ABSTRACT

of the dissertation for the degree of Doctor of Philosophy (PhD) on the specialty "8D05105 – Biotechnology"

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on the thesis: "**Enhanced oil recovery based on indigenous microorganisms from oil fields in Western Kazakhstan**"

General description of the work. The dissertation is dedicated to the study of the microbial diversity of formation water samples from oil fields in Western Kazakhstan and the screening of biosurfactant and biopolymer producing microorganisms isolated from these samples. Microbiological (morphological, cultural, physiological, and biochemical) and molecular genetic characteristics of the microbial strains were studied to determine their phylogenetic affiliation. Chemical analyses were conducted to investigate the properties of the biosurfactant and biopolymer producing microorganisms, and laboratory evaluations of the oil recovery efficiency of the promising strains were performed.

Relevance of the research topic. The oil and gas industry holds one of the key positions in Kazakhstan's economy. During the development of oil fields using traditional methods and under high water cut conditions, it is estimated that between 25% and 70% of the oil reserves remain trapped underground. Most of the country's oil fields are characterized by low reservoir permeability, high oil viscosity, and complex geological structures, classifying them as fields with hard-to-recover reserves.

In this context, against the backdrop of intensive oil production, the issue of enhancing oil recovery from reservoirs has become particularly relevant. Since the 1960s, pilot projects aimed at improving oil recovery have been implemented in Kazakhstan. Currently, both thermal and non-thermal enhanced oil recovery (EOR) methods are being applied.

Microbial enhanced oil recovery (MEOR) represents an attractive alternative approach in oil production. However, for the practical application of MEOR technologies under field conditions, there is still a lack of fundamental research in this area. While this method has been successfully employed abroad, oil composition and operational conditions vary significantly across regions. Therefore, research involving the application of MEOR technology is necessary for Kazakhstan's oil fields, the majority of which are at a mature stage of development.

MEOR technology employs microorganisms and their metabolites, among which biosurfactants and biopolymers are of particular interest. Biosurfactants contribute to enhanced oil recovery through mechanisms such as reducing interfacial and surface tension between oil and water, forming oil-in-water emulsions, and altering the wettability of rock surfaces from oil-wet to water-wet. Biopolymers can increase the viscosity of the displacing fluid, improve sweep efficiency, and selectively block high-permeability zones, promoting more efficient displacement of residual oil from low-permeability areas.

Moreover, biosurfactants and biopolymers offer several advantages over synthetic surfactants: they are less toxic, biodegradable, resistant to extreme

conditions (pH, temperature, salinity), and potentially more cost-effective, as they can be produced from renewable resources or industrial waste.

The aim of the work: Characterization of the microbial diversity of formation waters from the Akingen and East Makat oil fields and isolation of promising biosurfactant and biopolymer producing microorganisms for enhancing oil recovery.

To achieve this aim, the following tasks were solved:

1. Conduct physicochemical analysis of formation waters from oil fields in Western Kazakhstan;
2. Determine the microbial diversity of formation waters from oil fields in Western Kazakhstan;
3. Screen and identify biosurfactant producing microorganisms isolated from formation waters;
4. Detect genes encoding the synthesis of biosurfactants (*srfAA*, *srfAB*, *srfAC*, *srfAD*, *srfp*, *lchAA*) and the biopolymer (*sacB*) in the selected microbial strains;
5. Study the structural characteristics of biosurfactants and biopolymers produced by the selected microbial strains;
6. Determine the oil recovery efficiency based on biosurfactant and biopolymer producing microorganisms isolated from oil fields in Western Kazakhstan.

The object of study:

The objects of the study were formation water samples collected from oil fields in Western Kazakhstan for the investigation of microbial diversity, as well as pure cultures of microorganisms isolated from these samples.

Research methods:

Modern methods were applied in the dissertation work, including metagenomic analysis using the *Illumina MiSeq* platform, classical microbiological and physicochemical methods, molecular genetic methods (sequencing of the 16S rRNA gene fragment, detection of genes by PCR, gene expression analysis by qPCR), as well as tensiometer and FT-IR spectroscopy.

Scientific novelty of the research:

For the first time, a comprehensive characterization of the microbial diversity of formation waters from the Akingen and East Makat oil fields was carried out. Structural features of the microbial communities characteristic of each oil field were identified. At the class level, the dominant groups in the formation waters of the Akingen field were *Alphaproteobacteria*, *Actinobacteria*, *Clostridia*, *Gammaproteobacteria* and *Bacilli*. In the formation waters of the East Makat field, the dominant classes were *Gammaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Alphaproteobacteria*, *Actinobacteria*, *Clostridia* and *Methanomicrobia*.

For the first time, 33 microbial strains were isolated from the Akingen and East Makat fields. A qualitative and quantitative screening of these strains for biosurfactant production was performed. Six biosurfactant-producing strains were selected and identified as *Bacillus safensis* A2, *Bacillus subtilis* A8, A9 and A12, *Bacillus paralicheniformis* R4 and *Bacillus licheniformis* PW2. The presence and

expression of genes responsible for the synthesis of biosurfactants and biopolymers were confirmed in the selected strains:

- *srfAA* gene – detected in four strains: *Bacillus subtilis* (A8, A9, A12) and *Bacillus licheniformis* PW2;
- *srfAB* gene – detected in two strains: *Bacillus subtilis* (A8, A9);
- *srfAC* gene – detected in three strains: *Bacillus subtilis* (A8, A9, A12);
- *srfAD* gene – detected in three strains: *Bacillus subtilis* (A8, A9, A12);
- *srfp* gene – detected in four strains: *Bacillus safensis* A2 and *Bacillus subtilis* (A8, A9);
- *lchAA* gene – detected in *Bacillus licheniformis* PW2;
- *sacB* gene – detected in four strains: *Bacillus subtilis* (A8, A9, A12) and *Bacillus licheniformis* PW2.

The maximum biosurfactant yield was achieved by *B. subtilis* A8 (1.33 g/L) when cultivated on a medium using molasses as the carbon source. The maximum biopolymer yield was observed in *B. subtilis* A12 (0.42 g/L) when sucrose and soybean meal were used in the nutrient medium.

For the first time, laboratory evaluations using sand, natural core samples, formation water, and microbial consortia established oil recovery efficiencies of 23.2 ± 2.5% for the *B. subtilis* A9 strain and 24.4 ± 3.1% for the *B. subtilis* A8 : *B. subtilis* A9 consortium, significantly exceeding the control value of 4% observed without the application of microorganisms.

Provisions submitted for protection:

Biosurfactant producing strains were isolated and identified and registered in the international *GenBank* database: *Bacillus safensis subsp. safensis* A2 (OP565012), *Bacillus subtilis* A8 (OP565013), *Bacillus subtilis* A9 (OP565014), *Bacillus subtilis subsp. subtilis* A12 (OP565015), *Bacillus paralicheniformis* R4 (OP565016), and *Bacillus licheniformis* PW2 (OP565017).

All microbial strains were deposited in the LLP "Republican Collection of Microorganisms" (RCM) under the following registration numbers: №B-RKM 1109 *Bacillus safensis subsp. safensis* A2, №B-RKM 1110 *Bacillus subtilis* A8, №B-RKM 1111 *Bacillus subtilis* A9, №B-RKM 1112 *Bacillus paralicheniformis* R4, №B-RKM 1113 *Bacillus subtilis subsp. subtilis* A12 and №B-RKM 1114 *Bacillus licheniformis* PW2, as of December 29, 2023.

It was established that in model oil displacement experiments, the use of *B. subtilis* A9 achieved an oil recovery factor of 23.2 ± 2.5%, while the consortium of *B. subtilis* A8 : *B. subtilis* A9 achieved 24.4 ± 3.1%, which is significantly higher compared to the control samples, where oil recovery did not exceed 4%. This corresponds to an absolute increase of 19.2 and 20.4 percentage points, respectively.

The patent of the Republic of Kazakhstan has been obtained for the utility model titled "Biosurfactant producing strain *Bacillus subtilis* A9 for microbial enhanced oil recovery" №9060, dated February 2, 2024.

Provisions for defense:

1. The microbial diversity of the formation waters from the Akingen and East Makat oil fields was represented by *Alphaproteobacteria, Actinobacteria, Clostridia, Gammaproteobacteria,* and *Bacilli,* as well as

Gammaproteobacteria, *Deltaproteobacteria*, *Epsilonproteobacteria* and *Methanomicrobia*.

2. Biosurfactant producing strains were identified and registered in the *GenBank* database: *Bacillus safensis subsp. safensis* A2 (OP565012), *Bacillus subtilis* A8 (OP565013), *Bacillus subtilis* A9 (OP565014), *Bacillus subtilis subsp. subtilis* A12 (OP565015), *Bacillus paralicheniformis* R4 (OP565016) and *Bacillus licheniformis* PW2 (OP565017).
3. The maximum biosurfactant yield by *B. subtilis* A8 was achieved when grown on molasses as a carbon source, amounting to 1.33 g/L, compared to 0.1 g/L when using whey.
4. High oil recovery factors were obtained using *B. subtilis* A9 ($23.2 \pm 2.5\%$) and the consortium of *B. subtilis* A8 and *B. subtilis* A9 ($24.4 \pm 3.1\%$), which exceeded the control value (4%) by 19.2 and 20.4 percentage points, respectively.

Personal contribution of the dissertation candidate to the creation of the results of the scientific work proposed for defense:

The analysis of the literature on the research topic, formulation of the research aim and objectives, conducting of experimental work, statistical processing and analysis of the obtained results, writing of scientific articles and the dissertation text, and preparation of the manuscript were carried out independently by the author.

The connection of the dissertation work with scientific research:

The research was conducted within project AP19577160 “Study of biosurfactant-producing microorganisms and their applicability for enhanced oil recovery” (Project No. 0123RK00131, 2023–2025).

Approbation, approval of the results of the work and personal contribution of the author:

The results of the dissertation research were presented and discussed at the following international scientific conferences:

- VI International Scientific Conference of Students and Young Scientists "Farabi World", 2021, Almaty, Kazakhstan;
- VI International Scientific Conference of Students and Young Scientists "Farabi World", 2022, Almaty, Kazakhstan;
- XII International Scientific and Practical Conference "Modern Trends in the Development of Science and the Global Community in the Era of Digitalization", 2023, Moscow, Russia;
- VI International Scientific Conference of Students and Young Scientists "Farabi World", 2023, Almaty, Kazakhstan;
- International Forum "Modern Trends in the Sustainable Development of Biological Sciences", 2024, Almaty, Kazakhstan;
- ICASET-2024 International Conference on Advances in Science, Engineering and Technology, August 23–24, 2024, Hanoi, Vietnam.

Publications:

The main results of the dissertation have been published in 12 scientific works, including: 3 articles in national peer-reviewed journals recommended by the Committee for Quality Assurance in the Field of Science and Higher Education; 3

articles in journals indexed in international databases Scopus and Web of Science: Scientific Reports (92nd percentile, Q1), Frontiers in Microbiology (73rd percentile, Q2), and Journal of Environmental Management and Tourism; 4 abstracts in the proceedings of international conferences held in Kazakhstan; and 2 articles in the proceedings of international conferences held abroad and in Kazakhstan.

Structure of the dissertation:

The dissertation consists of 117 pages, including normative references, definitions, abbreviations, introduction, literature review, materials and methods, results and discussion, conclusion, and references (210 sources). It contains 26 figures, 15 tables, and 5 appendices.