ABSTRACT

of the dissertation for the degree of Doctor of Philosophy (Ph.D.) in the educational program "8D05105 – Biotechnology"

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on the thesis: "Stimulation of biogenic methane generation from Kazakhstani low-rank coals"

General description of work: This work is devoted to the enhancement of biogenic methane production from Kazakhstani low-rank coals. In the context of global energy transition and the growing demand for clean energy sources, special attention is being paid to the development of methods to stimulate the formation of methane, including its biogenic origin. This process is based on the activity of methanogenic microorganisms that are capable of decomposing organic coal compounds via the formation of methane.

Relevance of the research topic: Kazakhstan is one of the top ten countries in the world in terms of coal reserves, which exceed 33.6 billion tonnes, with about 64% of them being lignite and low-calorie coals. They have economic and environmental disadvantages that make them unsuitable for traditional use. However, they contain a significant amount of organic matter that is capable of methanogenesis during biological processing.

Coal combustion remains a major source of greenhouse gas emissions: about 80% of electricity in Kazakhstan is generated by coal-fired power plants, and CO_2 emissions reached 240 million tonnes in 2022, a significant part of the country's carbon footprint. Biogenic methane production from coal, compared to conventional combustion, can reduce CO_2 emissions by 30-50%, as it collects methane from coal seams and makes the process of methane production environmentally friendly.

In addition, biogenic methane production can be an important mechanism for the reclamation of abandoned mines and coal waste. There are more than 200 abandoned mines in Kazakhstan. The biological reclamation process not only reduces environmental load but also creates an additional source of energy. The experience of the USA, China and Canada shows that if optimal methods are implemented, 5-10 m^3 of methane can be produced from each tonne of coal in 3-5 years.

Despite the high potential of biogenic methanogenesis, it remains poorly studied in Kazakhstan. While studies on the microbiological decomposition of coal have been actively conducted worldwide for the last 20 years, in Kazakhstan, this direction is represented only by single works. The lack of fundamental data on the composition and activity of methanogenic communities, as well as the influence of geochemical factors on the process of methane formation, complicates the industrial implementation of methane-based methanogenesis. The present study aimed to fill this gap and create a scientific basis for further development of proposed technology. Thus, studying the stimulation of biogenic methanogenesis in low-rank coal in Kazakhstan is a strategically important direction that will contribute to increasing the economic value of coal resources, reducing carbon emissions and sustainable production of alternative energy.

The aim of the work: Increase methane yield by using various methods of stimulation of biogenic conversion of low-rank coal in Kazakhstan to methane.

To achieve this aim, the following tasks were performed:

1. To collect coal samples from various coal basins of Kazakhstan and study their physicochemical and microbiological characteristics.

2. To form aerobic and anaerobic microbial communities selected from various sources potentially containing exogenous microorganisms and to analyze their taxonomic composition using metagenomic approaches.

3. To cultivate and adapt the isolated microbial communities using coal as the sole source of carbon and energy.

4. To create a microcom by introducing selected aerobic and anaerobic microbial communities into coal samples.

5. To determine the intermediate organic compounds involved in the fermentation process and to establish the biogeochemical indicators of coal methanogenesis.

6. To study the bioavailability of coal and analyze the intensity of methane generation from coal.

7. Assess the impact of key environmental factors on the efficiency of coal-to-methane conversion.

Objects of the research: The objects of the study were coal samples and their microbial communities from three regions of Kazakhstan: Oikaragai coal basin of Almaty region (43°12'36.6""N. 80°35'48.3"E), Ekibastuz coal basin of Pavlodar region (51°73'08.5"N, 75°40'38.7"E) and Karaganda coal basin of Karaganda region (49°52'48.3"N. 73°06'16.5"E). The microbial community of activated sludge from the wastewater treatment system of Bostandyk district of Almaty city (43.21'90.78"N, 76.91'89.25"E) and the microbial community of cow manure from a livestock farm near the city of Kaskelen of Karasai district of Almaty region (43.22'88.84"N, 76.63'62.11"E) were used as a source of exogenous microflora.

Methods of the research: The thermophysical properties of coal samples (moisture content - W, ash content - A, calorific value - Q, volatile matter yield - V) were characterized using GOST standards. The elemental composition (C, N, H, S) was determined on a Vario EL Cube automatic analyzer (Germany) based on ISO 14820-3:2020 standards - "Fertilizers and leaching agents".

Metagenomic study methods based on 16S rRNA gene sequencing were used to determine the taxonomic composition. The community-level physiological profiling (CLPP) method was also used.

Fluorescence excitation-emission matrix (EEM) methods in combination with the parallel factor model (PARAFAC) were used to determine intermediates. X-ray diffraction analysis of the samples was performed on an Empyrean X-ray Diffraction System diffractometer (Malvern Panalytical, the Netherlands) with a wavelength of λ = 1.54056 Å from the reflection pattern of a crushed sample placed in a 25 mm diameter cuvette. The microstructure of the coal samples was studied using FE-SEM microscopy on a Hitachi S-4800 scanning electron microscope (Hitachi, Japan). The Fourier transform infrared spectroscopy method was carried out using an ALPHA II

QuickSnap spectrometer (Bruker Optics GmbH, Germany). The IR spectrum of the coal samples was recorded in the range from 400 to 4000 cm-1.

Agilent 7890A gas chromatograph with Agilent 5975C mass spectrometer was used to determine gases according to ASTM 1945 and GPA 2261.

Biosolubilization and acclimatization methods was used.

Scientific novelty of the research: This study is the first in our country to investigate and stimulate the potential for biogenic methane formation from low-rank coal in Kazakhstan, and is therefore the first work in this direction.

Cluster analysis of the microbial communities in coal basins and exogenous microbial sources (activated sludge and cattle manure) using the Majorbio Cloud platform was performed.

The microbial communities in the activated sludge were successfully adapted, and the isolated microorganisms were identified and registered in the GenBank database of the National Centre for Biotechnology Information (NCBI).

A microsystem for the optimal bioconversion of coal to methane was developed and its parameters were selected to achieve the maximum methane yield.

A method for increasing the bioavailability of coal was developed, on the basis of which a utility model patent was obtained (Method for dissolving low-rank coal, No. 10021).

Scientific and practical significance: The practical significance of the research lies in increasing energy efficiency by stimulating the formation of biogenic methane from low-rank coal. In the conditions of decreasing natural gas reserves, the microbiological transformation of coal is a promising direction of its processing. Biogenic methane can be used as an environmentally friendly fuel without complicated technological processes. In addition, the organization of microbiological gasification in the coal-mining regions of Kazakhstan is expected to create new jobs and improve the economic situation in these regions.

Provisions for defense:

1. The physicochemical and microbiological characteristics of coal samples from 3 different coal basins in Kazakhstan served as the basis for their classification as brown coal (type B). Their moisture, ash, volatile matter, carbon and sulfur contents were studied and found to be typical for brown coal. In addition, the presence of 8 main phylum of bacteria that predominated in the coal samples was recorded.

2. The possibility of using activated sludge as an effective inoculum for coal bioaugmentation is substantiated, since it contains a highly active community of methanogenic archaea capable of adaptation and functioning under conditions simulating the process of anaerobic fermentation with coal.

3. The feasibility of introducing the method of acclimatization of aerobic and anaerobic microbial communities for the effective implementation of the coal biogasification process has been determined.

4. The relationship between the physicochemical characteristics of coal and its methanogenic potential has been deduced: it has been established that coal from the Oiqaragay coal basin, under optimal environmental parameters (35°C, pH 7.0–

8.0, salt content 4.0 mg/cm³), demonstrates maximum structural transformations and methane yield up to 621 μmol/g.

The connection of the dissertation work with scientific research: The dissertation was carried out within the framework of the project IRN AP09057876 "Investigation of the potential for biogenic conversion of low-rank coals of Kazakhstan into methane."

The personal contribution of the dissertation candidate to the creation of the results of the scientific work proposed for defense: All stages of the research, including the analysis of literature data, formulation of research objectives and tasks, execution of experimental studies, processing and interpretation of the obtained results, as well as their statistical analysis, were conducted with the direct involvement of the author. Furthermore, the methods used in the study and the obtained data were thoroughly verified for reliability and scientific validity.

Approbation, approval of the results of the work: The results of the dissertation were presented at the following international scientific conferences:

- International scientific conference of students and young scientists "Farabi alemi", April 4-6, 2024, Almaty.
- International conference "Sustainable future-2024: Biotech, ecology, energy", November 20-21, 2024, Almaty.

Publications: The main provisions and results of the thesis research were presented at international scientific journals and conferences and published in 8 scientific papers, including 3 articles in journals indexed by Scopus: Scientific Reports (92%, SJR 0.9), Engineered Science (91%, SJR 0.87) and International Journal of Hydrogen Energy (93%, SJR 1.318). Also 1 article was published in a republican scientific journal recommended by the Committee for Control in the Sphere of Education and Science of the Republic of Kazakhstan, and 2 abstracts in conference proceedings. On the basis of the obtained results 1 monograph was published and patent was obtained from the Patent office of the Republic of Kazakhstan.

Structure of the dissertation work: The dissertation consists of 109 pages and includes a list of abbreviations, an introduction, a literature review, description of research objects, materials and methods, research results and their discussion, conclusion, list of used sources, 16 tables and 39 figures.