

**ABSTRACT**

The report consists of 52 pages, 31 figures, 20 tables, 73 sources, 3 applications.

OXIDIZED BROWN COAL, ELEMENTAL COMPOSITION, TECHNICAL CHARACTERISTICS, MICROBIOM, METAGENOMICS, BACTERIA, BIOSOLUBILIZATION.

Objects of research - Oxidized brown coals of the near-land areas of the Oy-Karagai coal deposit of the Almaty region, the Lengersky (Karatau) coal deposit of the Turkestan region and the Kiyakta coal deposit of the Karaganda region were used.

The purpose of the research is to create a multicomponent preparation "biohumus-plus" based on oxidized brown coal, cells of microorganisms with high target metabolic activity, with the participation of earthworms to increase the yield of potatoes, improve the quality of its tubers and soil fertility.

All experiments were carried out in laboratory conditions using physicochemical, genetic and microbiological research methods.

All the tasks of the stage were solved in full in accordance with the technical specification and the work schedule for 2018-2020.

The technical and economic indicators of this stage of research include the results obtained during the planned experiments and assessing their economic significance.

The results obtained in the study of the physicochemical and microbiological properties of oxidized brown coals of Kazakhstan coal deposits can be used in scientific research, publications, and educational materials.

Scientific and organizational work: number of published works - 14, of which scientific articles - 6, abstracts - 8. Participation in conferences - 8, number of reports presented - 8. Patent - 2, patent application - 1. Monograph - 1.

**ТҰЖЫРЫМ**

Есеп 52 беттен, 31 суреттен, 20 кестеден, 73 әдебиет көзінен, 3 қосымшадан тұрады.

ТОТЫҚҚАН ҚОҢЫР КӨМІР, ЭЛЕМЕНТТІК ҚҰРАМ, ТЕХНИКАЛЫҚ СИПАТТАМА, МИКРОБИОМ, МЕТАГЕНОМИКА, БАКТЕРИЯ, БИОСОЛЮБИЛИЗАЦИЯ.

Зерттеу объектілері – жұмыс барысында Алматы облысының Ой-қарақай көмір кені, Түркістан облысының Ленгер (Қаратау) көмір кені және Қарағанды облысының Қияқты көмір кені шахта маңының тотыққан қоңыр көмірлері пайдаланылды.

Зерттеудің мақсаты – картоптың өнімділігін арттыру, оның түйнектерінің сапасы мен топырақтың құнарлылығын жақсарту үшін жауын құрттарының қатысуымен тотыққан қоңыр көмір, мақсатты метаболизм белсенділігі бар микроорганизмдер жасушалары негізінде көп компонентті препарат жасау.

Барлық тәжірибелер зертханалық жағдайда физика-химиялық, биохимиялық, генетикалық және микробиологиялық зерттеулердің әдістерін қолдана отырып жүргізілді.

Жұмыс кезеңінің алға қойылған міндеттері техникалық сипаттамалар мен 2018-2020 жылдарға арналған жұмыс жоспарына сәйкес толығымен орындалды.

Ғылыми жұмыс кезеңінің техникалық-экономикалық көрсеткіші жоспарланған тәжірибелер мен экономикалық өзектілігін бағалау арқылы алынған нәтижелермен сипатталады.

Қазақстан көмір кен орынының тотыққан қоңыр көмірлерің физика-химиялық және микробиологиялық қасиеттерін зерттеу барысында алынған нәтижелер ғылыми мақсаттарда, жарияланымдар мен оқу құралдарында қолданылуы мүмкін.

Ғылыми - ұйымдық жұмыс: жарияланған жұмыстың саны – 14, соның ішінде ғылыми мақалалар – 6, тезистер – 8. Конференцияға қатысуы - 8, ұсынылған баяндама саны – 8. Патент – 2, патентке өтінім – 1. Монография – 1.

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**DEFINITIONS, DESIGNATIONS AND ABBREVIATIONS**

CFU – Colony Forming Units

TMC – Total Microbial Count

PCR – Polymerase Chain Reaction

XRC – X-ray crystal analysis

XRF – X-ray fluorescence analysis

NGS – Next Generation Sequencing

A – Ash-content, %

ACE – Abundance-based Coverage Estimator

EDX – Energy-dispersive X-ray spectroscopy

FTIR – Fourier-Transform Infrared Spectroscopy

KLE – Kiyakty Leonardite

LLE – Lenger Leonardite

OLE – Oi-Karagai Leonardite

OTU – Operational Taxonomic Unit

Q – Calorific value, MJ/kg

V – Volatile matter, %

W – Moisture content, %

bHS – bio-based Humic Substances

cHS – chemical-based Humic Substances

HA – Humic acid

HS – Humic substances

**INTRODUCTION**

Recent studies have established that the main raw material for obtaining highly effective organic fertilizers/amendments for farming and agriculture are brown coals oxidized in seams from different coal basins, as well as coal-containing waste generated in the process of coal mining and coal preparation. It was shown that oxidized brown coals contain a wide range of macro- and microelements, as well as a large amount of humic and fulvic acids, which are similar in composition to soil ones. In fact, they are a storehouse of organic matter and can be a reliable basis for the production of humus, a highly efficient organic fertilizer.

Meanwhile, coals weathered in seams and waste heaps, both brown and hard, are practically not used in industry and the national economy as fuel or raw materials. When coal is mined in an open way, they enter the dumps together with overburden grounds. The volume of oxidized brown coal is estimated for each deposit only during detailed exploration and development.

Thus, the estimated amount of oxidized brown coal from Kazakhstani deposits entering the dumps is tens of millions of tons annually. These coals, as well as coal waste, are stored in tailing dumps, where they are eroded in the atmosphere and today are practically not utilized, polluting the atmosphere and occupying hundreds of hectares of fertile land.

In connection with the above, research on the development of technology for obtaining a complex fertilizer "bio-humus plus" based on Kazakh oxidized brown coals are of theoretical and practical importance.

The goal of the research is to create a multicomponent product "bio-humus plus" based on oxidized brown coal, cells of microorganisms with a high target metabolic activity, with the participation of earthworms to increase potato productivity, tuber yield and soil fertility.

Research objectives:

1. Study of the physicochemical parameters and the microbial landscapes of selected materials.
2. Creation of a microbial consortium for intended purposes.
3. Conducting field experiments on the territory of the Kazakh Scientific Research Institute of Potato and Vegetable Growing. Inventory number 2018 0218РК00446, 2019 0219РК00573.

**MAIN PART**

**1 Selection of research areas**

Oxidation of various fossil coals in seams, i.e. weathering, occurs on a colossal scale and negatively affects not only the properties of the coals themselves, but also the composition and structure of the host rocks, contributing to their degradation, concentration, dispersion and secondary transfer of elements in the earth's crust.

Some authors have found [1] that "in the process of oxidation of hard coals with atmospheric oxygen, carboxyl groups are formed that are linked directly to condensed aromatic rings, in contrast to the original solid coal, in which carboxyl groups are mainly found in aliphatic blocks".

As a result of oxidation, there is a deterioration in the quality characteristics of fossil coals as fuel, while in some cases it is so great that these coals do not even come to energy and fuel use, due to the low heat of combustion and extreme fragmentation. Such coals are not taken into account when calculating economic profits, calculating reserves and are referred to as so-called off-balance coals.

When extracting solid fuel using opencast method, a significant part of it at the outcrops of layers under sediment is not used in agriculture and national economies and goes to dumps in the form of waste heaps, in connection with which, it becomes necessary to develop new methods of utilization. The reserves of oxidized brown and black coal are very large and reach billions of tons. They are scattered over large areas, which makes it difficult to separately extract and process them.

After studying their nature, oxidized coals can be used for the production of chemical raw materials, including fertilizers and for the reclamation of contaminated and disturbed lands.

On the territory of Kazakhstan there are such deposits of brown coal as the Oi-Karagai basin (Almaty province), Lenger basin (Turkestan province) and Kiyakty basin (Karaganda province). The possibility of using oxidized coals to obtain fertilizers, including production of humic substances, is being studied in different countries [2-5].

Weathered and metamorphosed coals contain a huge amount of humic acids, which in their properties and composition are close to the humic substances contained in fertile soils. This circumstance was the determining basis for a detailed study of the possibility of chemically obtaining humic acids (humates) for their use in the production of humus – a natural fertilizer for feeding vegetable crops.

Currently, a significant number of works are published, which show the positive effect of humic acids on soil fertility and crop productivity [6-8].

One of the ways to utilization of coal waste is to use them as fertilizers for crops and potatoes, and as a component of the reclamation layer in the restoration of contaminated and disturbed lands. Literature reviews that the introduction of oxidized coal as an ameliorant significantly increases the yield of seeds of perennial grasses. So, for example, in some crops up to 2 times, and the seed yield numerically amounted to a maximum of 11.1 centners/ha. Subsequently, with the accumulation of organic matter in soils, it is possible to cultivate other crops that are more demanding on soil fertility [9].

According to some data [10], brown coal and products obtained on its basis, when processed with biologically active substances, increase the productivity of agricultural crops without negatively affecting the humus state of chernozem, improving the mechanical structure of soils and supplying it with nutrients by increasing biomass and physiological activity of microorganisms. This study indicates that the introduction of brown coal promotes the migration of heavy metals into sedentary forms, and coal also promotes the binding of mobile compounds of heavy metals, which can have both positive and negative significance, depending on the level of concentration of trace elements in the soil and their physiological role.

Carbonaceous rocks contain various chemical elements necessary for the growth and development of crops, therefore they are one of the reserves of nutrients in the soil.

Studies [11] found that the use of oxidized coals as fertilizers requires liming (chalking), due to the content of pyrite in them. Once coming on the surface and forming a cover, pyrite can be oxidized to sulfuric acid, thus increasing the acidity of the soil.

The author of works [12] explains the mechanism of action of calcium carbonate during liming. It was pointed out that the application of this method on peat soils poor in calcium accelerates the decomposition processes, but at the same time contributes to a change in the composition of the soil due to the accumulation of humic acids associated with calcium.

According to other authors [13], liming has no pronounced effect on the concentration of humus; reduces the number of mobile forms of humic substances, increases the content of humates due to fractions associated with Ca, increases the content of non-hydrolysable residue and reduces the amount of fulvic acids.

When assessing the value of humic substances in the nature of the soil and its fertility, it is necessary to take into account the following factors: improving the physical, physicochemical structures of the soil, improving the supply of CO2 to plants for photosynthesis with nutrient elements and their entry into the plant, increasing the microbiological activity of the soil, regulating the presence of a number of biologically active substances.

The analysis of the studies [14] confirms that the use of humic fertilizers improves the water-physical properties of soils – the density and cohesion decrease, the structural coefficient, moisture capacity and moisture reserves in chernozem increase.

The study [15] note that oxidized brown coals improve the physical properties of soils, increasing their sorption capacity due to humified components, improving the mineral nutrition of plants and their supply with microelements. The interaction of different forms of nitrogen in the soil is mainly determined by the microbiological activity of the soil. The introduction of wastes from the coal industry has a great effect on the enzymatic activity and dynamics of mineral forms of nitrogen in the soil [16]. Oxidized coals contain about 50% carbon and, when applied to soils, can create a good reserve for conversion to soil humus.

Humus is the main indicator of soil fertility, largely determining their water and chemical-physical properties [5, 17]. There are evidences [18] indicating the positive effect of organic coal fertilizers on the content and reserves of humus in arable soils.

The use of fertilizers in regulated agroecosystems [19] improves the humus state of soils due to better development of the rhizosphere and its penetration into deeper horizons of the soil profile, and also causes changes in the qualitative composition of root residues. All this is directly related to the activity of microbiocenosis in the soil.

Studies [20] of the effect of various coal waste fractions on the microbiological activity of podzolic soil when applying fertilizer based on ammonia under aerobic conditions have shown that the organic part of carbonaceous rocks used as ameliorant can be consumed by soil microflora as a source of nutrients.

When carbonaceous rocks are introduced into soddy-podzolic soils, the number of microorganisms increases hundreds of times. When fertilizing the soil with carbonaceous rocks, those microbial communities that are involved in the mineralization of organic components are significantly activated. As a result, the soil is enriched with available nutrients.

The humic acids of oxidized coals, along with soil organic matter, are the basis of soil humus, which mainly determines the long-term level of soil fertility. The systematic use of organomineral fertilizers is necessary for the intensification of yield to maintain the humus content in the soil of agrocenoses at a high level [14, 19].

Numerous scientific works [5, 21] determined the identity of the properties of humic acids contained in coal shale and oxidized coals. One of the main criteria that determine the suitability of oxidized coals as raw materials for fertilizers is the content of humic acids.

**2 Materials and research methods**

**2.1 Research objects**

The objects used were:

1. The oxidized brown coals of the adjacent areas of the Oi-Karagai coal deposit of the Almaty province (1), the Lenger (Karatau) coal deposit of the Turkestan province (2) and the Kiyakty coal deposit of the Karaganda province (3);
2. Microorganism strains. The culture of aerobic bacteria *Bacillus sp*. RBK 7, *Acinetobacter pittii* RBK 1 and *Delftia sp*. RBK 5 isolated from brown coal samples;
3. Potato varieties. "Jelly" (*Solanum tuberosum* L. cv. *Jelly*) and "Agata" (*Solanum tuberosum* L. cv. *Agata*);
4. Earthworms. The adult *Eisenia fetida* worms (kept in plastic baths with soil at ~20°C and humidity 60-80% of full moisture capacity).

**2.2 Materials**

The following nutrient media for the growth of microorganisms were used:

Modified mineral medium (g/l): КН2РО4 – 0.9; К2НРО4 – 1.74; MgSO4 – 0.3; СаСl – 0.1; NaCl – 0.5; Н2О – 1.0 L. Oxidized brown coal was added as the sole source of carbon and nitrogen at a concentration of 5% in terms of dry matter. The coal was sterilized in an autoclave at 1 atm. Also, another mineral medium with powdered coal (5%) was used for the cultivation of bacteria. Composition of the medium (mg/l): NH4NO3 – 2.50, KH2PO4 – 1.75, K2HPO4 – 0.75, MgSO4 – 0.75, NaCl – 0.25 and (μg/l) ZnSO4 – 88.0, FeCl3 – 88.0, CuSO4 – 16.0, MnCl2 – 14.0, MoO3-7.0, Co (NO3) 2-5.0.

Luria-Bertani medium (g/l): tryptone - 10.0, yeast extract - 5.0, NaCl - 5.0.

Meat-peptone agar (g/l): peptone - 5.0, NaCl - 5.0, meat extract - 1.5, yeast extract - 1.5, agar - 15.0.

**2.3 Research methods**

*Technical analysis.* Technical analysis of oxidized coal samples was carried out in accordance with GOST [22-25]. The following characteristics were determined: moisture (W), ash content (A), heat of combustion (Q) and the yield of volatiles (V).

*Elemental analysis.* The content of elements was determined on an automatic Vario EL cube analyzer (Germany), as well as on a JEOL-6380LV scanning electron microscope (Jeol, Japan) equipped with an EDAX 2000 detector.

*Functional composition of coal combustion products.* The analytical lines of the elements were measured on an S6 JAGUARXRF wave X-ray spectrometer (Bruker, Germany); the spectrometer is equipped with an X-ray tube with an Rh anode, rated power - 4 kW.

*Elemental microanalysis.* Elemental analysis was performed using a JEOL-6380LV scanning electron microscope (Jeol, Japan) equipped with an EDAX GENESIS 2000 analyzer.

*X-ray structural analysis.* X-ray scattering curves were detected on an Empyrean X-ray Diffraction System (Holland) with a wavelength of λ = 1.54056 Å according to the scheme of reflection from a sample, preliminarily crushed and placed in a cuvette 25 mm in diameter.

*Raman spectrometry.* For this analysis, a Solver Spectrum Raman spectrometer (NT-MDT, Russia) was used. The spectra were obtained upon excitation with a solid-state diode laser with a wavelength of λ = 473 nm.

*Microstructural analysis.* The microstructure of oxidized brown coals was studied using electron microscopy on a scanning electron microscope FE-SEM, Hitachi S-4800 (Japan).

*Metagenomic analysis.* The analysis of the phylogenetic structure of microbiomes in the oxidized coal samples was carried out on a HiSeq device from Illumina (USA) at the University of Inner Mongolia (China) according to the standard protocol [26]. The full description of the Illumina platform is presented on the official website of the company https://www.illumina.com. The general process of metagenomic analysis includes several stages (Fig. 1). Each stage was performed according to the manufacturer's standard [27].

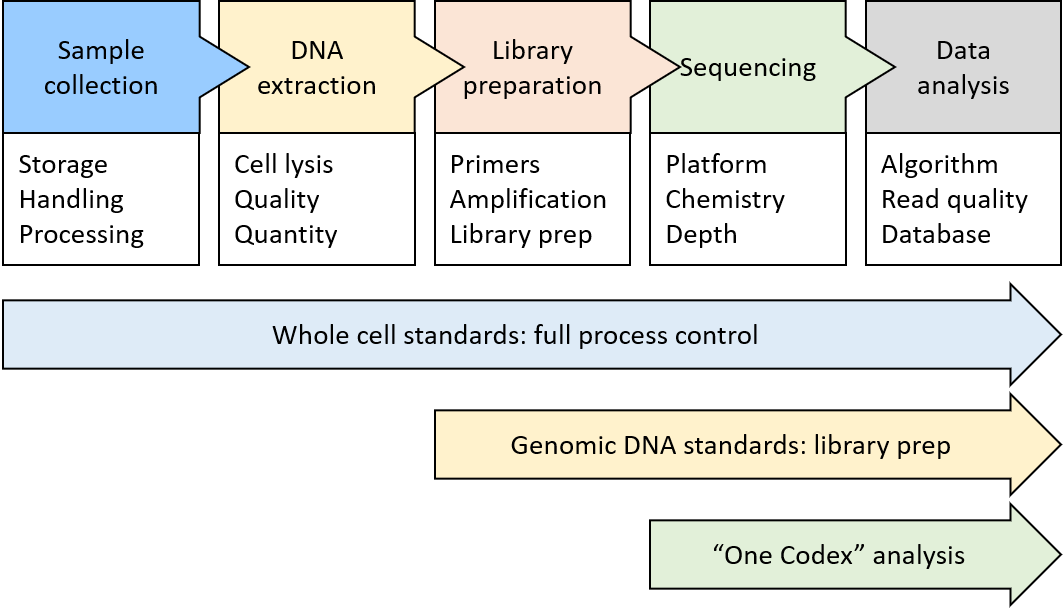
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Figure 1 - The process of conducting metagenomic analysis

*Isolation of bacteria from coal samples*. The coal sample was preliminarily ground in a porcelain mortar, moistened with saline, observing the rules of asepsis. To isolate the culture, the prepared samples were introduced into the meat-peptone broth, poured into 5 ml test tubes, and then incubated in a thermo shaker at 30°C for 2 hours. Then the culture was inoculated on the surface of nutrient media, poured into Petri dishes. Inoculation was performed with a sterile loop using the exhaustion stroke method. After inoculation, the dishes were placed in an incubator at 30°C for 3 days, then isolated colonies were selected. The purity of the culture of microorganisms was monitored by microscopy and observed by inoculating on solid media.

Selection of bacterial cultures with metabolic activity against oxidized coals:

1. *Agar diffusion method.* The agar diffusion method was used to study the biosolubilization capacity of the isolated microorganisms. The daily suspension of cultures was applied with a pipette to the surface of a Petri dish with LB in a volume of 1.0 ml, evenly distributed over the surface with a spatula. 15 min after drying, sterile coal was applied to the surface of the bacterial lawn at a rate of 1 g/cm2 and cultivated at 30°C for 5 days. Control Petri dishes without microbial cultures were placed in parallel.
2. *Submerged culture method.* Daily cultures were incubated in 200 ml of liquid LB medium until reaching OD600 = 0.1 (~ 24 hours) at 28°C on a rotary shaker at 150 rpm. Then, sterile coal at a concentration of 5% (w/v) was added to the cultures and incubation was continued for 15 days. A non-inoculated medium with coal was used as a control. During the solubilization process, culture aliquots were collected every day under aseptic conditions, centrifuged at 10,000 rpm for 15 min and filtered through membrane filters with a diameter of 10 cm with a pore size of 0.22 µm. The obtained supernatants without cells were measured at A450 using a LabTech UV-Vis spectrophotometer (UV-1000, China) to assess the intensity of biosolubilization.

*Methods for studying the phenotypic properties of isolates.* Physiological-biochemical and morphological-cultural properties of the obtained isolates were studied on the basis of microscopic data of specimen stained according to Gram, i.e. cell morphology, biochemical activity and cultural characteristics of microbial cells and the use of "Bergey's Bacteria Keys" [28]. Identification was carried out microscopically by morphological parameters (type of cell assemblage, shape, Gram stain, motility, sporulation) using microscopy. For biochemical analyzes of cultures, a *Vitek* analyzer with standardized test systems API 50 CH and API 20 E with *Apiweb* software manufactured by *BioMerieux* (France) was used.

*Identification of strains by genotypic traits*. The strains were identified by the method of determining the direct nucleotide sequence of the *16S rRNA* gene fragment, followed by the determination of the nucleotide identity, deposited in the international database Gene Bank. DNA was isolated using the Kate Wilson method [29]. Purified DNA samples were dissolved in 100 μl of a single TE buffer, and the DNA concentration was measured spectrophotometrically using a NanoDrop spectrophotometer at 260 nm. The PCR reaction was performed with the universal primers 1492R (5'-GGT TAC CTT GTT ACG ACT T-3 ') and 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') in a total volume of 30 μl. The PCR amplification program included prolonged denaturation at 95°C for 7 minutes; 30 cycles: 95°С - 30 seconds, 55°С - 40, 72°С - 1 minute; final elongation for 7 minutes at 72°C. The PCR program was performed using a GeneAmp PCR System 9700 thermocycler (Applied Biosystems). The nucleotide sequences of the *16S rRNA* gene of the 14 strains identified were analyzed and combined into a common sequence in the SeqScape 2.6.0 software (Applied Biosystems). The nucleotide sequence over 650 bp in length was identified in GeneBank using the BLAST algorithm.

*Cultivation of earthworms and obtaining their coprolites*. The isolation and cultivation of vermi-material was carried out using *E. fetida* worms. Vermiculture substrates are modified chernozem, duration of vermicomposting is 3 months. Coprolite samples were obtained by keeping the worms on sterile filter paper in Petri dishes at a temperature of ~ 5°C for 3 hours.

*Extraction of humic substances for solubilization of oxidized brown coal* (OBC). The OBC humic substances were obtained using a modified technique [5]. 1 g of pulverized OBC was suspended in 50 ml of 0.1 M NaOH and shaken at 20°C for 24 hours, after which it was centrifuged at 11,200 G-force for 15 minutes. The supernatant was filtered through Whatman # 1 filter paper and precipitated to adjust the pH to 2.0 using 11.6 M HCl. The solution was settled for 12 h, followed by centrifugation at 11 200 G-force for 15 min, then washed 3 times with distilled water and dried at 60°C. The resulting product was identified as cHS (Chemically Obtained Humic Substances). The bio solubilized suspension was filtered through Whatman # 1 paper and precipitated by adjusting the pH to 2.0 using 11.6 M HCl. The sediment was collected for the extraction of bacterial-transformed humic substances (bHS – humic substances obtained by a biological method). The extraction was processed as described above to obtain cHS.

*Characterization of solubilized products*. Fourier transform infrared spectroscopy (FTIR) of the samples were analyzed using a Nicolet 6700 FT-IR spectrometer. The IR spectrum of the samples was recorded in the range from 400 to 4.000 cm-1. The following data collection parameters were used: number of sample scans - 32; Resolution - 4.000; Zero filling - 2; He-Ne laser frequency: 15798.0 cm-1; The peak position of the interference - 8192; Apodization function - N-B strong; Background scans - 32; Background enhancement - 1.0; Optical fiber - 100.00; Sample growth - 1.0; High Pass Filter - 200.0000; Low pass filter - 20,000.0000; Data collection method - GC/IR; The final format is -% transfer. The elemental composition of humic substances was determined using a Vario EL cube analyzer (Elementar, Germany). A difference of 100% was attributed to oxygen content.

*UV spectroscopic analysis* of humic substances was carried out on a V-550 spectrophotometer (Jasco, Japan) using a quartz cuvette 1 cm in diameter and a phosphate buffer blank. Absorption spectra were recorded in the wavelength range from 200 to 900 nm after 1 hour of sample preparation.

*An FP-8500 fluorescence spectrometer* (Jasco, Japan) was used to measure the excitation-emission characteristics of samples of humic substances dissolved in phosphate buffer at a concentration of 10 mg/L at 20°C. Scanning was performed at lengths of excitation wave (Ex) in the range from 250 to 600 nm with an index of 5 nm and at lengths of emission wave (Em) from 260 to 650 nm with an index of 5 nm. The spectra were recorded at a scan rate of 1000 nm/min using an excitation bandwidth and an emission line of 5 mm.

*Planning an experiment in greenhouse conditions*. Research on the processes of growth, development and formation of the potato crop using the humic product II (without earthworms) was conducted in the greenhouse farm "al-Farabi KazNU" (Fig. 2). The application of vermicompost was carried out by processing (1.5% and 2.5%) before planting potato and during the growing season with a sprayer (0.01% and 0.05%). The solution was prepared in the following way: 0.1 ml or 0.5 ml of bHS is diluted in 1L of water. In this work, the effect of cHS in soil in doses of 1% or 5% on the growth and productivity of potatoes was also studied.

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| Figure 2 - Greenhouse farm "al-Farabi" | Figure 3 - Varieties of potatoes: a - "Jelly", b - "Agata" |

*Preparation of planting material*. Pure-grade potato tubers "Jelly" (*Solanum tuberosum* L. cv. *Jelly*) weighing about 70 g were taken for planting (Fig. 3a). Selected tubers were laid out in one layer in a well-lit place. For 3 days kept at 25°C, then germinated for 20 days at 15°C in the light without sun rays. The tubers were turned over every five days. 2 days before planting, the tubers were covered with a dark cloth and the temperature was lowered to 12°C. After 20 days, the germinated tubers were planted in boxes with soil in holes at a distance of 20-25 cm. The size of boxes was 50x60 cm, the height was 60 cm. After planting, the potatoes were watered every 6 days. In the experiments during the growing season, biometric measurements and phenological observations were carried out: 1. The area of ​​leaves was determined from their photographs using the UTHSCSA Image Tool software; 2. Plant height and number of stems were measured in the phase of mass flowering; 3. The dynamics of the accumulation of mass of tubers was taken into account according to the method [30]. Samples were collected from 5 bushes from each variant; 4. In the harvest, the number of tubers and their weight were determined according to GOST 7001-66 [31].

*Field experiments: stage No. 1*. For the experiment, the table potato variety Agata (*Solanum tuberosum* L. cv. *Agata*) was chosen (Fig. 3b). In the first quarter of the year, individual potato tubers of high sanitary quality were planted in the greenhouse in plastic pots (n = 15) with a volume of 6 liters, filled with sandy loam soil. The soil was steam pasteurized to kill pathogen and weed seeds as described in [32]. Then 10 g of organic fertilizer Bionic (containing N (150 g/m3), P2O5 (130 g/m3) and K2O (210 g / m3)) were mixed with 1.5 kg of soil. The potatoes were harvested after reaching the growth stage 909 (BBCH scale (German: Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) [33]). Plants formed enough tubers for further experiments in natural conditions.

*Field experiments: stage No. 2.* Field experiments at the pilot site were carried out in the second quarter of the year, i.e. in the best agrotechnical terms. Intact healthy tubers of approximately the same size were selected to obtain uniform plants. Tubers were randomly and blindly planted (to avoid unintentional manipulations) in (a) control soil, (b) soil enriched with product II (earthworms + bHS at a concentration of 1 g/kg-1), and (c) soil enriched with product I (earthworms + cHS – 1.5 g/kg-1). The potatoes were cultivated in 15 replicates for each soil type. The arrangement of the experimental variants was continuous, single-row and sequential. The total area of ​​the experimental plot is 500 m2. As in the previous stage, the plants were harvested when they reached the 909 stage of growth on the BBCH scale [33].

*Biometric measurements of plants*. Growth and yield phenotypic data were recorded during potato harvest for each variant. Growth observations, including the number of stems (009 on the BBCH) and plant height (805 on the BBCH), were recorded according to the Hacketal study. [33]. The number and weight of tubers as well as yield were measured at maturity. The harvested tubers were weighed and manually sorted into three categories (small: <80 g; medium: 81–150 g; and large:> 151 g).

*Soil cultivation.* The soil was supplemented with "Product I" (cHS + zoo-microbe) and "Product II" (bHS + zoo-microbe), and untreated soil was presented as a control. In more detail, bHS (in terms of dry weight) was introduced into the soil at a concentration of 1 g/kg-1 together with earthworms (50 individuals per 5 kg of soil) and mixed. The cHS dose (ratio of 1.5 g to 1 kg of soil) was determined in accordance with the characteristics of the soil to increase the nutrient content of the soil.

*Statistical analysis.* Data were presented as arithmetic mean ± standard deviation. Analysis of treatments for potato growth and tuber yield was performed using one-way analysis of variance (ANOVA) (SPSS Statistics, version 26.0, Chicago, Illinois, USA). The significance of differences between the means was assessed using Duncan's multiple range test with a significance level of 0.05.

**3 Research results and discussion**

**3.1 Study of the physicochemical parameters and the microbial landscapes of selected materials**

3.1.1 Sampling of oxidized brown coals from Kazakhstani deposits

Coal sampling was carried out in accordance with ISO 18283: 2006 “Hard coal and coke – Manual sampling and ISO 13909-4: 2016 Preview Hard coal and coke - Mechanical sampling - Part 4: Coal - Preparation of test samples” and GOST 10742-71 “Brown coals, coals, anthracite, oil shale and coal briquettes - Methods of sampling and preparation of samples for laboratory tests.

Sampling was carried out from a fixed layer of the surface of a coal waste heap (rock dump) by a mechanized method using a sampler. This method is specific to each coal type and involves taking spot samples, making a pooled sample and isolating an average sample.

Laboratory and analytical samples were placed in sterile jars, provided with labels, which indicated: 1. sample number; 2. date of sampling and processing of the sample; 3. name of the sample; 4. name of the area; 5. weight; 6. signature of the person responsible for the collection and processing of the sample.

For the research, oxidized brown coals of Kiyakty basin (Karaganda region) – KLE (1), Lenger basin (Turkestan region) – LLE (2), and Oi-Karagai basin (Almaty region) - OLE (3) were selected (Fig. 4).



Figure 4 - Schematic map of the sampling points of oxidized brown coals

Petrological characteristics of oxidized coal samples are given in Table 1.

Oi-Karagai coal deposit is located in the Narynkol region, Almaty province, 300 km east of Almaty city, near the border with China. The Middle Jurassic deposits, 45 to 110 m thick, enclose a coal seam of a simple structure from 4.5 to 23.5 m and form an overlaid monocline. Brown coal of class B3, low-ash (16%), high-calorie (5.4-7.9% thousand kcal/kg), low-sulfur, easily decomposes in the air. The reserves of the deposit are estimated at 80 million tons, of which 41 million tons suitable for open processing. The total volume of explored and calculated reserves according to the geological exploration commission is 124 million tons of brown coal. The deposit, according to the Code of the Republic of Kazakhstan "On Subsoil and Processing of Mineral Raw Materials", is classified as republican significance.

Lenger coal deposit is located in the Turkestan province, 35 km east of Shymkent city. In addition to the Lenger deposit itself (25 km2), which is divided into two coal areas: Promyshlennaya and Toguzskaya; 4 promising areas are distinguished: Yuzhnaya (about 10 km2), Georgievskaya (80 km2), Shymkent (60 km2) and Kazykurt (30 km2). Early Jurassic coal-bearing deposits, 350-500 m thick, include up to 10 coal seams, of which 5 have a working thickness of 1.5-2.5 to 14.7 m. Humus coals of class B3, medium ash (18-22%). High-sulfur coals up to (3%), prone to spontaneous combustion. The ash is fusible. Heat of combustion up to 7.3 thousand kcal. kg at semi-coking received up to 4-7% of resin. The deposit's reserves are significant; to a depth of 900 m are estimated at 750 million tons. The deposit is operated by mines under rather difficult mining and geological conditions [34].

Table 1 - Macroscopic description of coals

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No. | Description | OLE | LLE | KLE |
| 1 | Variety | C:\Users\Akimbekov.nuraly\Desktop\Гумус\Отчет_гумус_2018\OLE.png | C:\Users\Akimbekov.nuraly\Desktop\Гумус\Отчет_гумус_2018\LLE.png | C:\Users\Akimbekov.nuraly\Desktop\Гумус\Отчет_гумус_2018\KLE.png |
| 2 | Color | Dark brown | Light brown | Brownish |
| 3 | Fracture | Fibrous | Rough, earthy | Uneven, angular |
| 4 | Lustre | Fatty | Silky | Gummy |
| 5 | Texture | Uneven striated | Heterogeneous, fibrous | Heterogeneous, lignite |
| 6 | Parting/ cleavage | Exogenous | Exogenous | Exogenous |

The Kiyakty coal deposit is located in the Karaganda region, 98 km west of the village of Karsakbay. The deposit is represented by brown coals of the Jurassic period with an increased degree of coalification. The explored coal reserves of this deposit are 110 million tons. The geological exploration area, the central block covers an area of ​​3 km2. In the upper part of the coal seam there are interlayers rich in organic matter – humus with a thickness of up to 1.5 m. Its reserves in the central section of the deposit are about 3.5 million tons. In terms of the orohydrographic relation, the deposit area is a semi-desert rivers and arid climate.

The area of ​​the deposit is distinguished by a sharply continental climate, with temperature fluctuations from +40 to 38°С. The total average rainfall per year is 148.01 mm. The region of the Kiyakty field is distinguished by strong, up to 20 m/s constant winds, with a predominant north-east direction.

At the coal mine, emissions of dust and harmful substances into the environment occur during mining operations, in the process of dumping, ventilation from the open surfaces of waste dumps and a coal warehouse, as well as during transportation of coal and overburden rocks by road [35].

3.1.2 Study of the physicochemical and the structural properties of selected samples of oxidized brown coals

*Technical characteristics of oxidized brown coals.* In order to assess the thermal properties of coal samples on a laboratory basis, comprehensive studies of the physicochemical parameters of OLE, LLE and KLE coals were carried out, the results of which are presented in table. 2. Preparation and study of analytical samples were conducted in full accordance with standardized methods.

The moisture content in the working condition of all coal samples is significant, i.e. in the range from 9 to 12%. Such overview the course of research allows us to conclude that coals have a high tendency to drainage under natural conditions. The working ash content of the OLE and KLE samples falls within a narrow range with the limits of ~11%, which makes it possible to classify oxidized coals as a medium ash group, and a LLE high ash content - 22%.

Table 2 - Proximate and ultimate characteristics of oxidized coals

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Description of characteristics | Designation | Samples | | |
| LLE | OLE | KLE |
| Humidity, % | W | 9,1 | 11,8 | 9,8 |
| Ash content, % | A | 22,0 | 12,2 | 11,5 |
| Volatile matter yield, % | V | 40,8 | 35,8 | 41,8 |
| Calorific value, MJ / kg | Q | 7,8 | 15,6 | 21 |
| Elemental composition, % | C | 61,61 | 75,00 | 64,50 |
| H | 1,60 | 4,81 | 4,12 |
| N | 0,86 | 1,50 | 0,74 |
| S | 1,65 | 0,41 | 0,75 |
| O | 34.28 | 18.28 | 29.89 |

The concentration of carbon in dry ash-free mass of oxidized coal reaches 42%. This, in turn, determines a significant value of the heat of combustion of the dry ash-free mass, reaching more than 27 MJ/kg. Other components of the elemental composition of coals are found in insignificant amounts, with the exception of oxygen in LLE and KLE. However, it is necessary to pay due attention to the sulfur content, which in the LLE mass reaches 1.65%. For the purpose of additional analysis of the elemental composition, it was used X-ray spectral microanalysis by means of an energy dispersive (EDX) analyzer. The results of X-ray spectral microanalysis of oxidized coal samples (Table 2) and the results obtained in the course of studying the elemental composition (Fig. 5) of the samples were slightly different. Most likely, the differences are due to various methods of sample preparation for analysis and determination of the elemental composition.

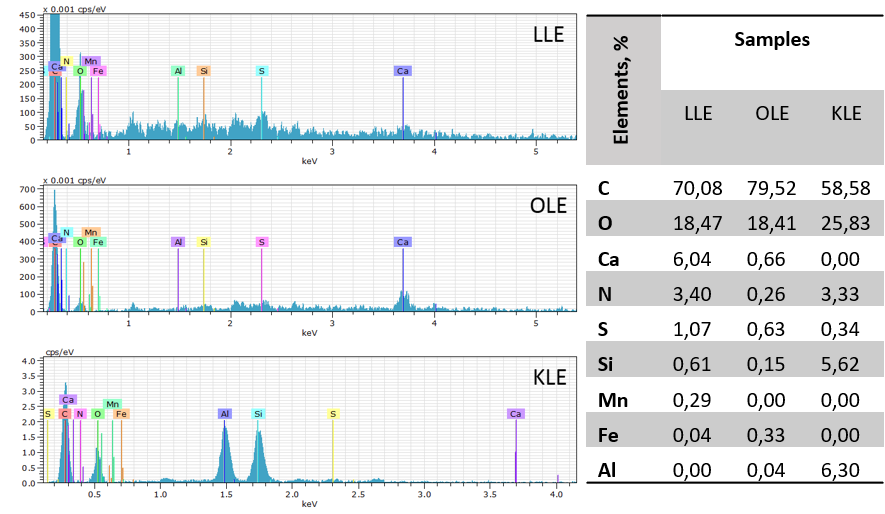


Figure 5 - Elemental microanalysis of oxidized brown coals in terms of Wt%

*Elemental composition of coal combustion products by X-ray fluorescence analysis.* The main component parameter characterizing the presence of various mineral and elemental compounds in coal is its ash content. Depending on the nature of the oxidized coal, the yield of volatiles and the chemical composition of combustion products are determined. In this work, the process of identifying the content of the main components and trace elements in the ash of OLE, LLE, and KLE by the XRD method was studied. Table 3 demonstrates the analysis data for coal ash samples collected after their combustion.

As the data show, the largest specific gravity belongs to oxides of silicon and aluminum, so for LLE - 59.4%; OLE - 27.75% and KLE - 41.7%. The ash from OLE and KLE coal seams contains calcium oxide, which indicates the presence of CaCO3 or other calcium minerals.

Table 3 - Chemical composition of ash from OLE, LLE and KLE

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component | Samples | | | Value |
| LLE | OLE | KLE |
| SiO2 | 37.94 | 23.61 | 27.5 | % |
| Al2O3 | 21.46 | 4.14 | 14.2 | % |
| Fe2O3 | 0.44 | 0.16 | 9.1 | % |
| CaO | 0.46 | 7.47 | 7.7 | % |
| MgO | 0.08 | 0.19 | 2.4 | % |
| K2O | 0.64 | 0.56 | 1.8 | % |
| Na2O | 0.62 | 0.61 | 2.8 | % |
| P | 244.8 | 16.4 | 48.8 | ppm |
| Ti | 0.446 | 0.090 | - | % |
| Mn | 58.6 | 112.3 | 91.0 | ppm |

*X-ray structural analysis of oxidized coals.* One of the important indicators of intermolecular bonds in the organic mass of coals is the spatial organization of structural fragments into quasi-crystalline formations, the presence of which leads to the formation of a number of characteristic reflections on the X-ray diffraction patterns of coals. The variety of the chemical composition of coals, as well as its regular change in the series of metamorphism, expand the possibilities of oxidative transformations of coal matter as a result of weathering. The change in the molecular organization as a result of oxidation also presupposes the transformation of the supramolecular structure of brown coal.

X-ray structural analysis is relatively widely used to analyze the transformation of the macrostructure of coals, both in the process of metamorphism and as a result of various chemical and physical effects.

With an increase in the chemical maturity rank of OLE and KLE coals, a significant decrease in the intensity of the ordered peripheral part (γ-band) and a gradual increase in the content of the condensed aromatic part of coal (002) are observed (Fig. 6).

However, the fraction of the crystalline phase in the case of LLE turned out to be more complex in contrast to OLE and KLE. The reason for this systematic discrepancy lies not only in the difference in the methods used for the decomposition of diffraction peaks, but also, apparently, due to the low content of inertinite components in the samples (vitrinite index 0.8-0.99%), the contribution of which to the formation of the crystalline phase the most significant.

Thus, the method of X-ray structural analysis revealed that in oxidized OLE and KLE coals, the γ-bands decrease and the intensity of the (002) bands in the X-ray diffraction patterns increases. This gives grounds for the assumption that the organic matter of these coals is closer in structure to amorphous polymers.

*Raman spectrometry.* Spectroscopy was used to study the microstructure of coal substances. Roman spectra were recorded in a wider (up to 3500 cm-1) range. Figure 7 shows the obtained spectra of oxidized brown coals.

The so-called D- and G-bands are recorded in the spectrum ranges from 1000 to 2000 cm-1; from 2700 to 3200 cm-1 - broad bands characteristic of C-H bonds; and from 3500 to 4000 cm-1 - a diffuse signal, the intensity of which increases with an increase in the emission of volatiles [36].

|  |  |
| --- | --- |
|  |  |
| Figure 6 - Diffraction patterns of LLE (a), OLE (b) and KLE (c) coals | Figure 7- Raman spectra of oxidized coals LLE (a), OLE (b) and KLE (c) |

As can be seen, the frequency position of the G-peak in different brands of oxidized coal practically does not change (~1592 cm-1), in contrast to the D-peak, whose frequency reaches from 1360 cm-1 in LLE to 1390 cm-1 in KLE. The high intensity of the D peak indicates an increase in the disordering of the carbon structure, as is expressed in the LLE coal grade.

The reason for the appearance of a diffuse band in the region of 2500-4000 cm-1 may be the presence of chemical bonds, which are formed by the atoms H, O, S and N present in the oxidized coal, as well as the presence of moisture.

*Microstructure of oxidized brown coals.* The method of scanning electron microscopy was applied to study the surface structure of oxidized coals and their features. The structure of oxidized coals is characterized by high density, heterogeneous parts of the coal separated by cracks are observed. The complexity of the structure of coal is associated with its heterogeneity, because it contains clearly expressed organic and mineral components [37]. The organic part of coal is characterized by the coexistence of condensed aliphatic and aromatic structures, each of which forms rather pronounced crystalline formations.

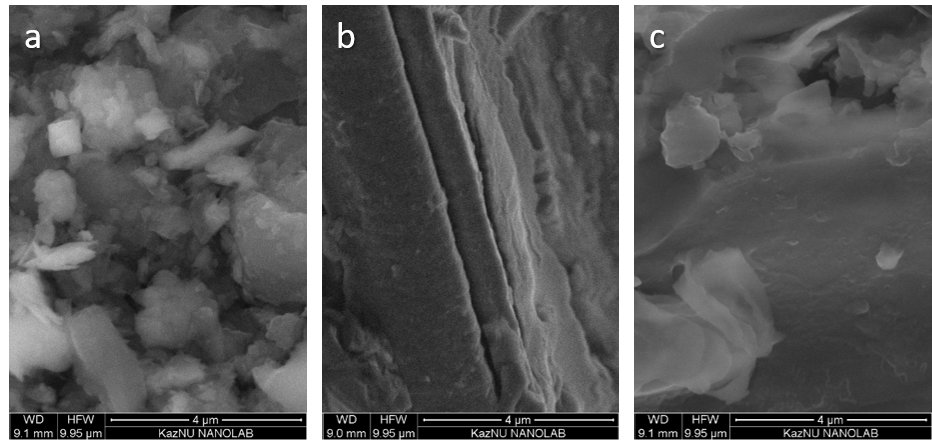


Figure 8 - SEM images of oxidized coals LLE (a), OLE (b), and KLE (c)

The structure of oxidized coals LLE and KLE is generally heterogeneous; in some places, inter-lump cracks are observed, as well as heterogeneous structures with local accumulations of isometric shapes. Especially, the LLE surface is loose and heterogeneous (Fig. 8). The surface of the OLE sample is homogeneous, the particles have regular shapes and sizes, and superimposed vein textures are widely developed, which indicates the presence of quartz.

Thus, the oxidized coals of the Oi-Karagai and Kiyakty deposits consist of the main organic matter and other mineral impurities. The main elements that make up the organic matter of coal are C, H, O, N and organic S. And they also contain chemical compounds of some metals - Ca, Fe, Mg, Mn, etc. Physicochemical analyzes showed that the coal from Oi-Karagai and Kiyakty are brown and belong to humus coals, which are promising raw materials for the production of bio-humus.

Considering the high concentration of sulfur in the oxidized brown coal of Langer origin (LLE), we consider it unpromising to carry out further research, since its rational use requires finding new ways. It is known from literary sources [38] that sulfur in coals is the most admixture, it reduces the value of fuel and worsens the quality of the final products of its processing.

3.1.3 Study of the physiological and the biochemical properties of microorganisms isolated from brown coal samples

*Metagenomic analysis.* Molecular methods that make it possible to study the properties of microbiomes *in situ*, without isolating inoculates into pure cultures, are promising in the determination and study of the uncultured majority of microorganisms. Metagenomics is the analysis of the total genetic material isolated from the whole biological system. The most priority in metagenomic systems is the analysis of the *16S rRNA* gene, on the structure of which the modern phylogenetic classification of bacteria is based.

As part of this study, a comparative metagenomic analysis of coal samples from Kazakhstani coal deposits was carried out. Despite the fact that coal basins occupy vast territories of Kazakhstan, there are no studies on metagenomic analysis of coal and soil of industrial zones. In total, two samples were analyzed: OLE and KLE. The characteristics of the primary data are given in table. 4.

The bacterial communities of coal samples are mainly formed by the phyla *Proteobacteria*, *Tenericutes*, *Acidobacteria*, *Firmicutes*, *Bacteroidetes*, *Nitrospirae*, *Chloroflexi*, *Gemmatimonadetes*, *Actinobacteria*, and *Fusobacteria* (Fig. 9). The *Proteobacteria*, which often occupies a dominant position in soil microbiota, has the largest ratio in micro-communities. In addition to these bacteria, representatives of the *Actinobacteria* constitute a significant proportion of the KLE.

Table 4 - Clustering statistics and OTE annotations

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Samples | Common tags | Taxon tags | Unclassified tags | Unique tags | ОТЕ |
| OLE | 81889 | 80420 | 0 | 1469 | 682 |
| KLE | 77015 | 75777 | 40 | 1198 | 1019 |

It is known from the literature that many *Actinobacteria*, especially their mycelial species - actinomycetes - are adapted to habitats with low humidity [39]. However, in the OLE sample, bacteria of the *Tenericutes* prevail, and actinobacteria have been obscured.

The prokaryotic KLE community is mainly composed of representatives of *Phyllobacterium* sp. (56.16%), *Mycobacterium celatum* (24.02%), *Bacteroides* sp. (2.05%), *Roseburia* sp. (1.95%).

The high share of *Candidatus* and *Bacilloplasma* in OLE is noteworthy. In the sample, they make up 28% of the total number of determined prokaryotes. And then - *Bacteroidaceae* (10.59%), *Shewanella* sp. (11.21%), *Phyllobacterium* sp. (2.28%) etc. (Fig. 10).

|  |  |
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| a | b |

Figure 9 - Taxonomic structure of OLE and KLE

(a - at the phylum level, b - at the class level)

The diversity of the prokaryotic community, according to the number of found OTUs and different indices (Table 5), in OLE and KLE is practically heterogeneous, because oxidized coals differ significantly in their physical and chemical properties.

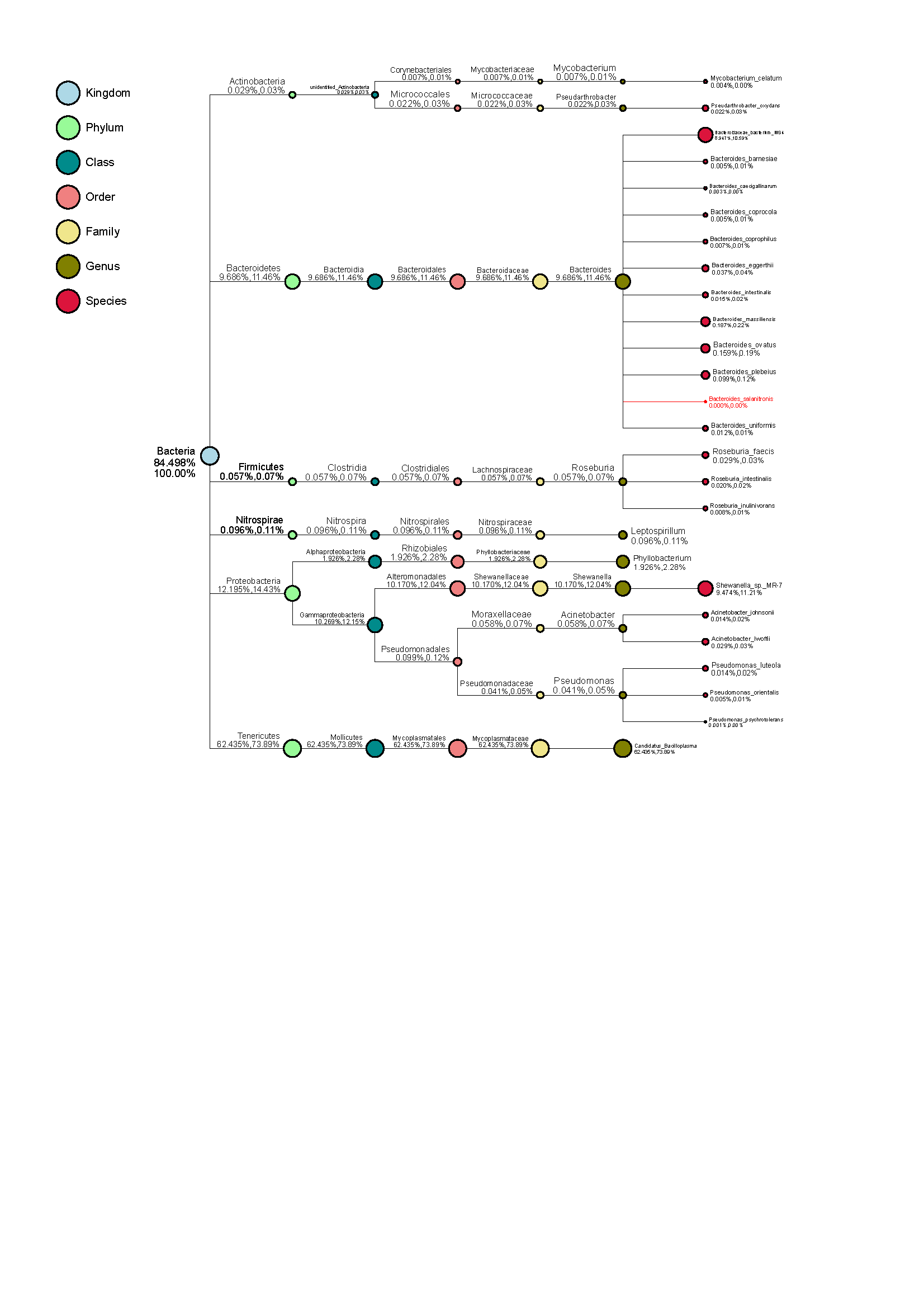


Figure 10 - Composition and ratio of individual species of OLE sample

Table 5 - Diversity indices of prokaryotes

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Samples | Discovered species | Shannon Index | The Simpson Index | chao1 | ACE |
| OLE | 572 | 2.637 | 0.619 | 633.008 | 658.940 |
| KLE | 931 | 6.413 | 0.967 | 1007.027 | 1006.286 |

The conducted research makes it possible to link the features of the structure and diversity of microbial communities, studied by sequencing the *16S rRNA* gene with the features of coal properties and their genesis.

*Isolation of bacterial cultures and study of their physiological and biochemical properties.* Aerobic bacterial cultures were isolated from samples of oxidized brown coals OLE and KLE. The method of enrichment cultures was used, the selection of bacterial isolates was carried out based on differences in cultural and morphological characters. Growth on nutrient media showed the dull, cream-colored, wrinkled colonies with raised folds, irregular shape, with jagged edges, <5 mm in size. 2 cultures from OLE and 1 culture from KLE were isolated.

To study the morphology of bacteria and spore, specimens were prepared according to Gram and Ozheshko. Microscopic examination of biological materials revealed that isolated cells are rods, 2.0-5.0 µm in size (Table 6).

Table 6 - Morphological and cultural characteristics of microorganisms

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristics | Strains | | |
| RKB1 | RKB5 | RKB7 |
| Description of the colonies | White, convex, smooth | Round, flat, smooth | Large, dull, fibrous |
| Cell morphology and size | Short, rounded, 1.0-2.0 µm | Rod shaped, ~ 2.5 µm | Large rods, <4 µm |
| Gram stain | Negative | Negative | Positive |
| Vegetative propagation | No spore | No spore | Intracellular spores |
| Generic affiliation | *Acinetobacter* | *Delftia* | Bacilli |

Table 7 - Physiological and biochemical properties of microorganisms

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Characteristics | | Strains | | |
| RKB1 | RKB5 | RKB7 |
| Catalase activity | | + | + | + |
| Oxidase activity | | - | + | + |
| Indole test | | - | - | - |
| Reduction of nitrates | | ± | + | + |
| Motility | | - | + | + |
| Hydrolysis | Casein | - | ± | + |
| Gelatins | - | ± | + |
| Starch | - | ± | + |
| Assimilation | Glucose | + | - | + |
| Maltose | ± | - | + |
| Mannita | - | - | + |
| Arabinose | - | - | - |
| Xylose | + | - | + |
| Lactose | ± | - | + |
| Mannose | + | - | + |
| Sorbita | ± | - | - |

Phenotypic identification based on cultural and morphological characters made it possible to presumably assign the strains to the genera *Acinetobacter*, *Delfia*, and *Bacillus*. As a result of this work, the physiological and biochemical characteristics of the isolates were also determined (Table 7).

Based on the results obtained, the bacteria were assigned to the genera *Acinetobacter*, *Delfia* and *Bacillus*.

3.1.4 Selection of the bacterial strains with target metabolic activity

The selection of microorganisms with target activity in relation to biosolubilization was carried out in two ways using methods: agar-diffuse and submerged culture.

At the first stage, the metabolic activity of the isolates was determined by the agar diffusion method. Sterile dry coal was applied to a solid nutrient medium with a bacterial lawn and incubated for 5 days. After the incubation period, the presence or absence of biosurfactants was visually determined.

As seen from Fig. 11 bacterial cultures quickly solubilized samples of oxidized coals on solid culture medium LB after 2 days.

|  |  |  |  |
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| а | b | c | d |
| Figure 11 - Biosolubilization of oxidized coals: a - RKB 1,  b - RKB 5, c - RKB 7 and d - control | | | |

In the control, the brown band around the coal was not observed due to the absence of inoculum. Thus, it can be assumed that all the isolated cultures have high solubilized activity against oxidized brown coal.

In the second stage, the cultures were used to solubilize the oxidized coal samples in liquid culture. Solubilizing indicators are shown in Fig. 12. At a coal concentration of 5%, the degree of OLE solubilization increased significantly with an increase in incubation time up to 10 days, but subsequently the indicator remained unchanged in the stationary phase.

|  |  |
| --- | --- |
|  |  |
| Figure 12 - Level of biosolubilization of OLE | Figure 13 - The level of biosolubilization of KLE |

Higher metabolic activity using OLE was shown for the RKB 7 culture, since the spectral absorption of biosurfactants in the supernatant is 3.75±0.8. A similar result was observed upon solubilization of KLE, where the absorbance reaches 3.05±0.4. Cultures RKB 1 and RKB 5 showed no less significant results after 8 days of incubation (Fig. 13).

Since it seemed advisable to select only those bacterial strains that had a high level of metabolic activity in relation to OLE and KLE carbons, we took all 3 inoculums as promising solubilizing strains for further identification.

To clarify the species, genotyping of the isolated strains was carried out. Nucleotide sequences of the *16S rRNA* gene were analyzed and combined into a common sequence in SeqScape 2.6.0 (Applied Biosystems), after which the terminal fragments were removed, which made it possible to obtain a nucleotide sequence longer than 650 bp, identified in GeneBank by the BLAST algorithm. Nucleotide sequences and identification results are presented in table. 8.

Table 8 - Results of identification of bacterial strains by analysis of the nucleotide sequence of the *16S rRNA* gene using BLAST

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strain name | Sequence of a fragment of the *16S r RNA* gene | Identification of nucleotide sequences in the international database BLAST algorithm | | |
| GeneBank stock number | Strain name | % match |
| RKB 1 | CACCGATGGGTACCGCCCTCTTTGCAGTTAGGCTAGCTACTTCTGGTGCAACAAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATTCTGATCCGCGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGATCGGCTTTTTGAGATTAGCATCCTATCGCTAGGTAGCAACCCTTTGTACCGACCATTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGCCATGATGACTTGACGTCGTCCCCGCCTTCCTCCAGTTTGTCACTGGCAGTATCCTTAAAGTTCCCGACATTACTCGCTGGCAAATAAGGAAAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTATGTAAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTTACTATGTCAAGGCCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAGTCTTGCGACCGTACTCCCCAGGCGGTCTACTTATCGCGTTAGCTGCGCCACTAAAGCCTCAAAGGCCCCAACGGCTAGTAGACATCGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCATGCTTTCGCACCTCAGCGTCAGTGTTAGGCCAGATGGCTGCCTTCGCCATCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCATCCTCTCCCACACTCTAGCTAACCAGTATCGAATGCAATTCCCAAGTTAAGCTCGGGGATTTCACATTTGACTTAATTAGCCGCCTACGCGCGCTTTACGCCCAGTAAATCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGCTTATTCTGCGAGTAACGTCCACTATCTCTAGGTATTAACTAAAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCATAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACTTAGGCTCATCTATTAGCGCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATTCCTTTCGAAATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACCCGTCCGCCGCTAAGATCAGTAGCAAGCTACCTCTCTCCGCTCGACTGCATGTGTAAGCTGC | NR\_117930.1 | *Acinetobacter pittii* | 100 |
| NR\_042387.1 | *Acinetobacter calcoaceticus* | 99 |
| NR\_102814.1 | *Acinetobacter oleivorans* | 99 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| RBK 5 | CGCTCCCATGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACGACTGGTTTTATGGGATTAGCTCCCCCTCGCGGGTTGGCAACCCTCTGTACCAGCCATTGTATGACGTGTGTAGCCCCACCTATAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCATTAGAGTGCTCAACTGAATGTAGCAACTAATGACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTGCAGGTTCTCTTTCGAGCACGAATCCATCTCTGGAAACTTCCTGCCATGTCAAAGGTGGGTAAGGTTTTTCGCGTTGCATCGAATTAAACCACATCATCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGCCGTACTCCCCAGGCGGTCAACTTCACGCGTTAGCTTCGTTACTGAGAAAACTAATTCCCAACAACCAGTTGACATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTACAGGTCCAGGGGATTGCCTTCGCCATCGGTGTTCCTCCGCATATCTACGCATTTCACTGCTACACGCGGAATTCCATCCCCCTCTACCGTACTCTAGCCATGCAGTCACAAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCTGTCTTACATAACCGCCTGCGCACGCTTTACGCCCAGTAATTCCGATTAACGCTCGCACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTTATTCTTACGGTACCGTCATGGGCCCCTTGTATTAGAAGGAGCTTTTTCGTTCCGTACAAAAGCAGTTTACAACCCGAAGGCCTTCATCCTGCACGCGGCATTGCTGGATCAGGCTTTCGCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAGACCAGCTACAGATCGTCGGCTTGGTAAGCTTTTATCCCACCAACTACCTAATCTGCCATCGGCCGCTCCAATCGCGCGAGGCCCGAAGGTCCCCCGCTTTCATCCTCAGATCGTATGCGGTATTAGCTACTCTTTCGAGTAGTTATCCCCCACGACTGGGCACGTTCCGATGTATTACTCACCCGTTCGCCACTCGTCAGCGTCCGAAGACCTGTTACCGTTCGACTGCATGTGTAAGGCATGC | NR\_116495.1 | *Delftia lacustris* | 99 |
| NR\_113870.1 | *Delftia tsuruhatensis* | 99 |
| NR\_024786.1 | *Delftia tsuruhatensis* | 99 |
| RBK 7 | CTTCGGGCGGCTGGCTCCTAAAGGTTACCTCACCGACTTCGGGTGTTGCAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTATGGGATTGGCTAAACCTTGCGGTCTTGCAGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGTCCCCGAAGGGAAAGCCCTATCTCTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGAGCCCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTTCGCTCGACTNGCATGATAGCACGCG | NR\_113945.1 | *Bacillus safensis* | 99 |
| NR\_148787.1 | *Bacillus australimaris* | 99 |
| NR\_112637.1 | *Bacillus pumilus* | 99 |

Based on the analysis of the nucleotide sequence, the RKB 1 strain was assigned with a 100% probability to the *Acinetobacter pittii* species, and the RKB 5 and RBK 7 strains with a 99% probability were assigned to the *Delftia* and *Bacillus* genera, respectively.

**3.2 Creation of a special-purpose microbial consortium**

3.2.1 Isolation and cultivation of vermiculture

The excavation method was used to obtain earthworms [40]. An area of ​​25×25 cm was selected. Initially, the litter layer was examined, from which the detected earthworms were selected, then, using a clean shovel, layer-by-layer excavations were carried out from the surface to a depth of 10 cm. Sexually mature individuals of earthworms and, if possible, cocoons were selected from the soil. The collected worms were placed together with the info labels in soil-loaded breathing bags (15×15 cm). To determine the species of the worms, an illuminator, a magnifying glass, Petri dishes, tweezers, and a guide were used [41]. When identifying species, only external morphological characters of worms were noted.

A mixture of lowland peat and floodplain soil was used as substrates for the cultivation of vermicultures. The mixture was placed in boxes, then mixed and moistened with settled tap water to a moisture content of 80% of TMC. The experiments were conducted in plastic boxes 15×10 cm in size and 15 cm height. The boxes had drainage holes at the bottom for draining excess water. Earthworms were introduced in each box at 10 individuals/kg. The optimum temperature for dilution is 20-28°C, illumination - 200-400 lux, humidity - 50-70%, pH 6.0-8.0. Cultivation was carried out in dynamics for 3 months (Fig. 14).

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| Рисунок1 |  |
| Figure 14 – Cultivation of vermiculture | |

During the maturation of the model vermicompost, the mass of earthworms was monitored. According to the results, their mass was about 450 mg/kg soil. As a result, six batches of ready-made vermicultures were obtained for modeling and analysis of zoo-microbial complexes. Additionally, the studies to assess the functional diversity of microbial communities of soils and coprolites of earthworms were analyzed.

*Taxonomic composition* of microbial communities of soils and coprolites of earthworms. Changes in the composition of bacteria during the passage of soil through the digestive tract of worms are usually considered by comparing the complexes of microbial cells of the soil consumed by the worms and coprolites [42]. To understand the mechanisms of changes in the soil microbiome passing through worms, it is also necessary to know the composition of bacteria. This allows a more complete assessment of the effect of the passage of soil through the intestines of worms on the composition and abundance of its microbial inhabitants. In this regard, at the first stage of the study, the taxonomic composition and abundance of bacterial species in soil (Sample I) and coprolites of earthworms (Sample II) were studied.

In the digestive tract of earthworms, structural changes occur in the composition of the soil microbial complex. Some species disappear, others appear (Fig. 15a). Experiments with *E. fetida* worms showed that *Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi,* and *Bacteroldetes* dominate at the type level in coprolite (Sample II). In the control variants (Sample I), these groups are preserved, but the structure of the complex changes. Gram-positive actinobacteria always retain their dominance, and their proportion may even increase in experimental tests. Circos analysis revealed the similarities and differences observed between samples I and II. At the family level, both test samples have similar 277 families as visualized in the Venn (Figure 15b) and Co-occurrence Network (Figure 15c) plots.

The ingestion of soil into the digestive tract of the worms caused significant changes in the abundance of the microbiome. Facultative-anaerobic chemoorganotrophs, such as *Rhodobacteraceae, Steroidobacteraceae, Iamiaceae, Azospirillaceae, Flavobacteriaceae,* etc., are fundamentally different. (Fig. 16).

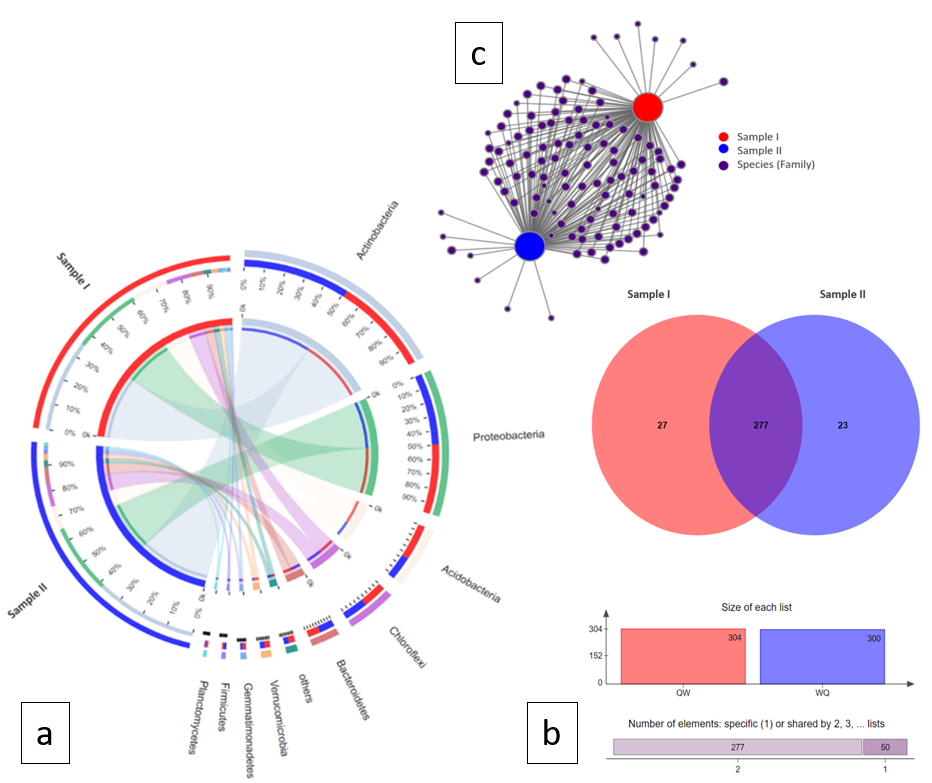


Figure 15 - The relative abundance of the microbial community at the family level. a. Circos analysis; b. Venn diagram; c. Co-occurrence Network

It was found that the strict aerobes *Microscillaceae* were not present at all in the digestive tract, despite the fact that there was a high abundance of them in the soil. Minor changes in the composition of the bacterial community occurred in the families *Ilumatobacteraceae, Xanthobacteraceae, Nocardioidaceae,* and *Propionibacteriaceae*. The ratio of *Gaiellaceae* also decreased significantly in coprolites.

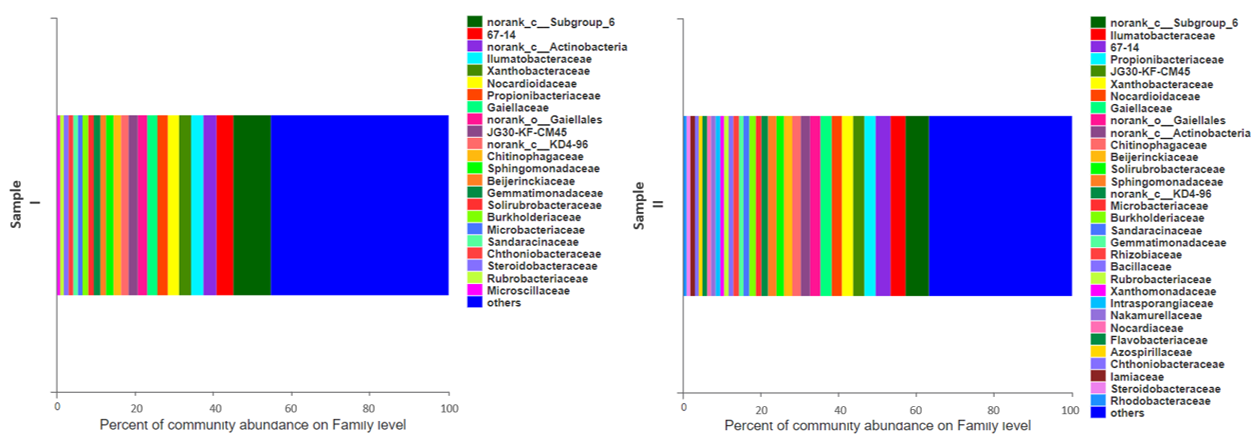


Figure 16 - Bar-plot analysis of microbial communities

The data show that the composition of bacteria in coprolites is largely determined by the specificity of the microbiome of the consumed substrate. The main mechanism in the formation of the coprolite microbiome is the aggregation of organic matter and the mineral fraction of the soil in the digestive tract of worms [43, 44].

Since the taxonomic composition of the microbiome in coprolites is largely determined by the composition of the consumed food. Section 3.2.3. in this study presents the results of a metagenomic analysis of the dynamics of changes in soil microbiome in the presence of humic and brown coal samples.

3.2.2 Selection of components of experimental humus (zoo-microbial complex)

Since low-grade/low-rank coals have a lignocellulose-like structure, one can expect their high transformation by lignin-transforming microorganisms. Most studies have demonstrated the key role of bacteria in the biosolubilization of brown coal [45].

The study of the processes of biodegradation of coal by bacteria using modern methods will allow a more accurate approach to assessing the state of humic substances in coal; will expand the knowledge about biosolubilization by active strains of bacteria; will help in the development of biofertilizers/bioamendments based on humic acids from brown coal and practical substantiation of the action of these products on plants.

In this regard, one of the main objectives of this stage was to carry out a comprehensive analysis (IR spectroscopy and elemental analysis) to study the physicochemical properties of products (in particular, humic substances) of biosolubilization of brown oxidized coals. The study used a highly active strain of *Bacillus* sp. RBK 7 with solubilizing activity against OBC. This culture was isolated in previous works and selected as a promising producer of biosurfactants.

*IR spectroscopy.* IR spectra of solubilization products of samples of untreated (OBC) and biotreated products (supernatant and sediment) revealed different characteristic absorption bands, which indicates the diversity of their functional groups (Fig. 17). The main spectral regions on the samples can be attributed to stretching vibrations -OH (3700-1220 cm-1), accompanied by -NH3+, -NH2+, -CO-NH2+, -CO-NH-; aliphatic bands (C-H) at 2980-2845 cm-1; carbonyl vibrations COOH, -CHO, -CO- (1850-1650 cm-1); stretching vibrations of aromatic bands (C = C, COO-) (1635-1600 cm-1), less substituted rings appear at 873-728 cm-1, organic halides and minerals (430-550 cm-1).

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|  | Рисунок23 |
| Figure 17 - IR spectra of samples | Figure 18 - UV spectra of humic acids |

The characteristic intensity for raw OBC at 2845 cm-1 may be due to aliphatic groups. The absorption bands of the products at 1700 cm-1 are characteristic of the carbonyl vibrations of aldehydes, ketones, and carboxyl groups. Vibrations due to the presence of hydroxyl groups are observed between 1410 and 1310 cm-1. The bands at 1450, 1429 and 1360 cm-1 refer to aliphatic C-H, C=N primary amides and -CO-CH3, respectively. The bands in the supernatant are more intense, which suggests that the metabolites secreted by *Bacillus sp.* RKB7 can cause significant changes in the composition of a biosolubilized product. The minimum in the wave number around 2845 cm-1 disappeared, indicating the degradation of aliphatic chains under the influence of bacterial metabolism.

Thus, the results indicate that the supernatant, i.e. bHS and OBC have a low correlation with each other. The IR spectra showed that, under the influence of bacterial activity, changes in OBC samples are caused by the appearance of new bands. This also justifies the theory that the biosolubilization products can be heterogeneous molecules with different functional groups compared to untreated OBC.

*Elemental analysis.* The humic substances were subjected to elemental analysis (Table 9). The elemental composition of HS changed in response to biosolubilization. It is noteworthy that bacterial activity on coal samples generates a higher N and O content due to metabolic processes that cause structural changes in HS [46]. The H/C atomic ratio indicates the degree of aromaticity, since its value in cHS is lower, which is explained by the higher molecular weight and the content of condensed aromatic structures [47, 48]. The O/C atomic ratio is regarded as an indicator of the amount of oxygen-containing groups (carbohydrates and carboxyl groups) in organic matter; its typical value for HS is about 0.4. Lower values ​​indicate a higher degree of aromatic condensation in the HA. The atomic ratio N/C reflects the amount of nitrogen in organic matter; higher values ​​are characteristic of bHS. The N/O ratio also indicates a lower nitrogen composition in the biosolubilization product and a lower molecular weight [2].

Table 9 - Elemental analysis of HS samples.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Samples | Elements, % | | | | | | Atomic ratio | | | |
| C | H | N | S | Odiff. | Ash | H/C | O/C | N/C | N/O |
| cHS | 55.71 | 3.90 | 1.55 | 0.14 | 34.24 | 4.46 | 0.84 | 0.46 | 0.02 | 0.05 |
| bHS | 54.42 | 4.05 | 3.10 | 0.17 | 35.66 | 2.60 | 0.89 | 0.49 | 0.04 | 0.09 |

Elemental analysis demonstrated that the chemical composition (N and O) of biosolubilization products increased, indicating a low molecular weight and the content of condensed aromatic structures. A higher composition of nitrogen (values ​​of atomic ratios) in bHS was also revealed.

*UV-Vis spectroscopy.* UV spectroscopy is an analytical method used to investigate the various properties of humic substances, including their aromaticity, hydrophobic content, and molecular weight [49]. The absorption spectra of samples of humic substances obtained from OBC are shown in Fig. 18.

In general, the spectra are relatively similar to the typical spectra of humic acids. The spectral curves have maximum bands around 256 and 312 nm due to the electronic transitions π → π \*, indicating the presence of aromatic fragments. The intensity and location of these bands is mainly influenced by the degree of substitutions in the aromatic ring, such as phenols, carboxylic acids and aliphatic chains. The absence of maxima on the right side of the spectra may indicate their overlap due to the complex structure of humic acid molecules.

The presented results show that humic substances obtained from OBC in the experiments were analyzed in detail and described using analytical methods; the results obtained using various approaches demonstrated a high correlation with each other.

Table 10 - Selection of experimental humus components

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variants | Soil | cHS | bHS | *Eisenia fetida* |
| Sample I | + |  |  |  |
| Sample II | + |  |  | + |
| Sample III | + |  | + |  |
| Sample IV | + |  | + | + |
| Sample V | + | + |  |  |
| Sample VI | + | + |  | + |

According to the results, as the main component in the production of a complex amendment on the basis of a mix-consortium, bHS and cHS of OBC were selected from the Oi-Karagai coal deposit (Almaty region, Kazakhstan). To obtain experimental humus based on the zoo-microbial complex, the following options were selected (Table 10).

Thus, as a result of the study, microbial consortia for targeted purposes were constructed based on humic substances, oxidized brown coal, strains of microorganisms with desired metabolic activity, and earthworms.

3.2.3 Construction of a mixed consortium based on the zoo-microbial community and oxidized brown coals

At the present stage of the study, an attempt was made to analyze the composition of microbial communities that are responsible for the internal regulation of the zoo-microbial complex. It is becoming evident that the taxonomic structure of prokaryotes is based on close interactions between microorganisms and earthworms.

The effectiveness of the introduction of bHS and cHS was studied against the background of the use of vermicompost. The experiment was carried out in 4-fold repetition according to the scheme (Fig. 19).

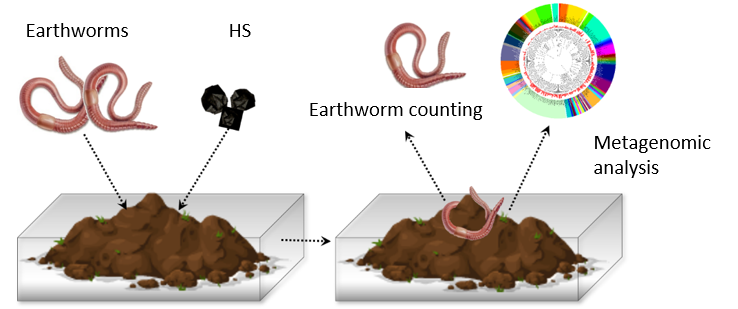


Figure 19 - Conceptual diagram of the integrated construction of the zoo-microbial community

A month after the construction of the mix consortium, the earthworms were counted. According to the results of the analysis, the total number of earthworms was found to be ~30. The largest number of individuals is 34, was found in sample IV. Almost 40% of the detected earthworms were juvenile individuals, which indicates the beginning of the process of reproduction of this species in new conditions. Sample II contained 30 earthworms. Of these, 25% were juvenile. Our experiment on the cultivation of earthworms on soils with bHS showed that the worms quickly adapt the environment and actively settle.

*Taxonomic structure of the bacterial community.* The study of the taxonomic characteristics of the microbial community of vermicompost by molecular-genetic methods shows that earthworms and the nature of raw materials have a significant contribution to the formation of the microbiome of vermicompost.

To study the micro-taxonomic structure of model variants of zoo-microbial communities, a comparative metagenomic analysis was conducted using a HiSeq system (Illumina, USA). The main indicators of the alpha diversity of microbial communities are presented in Table 11.

Table 11 - Main indicators of α-biodiversity of zoo-microbial complexes (phylum level).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variants | Sobs | Shannon index | Simpson index | Ace | Chao index |
| Sample I | 24 | 1.752766 | 0.246472 | 24.412109 | 24 |
| Sample II | 26 | 1.813435 | 0.227488 | 26.475795 | 26 |
| Sample III | 25 | 1.803599 | 0.23201 | 25 | 25 |
| Sample IV | 25 | 1.788933 | 0.239779 | 25.39053 | 25 |
| Sample V | 28 | 1.839306 | 0.218615 | 28.92667 | 28.5 |
| Sample VI | 24 | 1.542355 | 0.307467 | 24.53669 | 24 |

The data indicate the functional (trophic) differences of the microbial community depending on the substrate used for vermiculture. Figure 20a and b show the results of the analysis of beta-diversity data (Hierarchical Clustering Tree (HCT) and Principal Coordinates Analysis (PCoA)), which demonstrate that the taxonomic structure of prokaryotes is determined mainly by the nature of the raw materials.

|  |  |
| --- | --- |
|  |  |
| a - Hierarchical clustering (HCT) at the OTU level | b - Normal coordinate analysis (PCoA) at the OUT level |

Figure 20 - Results of comparison of the bacterial taxonomic structure

Other analyzes give a similar result. The composition of the prokaryotic community changes at each taxonomic level, which can be displayed using a heat map (Fig. 21).

The earthworm’s intestine is a specific habitat for microorganisms. Physicochemical (composition of nutrients, moisture, pH, redox) and biological factors (enzymatic activity) form microbial communities atypical for soil.

Bacterial communities of all zoo-microbial complexes are formed mainly by the phyla *Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi,* and *Bacteroidetes* (Fig. 22). The *Actinobacteria* has the largest proportions in microbial communities (total proportion in samples ~35%), which often occupies a dominant position in the soil microbiome; their ecological role is most often associated in the decomposition and synthesis of humic substances. Representatives of the groups of true symbionts *Proteobacteria* (~23%), *Acidobacteria* (~10%), and *Bacteroidetes* (~5%) make up a significant proportion of the communities of these complexes. In addition, in all samples, bacteria of the *Verrucomicrobia* have a certain representativity (~3%). Many bacteria species of these phylum can act as symbionts of invertebrates, in particular, earthworms.

However, individual groups of bacteria are also found in the samples, the results were visualized in the form of a Venn diagram, reflecting the distribution of groups of microorganisms at the family level (Fig. 23).

|  |  |
| --- | --- |
|  |  |
| Figure 21 - Heat map of the taxonomic composition of zoo-microbial complexes | Figure 22 - Phylogenetic tree at the phylum level |

Network analysis was constructed to visualize statistical correlations about the size of families between samples. As shown in Figure 24, the mechanism for the formation of phenotypic differences between samples is more complex and non-unique.

|  |  |
| --- | --- |
| C:\Users\akimb\AppData\Local\Microsoft\Windows\INetCache\Content.Word\Рисунок13.png | Рисунок28 |
| Figure 23 - Venn diagram | Figure 24 - Results of network analysis of samples |

The zoo-microbial association, to one degree or another, determines the course of the main soil physicochemical processes and the state of the biotic balance. The effects of interactions between animals and microorganisms are realized in the processes of decomposition and/or mineralization of the organic part of the OBC, mobilization and/or immobilization of HS. Close interactions determine the activity of microbial populations as well as fauna populations. The consequence of these interactions is the formation of a complex of soil fauna and microbiota, the dynamics of biomass and their profile distribution.

Earthworms are obligatory dependent on bacteria as a source of essential amino acids. They also need organic nitrogen and phosphorus, the supply of which is concentrated in the soil and microbial biomass. Microorganisms can be the main source of nitrogen, but not carbon. The efficiency of carbon assimilation can be increased by adding various readily available organic substances, such as HS, to the medium. However, these compounds are formed or transformed as a result of the activity of microorganisms and their exoenzymes.

Thus, as a result of the study, humus-zoo-microbial complexes were obtained: "Product I", i.e. sample IV (cHS+zoo-microbe) and "Product II", i.e. sample VI (bHS+zoo-microbe). A laboratory-prepared sample of the humic product bHS is shown in the Figure 25.

|  |  |
| --- | --- |
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Figure 25 - Laboratory-prepared sample of bio-humus product

*Fluorescence spectroscopy of products based on humic substances and zoo-microbial communities.* A typical EEM (excitation-emission matrix) contour map is represented by fluorescence peaks of maximum intensity and corresponds to specific fluorophores or functional groups. Fig. 26 demonstrates the EEM spectra: Sample II, Sample IV and Sample VI. Strong fluorophores are observed at excitation/emission around 270/445 nm in sample IV. Coble P. [50] proposed eight main types of fluorescence peaks, where humic, protein-like, and pigment-like fluorescence are the main values. In the present study, the maxima of the peaks were within the region called peak A, denoting its humic nature. In the case of sample VI, the peak was in a wide excitation/emission range of 275/440-450 nm due to its inhomogeneous nature.

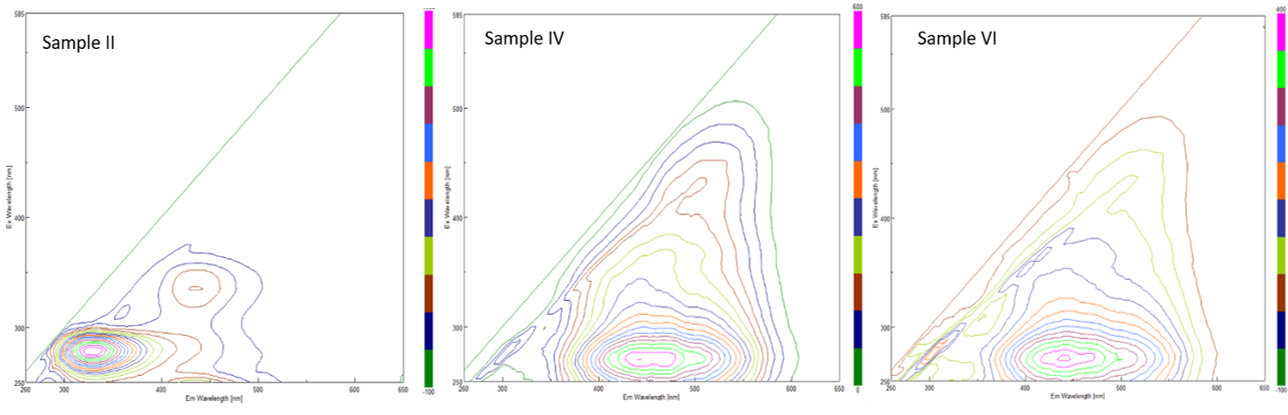


Figure 26 - EEM spectra of the samples of zoo-microbial complexes

Thus, the general similarity between Sample IV and Sample VI is the reason for designating all peaks as humic. However, slight differences in peak positions are evident, suggesting that there are slight differences in humic composition between samples. In the case of sample II, the EEM has a different view with a wavelength of 340 nm. According to the reference [51], lower radiation wavelengths in the range of 340-430 nm can be associated with non-humic structures.

3.2.4 Study of the biological activity and the effectiveness of the obtained product "bio-humus plus" in greenhouse conditions

The available data on the effectiveness of products based on humic substances indicate that their amendment is important at all stages of crop development, but especially at early stages [30, 52]. The rational development of potato and vegetable production requires promising scientific solutions to provide the human population with the most important food products and raw materials for the processing industry. In modern conditions, the development and implementation of environmentally friendly, resource-saving technologies for growing potatoes is of great importance.

During the study period, the decisive factor was the application mode of humic substances. This was reflected in the growth of potatoes in height (Table 12).

Table 12 - Potato height in the dynamics of plant development during the growing seasons.

|  |  |  |  |
| --- | --- | --- | --- |
| Variants | Plant development phase, cm | | |
| The beginning of budding | Blossoming | End of flowering |
| Control | 25.6 | 29.4 | 35.1 |
| Seed treatment with bHS 1.5% | 29.1 | 38.1 | 41.3 |
| Seed treatment with bHS 2.5% | 28.4 | 37.8 | 40.9 |
| Spray treatment with bHS 0.01% | 27.4 | 38.5 | 43.1 |
| Spray treatment with bHS 0.05% | 28.0 | 37.5 | 44.3 |
| cHS 1% | 27.5 | 33.5 | 42.5 |
| cHS 5% | 26.9 | 32.1 | 40.5 |

So, in the control, the height of plants at the beginning of budding was on average 35 cm, and in variants with pre-planting treatment of tubers with bHS it was in the range from 28.4 to 29.1 cm. By the end of flowering, the indicators increased: in the control - 35.1 cm, in variants with the treatment of tubers, the plant height was from 40.9 to 41.3 cm. Spraying potato plantings during the growing season with bHS also had a positive effect on plant growth. At a dose of 0.01%, the height of plants by the end of the growing season reached an average of 43.1 cm, while at 0.05% - 44.3 cm (Fig. 27). The introduction of cHS into the soil at a concentration of 1% promoted the formation of potatoes with an average height of 42.5 cm, which is 21% more than in the control.

The use of humic substances had a stimulating effect, as a result of which the height of the potato with all methods of its use increased significantly in comparison with the control. The best effect was achieved when spraying in increasing doses of the product (0.05%). The use of cHS as a source of humus at 1%, although significantly inferior to the best option (spraying 0.05%), in comparison with the control, caused the formation of tall potatoes.

Thus, in the greenhouse conditions, similar patterns were revealed: the greatest positive effect from the use of humin based on brown coals was achieved when spraying plants with increasing doses, so the best option is spraying potatoes with a solution of 0.05%.



Figure 27 - Development phases of experimental potatoes

Observations of the onset of phenological phases demonstrated that the humic substances used in the experiments did not cause differences in the timing of the onset of the phases of potato development (Table 13).

Table 13 - The interval of interphase periods depending on the use of humic substances

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variants | Days from planting to: | | | |
| sprouting | budding | blooming | dieback of halm |
| Control | 25 | 43 | 53 | 105 |
| Seed treatment with bHS 1.5% | 25 | 43 | 53 | 105 |
| Seed treatment with bHS 2.5% | 25 | 43 | 53 | 105 |
| Spray treatment with bHS 0.01% | 25 | 43 | 53 | 105 |
| Spray treatment with bHS 0.05% | 25 | 43 | 53 | 105 |
| cHS 1% | 25 | 43 | 53 | 105 |
| cHS 5% | 25 | 43 | 53 | 105 |

The duration of time from planting to sprouting of plants for all variants of the experiment was 25 days. The period from planting to budding, also for all variants, regardless of the dose and method of application of humic substances, was 43 days. The duration of the blooming phase was 53 days, and the complete dieback of the halm was 105 days.

The vegetative mass of potato determines the final yield of tubers. The results of the formation of potato productivity in the dynamics of development (Table 14) show that the use of various methods of humic substances caused differences in the formation of the area of ​​the assimilation surface of potatoes.

Table 14 - Dynamics of the growth of the leaf surface area of potatoes

|  |  |  |  |
| --- | --- | --- | --- |
| Variants | The leaf surface area, thousand m2/ha | | |
| 30 days after planting | 46 | 62 |
| Control | 31.1 | 24.5 | 21.5 |
| Seed treatment with bHS 1.5% | 39.7 | 45.3 | 31.2 |
| Seed treatment with bHS 2.5% | 38.7 | 44.3 | 33.5 |
| Spray treatment with bHS 0.01% | 44.2 | 46.1 | 37.0 |
| Spray treatment with bHS 0.05% | 49.4 | 51.2 | 45.1 |
| cHS 1% | 38.1 | 39.5 | 37.1 |
| cHS 5% | 34.5 | 35.9 | 31.5 |

The pre-planting treatment of potato tubers, although it caused an increase in the leaf surface, was less effective in comparison with the treatment of vegetative potato plants. Spraying potatoes with a humic substance resulted in a high effect in the formation of the leaf surface of potatoes. So, in comparison with the control, its value increased by 45.1 thousand m2/ha.

Plant productivity is mainly determined not only by the leaf area, but also by the photosynthetic activity of plants, i.e. the net productivity of photosynthesis (Net Primary Productivity - NPP), which shows the increase in dry weight of potatoes in grams over a certain period of time per unit of leaf area (Table 15). The most positive NPP was in the variants with the spraying of vegetative plants with bHS. The more funds are spent on increasing the yield, the more noticeably the NPP decreases. Thus, there is a positive relationship between the assimilation surface of leaves and plant productivity.

Table 15 - Net productivity of photosynthesis, g/m2 day

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Variants | Control | Seed treatment with bHS 1.5% | Seed treatment with bHS 2.5% | Spray treatment with bHS 0.01% | Spray treatment with bHS 0.05% | cHS 1% | cHS 5% |
| NPP | 3.8 | 3.6 | 3.3 | 3.2 | 2.9 | 3.6 | 3.5 |

The growth of tuber biomass is determined by the development and activity of the photosynthetic apparatus of plants, which was significantly influenced by the methods of application and doses of humic substances. Under greenhouse conditions, by the beginning of flowering of plants, the total mass of tubers, depending on the variants, ranged from 97 to 123 centners, while the number of commercial tubers also significantly changed from 43 to 57% (Table 16).

With the dying off of the tops, the total mass of tubers increased and amounted to 217 centners in the control, while in the variant with spraying the plants with humin at 0.05% - 285 centners, and the marketability increased to 77%. The pre-planting treatment of the planting material, as well as the spraying of plants with the product, provided a significant difference in the accumulation of the mass of tubers in dynamics. Although the mass of tubers in the cHS variant at 1% increased by 234 centners in comparison with the control, the marketability reached only 67%. It should be noted that with all methods of using humic substances, even in the form of cHS, more optimal conditions were created for the formation of potato productivity and the quality of its tubers.

Table 16 - Dynamics of tuber biomass growth

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variants | Potato development phase | | | |
| The beginning of flowering | | The withering away of halms | |
| Total mass of tubers, c/ha | Marketability, % | Total mass of tubers, c/ha | Marketability, % |
| Control | 97 | 43 | 217 | 66 |
| Seed treatment with bHS 1.5% | 109 | 46 | 233 | 67 |
| Seed treatment with bHS 2.5% | 113 | 57 | 251 | 75 |
| Spray treatment with bHS 0.01% | 116 | 46 | 257 | 70 |
| Spray treatment with bHS 0.05% | 123 | 51 | 285 | 77 |
| cHS 1% | 108 | 45 | 234 | 67 |
| cHS 5% | 105 | 44 | 229 | 65 |

Thus, the regularities of the effect of various doses and methods of using the humic products on the accumulation dynamics of the mass of potato tubers were identical. The maximum dose of humic substances during spraying for the growing season of potatoes had an advantage in all the studied variants. The introduction of humic substances into the soil contributed to the intensification of the production process, the formation of higher bushes in height, as well as an increase in the yield of tubers by an average of 234 c/ha. The results obtained deserve attention in the context of a shortage of high-quality organo-mineral fertilizers.

**3.3 Conducting field experiments on the territory of the Kazakh Research Institute of Potato and Vegetable Farming**

3.3.1 Preparation of experimental soils and determination of their physicochemical parameters and biological properties

Due to the introduction of robust lookdown regime from March 19, 2020, the experiment on the territory of the Kazakh Research Institute of Potato and Vegetable Farming (Kaz RI PVF) failed. However, this experiment was agreed with the Agrobio-station of the al-Farabi KazNU, located in the village "Zhana-Talap", Baiserke rural district of Ili district, Almaty province. There is an agreement from all sites (Kaz RI PVF, Agrobio-station and host institute) for conducting field experiments on the above-mentioned territory (APPENDIX С).

Loamy soil was taken on the territory of the Agrobio-station of the al-Farabi KazNU (43 ° 27'57.4 "N 76 ° 58'32.5" E). Soil samples were collected randomly from a depth of 0-20 cm at a distance of at least 5 m from the nearest trees. The soils were then air dried and sieved to 2 mm, collected on-site, and stored at 4°C for no more than 2 weeks prior to the start of the experiment. The physicochemical properties of the soil were characterized by the Berndt-Michael Wilke method [53]. The pH values ​​for each soil group were measured 3 months after application of the product and before growing the plants. The pH measurements were carried out on suspensions of 5 g of air-dried soil samples in 25 ml of dH2O using a pH meter 781 (Metrohm AG, Herisau, Switzerland).

Physicochemical properties of soil samples are shown in Table 17. The soil had a clay-loamy structure and a pH of 8.0.

Table 17. The main characteristics of the composition and properties of soils

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Physical properties of soil | | | Chemical properties of soil | | |
| Sand, % | Dust, % | Silt, % | pH (1:2) | Salts, g·kg−1 | Organic matter, g·kg−1 |
| 24.8 | 69.4 | 5.8 | 8.0 | 0.03 | 1.8 |

The addition of bHS and cHS had a significant effect on soil pH, as the pH of the soil treated with product I (7.2±0.2) was lower than that of the control soil (8.0±0.5). However, the addition of product II to soil pH had less effect (7.8±0.3). The effect of HS on the absorption of essential anionic macro-elements (such as nitrates, sulphates, and phosphates) was discussed in [54], which indicates a large role for pH. It is known that a change in soil pH leads to changes in soil microbial communities [5, 55]. The biological properties of soil samples were studied at the next stage of the study.

3.3.2 Study of the effectiveness of various doses and methods of using a humic product on the formation of potato yield

The reported concentrations of humic substances used for soil cultivation vary considerably. Chen and Aviad [56] determined an average dosage for field applications of 75 kg of humic substances per hectare (values ​​varied between 20-225 kg·ha-1). Thus, when using coals with 70% humic substances, the required amount will be approximately 110 kg·ha-1, which is in the range of 30-350 kg·ha-1. Unlike commercially available humic substances, which have been extensively studied in greenhouse and field conditions, there are few data on humic substances obtained from coal. The concentration of coal-based humic formulations that can be used may vary greatly, making it difficult to determine effective treatment doses. In addition, the structural/compositional characteristics of mineral humic substances may differ from those of the soil [57]. Other factors such as extraction/purification methods, pretreatment and application of humic acids can also have a significant impact on the overall yield [58].

In our case, a dose of bHS 1 g·kg-1 was selected for further stages of analysis for the following reasons: (1) the studied soils had a relatively low content of organic matter and were treated with only one dose of HS throughout the experiment, and (2) the bioavailability of humic acids obtained from coal may differ from those in soil/peat. Similarly, Asik et al. [59] proposed to treat saline soil with humic acid obtained from leonardite at a dose of 1 g·kg-1 for wheat growth and productivity. In our case, 1.5 g·kg-1 cHS was used, since it had a high potential for the yield of humic acid (71.1%).

Mechanical soil cultivation with HS was chosen as the main and most effective way to achieve results.

The impact of organic matter on the soil ecosystem is primarily associated with the activation of the metabolism of microbial communities in the soil. The available organic matter in the soil ecosystem is decomposed by microorganisms that retain C and N in their biomass and release CO2, CH4, and NO2 into the atmosphere [60]. Many chemical transformations of humic soil additives are mediated by heterotrophic microorganisms [61]. Studies show that the introduction of HS into the soil usually affects the composition of the community and the number of soil bacteria and, to a lesser extent, soil fungi, actinomycetes, and microalgae [5, 62]. To date, metagenomic approaches have become a valuable method of choice in establishing the structure and diversity of microbial populations. Studies of the effect of coal-based humic substances on soil microbiome are limited. However, the published data obtained using phylogenetic microarrays based on the *16S rRNA* gene revealed a large effect of commercial humic products on the resident bacterial community in various soil profiles [63].

Differences in microbial communities of soil samples were revealed before potato cultivation by comparing indicators of species richness and diversity. A total of 182,470 high quality 16S sequences were obtained from all samples, including the control, soils treated with "Product I" (sample IV) and "Product II" (sample VI). These sequences were distributed among 7371 operational taxonomic units (OTU) in all samples, of which 2172 (sample IV), 2630 (sample VI), and 2569 (control) are OTUs (Table 18).

In total, 1681 OTUs were split across all soil samples, while individual OTUs accounted for 168 (sample IV), 359 (sample VI), and 253 (control) of the total OTUs. A total of 184 OTUs were split between IV and control, 139 OTUs between IV and VI, and 451 OTUs between VI and control (Fig. 28).

Table 18. Number of observed OTUs, species richness and diversity of soil samples

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Samples | OTUs | Shannon's Index | Simpson's Index | ACE | Chao1 |
| Control | 2569 | 6.65 | 0.0027 | 3070 | 3069 |
| Sample VI | 2630 | 6.69 | 0.0024 | 3096 | 3068 |
| Sample IV | 2172 | 6.40 | 0.0036 | 2738 | 2706 |

In addition, Shannon's curves showed similar trends, with the greatest diversity of microbes observed in sample VI, and the smallest value in sample IV (Fig. 29).

Diversity indices revealed the highest bacterial diversity and richness in the bHS-treated soil samples. This phenomenon can be explained by the fact that humic acid can serve as a source of nutrients for microbial communities, which can stimulate local microorganisms by increasing the intensity of their growth and distribution [60].

|  |  |
| --- | --- |
|  |  |
| Figure 28 - Venn diagram showing specific and common OTUs | Figure 29 - Shannon curves for soil samples |

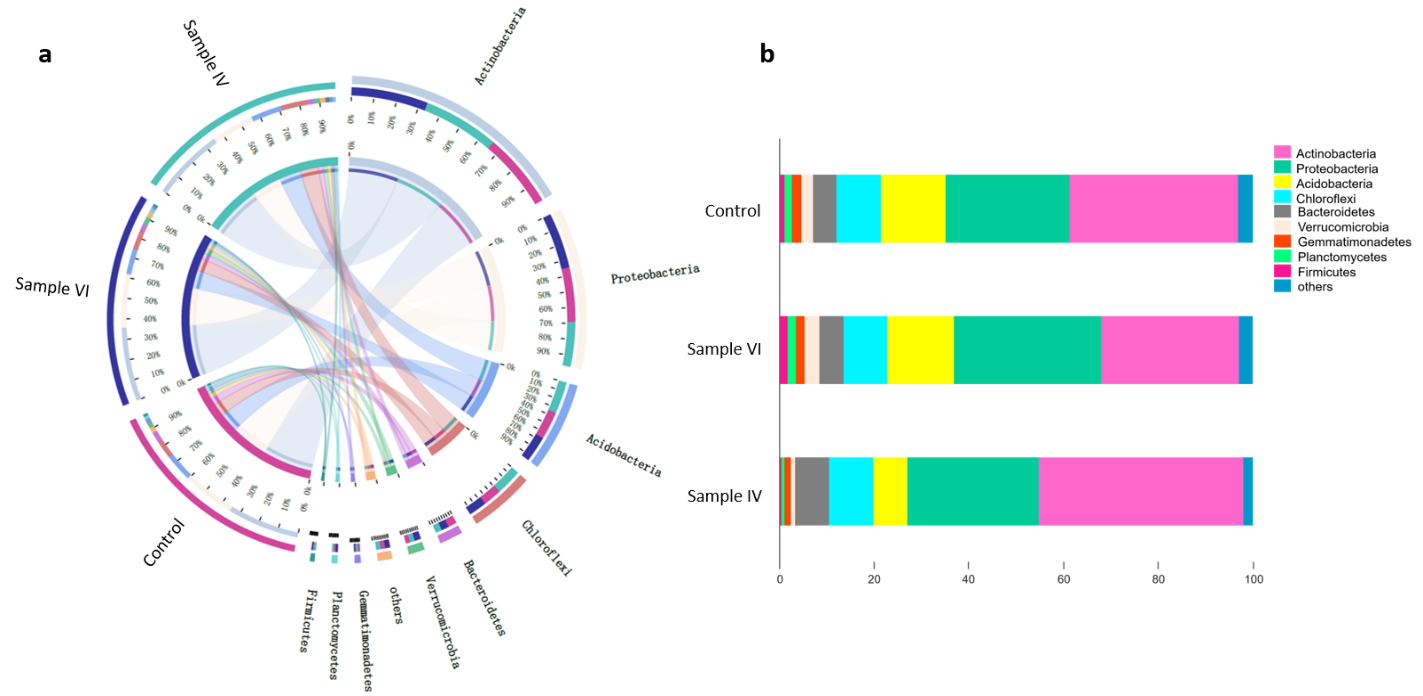


Figure 30 - The relative abundance of the microbial community for each group at the phylum level. (a) Circos analysis displays the corresponding abundance relationship between samples and bacterial communities; (b) Bar-plot analysis displays the average relative abundance of soil microbiota in each group.

Circos analysis was applied to visualize the corresponding abundance relationship between soil samples and bacterial communities at the phylum level, which confirmed the results of histogram analysis (Fig.30a).

The composition of the microbial community in sample VI contained mainly proteobacteria, which could have properties promoting plant growth, providing nutrients that are easily assimilated by plants [64]. The predominance of *Proteobacteria* in samples IV can also be associated with the depolymerization of humic substances in the digestive tract of earthworms [65].

Fig. 5b shows a Bar plot analysis describing the effect of humic additives (cHS and bHS) on the structure of bacterial communities at the phylum level in the zoo-sphere. All samples were dominated by five phyla, including *Actinobacteria*, *Proteobacteria, Acidobacteria, Chloroflexi,* and *Bacteroidetes.* However, the use of cHS led to significant changes in the structure of bacterial populations in comparison with the control. The type of *Actinobacteria* decreased from 35.72% to 29.08%, while *Proteobacteria* grew from 26.15% to 31.17%. The structure of the bacterial population also changed under the influence of cHS. The *Actinobacteria* was found to be particularly favorable to an environment rich in cHS (43.26%).

3.3.3 Conducting field experiments, analysis and evaluation of the results

Maintaining the functional integrity and stability of the soil is a priority in the intensive development of agriculture. Long-term use of non-renewable chemical fertilizers and pesticides adversely affects soil health and causes environmental problems. Thus, the current concern in agriculture relates to the gradual replacement of chemicals with organic additives and to increase their effectiveness through the adoption of appropriate methods. [66] Biomodified OBC, due to the presence of humic acids in it, can be promising for soil improvement [67].

Field experiments showed that the growth of potatoes and the yield of tubers were significantly influenced by products based on the zoo-microbial complex. The growth characteristics of potatoes and the number of grown tubers in samples IV and VI compared to the control are shown in Table 19. The addition of product II tended to increase the plant height, as well as the number of stems per plant (plant height increased by 25.3%, and the number of stems by plant by 46.1% compared to control) (рис. 31).



Figure 31 - Potato development phases in the field

The humic products also significantly influenced the total number of tubers classified by size. The largest number of potato tubers was obtained in sample VI (90.3% more than in the control group).

Table 19. The effect of humic products on potato growth and tubers yield

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Plant height, cm | Number of stems per plant | Number of tubers per plant | | | |
| Small | Medium | Large | Total |
| Control | 37.6 ± 0.7c,\* | 2.6 ± 0.4 b | 3.3 ± 0.1 | 2.1 ± 0.1 | 1.8 ± 0.4 | 7.2 ± 0.2b |
| Sample IV | 43.8 ± 1.0b | 3.1 ± 0.6b | 3.9 ± 0.4 | 2.5 ± 0.4 | 3.6 ± 0.2 | 10 ± 0.3b |
| Sample VI | 47.1 ± 0.7a | 3.8 ± 0.4a | 4.5 ± 0.3 | 4.8 ± 0.2 | 4.4 ± 0.1 | 13.7 ± 0.2a |

\* A significant difference according to the Duncan's rank test at p <0.05 is indicated by different letters (mean ± standard deviation; n = 15).

The observed increase in the yield of tubers in response to treatment with humic product I and II can obviously be explained by an increase in the relative number of stems and tubers. Our data on the stimulating effects of bHS are consistent with the results presented by Z. Ekin [68] and R. Selladurai et al. [69], who revealed that treatment with humic acid significantly increases the yield of potatoes in comparison with control in both greenhouses and field conditions.

3.3.4 Conducting an ecological and economic assessment of the use of a humic product on potatoes

The highest marketable yield was obtained with the treatment with humic product II, showing an increase of 66.4% compared with the control (Table 20).

Table 20. The effect of humic products on the yield of tubers and economic efficiency.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Tuber yield per plant, kg | | | | Marketable yield, kg | Marketable yield, % |
| Small | Medium | Large | Total |
| Control | 0.41 ± 0.1 | 0.28 ± 0.1 | 0.19 ± 0.2 | 0.88 ± 0.1b,\* | 0.47 ± 0.1\*\* | 53,4 |
| Sample IV | 0.40 ± 0.2 | 0.27 ± 0.2 | 0.35 ± 0.1 | 1,02 ± 0.2b | 0.62 ± 0.1 | 60,8 |
| Sample VI | 0.42 ± 0.2 | 0.39 ± 0.1 | 0.44 ± 0.1 | 1.25 ± 0.1a | 0.83 ± 0.1 | 66,4 |

\* A significant difference according to the Duncan rank test condition at p <0.05 is indicated by different letters (mean ± standard deviation; n = 15). \*\* Marketable yield is the sum of the average and large size of tubers.

The application of humic product I to the soil had a less significant effect on the potato growth of and the tuber yield. However, due to the complex nature of HS, it is difficult to characterize all the reactions involved in the bioconversion and micro-degradation of HS in the soil. It is noteworthy that in the samples with humic product II, a high number of *Actinobacteria* was observed. Due to the filamentous nature, *Actinobacteria* can penetrate into the smaller pores of the carbon matrix; in addition, many *Actinobacteria* are found in coprolites of earthworms and produce biosurfactants that facilitate the solubilization of hydrocarbons [70]. The reaction of these bacterial communities indicates a high potential for biodegradation of coal HS in the soil. Recent studies by S.J. Robbins et al. [71] and A. Detman et al. [72] also suggested that methods such as bioaugmentation (inoculation of exogenous microorganisms into the soil) [73] and biostimulation (stimulation of the degrading capacity of local microorganisms by adding nutrients) [ 73] can be interesting options for stimulating the decomposition of coal HS.

Simplified estimates of economic efficiency were carried out on the basis of determining the costs of using "Product II". Calculations of economic efficiency under steady-state conditions have shown that all costs associated with the use of HS based on coal and a zoo-microbial complex are fully paid off. Our results showed a stimulating effect of HSs obtained from a zoo-microbial consortium on plant growth and tuber yield. Formulations based on HS and zoo-microbial complex may provide useful opportunities in the development of sustainable agricultural technologies and organic fertilizers to improve the soil in an environmentally responsible manner.

**CONCLUSION**

Based on the results of the research, the following conclusions were made:

1.Sampling of oxidized brown coals was carried out in accordance with the requirements on the territory of the Karaganda, Turkestan and Almaty provinces. In total, 3 weathered coal samples were collected, processed and investigated in the near-land areas of the Oi-Karagai (OLE), Lenger (Karatau) (LLE) and Kiyakty (KLE) coal deposits.

According to the results of physicochemical and structural analyzes of oxidized brown coal samples, the following characteristics have been established: coal grade OLE, % - W: 11.8, A: 12.2, V: 35.8, Q: 15.6 MJ/kg; LLE grade coal, % - W: 9.1, A: 22.0, V: 40.8, Q: 7.8 MJ/kg and KLE grade coal, % - W: 9.8, A: 11.5, V: 41.8, Q: 21 MJ/kg. The chemical composition, molecular structure and macro- and microscopic properties of the analyzed coals were characterized by different spectral and microscopic methods.

It was found that the bacterial communities of coal samples, according to metagenomic data, are formed mainly by nine phyla of *Proteobacteria, Tenericutes, Acidobacteria, Firmicutes, Bacteroidetes, Nitrospirae, Chloroflexi, Gemmatimonadetes, Actinobacteria,* and *Fusobacteria. Proteobacteria* is the dominant phylum of bacteria in all analyzed coal microbiomes, regardless of the type of coal. Indigenous bacteria were isolated and their morphological-cultural and physiological-biochemical properties were studied.

Three cultures of microorganisms producing biosurfactants with target metabolic activity were selected, i.e. the ability to biosolubilize oxidized brown coal. They were identified as *Acinetobacter pittii* RKB 1, *Delftia* sp. RKB 5 and *Bacillus* sp. RBK 7.

2. *Eisenia fetida* earthworm cultures were obtained. Vermiculture was cultured in laboratory conditions (temperature - 20-28 ° С, illumination - 200-400 lux, humidity - 50-70%, pH 6.0-8.0, regular oxygenation of the air), the maximum amount of biomass for creation of a zoo-microbial complex is received. At the beginning of the experiment, the biomass of worms was ~ 100 mg per 1 kg of soil, and after cultivation, an increase in mass by ~ 450 mg was observed. As a result of a comparative metagenomic analysis (*16S rRNA*) of samples of earthworms, it was concluded that there are dominant phyla of *Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, Bacteroidetes,* and *Verrucomicrobia.*

Cultures of *E. fetida* earthworms, OBC samples from the Oi-Karagai (OLE), bHS and cHS deposits based on them, as well as a producer of biosurfactants (*Bacillus* sp. RBK 7) with the target metabolic activity were taken as components. In this work, studies were additionally carried out to study the physicochemical properties of bio-humus obtained using spectrometric methods.

Zoo-microbial complexes were obtained: cHS+zoo-microbe (Product I) and bHS+zoo-microbe (Product II). Metagenomic analysis of these complexes (Sample IV and Sample VI) produced by one species of earthworm showed that the components of zoo-microbial communities significantly affect the structure of the microbial community. Modern fluorescence spectroscopy has revealed small differences between samples IV and VI, as well as that humic substances have similar characteristics in the EEM spectra at excitation/emission around 270-275/440-445 nm.

The reliable efficiency of the use of zoo-microbial communities in terms of the effect on the growth and development of potato plants has been revealed. Depending on the concentration and method of application of the humic products, the height of plants increases by 20-26%. Treatment of plants with the humic product in increasing doses led to the intensification of the morphophysiological processes of potatoes - the leaf area increased by 1.15-1.26 times and the photosynthetic potential by 1.05-1.31 times, which led to an increase in productivity. According to the research results, it should be noted that in all variants with the use of humic substances based on OBC, regardless of the method of its use, potato plants were significantly higher than the control ones.

1. The main physicochemical and biological parameters of the experimental soils are characterized: the ratio of sand, dust and silt was 24.8, 69.4 and 5.8. The organic matter content was 1.8 g·kg-1, the salt content was 0.03 g·kg-1, and the pH was 8.0. According to metagenomic analysis, five phyla predominated in soil samples, including *Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi,* and *Bacteroidetes.*

The effective dose of HS for the treatment of potato plantings was determined, so for bHS it was 1 g·kg-1, and for cHS - 1.5 g·kg-1.

The high efficiency of the use of the biological product was revealed in terms of the effect on the growth and development of plants: in comparison with the control, the height of potatoes increased by 25.3%, the number of stems per plant by 46.1%, the number of tubers by 90.3%.

The economic efficiency and ecological feasibility of the use of biological products are shown, which contributed to the growth of potatoes and an increase in the yield of tubers, thereby increasing the cost of gross production. Considering the fact that its use significantly improves the composition and structure of the soil, the use of "Product II" led to a high profit.

The assigned research tasks were fully completed.

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**APPENDIX A**

**List of published works on the project**

Articles and abstracts in foreign rating publications

1 Zhubanova A.A., Ualieva P.S., Abdieva G.Zh., Kayrmanova K.T., Akimbekov N.Sh., Xiaohui Q., Tastambek K.T. Metagenomic Analysis Reveals Correlation Between Microbiome Structure and Leonardite Characteristics from Kazakhstan Coal Deposits. //Eurasian Chem. Tech. J., 2019, vol. 21, no. 2, pp. 135-141. (Status: Printed. SJR=0.11)

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8 Patent "Method of coal processing" No. 2018 / 0682.1 dated 01.10.2018

9 Patent "Method of coal processing" No. 2018 / 0681.1 dated 01.10.2018

10 Patent application “Method for obtaining biologically active products of brown coal solubilization by bacterial strains of Acinetobacter pitti. RKB1 "№2019 / 0504.1 from 15.07.2019

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14 Akimbekov N. Qiao X. Digel I. Abdieva G. Ualieva P. Zhubanova A. The Effect of Leonardite-Derived Amendments on Soil Microbiome Structure and Potato Yield. Agriculture (Switzerland), 2020, 10(5), 147.

**APPENDIX В**

**Work schedule**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| # | The name of the objectives, activities for the implementation of the tasks of the project | Duration (in months) | Beginning and completion of research (dd/mm/yy.) | Years of the project implementation, expected results (in terms of objectives and activities) | | |
| 2018 | 2019 | 2020 |
| 1. | The study of physical and chemical parameters and microbial landscapes of selected materials (soils and coals). | 12 | 03.01.  2018-31.12.  2018 | The physical and chemical parameters and microbial landscapes of selected materials (soils and coals) will be studied. |  |  |
| 1.1 | Acquaintance with the objects of research and selection of coal (brown oxidized) samples for further experiments. | 3 | 03.01.  2018-31.03.  2018 | The objects of research will be acquainted and selection of coal (brown oxidized) samples for further experiments will be conducted. |  |  |
| 1.2 | The study of the physico-chemical and structural properties of oxidized brown coals. | 3 | 01.04.  2018-31.06.  2018 | The physico-chemical and structural properties of oxidized brown coals will be studied. |  |  |
| 1.3 | The study of physicochemical parameters, cultural, metabolic and antimicrobial properties of collection microorganisms and isolated strains. | 3 | 01.07.  2018-31.09.  2018 | The physicochemical parameters, cultural, metabolic and antimicrobial properties of collection microorganisms and isolated strains will be studied. |  |  |
| 1.4 | Obtaining and growing of vermiculture. | 3 | 01.10.  2018-31.12.  2018 | Vermiculture will be obtained and grown. |  |  |
| 2. | The creation of a microbial consortium of special-purpose use on the basis of humus, strains of microorganisms with the target metabolic activity. | 12 | 03.01.  2019-31.12.  2019 |  | A microbial consortium of special-purpose use on the basis of humus, strains of microorganisms with the target metabolic activity will be created. |  |
| 2.1 | The creation of experimental humus, based on zoo-bacterial complex. | 3 | 01.01.  2019-31.03.  2019 |  | The experimental humus, based on zoo-bacterial complex will be created. |  |
| 2.2 | The construction of a mix consortium based on the zoo-microbial community and brown oxidized coals. | 3 | 01.04.  2019-31.06.  2019 |  | A mix consortium based on the zoo-microbial community and brown oxidized coals will be constructed. |  |
| 2.3 | The study of biological activity and effectiveness of the obtained preparation "bio-humus plus". | 3 | 01.07.  2019-31.09.  2019 |  | The biological activity and effectiveness of the obtained preparation "bio-humus plus" will be studied. |  |
| 2.4 | The determination of physicochemical parameters and biological properties of experimental soils. | 3 | 01.10.  2019-31.12.  2019 |  | The physicochemical parameters and biological properties of experimental soils will be determined. The biological activity and effectiveness of the obtained preparations "vermicompost plus" in greenhouse conditions will be studied.  1 (one) article will be published in a peer-reviewed foreign scientific journal, indexed in the Web of Science database or  Scopus with non-zero impact factor. Also 2 (two) will be published  articles in journals recommended by KKSON MES RK. |  |
| 3. | The determination of the influence of new forms of fertilizers - humic product on growth, development and indices of photosynthetic potato plant productivity. | 12 | 03.01.  2020-31.12.  2020 |  |  | The influence of new forms of fertilizers - humic product on growth, development and indices of photosynthetic potato plant productivity will be determined. |
| 3.1 | Identification of the effectiveness of different doses and ways to use humic product on potato yields. | 3 | 01.01.  2020-31.03.  2020 |  |  | The effectiveness of different doses and ways to use humic product on potato yields will be identified. |
| 3.2 | Identification of the optimal doses and methods for application of humic product in potatoes crop. | 3 | 01.04.  2020-31.06.  2020 |  |  | The optimal doses and methods for application of humic product in potatoes crop will be identified. |
| 3.3 | Conducting an environmental and economic assessment of the application of humic product on potato plantings. | 3 | 01.07.  2020-31.09.  2020 |  |  | An environmental and economic assessment of the application of humic product on potato plantings will be conducted. |
| 3.4 | Conducting field experiments in the fields of the Kazakh research institute on potato and vegetable growing, analysis and evaluation of the obtained results. | 3 | 01.10.  2020-31.12.  2020 |  |  | The field experiments in the fields of the Kazakh research institute on potato and vegetable growing will be conducted, the obtained results will be analyzed and evaluated.  An ecological and economic assessment of the use of a humic preparation on potato plantings will be carried out.  2 (two) articles will be published in peer-reviewed foreign scientific journals |

**APPENDIX С**

**Consent letter**

