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Ecologically Safe Directions of the Low Rank Coal Bioconversion

Chernysh Y.[1*], Plyatsuk L.[1], Ablieieva I.[1], Yakhnenko E.[1],
Roubik H.[2], Miakaieva H.[1], Bataltsev E.[1], Lis D.[1]

[1] Sumy State University, 2 Rymskogo-Korsakova St., 40007 Sumy, Ukraine;
[2] Czech University of Life Sciences Prague, 129 Kamycka St., 165 00 Prague, Czech Republic

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[*]Corresponding Author's Address:
e.chernish@ssu.edu.ua

Abstract. This paper deals with theoretical research of the biochemical features of low rank coals (LRC) treatment. The biochemical bases of the organic component degradation of LRC and the biochemical principles of bioleaching of sulfur compounds and metals from LRC are presented. The analysis of microorganisms' groups was carried out and the optimal conditions of their cultivation were determined. Electronic databases such as KEGG, BacDive, and EAWAG-BBD were used to identify the necessary ecological and trophic groups of microorganisms and to realize the patterns of trophic interactions in associations of different groups of microorganisms both under anaerobic and aerobic conditions. The methodological approach was applied and principle diagram of biochemical research of LRC processing was formed in order to develop the environmentally friendly direction of biogas and humic products production.

Keywords: low rank coal, biochemical features, biogas, humic substances, ecological-trophic groups of microorganisms.

1 Introduction

The past fifteen years have seen unprecedented change in the consumption of energy resources. Increasing the share of renewable energy in Central and Eastern Europe is one of the factors that improve the quality of economic growth [1]. Unexpected high growth in the renewables market, in terms of investment, new capacity and high growth rates in different countries have changed the landscape for the energy sector. However, coal still provides around 40 % of the world's electricity [2].

The coal industry was and remains to be an important basis for the Ukrainian economy. Coal is the main energy source for this country. Today the importance of the coal issue has risen more than ever because of the objective necessity of Ukraine's inclusion into the world economy. The rise of the coal consumption is accompanied by an increase in the anthropogenic impact on the environment, because coal burning, and processing produce more harmful by-products compared to oil and gas [3].

One of the ways of solving this problem is expanding the scope of use new technological treatment decisions of low rank coals (LRC), which will contribute to stabilize the fuel and energy balance and create a stock of time for the coal industry development.

In [4] it was shown that with an increase in the duration of the process of aerobic treatment brown coal by the bacterial strain Acinetobacter calcoaceticus VKPM B-4833 the relative hydrogen content decreases (by 5.9–18.6 wt. %) and the oxygen content increases (by 6.4–11.5 wt. %) in biomodified coals relative to the original coal. The most noticeable changes in the content of these elements occur when the duration of the biotreatment process is 10 h [4].

Biomodification of brown coal leads to an increase of the humic acids formation by 22.9–30.6 % compared with the original LRC. The maximum humic acids formation (32.4 wt. %) was received for coal that was bioprocessed for 10 h write as [4]. It was determined by Ivanov et al that the brown coal biotreatment is accompanied with a change its thermochemical properties in the temperature range of 30–900 °C.

The LRC such as lignite have a soft, friable consistency, opaque appearance, humidity of 30–45 %, high ash content, low fixed carbon content (low energy content) and are considered by-products of open pit mining. LRC as understood by its low degree of carbonization is a great source of humic substances (HS) and has high contents of elements that stimulate microbial growth and development, and, through different mechanisms, its macrostructure allows the release of HS. Consequently, LRC could be

used as an organic amendment for the management of degraded soils. Among the microorganisms that can solubilize LRC to generate substances with similar characteristics to HS obtained from LRC by chemical extraction are bacteria isolated from coal samples; some genera and species of Escherichia freundii, Pseudomonas rathonis, Pseudomonas fluorescens, Streptomyces setoni, Pseudomonas putida, Bacillus sp., Staphylococcus, Rhodococcus and others have been reported. Three new LRC biotransformers were reported: Bacillus mycoides, Acinetobacter baumannii and Microbacterium sp.; these were isolated from environmental samples with coal residues, with the ability to solubilize LRC, producing up to 300 mg/l of HS in liquid medium (Figure 1) [5].

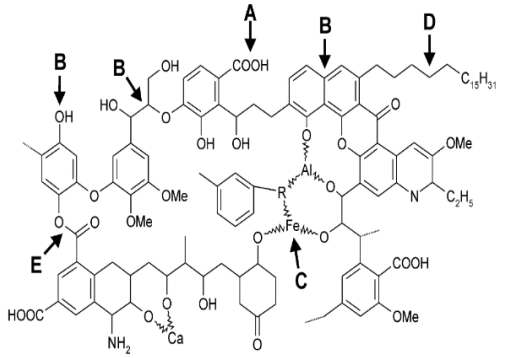


Figure 1 – The so-called ABCDE-mechanism of biological conversion of brown coal (modified after Fakoussa, 1991). The arrow syndicate structures that can be attacked by different microbial agents: A – alkaline substances; B – biocatalysts (oxidative enzymes); C – chelators; D – detergents; E – esterases [5]

The nature and direction of the processes occurring during diagenesis can be viewed by comparing the properties of peat and LRC. The most noticeable differences are in their group composition. Peat still contains the constituent parts of the original plants (carbohydrates, lignin, etc.), but they are almost absent in LRC. Humic acids are present in both peat and LRC. However, humic acids are formed and accumulated during peat formation and its' quantity decreases in the diagenesis process (Table 1) [6].

Table 1 – Group composition of fossil fuels (wt. %)

Substance groups	Fossil fuels				
	Peat	Coal			
		Soft brown coal (LRC)	Solid brown coal (LRC)	Bituminous coal	Anthracite
Bitumoids	8	12	6	5	2
Polysaccharides	29	3	–	–	–
Humic acids	47	65	22	–	–
Humin (residual coal)	16	20	72	95	98

There are three groups in the brown coal group composition: bitumoids, humic acids and residual coal. During carbonization, humic acids of earthy brown coal are transferred to residual coal of solid brown coal [7].

Table 2 shows the comparative characteristics of brown coal and HS by elemental composition [8].

Table 2 – LRC and HS similarities

Element	Humic Acid, %	Coal
Carbon	53.8–58.7	60–70
Hydrogen	3.2–6.2	6.0–5.8
Oxygen	32.8	34
Nitrogen	0.8–4.3	1.5
Sulfur	0.1–1.5	0.2–10

The paper focused on theoretical research of the biochemical features of LRC treatment. To achieve the aim, the following tasks were set:

– theoretical analysis of biochemical features of coal treatment with the methodological approach forming;

– review of bioactivators of intensification processes of LRC bioconversion.

2 Research Methodology

2.1 Biochemical principles of metals leaching from coal

Biological leaching is one of the most successful biotechnological approaches of heavy metals' removal [9]. Unlike other environmental pollutants, the removal of heavy metals from the environment is difficult. These metals cannot be chemically or biologically degradable. Various methods of physico-chemical treatment are effectively used to extract toxic heavy metals from soil and water, but they have some unavoidable disadvantages, such as low productivity and a huge price, unlike the biological methods. In the research works [10, 11] several principles and mechanisms of the bio-leaching process were determined.

Fig. 2 shows the flowchart of features of the bio-leaching process.

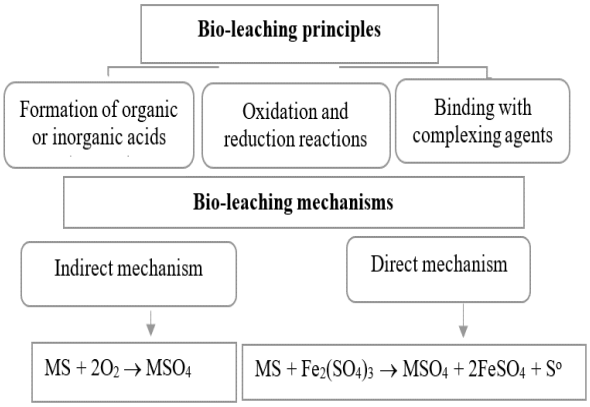


Figure 2 – Principle diagram of biochemical features of leaching

There are two types of mechanisms such as direct and indirect. First needs physical contact between bacteria and metal sulfide particles. Under the indirect mechanism, bacteria oxidize the ferrous ion to the ferric ion state, regenerating the ferric ion, which is necessary for the chemical oxidation of the sulfide mineral [9].

Figure 3 shows the contact, contactless and cooperative mechanisms of the bio-leaching process.

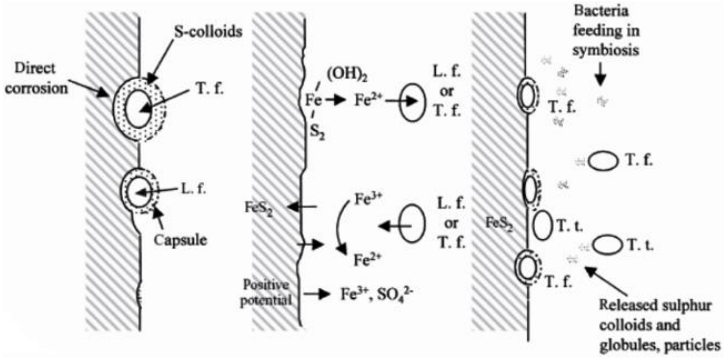


Figure 3 – Patterns of direct and indirect interaction of the bacteria with pyrite: a – contact leaching; b – non-contact leaching; c – cooperative leaching [12]

Sulfuric acid is the main inorganic acid that is found in leaching conditions. Many organic acids are formed by bacterial (and fungal) metabolism that leads to organic acidosis, the complexes and chelates formation [13].

The bacteria then enter the reaction by oxidizing ferrous ions to the ferric state, thereby regenerating the primary oxidant. The direct contact mechanism is independent of the action of ferric ions, requiring only intimate physical contact between the bacteria and the sulphide mineral under aerobic conditions.

The dissolution of metal sulphides is controlled by two different reactions, i.e. the thiosulfate and polysulfide pathways.

The thiosulfate pathway is applicable only to acid insoluble sulfides of metals, such as pyrite.

The mechanism of the pyrite bio-leaching is given below [13]:

$$FeS_2 + 6Fe^{3+} + 3H_2O \rightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+ \quad (1)$$

$$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \rightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+ \quad (2)$$

In total, these two equations can be presented in this form:

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 2SO_4^{2-} + 15Fe^{2+} + 16H^+ \quad (3)$$

The main role of microorganisms in this mechanism is to catalyze the regeneration of ferric ions by aeration. It's shown in the equation:

$$14Fe^{2+} + 3.5O_2 + 14H+ \rightarrow 14Fe^{3+} + 7H_2O \quad (4)$$

Equations (3) and (4) describe the indirect mechanism.

The general reaction based on the primary of oxygen oxidation is given below:

$$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (5)$$

Equation 5 represents the direct mechanism of pyrite removal from coal.

The polysulfide pathway is applicable to acid-soluble metal sulfides, such as PbS, ZnS, FeAsS and $CuFeS_2$.

The mechanism of the zinc sulfide bio-leaching reaction is shown below:

$$8ZnS + 14Fe^{3+} + 2H^+ \rightarrow 8Zn^{2+} + 14\,Fe^{2+} + H_2S_8 \quad (6)$$

$$H_2S_8 + 2Fe^{3+} \rightarrow S_8 + 2Fe^{2+} + 2H^+ \quad (7)$$

The role of the microorganisms in this mechanism is twofold:

– catalyzation of the regeneration of ferric ions used for the chemical oxidation of intermediate hydrogen sulfide to elemental sulfur through the formation of polysulfides;

– catalyzation of the generation of sulfuric acid in order to maintain the required number of protons in the first stage of the mineral dissolution reaction.

It is obvious that the high oxidation rate from ferrous to ferric iron is important for an efficient biological leaching process of sulfide minerals.

2.2 Bio-desulphurization of coal

The organisms can be classified based on whether they can remove inorganic or organic sulfur from coal: (a) obligate autotrophs oxidize only pyritic sulfur, (b) facultative autotrophs oxidize pyritic sulfur and some organic sulfur compounds, (c) heterotrophs oxidize only some organic sulfur compounds [14].

Bioleaching process using acidophilic sulfur oxidizing bacteria (Acidithiobacillus ferrooxidans, Acidithiobacillus thioosidans) and neutrophilic microorganisms (Aspergillus niger) has been intensively investigated for successful removal of metals from sediment, municipal solid, and sludge [15].

Mesophilic and moderately thermophilic acidophilic chemolithotrophic bacteria (ACB) and archaea, particularly, mesophilic representatives of the genus Acidithiobacillus – Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans dominate in the process of inorganic sulfur's removing. Information about the use of other ACB's representatives in the process of coal desulfurization is not enough. There is information about the use for these purposes of a representative of the genus Acidithiobacillus – a strain of Acidithiobacillus ferrivorans, which has distinctive ability to grow on tripton and soy broth along with the properties common to typical representatives of Acidithiobacillus. The strain isolated from the acidic drainage waters of the Balıkesir field (Turkey) and identified by molecular genetic analysis using 16S rRNA as Acidithiobacillus ferrivorans was able to oxidize sulfur and iron [16].

The data showed that 26.7 % of sulfur was removed by Alicyclobacillus in a few days; however, 49.1 % of sulfur was removed by Acidithiobacillus in 30 days. This was interesting since the leachings of zinc, strontium, titanium,

and iron by Alicyclobacillus, obtained in a few days, were almost the same as the leachings by Acidithiobacillus in 30 days. The results obtained also showed that the Alicyclobacillus cells growing at 55 °C removed most of the coal impurities without any change in the carbon content of this fuel. To the best of our knowledge, coal leaching by Alicyclobacillus is reported for the first time [17].

According to study [18] Acidithiobacillus ferrooxidans had significantly promoted the biodesulfurization of coal and bioleaching of coal's pyrite. After 16 days of processing, the total sulfur removal rate of coal was 50.6 %, and among them the removal of pyritic sulfur was up to 69.9 %.

The adapting of the Acidithiobacillus ferrooxidans population at higher concentrations of ferrous sulphate (18g/l Fe^{2+}) determined a raised efficiency of coal desulphurization at values between 63.1–88.5 %. In addition, raising the solid/liquid ratio from 0.05 to 0.1 g/ml determined the increasing of the coal biodesulphurization efficiency, which gets to 57.3–76.4 % for the pit coal and 72.2–82.5 % for lignite. The comparative results regarding the efficiency of coal desulphurization in the presence of A ferrooxidans cultures illustrated that the P7 population oxidized the highest percentages of sulphur from coal (54.8–63.1 %) [19].

In study [20], low-rank lignite coal sample collected from Jining coalfield of Shandong province in China was subjected to desulphurization by using a new bacteria and Acidithiobacillus ferrooxidans isolated from the native coal mine site. The molecular identification of the 16S rRNA gene showed that the new native bacteria was Pseudomonas sp., denoted as NP22, and it is reported for the first time for the capability to remove about 46 % of total sulphur from the lignite coal. Analytical characterization indicated that total sulphur content of lignite coal was reduced to 2.8 % and 3.2 % by using two microorganisms. In addition, the calorific value of lignite coal was not affected adversely after two microorganisms' desulphurization but rather its calorific value increased from 6.2 to 6.4 kcal/g, and 6.3 kcal/g, however, the ash content of the lignite coal was eliminated.

Accordingly, the removal of sulfur compounds and heavy metals from LRC occurs in the same biochemical process of the coal components transformation by sulfur-oxidizing microorganisms under aerobic conditions.

With certain coals, the direct mechanism for oxidation of pyrite may be limited because the microorganisms are too large to enter most of the coal pores as shown in Figure 4.

The process is accompanied by the acid's formation, which supports the low pH values, favorable for the vital and oxidative activities of ACB [16].

Bio-desulphurisation of organic sulphur was carried out in most of the literature by using model compounds, which are frequently recognized as the organo-sulphur compounds in coal. Various organisms such as Rhodococcus erythropolis IGTS8 (ATCC 53968), Shewanella putrefaciens, Brevibacterium, Rhodococcus sp. IGTS8, thermophilic Paenibacillus sp. A11-2 and B. Subtilis WUS2B, Mycobacterium sp., Gordonia sp., Microbacterium, Lysinibacillus sphaericus and so forth, were also explored for bio-desulphurisation of coals. Most of these investigations stressed upon the relevance of bio-desulphurisation of coal with the microbes while testing their potency to degrade the organic S-compounds expected in coals [21].

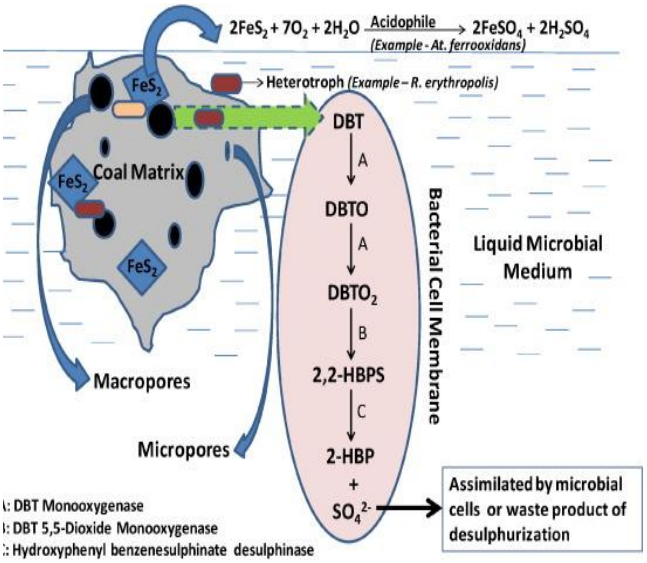
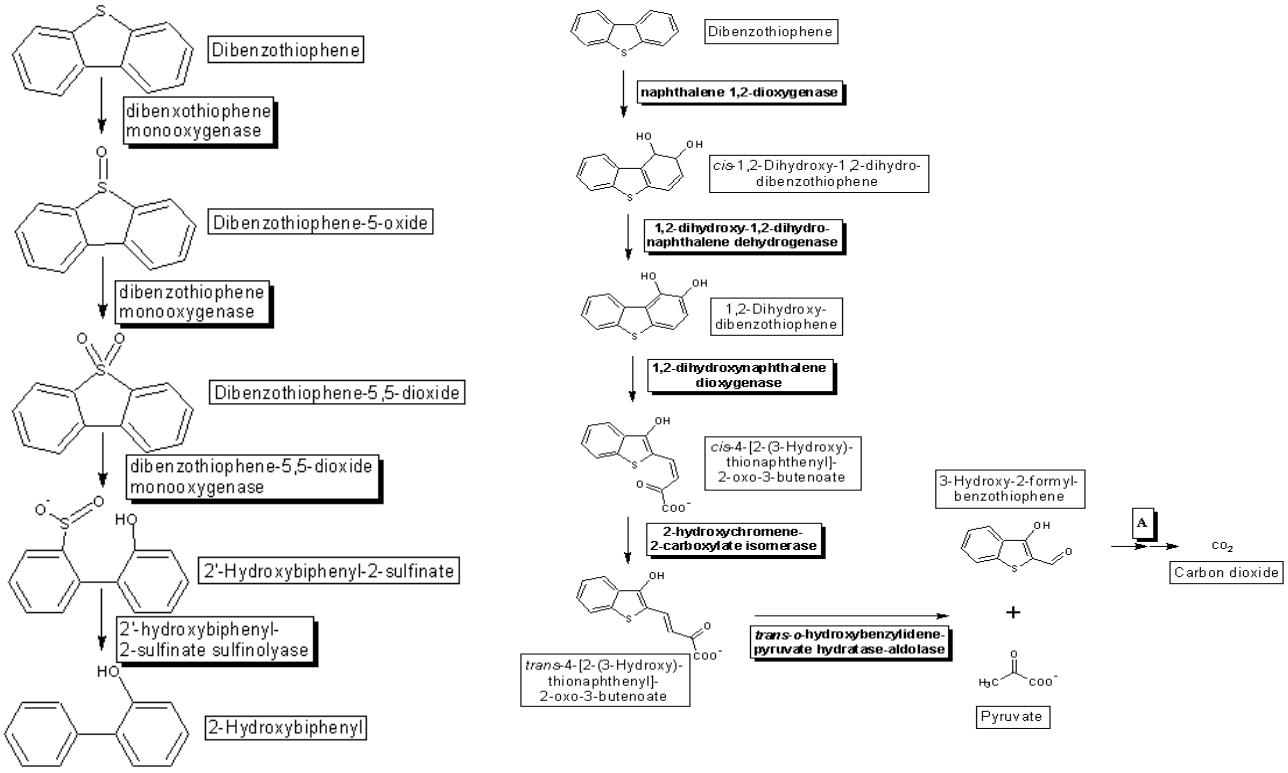


Figure 4 – Bimodal pore structure of coal and pyrite oxidation [7, 8]

Dibenzothiophene (DBT) is a model compound for organic sulfur in fossil fuels, and its desulfurization pathway removes this sulfur. Rhodococcus sp. IGTS8 can use dibenzothiophene as a sole source of sulfur [22].

Pseudomonas sp. C18 can metabolize dibenzothiophene, naphthalene and phenanthrene by a single pathway. The names of the enzymes in the pathway are not given, only the DOX operon, which encodes them in its 9 open reading frames (ABDEFGHIJ). The pathway is very similar to the one of naphthalene and the enzymes have high sequence identity with the naphthalene enzymes, thus the corresponding names have been used for the enzymes. Naphthalene dioxygenase, the enzyme that initiates this pathway, is used in a biotechnological process to synthesize the blue jean dye indigo. This versatile enzyme has many other catalytic abilities, which are documented in a table of the Reactions of Naphthalene 1,2-Dioxygenase [23] (Figure 5).



a b

Figure 5 – Graphic representation of biotransformation paths in coal desulphurisation processes:
a – dibenzothiophene desulfurization graphical pathway map; b – dibenzothiophene degradation graphical pathway map

2.3 Development of the methodological approach of the biotechnological integrated processing of LRC with biogas production

Bioconversion is accomplished by adapting microorganisms to coal in the presence of other appropriate nutrient components. This processing can have several step that includes different type of transformation LRC under aerobic and anaerobic conditions (Figure 6).

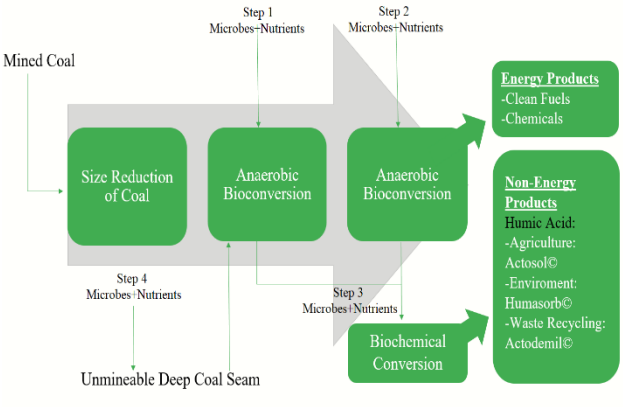


Figure 6 – Integrated MicGAS™ biotechnology process flow scheme [8]

The model for biochemical parameters determining has been developed (Figure 7) to assess the effectiveness of the coal use as a mineral substrate by various groups of microorganisms.

In the work [24], mixed cultures of T. ferrooxidans AM and T. thiooxidans AM were used for desulphurization of coal. These cultures were isolated from sediments of river, which flows through a coal belt containing high sulphur. Isolated cultures were stored and maintained on a nutrient medium with coal containing sulphur.

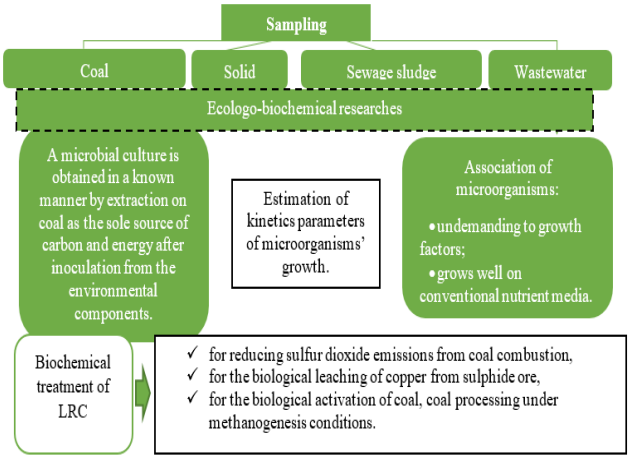


Figure 7 – Principle model of biochemical researches

Seeding methods on dense and in liquid nutrient substrates consider only viable cells of microorganisms. If we need to isolate and consider the widest possible range of microorganisms inhabiting this substrate, we use the Koch method and at the same time select a substrate where microorganisms with different properties can grow. However, it is not possible to identify all groups of microorganisms on the same substrate due to significant physiological and biochemical differences between them. The basis for isolating and determining the number of representatives of microorganisms' individual groups is the obtaining of accumulative cultures by creating elective conditions. This was in similarity with findings in different studies by [25, 26].

Bacteria are isolated from the soil in the following way. Individual soil samples are taken with a clean tool from each horizon, mixed samples are taken across several horizons by a cylindrical drill from no less than five points of the field along one or two diagonals. The larger the field area, the more individual samples are needed. According to [27] numbers of colonies appearing on solid media were determined by examining the plates at a magnification of ×10 with a stereomicroscope. Each count represents the mean for a series of dilution steps with three plates at each dilution and was calculated based on the dry weight of the soil and dilution factors.

Further, a soil suspension is prepared from water and the soil protected from impurities, and it is used to prepare dilutions. Dilutions are made in sterile 0.5 % aqueous solution of NaCl. Decimal dilutions are most often made; that was confirmed in [28].

The suspension of microorganisms obtained after dilution is used to determine the number of microorganisms by sowing on various nutrient substratum and/or to consider the number of microorganisms using direct microscopy, as well as to study the qualitative composition and morphology of microorganisms by microscopic methods.

Vinogradsky–Shulgin–Brida method is used in various modifications to determine the number of microorganisms in a variety of natural substrates – in soil, polluted water, optically opaque environments. Counting cells on fixed stained smears is reduced to the fact that in a certain volume of the investigated suspension, directly under the microscope, the number of microorganism cells is counted. The use of fixed smears makes it possible to save preparations for a long time and to count not in the course of experience, but at a time convenient for the researcher.

Meat-peptone agar (MPA) is often used as a nutrient substrate for cultivating soil microorganisms. It is suitable for cultivating of many heterotrophic microorganisms. Microorganisms of various systematic and physiological groups grow on MPA after seeding from the soil: Gram-negative bacteria of the genus Pseudomonas, Flavobacterium, gram-positive spore-forming bacillus of the genus Bacillus, cocci of the Micrococcus and Sarcin genus, various mycobacteria (Mycobacterium genus) and some higher actinomycetes (Streptomyces genus).

The microbes that are present in the test material and grow on a selective substrate can be isolated into pure culture. This can be confirmed by microscopy. The Gram stain was carried out in accordance with the generally accepted method [29]. Identification of cultures was carried out according to Bergey's Manual of Determinative Bacteriology [30] based on data from morphology, physiology and biochemical properties of microbial cells.

The research is carried out in two directions: the first one – the allocation of accumulated culture from the soil or another environment; the second is the research of the development's dynamics of the required ecological and trophic microorganisms' groups on the proposed substrate (LRC, etc.).

The advantage of deep cultivation is that this method does not require large areas and cumbersome equipment, the capacity of fermenter can be increased by the height enlargement. Easy maintenance, the possibility of automation, convenience of removing the intact solid product from the culture fluid are also the advantages of this method.

In this case the removal of aerobic bacterias occurs on the surface in a laboratory thermostat TGU-01-200, where microorganisms receive oxygen directly from the air.

Chemical methods include:

1) the use of chemicals that absorb molecular oxygen. Pyrogallol alkaline solution, sodium dithionite ($Na_2S_2O_4$), metallic iron, monovalent copper chloride and some other reagents are used as molecular oxygen absorbers in laboratory practice.

2) the use of reducing agents that are added to most substrates for decreasing their redox potential: sodium thioglycolate, cysteine, ascorbic acid.

The methods that limit the access of air to the growing culture for the cultivation of anaerobic bacteria use:

– growing in a high layer of substrate;

– growing in a dense substrate's layer;

– cultivation in viscous substrates where the diffusion of molecular oxygen into a liquid decrease with an increasing its density;

– filling the substrate with a high layer of sterile vaseline oil or paraffin.

Research has been continued on seeking new and mutant microorganisms for different biotransformations and on biodegradation of organic compounds as models for microbial studies such as destructions of lignin, organic sulfur compounds, car-boxyl groups and organic nitrogen bonds [31].

The most common compound used as a model for organic sulphur compounds in coal is dibenzothiophene (DBT) [21]. Two new bacterial isolates capable of dibenzothiophene (DBT) degradation to benzoic acid through the 4S-pathway were isolated from different Egyptian hydrocarbon polluted soil samples. These organisms, designated NShB1 and NShB2, were tentatively identified as Aureobacterium sp. and Enterobacter sp., respectively as determined by 16S rDNA gene sequence analysis. DBT degradation pathway has been identified by GC-MS. The NShB1 and NShB2 strains were capable to degrade up to

approximately 49 % and 36 % of 1 000 ppm DBT, respectively within 7 days of incubation at 30 °C and pH 7 [32].

However, the important direction is the research of the natural microorganisms' associations capable to effectively carry out complex bioconversion of LRC with the formation of an integral biochemical model of the process in artificially created cultivation conditions.

The analysis of the ecological-trophic groups of microorganisms was carried out and Table 3 with the conditions of their cultivation was formed with using electronic databases such as KEGG database, BacDive and EAWAG-BBD. Thus, the main groups of microorganisms and the conditions in which they can be cultivated were analyzed, which will further help to choose the best option for combining them for carrying out a complex bioconversion of brown coal.

Table 3 – Analysis of the cultivation conditions of different ecological-trophic microorganisms' groups involved in the desulfurization and methanogenesis in the LRC processing

Genus	Bacterium species	Culture substrate	pH	Temperature	Aerobic or anaerobic
Acinetobacter	Acinetobacter calcoaceticus	strain is undemanding to growth factors, grows well on conventional nutrient substrates (meat peptone broth (MPB), meat peptone agar (MPA), dry nutrient agar (SPA)). It forms small, round, non-pigmented colonies, shiny, with a smooth edge on agar substrates	7.0–7.2	30 °C	Aerobic
Acidithiobacillus	Acidithiobacillus ferrooxidans	strain is cultivated on substrates with iron (II), manganese (II) ion, elemental sulfur, thiosulfate and sulfide ions as the sole source of energy. It is not able to use sugar and peptone	1.0–4.0	25–30 °C	Aerobic
	Acidithiobacillus thiooxidans		1.5–2.15	20–23 °C	Aerobic
Aureobacterium	–	strain is cultivated with constant mixing (150 rpm) during 12 days in an environment that does not contain phosphates, and which is prepared on 50 mm HEPES buffer	7.2	28 °C	Aerobic
Desulfobacter	–	cultivated in the substrate of Postgate C	7.0–7.6	28 °C	Anaerobic
Methanosaeta	Methanosaeta concilii	De Ley Method	8.0	37 °C	Anaerobic
Methanosarcina	Methanosarcina barkeri	tested with 0.1 M sodium formate or with a headspace of 200 kPa H_2-CO_2 (80:20) substituted for trimethylamine.	6.0–7.0	25 °C	Anaerobic
Rhodococcus	Rhodococcus erythropolis	agar medium (meat-peptone agar, wort agar, glucose-potato agar agar)	6.0–8.5	28–30 °C	Aerobic
Lysinibacillus	Lysinibacillus sphaericus	cultivation on the Munz's agar substrate	6.5–8.0	10–37 °C	Aerobic

The goal of some coal bed producers is to extend coal bed methane productivity and to utilize hydrocarbon wastes such as coal slurry to generate new methane. However, the process and factors controlling the process, and thus ways to stimulate it, are poorly understood. Subbituminous coal from a nonproductive well in south Texas in [33] was stimulated to produce biogas in microcosms when the native population was supplemented with nutrients (biostimulation) or when nutrients and a consortium of bacteria and methanogens enriched from wetland sediment were added (bioaugmentation). The native population enriched by nutrient addition included Pseudomonas spp., Veillonellaceae, and Methanosarcina barkeri. The bioaugmented microcosm generated methane more rapidly

and to a higher concentration than the biostimulated microcosm. Dissolved organics, including long-chain fatty acids, single-ring aromatics, and long-chain alkanes accumulated in the first 39 days of the bioaugmented microcosm and were then degraded, accompanied by generation of methane. The bioaugmented microcosm was dominated by Geobacter sp., and most of the methane generation was associated with growth of Methanosaeta concilii.

The ability of the bioaugmentation culture to produce methane from coal intermediates was confirmed in incubations of culture with representative organic compounds.

Thus, methane production could be stimulated at the nonproductive field site and that low microbial biomass may be limiting in situ methane generation. In addition, the microcosm study suggests that the pathway for generating methane from coal involves complex microbial partnerships.

Figure 8 shows that only completely unspecific enzymes have a chance of attacking the coal structure recently according to [35].

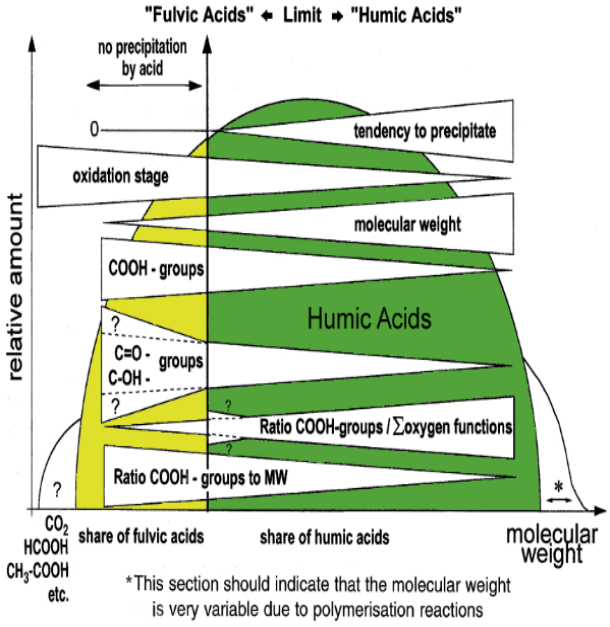


Figure 8 – Flow diagram of different features of coal-derived humic and fulvic acids, modelling their heterogeneity (the relation of humic to fulvic acid is optional) [35]

The population isolated in [34] includes strains of the genera Rhodococus sp., Pseudomonas sp., Mycobactenum sp. and sulfate-reducing microorganisms. Clostridium sp. and Bacillus sp. were morphologically defined.

The concentration of microorganisms in the mixture injected into the LRC is equal to the sum of their concentration in the sludge and in the accumulative culture, diluted 20 times during preparing the mixture of accumulative culture and sludge from digester in a ratio of 5:95.

Should be noted, in Ukraine humic products are mainly used as soil regulator for fruit trees, oil vegetables and short-term nutrition absorbing crops such as rice, wheat, etc. It can also be used as multi-functional high effective compound fertilizer after combination with the elements of nitrogen, phosphorus and potassium.

Further research is expected to lab-scale investigate ability to support biomethane production under brown coal bioconversion. The important direction of the research is microbiological investigation of the natural microorganisms' associations capable to effectively carry out complex bioconversion of LRC with the formation of an integral biochemical model of the process in artificially created cultivation conditions.

3 Conclusions

The biochemical parameters of LRC treatment were analyzed and methodological model of their using was formed to assess the effectiveness of the coal use as a mineral substrate by various groups of microorganisms. Thus, biotechnological approach is the promising way for the integrated processing of LRC to produce biogas and humic substances. At the same time, an important technological aspect is the removal of sulfur compounds and heavy metals from processing products. In this way, it is important to expand the bioenergy potential of brown coal to produce methane after biogas purification from impurities.

Different bacteria genera were considered under LRC biotreatment with biogas production and their cultivation conditions such as Desulfobacter sp., Methanosaeta sp., Methanosarcina sp., Rhodococcus sp., Lysinibacillus sp. etc. Thus, the main groups of bioactivators were analyzed, which will further help to choose the best option for combining them for carrying out a complex bioconversion of brown coal.

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Екологічно безпечні напрямки біоконверсії низькоякісного вугля

Черниш Є.[1*], Пляцук Л.[1], Аблєєва І.[1], Яхненко О.[1],
Рубік Г.[2], М'якаєва Г.[1], Батальцев Є.[1], Ліс Д.[1]

[1] Сумський державний університет, вул. Римського-Корсакова, 2., 40007, м. Суми, Україна;
[2] Чеський агротехнічний університет, вул. Камицка, 129, 165 00, м. Прага, Чехія

Анотація. У цій статті наведені теоретичні дослідження біохімічних особливостей оброблення низькоякісного вугілля. Представлені біохімічні основи деградації органічних компонентів низькоякісного вугілля і біохімічні принципи біологічного вилучення з нього металів та сполук сірки. Проведено аналіз груп мікроорганізмів і визначені оптимальні умови вирощування останніх. Для ідентифікації необхідних екологічних і трофічних груп мікроорганізмів, а також для реалізації закономірності трофічних взаємодій у асоціаціях різних груп мікроорганізмів як в анаеробних, так і в аеробних умовах використовувались електронні бази даних KEGG, BacDive і EAWAG-BBD. З метою розроблення екологічно безпечного напрямку виробництва біогазу і гумінових продуктів застосовано методологічний підхід та сформована принципова схема біохімічних досліджень переробки низькоякісного вугілля.

Ключові слова: низькоякісне вугілля, біохімічні особливості, біогаз, гумінові кислоти, еколого-трофічні групи мікроорганізмів.