Clay- and zeolite-based biogeosorbents: modelling and properties

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Abstract
This work presents data on the mineral and chemical composition of zeolite-containing and clay rocks considered as carriers for oil-oxidizing microorganisms, as well as their textural characteristics. The authors proved the use of mineral sorbents based on clay and zeolite-containing rocks for design of a biogeosorption material of destructive type. The minerals met the requirements for oil sorbents (environmental compatibility, oil capacity, feasibility), and were biocompatible with Biotrin consortium. Hydrocarbon-oxidizing microorganisms combined with mineral sorbents had a significant destructive potential. The degradation of petroleum hydrocarbons by biogeosorbents modeled on 551, 541-31, 539-40 samples were 77, 60 and 65.5 % for 4 days, respectively.
Keywords: biogeosorbents, clays, zeolites, Biotrin, petroleum hydrocarbons, n-alkanes, biodegradation, sorption
Kulcsszavak: biogeoszorbensek, agyagok, zeolitok, Biotrin, ásványolaj-szénhidrogének, n-alkánok, biodegradáció, szorció

1. Introduction
Today, zeolite and zeolite-alumina composites, based on zeolite and clay rocks, are used in a wide area of industry and everyday life primarily as sorbents because of their technical, chemical, biological and medical properties. Water and soil pollution with hydrocarbons during exploration, mining, transportation, storage and processing should be noted to remain a significant environmental problem. Many oil sorbents [1-8] are known to be divided into inorganic, natural organic and organic-mineral, and synthetic. Sorbents are evaluated mainly by their capacity to hydrocarbon pollutant under static conditions, the degree of hydrophobicity, the potentials for desorption, regeneration or utilization of the sorbent. Of course, the availability of raw materials and their cost must not be forgotten. Inorganic both sorbing and filtering materials include various types of clays, diatomite rocks, sand, zeolites, tuffs, pumice, etc. The methods for regenerating sorbents are processing with extractants or surfactants and burning, which causes secondary environment pollution [9].
The experience of the authors [10-13] shows that decontamination techniques using sorbents, biopreparations and zeolite and clay based biogeosorbents, which correlate with similar studies of zeolite and zeolite-alumina composites [14-16] are economically and environmentally desirable. Biopreparations composed of viable cells of microorganisms able to degrade hydrocarbon pollutants set the new trend in these studies. Such microorganisms are characterized by different physiological and biochemical properties, such as thermotolerance, osmophilicity, optimal pH for growth, capability of metabolize different classes of hydrocarbons.
The production of biopreparations involves several disadvantages of freeze drying. Firstly, the supernatant fluid, containing pool of extracellular enzymatic activities, is removed, and secondly, the freeze drying may cause death of the most of the microorganisms. Thirdly, not all associations of microorganisms can restore destructive activity after freezing exposure, which eliminates the feasibility of the use of many biological products in winter [17].

The production of biogeosorbents based on mineral raw materials solves problems both of increasing efficiency of microorganism cell immobilization, preserving their viability and biochemical activity by replacing freeze drying with the immobilization of microorganisms with a sorbent, and utilization of sorbent materials. In environmentally unfavorable conditions, sorbents act as a transport base for microorganisms, which allows up to 10 years anabiosis. Another advantage, compared with biopreparations, is the economic efficiency associated with a decrease in the amount of biogeosorbent used in the process of bioremediation [8, 18].

High ion-exchange and sorption capacities of clay and zeolites result in their wide use in environmental protection: to purify drinking water and industrial effluents, to regenerate soil, to remove harmful gases from industrial emissions [19-24]. The ability of clays and zeolites to absorb and retain water allows effectively immobilizing microorganisms and their metabolites on the surface. The sorption binding of hydrocarbons reduces toxicity of the environment to the microorganisms and fixes the substrate in close proximity to the microbial community. As a result, this raw material is a mineral fertilizer; it is not necessary to utilize the biogeosorbent [25].

To describe the interaction of oil and clay particles in aquatic microcosms, Lee et al. [26] suggested the term oil-mineral aggregates (OMA) and identified three types of the aggregates: droplet, solid, and flake aggregates. Droplet aggregates are represented by the smallest oil droplets surrounded by individual mineral particles or their floccules. In solid aggregates, oil and mineral bodies of various shape up to tens of micrometers are mixed. Flake aggregates are ordered thin sheets and can reach the millimeter size range. Solid oil-mineral aggregates, formed in microcosms with the participation of clays and zeolites, attach to minerals, which increases their size [27].

The aim of the work is to model biogeosorbents based on clay and zeolite rocks and Biotrin biopreparation, to study their sorption and oil degrading properties.

2. Objects and methods of research

The objects of study were:

1. Zeolite-containing and clay rocks of the European Northeast of Russia (Table 1).

| Sample No | Rock Description | Deposit/occurrence |
|-----------|------------------|-------------------|
| 551       | Analcite-containing Brownish-gray argillite | "Veslyana", Koirinskaya zeolite area |
| 541-31    | Clinoptilolite-containing clay Dark gray clay | Chim-Lop'tyuga oil shale deposit, Yarenga oil shale region |
| 539-40    | Glaucosite-containing Green calcareous clay | Chim-Lop’tyuga oil shale deposit, Yarenga oil shale region |

Table 1. Collection of zeolite-containing and clay rocks for use as mineral carriers of oil degrading microorganisms

2.1 Biogeosorbent Modeling

The biomass of bacteria was cultivated meat-peptone broth: per 1 dm$^3$ of tap water: peptone – 20 g, NaCl – 3.0 g; KCl – 1.0 g; MgSO$_4 \times 7H_2O$ – 0.5 g at a temperature of 15–25 °C for 3-5 days of liquid phase fermentation process.

Yeast biomass was cultivated on the Czapek medium: per 1 dm$^3$ of water: sucrose – 20 g, NaNO$_3$ – 5.0 g; KH$_2$PO$_4$ – 1.0 g; KCl – 0.5 g; MgSO$_4 \times 7H_2O$ – 0.5 g, at a temperature of 15–25 °C for 5 days of liquid phase fermentation process.

Microalgal biomass was cultivated on the Tamiya medium: per 1 dm$^3$ of water: KNO$_3$ – 5.0 g; FeSO$_4 \times 7H_2O$ – 0.003 g; MgSO$_4 \times 7H_2O$ – 2.5 g; KH$_2$PO$_4$ – 1.25 g, at a temperature of 15–25 °C for 10 days of liquid phase fermentation process.

The suspensions obtained were combined into Biotrin biopreparation. The biogeosorbents were produced by application of Biotrin biopreparation (cell titer 10$^9$) directly to the mineral carriers with a particle size of 0.1–0.25 mm in the ratio of 1 part of the biopreparation to 6 parts of the sorbent.

2.2 Preparation of oil polluted water (model water sample)

0.5 g of oil was added to 100 cm$^3$ of Czapek’s medium without sucrose. It was aerated in a shaker at 180 rpm for 4 days at room temperature and natural light. The water was filtered. The total petroleum hydrocarbon (TPH) content in the reference sample was 0.121 ppm, which was 2.4 times higher than the standards of maximum permissible concentrations in the water of fishery reservoirs in Russia [31].

2.3 Experimental design

Prepared water polluted with oil was poured into 250 cm$^3$ flasks and 1 g of initial sorbents and biogeosorbents based on them were added. The experiment was carried out for 4 days at room temperature, natural light, aeration in a shaker (180 rpm).

TPH in the model water sample, in the oil-mineral aggregates formed was analyzed fluorimetrically using Fluorat-02 liquid analyzer [32].

The qualitative and quantitative determination of the content of n-alkanes was carried out by Trace DSQ (Thermo) chromatograph in the mode of selective ion detection (SIM) at an electron energy 70 eV. SIM scanning was carried out.
out in three ions with masses 57, 71 and 85, characteristic of saturated hydrocarbons. N-alkanes in the chromatogram were identified previously in the mode of the full ion current using their standard solutions. The quantitative content of n-alkanes was studied by the internal standard method (the internal standard – n-decane, 0.05 mg/cm$^3$) [33].

3. Results and discussion

The mineral and chemical compositions of the initial mineral carriers are previously published by Shchemelinina et al. [34].

Cultured strains of microorganisms were immobilized on the sorbents, and their surface was studied using a scanning electron microscope. As seen in Fig. 1, there are clusters of Biotrin microorganisms, fixed on the surface of mineral carriers.

The sorption was evaluated in relation to TPH of the initial sorbents and biogeosorbents modeled. Earlier researches pointed out analcime-containing rock 551, clinoptilolite-containing clay 541-31, and glauconite-containing rock 539-40 as the most promising as sorbents and carriers for Biotrin biopreparation.

The analysis of the water in the microcosms after application of both analcime-containing rock 551, and biogeosorbent based on it confirms the reliable high water purification levels, but variations in treatments with sorbent/biogeorbent are insignificant, and important differences are noted in sample 551. Samples of clinoptilolite-containing rock 541-31 and biogeosorbent based on it showed the efficiency of TPH reduction. Significant differences in TPH content in both water and OMAs of 539-40 glauconite-containing sample are observed (Table 2).

The experiment showed (Fig. 2) that the initial samples of these sorbents exhibited adsorption activity with respect to TPH. As a result of the application of these samples into oil-contaminated water, the content of pollutant in water decreases in 4 days by 63, 71 and 80% for samples 551, 541-31, 539-40, respectively. When the biogeosorbents based on clay and zeolite-containing rocks are applied into polluted water, water purification efficiency decreases (8, 52, 40%, respectively), which indicates a decrease in the sorption properties of mineral carriers after immobilization of microorganism cells. The decrease in the sorption properties of the biogeosorbents is probably caused by decreasing specific surface because of adhesively fixed biomass of microorganisms. This is confirmed by experimental data on the specific surface area of sorbents and biogeosorbents (Table 3). In almost all the cases, the specific surface area of the raw sorbents is higher than the specific surface area of the biogeosorbents. When microorganisms are immobilized on the sorbents, the specific surface area decreases by 1.01–8.75 times.

The hydrocarbon biodegradation in samples 551, 541-31, 539-40 is 77, 60 and 65.5% for 4 days, respectively (Table 2).

| Sample No. | Specific surface area, m$^2$/g |
|------------|-------------------------------|
|            | without degassing | with degassing at 25 °C, 2 hours |
| 551        | 13.65                | 8.24                     |
|            | 13.79                | 16.67                    |
| 541-31     | 8.56                 | 11.32                    |
|            | 9.60                 | 10.03                    |
| 539-40     | 2.17                 | 2.72                     |
|            | 8.19                 | 23.80                    |

Table 2: Specific surface area of sorbents and biogeosorbents

| Sample No. | TPH content in water, ppm | TPH content in OMA, mg/g |
|------------|---------------------------|--------------------------|
| 551        | 0.111±0.015               | 67±9                     |
|            | 0.044±0.005               | 250±30                   |
| 541-31     | 0.058±0.008               | 100±16                   |
|            | 0.035±0.004               | 250±50                   |
| 539-40     | 0.072±0.009               | 20±3                     |
|            | 0.024±0.003               | 58±10                    |

Table 2: TPH content in the microcosms when the initial sorbents and biogeosorbents based on them applied

Note: in the numerator – biogeosorbent applied, in the denominator – sorbent applied. The indicated values did not exceed 1% variance for TPH.

3. táblázat: A TPH-tartalom a vízmintákban különböző szorbensek és biogeoszorbensek alkalmazása esetén

Fig. 2 TPH content in water samples when applying various sorbents and biogeosorbents based on them

2. ábra: A TPH-tartalom a vízmintákban különböző szorbensek és biogeoszorbensek alkalmazása esetén
We identified C16-C33 \( n \)-alkanes in the water samples studied (Table 4).

| Sample | \( \sum_{C_{16-33}} \mu g/dm^3 \) | \( \sum_{C_{16-23}} \) | \( \sum_{C_{24-36}} \) |
|--------|-----------------|------------|------------|
| 551    | 5.54            | 0.22       | 0.42       |
| 541-31 | 2.28            | 0.15       | 0.29       |
| 539-40 | 0.7             | 0          | 0.23       |
| Reference | 13.53          | 0.52       |            |

Table 4 Alkanes in oil-contaminated water after application of sorbents and biogeosorbents

Note: in the numerator – biogeosorbent, in the denominator – sorbent

The major part of normal alkanes covers the homological series in the range C16 to C33 with maxima of C29 and C31. Odd alkanes in the range of C25, C27, C29, C31, C33 prevailed. The ratio of light (\( \sum_{C\leq23} \)) to heavy molecules (\( \sum_{C>23} \)) is 0.52 (Table 4, Fig. 3).

When sorbents applied, the qualitative and quantitative distribution of alkanes is significantly different in oil-contaminated water. After analcime-containing rock 551 application (Table 4, Fig. 3A), the alkane content decreased 2.1 times compared with the reference. Mass concentrations of C28 and C30 prevailed over all other individual compounds. Light (\( \sum_{C\leq23} \)) to heavy molecules (\( \sum_{C>23} \)) ratio was 0.42. When the biogeosorbent applied, no important differences were observed in the total content of \( n \)-alkanes, while light to heavy molecules ratio was 1.9 times lower, and the ranked homological series are dominated by hydrocarbons with the numbers of carbon atoms C27, C29, and C31.

Application of sorbent and biogeosorbent based on clinoptilolite-containing clay 541-31 reduces the amount of \( n \)-alkanes by more than 5 times (Table 4, Fig. 3B), but the differences within the variants are not significant. The distribution curve of the mass concentrations of alkanes in water after applying of biogeosorbent became identical to the curve after applying of sorbent.

The bulk of alkanes in water after processing with glauconite-containing rock 539-40 is represented by C17 – C33 homologues and amounts to 8.97 \( \mu g/dm^3 \) (Table 4, Fig. 3C), reduced by 34% relative to the reference with a reliable concentration of C27, C29, C31 homologues in the microcosm. Due to the low mass concentrations of homologs, the identification of patterns of distribution is difficult.

Our experiments confirm the data obtained by Netzer et al. [35] that the formation of aggregates contributed to TPH removal from the water because of accumulation and
biodegradation. Solid oil-mineral aggregates can be attached to minerals, which increase their size [27]. It was noted (Fig. 4) that TPH accumulation in the aggregates formed by biogeosorbents was significantly lower (by 77, 60, 66% for biogeosorbents modeled on 551, 541-31, 539-40 samples, respectively) than in the aggregates based on the minerals studied, which is most likely related to the biodegradation of hydrocarbons accumulated by the cells of Biotrin consortium.

In OMA sample of the analcime-containing rock 551 (Table 5, Fig. 5A), we identified C16–C32 n-alkanes, the total content was 11.84 µg/g, the proportion of light alkanes in the composition was 2.33. C17, C18 homologues dominated. The data indicate that the sample is capable of holding light alkanes, which under natural conditions undergo weathering processes. In OMA of 551B, the amount of alkanes is threefold decreased (3.89 µg/g), C19–C31 compounds were identified without a clear predominance of any homologs. The light (ΣС≤23) to heavy molecules (ΣC>23) ratio was 0.57.

The decrease in the n-alkane content in the water of the microcosm was also accompanied by the accumulation in OMAs [26, 36, 37]. Different homolog sequences were identified in the samples.

| Sample  | ΣС16–32 µg/dm² | ΣС16–23 | ΣС24–32 |
|---------|----------------|---------|---------|
| 551     | 3.89           | 0.57    | 7.23    |
| 541-31  | 5.14           | 0.34    | 1.14    |
| 539-40  | 9.11           | 0.19    | 0.31    |

Table 5: Alkanes in oil-polluted water after the use of sorbents and biosorbents

The major part of normal alkanes in the OMA sample based on clinoptilolite-containing clay 541-31 covers the homological series in the range of C16 – C30, the mass concentrations of C17, C19 prevail over other individual compounds. The light (ΣC≤23) to heavy (ΣC>23) ratio was 1.14 (Table 5). In OMA of the biogeosorbent, the amount of alkanes is lower by 42% and amounted to 5.14 µg/g, the pert of light alkanes is 0.34. Fig. 5B shows that the distribution of alkanes C>23 in OMA of the sorbent and the biogeosorbent is identical.

In OMA of sorbent and biogeosorbent of glauconite-containing rock 539–40, C19 – C31 n-alkanes were identified, there were no significant differences in the total content of alkanes (8.28 and 9.11 µg/g, respectively (Table 5)). However, the distribution pattern of individual compounds is different (Fig. 5C): OMA of the biogeosorbent accumulates C24–C28 compounds, while C27 homologue prevails in OMA of the sorbent.

Thus, the course of the process of biodegradation of hydrocarbons can be traced quite clearly by the distribution
of alkanes in OMAs. The ratio of normal alkanes changes, lighter alkanes degrade more quickly. The predominance of odd chains occurs when alkanes are formed in the microcosms as a result of microbiological transformation of organic matter [37]. The dominance of odd long-chain homologues of alkanes indicates that both oil and biomass characterized by a similar type of \( n \)-alkanes spectrum are the main sources in OMAs of biogeosorbents [39]. Probably, microorganisms convert them into the corresponding fatty acids, followed by incorporation directly into cellular lipids.

4. Conclusion

Our experiments resulted in modelling of biogeosorbents, in which clay and zeolitic rocks of the European Northeast of Russia are carriers for oil-oxidizing microorganisms of Biotrin biopreparation immobilized on them. Among the samples studied, the most promising as a sorbent and carrier of Biotrin biopreparation are analcime-containing rock 551, clinoptilolite-containing clay 541-31, and glauconite-containing rock 539-40. Samples of the initial sorbents show high adsorption of hydrocarbons.

During the immobilization of microorganism cells on the surface of mineral carriers, we revealed decreasing sorption properties, but at the same time, the microorganisms supported the oxidation of hydrocarbons. The TPH biodegradation in the biogeosorbents modeled on 551, 541-31, 539-40 samples for 4 days is 77, 60 and 65.5%, respectively. The analcime-containing rock 551, clinoptilolite-containing clay 541-31 are capable of holding volatile low molecular weight alkanes.

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