

УДК 504.53.054  
МРПТИ 34.39.15  
DOI 10.56525/KOPX8394

## ADSORPTION IMMOBILIZATION OF MICROORGANISM CELLS ON MINERAL CARRIERS

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**Abstract.** This paper presents the results of laboratory work to determine the effectiveness of adsorbing immobilization of natural minerals as carriers of destructor microorganisms. A consortium of microorganisms of *Dietzia maris* strains was immobilized on the minerals haydite and zeolite. The problem of eliminating oil pollution from soil surfaces has important economic and environmental significance due to increased emergency situations in hydrocarbon extraction areas. The controlled biological method of bioremediation using immobilized microorganisms offers a cost-effective solution for removing petroleum products to background levels. The experimental methodology involved preparing mineral carriers (zeolite and haydite), cultivating a consortium of *Dietzia maris* strains (U2.1, U2.6, MB3, KU1), and measuring adsorption capacity through spectrophotometric analysis. According to the calculated results, zeolite demonstrated superior adsorption capacity with 19.69 mg of dry cells per gram of carrier, while haydite showed 17.92 mg per gram. The effectiveness of adsorption depends on properties of the microorganism, sorbent characteristics, and environmental conditions. This experiment demonstrated that zeolite sorbent enables high efficiency in biological remediation of hydrocarbons, making it an effective biocatalyst for petroleum hydrocarbon bioremediation processes. Natural minerals available in Kazakhstan, particularly zeolite, represent promising and economically viable carriers for immobilized microorganisms in oil-contaminated soil remediation.

**Keywords:** adsorption; immobilization; microorganisms; *Dietzia maris*; zeolite; haydite; bioremediation; oil pollution; mineral carriers.

### Introduction

The problem of eliminating oil pollution from the soil surface currently has important economic and environmental significance due to increased emergency situations, especially in hydrocarbon extraction areas. In Kazakhstan, where the oil and gas industry plays a crucial role in the national economy, addressing petroleum contamination of soils is particularly urgent. The increasing frequency of oil spills and accidental releases during extraction, transportation, and storage operations necessitates development of effective, economically feasible remediation technologies.

Currently, among companies engaged in oil waste processing, the most popular method is the controlled biological method of bioremediation, which is based on microorganisms capable of using petroleum hydrocarbons as a source of nutrition. The use of microorganisms immobilized on various carriers makes it possible to remove oil and petroleum products to background levels with low operating costs and simplicity of solutions. Immobilization technology offers several advantages over traditional bioremediation approaches, including enhanced microbial stability, increased degradation rates, and ability to reuse biocatalysts [1].

Various methods are known for cleaning soil from oil and petroleum products, including the use of various sorbents of artificial and natural origin. Artificial sorbents include polyurethane, carbamide and phenolic resins, which often have high production costs and limited biodegradability. Natural sorbents include peat, ash, coal, zeolite, vermiculite, shungite rocks, haydite, and other materials. Natural sorbents offer advantages of environmental compatibility, abundance, and cost-effectiveness [2].

Recently, immobilization methods have been increasingly used in bioremediation processes. Immobilization is the limitation of mobility, fixation on a carrier while maintaining cell viability and catalytic functions. The immobilization process significantly reduces the costs of bioremediation processes and increases the efficiency of biotreatment. Immobilized cells demonstrate greater resistance to environmental stresses, including temperature fluctuations, pH changes, and presence of toxic compounds, compared to free-living cells [3].

The process of attaching microorganisms to a solid surface is denoted by the term "adsorption of microorganisms." This approach to studying the interaction of microbial cells with solid surfaces is very promising and gives good results, since the adsorption method is one of the simplest and most accessible methods for immobilizing microbial cells. One of the promising sorbents for immobilization are natural minerals – zeolites and haydite, which have mechanical strength and sufficient substrate permeability [4, 5]. It is also important that deposits of these minerals are located on the territory of Kazakhstan, making them readily available and economically attractive for large-scale applications. However, currently insufficient attention is paid to the use of these minerals for microbial cell immobilization in Kazakhstan's environmental remediation sector.

Based on the above, the purpose of this work is to study the sorption capacity of natural minerals zeolite and haydite in relation to cells of *Dietzia maris* strains, which are known petroleum hydrocarbon degraders.

**Materials and Methods of Research.** **Microorganism Strains.** The object for the experiment is a consortium of strains *Dietzia maris* U2.1, *Dietzia* sp U2.6, *Dietzia* sp MB3, *Dietzia maris* KU1, cultivated in dry nutrient medium and on nutrient agar. These strains were selected based on their demonstrated ability to degrade petroleum hydrocarbons and their suitability for immobilization applications.

**Mineral Carriers.** The sorbents taken for the experiment were characterized by the following physical, chemical and biological properties:

*Zeolite (clinoptilolite)* – a mineral whose crystals are permeated with a system of channels or cavities, have a well-developed internal surface. This structure allows zeolites to selectively sorb molecules, for example, halogenated halogens, components of waste and drinking water, that is, to play the role of molecular sieves. In the dehydrated state, these minerals sorb ammonium, alcohol, nitrates and other substances. The sizes of the channels are sufficient for organic molecules and cations to penetrate into them, and their total volume together with pores reaches 50%. Properties of zeolites include abundance, accessibility, low cost, and the possibility of repeated use. The ion exchange capacity of zeolites is one of the main parameters characterizing their sorption and technological properties. The maximum ion exchange capacity corresponds to the complete replacement of one ion by another in all crystalline positions, which corresponds to the maximum sorption capacity of zeolite.

*Haydite* – produced from clay by high-temperature firing conducted at specialized enterprises. The outer surface of clay conglomerates is fused, which ensures its smoothness and specific coloration. The formation of a porous structure occurs due to gases released during firing. Gravel and crushed stone have sizes from 5 to 40 millimeters, and haydite sand consists of particles less than 5 millimeters. Small crushed fractions of haydite are used in water purification (filtration) systems, as well as bedding in terrariums and aquariums. Such use is one of the evidence of low toxic qualities, allowing haydite to receive high marks for environmental friendliness [6].

**Experimental Methodology. Preparation of Carriers.** Sorbents (zeolite, haydite) were weighed out in 1 gram portions and placed in 250 ml flasks. After adding 100 ml of distilled water, the mixture was sterilized for 30 minutes at 1 atm. After the sterilization procedure, drying was carried out in a drying cabinet at 105°C until constant weight.

**Preparation of Microbial Suspension.** The microorganism consortium was centrifuged 3 times at a speed of 4500 rpm for 5 minutes to concentrate the cells and remove culture medium. The remaining liquid after centrifugation was poured into a sterile flask, and 3 ml of potassium phosphate buffer solution and 200 ml of acrylamide (1M solution) were added to stabilize the suspension.

**Spectrophotometric Analysis.** The density of the microorganism suspensions was measured on a UNICO spectrophotometer using cuvettes 0.5 cm thick at a wavelength of 540 nm. Buffer solution was introduced into one cuvette as a control, and 0.5 ml of prepared suspension and 3.5 ml of buffer solution were introduced into another. Results for two separately prepared suspensions:  $d_{\text{initial}} = 1.085$ ;  $d_2 = 0.431$ ;  $d_{\text{buffer}} = 0$ .

**Determination of Dry Cell Mass.** The next stage for determining the number of dry cells in one milliliter of suspension was to weigh three Eppendorf tubes on analytical scales; the average value of their mass was  $m = 1.1953$  g. Then 2 ml of suspension was introduced into the tubes and centrifuged at 10,000 rpm. Under the force of gravity, the cells settled. The tubes were dried in a thermostat at 30°C to constant mass. The mass of the tube with sediment was also determined on analytical scales. The mass was 1.1968 g; optical density  $d_2$  at 0.431 opt. units corresponds to 0.41 mg of dry cells in 1 ml of suspension [7].

**Adsorption Process.** The adsorption process was carried out for 25 minutes on 10 ml of suspension with the studied adsorbents (zeolite, haydite) by mixing on a shaker at 100 rpm. After mixing, the sorbent was separated using a fast-acting filter (red ribbon). The optical density of the filtered liquid  $d_{\text{filter}}$  was determined. After adding potassium phosphate buffer, the solution was filtered again and the optical density  $d_{\text{wash}}$  was also determined.

**Calculation of Adsorption Capacity.** Cell adsorption on the carrier was determined using the formula [8]:

$$X = [(d_{\text{sorb}} \cdot 0.41) / d_2] \cdot 10 \quad (1)$$

$$d_{\text{sorb}} = d_{\text{initial}} - (d_{\text{filter}} + d_{\text{wash}}) \quad (2)$$

Where:  $d_{\text{sorb}}$  - is the optical density of the suspension (when conducting experiments with zeolite, haydite);  $d_2$  - is the optical density of the initial suspension (opt. units);  $d_{\text{initial}}$  is the optical density, 1.085 opt. units;  $d_{\text{filter}}$  is the optical density of the filtered suspension (opt. units);  $d_{\text{wash}}$  is the optical density after the second wash (opt. units).

**Research Results.** The experimental results of adsorption of the microorganism consortium on mineral carriers are presented in Table 1.

Table 1 – Data on adsorption of microorganism consortium on mineral carriers

Sorbent type	$d_{\text{filter}}$	$d_{\text{wash}}$	$d_{\text{initial}}$	$d_{\text{sorb}}$	Cell concentration X, mg/ml	Adsorption on carrier, mg/g
Zeolite	0.036	0.014	1.085	1.035	9.5603	19.6914
Haydite	0.124	0.019	1.085	0.942	8.961	17.922

The data presents results for adsorption of cells of a microorganism consortium consisting of strains *Dietzia maris U2.1*, *Dietzia sp U2.6*, *Dietzia sp MB3*, *Dietzia maris KU1*, immobilized on mineral carriers zeolite and haydite.

According to the experimental data, zeolite demonstrated superior adsorption capacity compared to haydite. The zeolite sorbent showed adsorption capacity of 19.69 mg of dry cells per gram of carrier, while haydite exhibited 17.92 mg per gram. This represents approximately 10% higher adsorption capacity for zeolite compared to haydite.

The higher adsorption capacity of zeolite can be attributed to its unique structural properties. The well-developed internal pore system of clinoptilolite provides greater surface area available for microbial cell attachment. The ion exchange properties of zeolite may also facilitate stronger interactions between negatively charged bacterial cell surfaces and the mineral carrier. Additionally, the channel dimensions of zeolite are particularly suitable for accommodating bacterial cells while maintaining their viability and metabolic activity.

Haydite, despite showing slightly lower adsorption values, still demonstrated good capacity for cell immobilization. The porous structure of haydite, formed during high-temperature firing, provides adequate sites for bacterial attachment. The mechanical strength of haydite offers advantages for applications requiring carrier durability and resistance to physical stress.

Both mineral carriers showed optical density values that confirm successful microbial adsorption. The relatively low values of  $d_{\text{filter}}$  and  $d_{\text{wash}}$  indicate that most cells were successfully immobilized on the carriers rather than remaining in suspension or being lost during washing procedures.

**Conclusion.** Based on the results of comparative experiments, it can be stated that zeolite showed superior adsorption performance as a carrier for immobilized microorganisms. The zeolite sorbent demonstrated adsorption capacity of 19 mg of dry cells per 1 gram of carrier, while haydite showed 17 mg of cells per 1 gram of carrier, respectively. This difference, while modest, may become significant in large-scale bioremediation applications.

The effectiveness of adsorption depends on several key factors:

- properties of the microorganism (cell surface characteristics, size, metabolic state);
- properties of the adsorbent (surface area, pore size distribution, surface chemistry, ion exchange capacity);
- chemical and physical properties of the medium in which adsorption occurs (pH, ionic strength, temperature, presence of competing substances);
- conditions determining the possibility of contact between bacterial cells and adsorbent particles (mixing intensity, contact time, cell concentration).

This experiment demonstrated that the use of zeolite sorbent will allow achieving high results in the biological remediation of petroleum hydrocarbons. Based on *Dietzia maris* cells immobilized on natural zeolite, an effective biocatalyst for petroleum hydrocarbon bioremediation processes was obtained. The availability of zeolite deposits in Kazakhstan makes this approach particularly attractive for practical implementation in the country's oil and gas sector.

Future research should focus on evaluating the long-term stability and reusability of zeolite-immobilized consortia under field conditions, optimizing immobilization protocols for maximum cell viability and degradation activity, and conducting pilot-scale studies on actual oil-contaminated soils in Kazakhstan. Additionally, investigation of zeolite pretreatment methods that could further enhance adsorption capacity would be valuable for improving the technology.

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## МИНЕРАЛДЫ ТАСЫМАЛДАҒЫШТАРДА МИКРООРГАНИЗМ ЖАСУШАЛАРЫНЫҢ АДСОРБЦИЯЛЫҚ ИММОБИЛИЗАЦИЯСЫ

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**Аннотация.** Бұл жұмыста табиғи минералдардың адсорбциялық иммобилизациясының тиімділігін анықтау бойынша зертханалық жұмыстардың нәтижелері келтірілген. *Dietzia maris* штамдарынан құралған микроорганизмдер консорциумы керамзит және цеолит минералдарына иммобилизацияланды. Топырақ бетінен мұнай ластануын жою мәселесі көмірсутек өндіру аймақтарындағы төтенше жағдайлардың жиілеуіне байланысты маңызды экономикалық және экологиялық маңызға ие. Иммобилизацияланған микроорганизмдерді пайдалана отырып биоремедиацияның бақыланатын биологиялық әдісі мұнай өнімдерін фондық деңгейге дейін жоюдың тиімді шешімін ұсынады. Эксперименттік әдістеме минералды тасымалдағыштарды (цеолит және керамзит) дайындауды, *Dietzia maris* штамдарының консорциумын (U2.1, U2.6, MB3, KU1) өсіруді және спектрофотометриялық талдау арқылы

адсорбциялық қабілетті өлшеуді қамтыды. Есептік нәтижелерге сәйкес, цеолит тасымалдағыштың грамына құрғақ жасушалардың 19,69 мг-мен жоғары адсорбциялық қабілетін көрсетті, ал керамзит грамына 17,92 мг көрсетті. Адсорбцияның тиімділігі микроорганизмнің қасиеттеріне, сорбенттің сипаттамаларына және қоршаған орта жағдайларына байланысты. Бұл эксперимент цеолит сорбенті көмірсутектердің биологиялық ремедиациясында жоғары тиімділікті қамтамасыз ететінін көрсетті, бұл оны мұнай көмірсутектерінің биоремедиация процестері үшін тиімді биокатализатор етеді. Қазақстанда қолжетімді табиғи минералдар, әсіресе цеолит, мұнаймен ластанған топырақты қалпына келтіруде иммобилизацияланған микроорганизмдер үшін перспективалы және экономикалық тұрғыдан тиімді тасымалдағыштар болып табылады.

**Түйін сөздер:** адсорбция; иммобилизация; микроорганизмдер; *Dietzia maris*; цеолит; керамзит; биоремедиация; мұнай ластануы; минералды тасымалдағыштар.

## АДСОРБЦИОННАЯ ИММОБИЛИЗАЦИЯ КЛЕТОК МИКРООРГАНИЗМОВ НА МИНЕРАЛЬНЫХ НОСИТЕЛЯХ

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**Аннотация.** В работе приведены результаты лабораторных работ по определению эффективности адсорбирующей иммобилизации природных минералов в качестве носителей микроорганизмов-деструкторов. Консорциум микроорганизмов штаммов *Dietzia maris* был иммобилизован на минералы керамзит и цеолит. Проблема ликвидации нефтяного загрязнения с поверхности почвы имеет важное экономическое и экологическое значение в связи с участвовавшими аварийными ситуациями в районах добычи углеводородного сырья. Управляемый биологический метод биоремедиации с использованием иммобилизованных микроорганизмов предлагает экономически эффективное решение для удаления нефтепродуктов до фоновых значений. Экспериментальная методика включала подготовку минеральных носителей (цеолита и керамзита), культивирование консорциума штаммов *Dietzia maris* (U2.1, U2.6, MB3, KUI) и измерение адсорбционной емкости методом спектрофотометрического анализа. Согласно расчетным результатам, цеолит продемонстрировал превосходную адсорбционную способность с 19,69 мг сухих клеток на грамм носителя, в то время как керамзит показал 17,92 мг на грамм. Эффективность адсорбции зависит от свойств микроорганизма, характеристик сорбента и условий окружающей среды. Данный эксперимент показал, что использование сорбента цеолита позволяет достичь высоких результатов при биологической ремедиации углеводородов, что делает его эффективным биокатализатором для процессов биоремедиации нефтяных углеводородов. Природные минералы, доступные в Казахстане, особенно цеолит, представляют собой перспективные и экономически выгодные носители для иммобилизованных микроорганизмов при ремедиации загрязненных нефтью почв.

**Ключевые слова:** адсорбция; иммобилизация; микроорганизмы; *Dietzia maris*; цеолит; керамзит; биоремедиация; нефтяное загрязнение; минеральные носители.