАЦИИ УГЛЯ С ИСПОЛЬЗОВАНИЕМ МАТЕМАТИЧЕСКИХ МЕТОДОВ

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Целью работы была оптимизация процесса бактериальной десульфуризации энергетического угля, в частности определение влияния компонентного состава питательной среды и условий осуществления процесса, обеспечивающих максимальное развитие и активность аборигенной ассоциации ацидофильных хемолитотрофных бактерий и, как следствие, максимальный показатель снижения серы в угле за минимальный срок. Для оптимизации использовали метод математического планирования эксперимента, адаптированный к плану греко-латинских квадратов, расчеты в котором основываются на дисперсионном анализе (ANOVA). Формальное планирование эксперимента проводили с четырьмя действующими факторами (компонентами питательной среды) на четырех уровнях (концентраций). Расчеты были выполнены в программе Excel. Выбор действующих факторов и их комбинаций проводили с использованием однофакторного дисперсионного анализа, корреляционного анализа и метода главных компонент в программе R 3.4.0 на основании результатов предварительно проведенных оценочных экспериментов. Для получения наиболее значимого эффекта десульфуризации использовали аборигенный штамм *Acidithiobacillus ferrooxidans* Coal 17, выделенный из исследуемого угля. Для каждого компонента питательной среды осуществлен анализ значимости уровня фактора на основании множественного рангового критерия Дункана, проверки однородности дисперсий с помощью критерия Кохрена, а также значимости факторов по критерию Фишера. В результате для оптимальной питательной среды рекомендовали комбинацию факторов и их уровней, соответствующих составу, г/дм^3: $(NH_4)_2SO_4$ — 0,15; K_2HPO_4 — 0,50; $FeSO_4{\cdot}7H_2O$ — 44,50; KCl — 0,10; $MgSO_4{\cdot}7H_2O$ — 0,10; $Ca(NO_3)_2$ — 0,10; дрожжевой экстракт — 0,025% (об.); штамм *A. ferrooxidans* Coal 17 (титр $1{\cdot}10^8$ КОЕ/мл) — 1,60% (об.). Это дает возможность снизить содержание серы в угле на 66,31% за короткий срок (семь суток), чего нельзя было достичь ранее.

Ключевые слова: десульфуризация, аборигенная ассоциация, ацидофильные хемолитотрофные бактерии, план греко-римских квадратов, дисперсионный анализ.