ANNOTATION

dissertations for the degree of Doctor of Philosophy (PhD) in the specialty 8D07108 – "Basic processes of synthesis and production of new organic and polymeric materials"

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BIODESULFURIZATION OF HIGH-SULFUR OIL USING DEEP EUTECTIC SOLVENTS

Relevance. The relevance of developing new sulfur removal technologies for fuels is driven by increasingly stringent environmental regulations. In the EU and Japan, the sulfur content in gasoline and diesel fuel must not exceed 10 ppm, while in the United States the limit is 15 ppm. At the same time, some crude oils produced in Kazakhstan contain as much as 1.6-2.2 wt.% sulfur, which is several orders of magnitude higher than the established standards. This reduces the competitiveness of Kazakhstani crude oil on the international market and limits the country's export potential. The high content of sulfur-containing compounds in Kazakhstan's heavy oils deteriorates fuel quality, causes equipment corrosion, and leads to SO_2 emissions, thereby negatively affecting the environment. Existing industrial technologies provide effective removal of light sulfur forms; however, they require substantial costs, including high temperatures (300 to 400 °C) and pressures (2-8 MPa), the use of hydrogen and expensive catalysts, excess oxidants, as well as product separation stages, regeneration, and solvent loss control.

These factors reduce their economic feasibility for the processing of heavy oils. In this regard, the development of alternative, more environmentally friendly and economically justified desulfurization methods capable of selectively removing sulfur with minimal destruction of the hydrocarbon matrix is of particular importance. One of the most promising approaches is biodesulfurization, which is based on the ability of microorganisms, under optimal conditions, to selectively oxidize sulfur present in organic compounds. In this work, it is proposed to use a technology for the biodesulfurization of crude oil and petroleum products in combination with deep eutectic solvents (DESs) incorporated into nutrient media, together with an optimal operating regime, in order to stimulate the growth of sulfur-oxidizing microorganisms and increase the bioavailability of organosulfur compounds. This approach combines environmental safety, technological efficiency, and the possibility of integrating the developed technology into existing oil refining schemes, which determines its high relevance for solving the problem of deep desulfurization of high-sulfur oils in Kazakhstan and aligns with Sustainable Development Goal 7 – "Affordable and Clean Energy."

Research objective. Development and substantiation of a chemicaltechnological approach to the biodesulfurization of Kazakhstan's heavy high-sulfur crude oils based on synthesized deep eutectic solvents and their

incorporation into desulfurization systems for the intensification and optimization of the biodesulfurization process.

Research Tasks:

1. Synthesis and characterization of deep eutectic solvents, as well as evaluation of their effects on the growth and metabolic activity of isolated and collection microorganisms during the biodesulfurization of heavy high-sulfur oil.

2. Isolation and study of microbial strains from crude high-sulfur oil samples of Kazakhstan, and identification of their cultural and technological properties through aerobic processes.

3. Investigation of the desulfurization activity of microorganisms in the treatment of high-sulfur oil and determination of their role in the oxidation and reduction processes of sulfur-containing compounds.

4. Evaluation of the efficiency of oil desulfurization through the combined application of deep eutectic solvents and microorganisms.

Within the framework of this study, deep eutectic solvents are considered not only as auxiliary reagents but also as key elements of the methodology, providing a dual effect:

– stimulation of the growth and metabolic activity of isolated and collection microorganisms by modifying the conditions of the desulfurization medium;

– enhancement of the availability of organosulfur compounds in the oil matrix for biocatalytic degradation.

Such a combined approach enables the integration of chemical and biotechnological advantages, facilitating the development of more efficient and environmentally safe technologies for the desulfurization of heavy high-sulfur oils.

Research methods.

The research was based on the application of the following methods:

– gas chromatography with a flame-ionization detector (GC-MS with FID Agilent 7890B) for qualitative and quantitative analysis of sulfur-containing compounds in crude oil samples;

– microbiological isolation and cultivation techniques for obtaining strains of sulfur-desulfurizing microorganisms from crude oil samples and their identification using morphological, cultural, and biochemical characteristics;

– spectrophotometric analysis (Jenway 7315), based on measuring optical density (D_{opt}) at 600 nm, to assess the influence of heavy metals on the growth of isolated bacterial cultures;

– genetic identification of microorganisms through analysis of the nucleotide sequence of the 16S rRNA gene fragment;

– synthesis of deep eutectic solvents (DES) to obtain target compositions at defined molar ratios;

– experimental biodesulfurization assays to evaluate the efficiency of sulfur removal under laboratory conditions and to optimize the technological parameters of the process;

– energy-dispersive X-ray fluorescence (EDXRF) analysis for determining the mass fraction of sulfur in the DES-based biodesulfurization system using the “Spectroscan S” energy-dispersive X-ray fluorescence analyzer.

The following statements are submitted for defense:

1. The composition of the obtained deep eutectic solvents based on betaine:glycerol (Bet+Gly) (1:2), citric acid:glycerol (CA+Gly) (1:4), tetrabutylammonium chloride:lactic acid (TBAC+La) (1:2), choline chloride:ethylene glycol (ChCl+EG) (1:2), and tetrabutylammonium bromide:isopropanol (TBAB+isopropanol) (1:2);

2. Results of studying oil colonization by hydrocarbon- and sulfur-oxidizing microorganisms, determination of their cultural and technological properties, genetic identification of active microbial strains based on nucleotide sequence analysis of the 16S rRNA gene fragment, and spectrophotometric analysis (λ = 600 nm) of the effect of heavy metals on the growth of isolated microorganisms;

3. The use of deep eutectic solvents based on betaine:glycerol (Bet+Gly) (1:2) and citric acid:glycerol (CA+Gly) (1:4) in combination with indigenous microbial strains *B. cereus* (SFN-2) and *B. thuringiensis* (SFN-3) for oil desulfurization (up to 82.35%), whereas strains SFN-1, H-1, H-4, F-1, F-2, E-1, and H-2 demonstrated sulfur-reducing capabilities;

4. Results of applying a composition of deep eutectic solvents based on betaine:glycerol (Bet+Gly) (1:2) and citric acid:glycerol (CA+Gly) (1:4) in combination with indigenous sulfur-oxidizing microorganisms *B. cereus* (SFN-2), *B. thuringiensis* (SFN-3), and collection microorganisms *P. aeruginosa* B-5807, *Rh. erythropolis* AC-1039, *P. putida* B-1827 in the biodesulfurization of oil (sulfur removal up to 96.3%).

Scientific novelty of the obtained results.

For the first time:

– the possibility of using deep eutectic solvents as components of desulfurization nutrient systems for isolated and collection microorganisms has been established;

– the intensification of biodesulfurization processes of heavy high-sulfur crude oils has been achieved through the application of deep eutectic solvents within desulfurization systems.

For the first time, it has been shown that the introduction of DES into the nutrient medium ensures:

– the formation of a desulfurization environment;

– a significant increase in the growth rate and metabolic activity of oildegrading and sulfur-desulfurizing microorganisms due to the optimization of the physicochemical conditions of their cultivation.

Based on comprehensive studies of crude oils from the Karazhanbas field, it has been revealed for the first time that the use of DES promotes an increase in biomass, enhances the activity of enzyme systems involved in desulfurization, and broadens the spectrum of organosulfur compounds subjected to biocatalytic degradation. The results obtained confirm that the developed approach holds high potential for the creation of environmentally safe and energy-efficient technologies for deep desulfurization of crude oil.

Practical significance of the work.

The biodesulfurization method proposed in this work, using deep eutectic solvents, opens new opportunities for improving the efficiency of high-sulfur crude oil purification processes. The addition of DES does not alter the oil matrix itself but creates optimal conditions for the growth and activity of sulfur-desulfurizing microorganisms, which ultimately facilitates their access to organosulfur compounds and accelerates their degradation.

This approach allows:

– reducing energy consumption;

– avoiding the use of aggressive reagents, thereby minimizing negative environmental impacts;

– maintaining a high degree of removal of organosulfur compounds from crude oils.

The obtained results can be applied at the macro level for sulfur removal based on the developed chemical-biotechnological desulfurization method for high-sulfur heavy crude oils.

Author's personal contribution, publications, and testing of practical research results.

The author's personal contribution consists of analyzing and critically evaluating scientific literature, conducting experimental studies, applying physico-chemical and biotechnological analytical methods, as well as systematizing and interpreting the obtained results, followed by the formulation of substantiated scientific conclusions.

Approbation of the work: The research results were presented and validated at international and national scientific forums: **Frontier Symposium of Engineered Science**, June 23-29, 2024, Astana, Kazakhstan. Biodesulfurization of high-sulfur oil from the Karazhanbas field by Deep Eutectic Solvents. Authors: **Akimbek A.O.,** Jamalova G.A., Rafikova Kh.S., Yernazarova A.K., Yelikbayev B.K., Kaiyrmanova G.K., Pagano M.C., Islam S., Nauryzova S., Kerimkulova A., Selenova B. (poster).

Publications:

1. **Akimbek A.O.,** Jamalova G.A., Yernazarova A.K., Kaiyrmanova G.K., Yelikbayev B.K., Pagano M.C., Zazybin A.G., Rafikova K.S. Biodesulfurization of high-sulfur oil from the Karazhanbas field of Kazakhstan with deep eutectic solvents. Heliyon, 2025, Volume 11, Issue 2, 30 January 2025, e41877. DOI: 10.1016/j.heliyon.2025.e41877. **(Scopus, WoS).**

2. In the journal CiteScore (Scopus, 2025), the quartile of the journal is Q2, the percentile is 73 %. Uzcan F., Joldybayeva S., **Akimbek A.,** et al. A very simple and sensitive pelargonic acid based liquid phase microextraction of erythrosine from food and water samples. Environmental Monitoring and Assessment, 197, 569 (2025). DOI: 10.1007/s10661-025-13954-2. **(Scopus, WoS).**

3. In the journal ChemistrySelect, the quartile of the journal is Q3, the percentile is 45 %. Uğur Işik, Khadichakhan Rafikova, Nermin Meriç, Remziye Güzel, Aygul Kerimkulova, **Arailym Akimbek**, Veysi Okumuş, Feyyaz Durap, Cezmi Kayan, Murat Aydemir. Half-sandwich ruthenium (II) and iridium (III) complexes of imidazole based phosphinite ligands: antioxidant and antibacterial

activities as well as electrochemical properties. ChemistrySelect, Volume 9, Issue 6, February 12, 2024, e202304785. DOI: 10.1002/slct.202304785. **(Scopus, WoS).**

The structure and scope of the dissertation. The dissertation work includes an introduction (9 pages), 3 chapters (158 pages), conclusion (3 pages) and a bibliographic index of literature, including 507 titles (37 pages). The total volume is 208 pages of computer text, which contains 38 tables and 70 figures.