Biogeosorbents for solving ecological problems

T Shchemelinina¹, O Kotova², M Harja³ and E Anchugova¹

¹ Institute of Biology, Komi Science Center, Ural Branch of RAS, Syktyvkar, Russia
² Institute of Geology, Komi Science Center, Ural Branch of RAS, Syktyvkar, Russia
³ «Gheorghe Asachi» Technical University of Iasi, Iasi, Romania

E-mail: tatyanakomi@mail.ru

Abstract. Among the variety of the ecotoxicants the petroleum products are the most environmentally dangerous, since the intensive migration of pollutants from them can occur because of the solubility of ionic components. Zeolites have high adsorption and cation exchange capacity and can be used as adsorbents for hydrocarbons polluting water bodies and soil. The disadvantage of their application is that after the adsorption of pollutants the secondary waste requiring costly disposal methods would be formed. Developing biogeosorbents via immobilization of petroleum oxidizing microorganisms on glauconites can provide the solution to the problem of immobilization of hydrocarbons polluting water bodies and soil. The obtaining zeolite-based biogeosorbents through immobilization, rather than freeze drying, solve the problem of sorbing material utilization, moreover, of increasing the efficiency cell retention, cell viability and extracellular enzyme activity. In extremely poor environmental conditions zeolite carrier provides transport of bacteria, allowing to exist in state suspended animation for up to 10 years.

1. Introduction

Petroleum products are one of the most dangerous ecotoxics for the environment, because migration of pollutants from them can be the most intensive, because compounds in their composition are in molecular and ion-soluble state [1]. Zeolites have high adsorption and cation exchange properties and can be used as adsorbents of petroleum products polluting water bodies and soil. The problem of their use is that adsorption of petroleum products results in the secondary pollution, which requires application of expensive utilization methods. The development of biogeosorbents by immobilizing oil-oxidizing microorganisms on glauconites can be the response to this problem. Glaucnites has such unique properties as a high dispersity and physical-chemical activity. Its cohesive-adhesive properties and ability to regenerate are very important [7]. The adsorption activity of glauconite is primarily related to ion-exchange properties. The ion exchange takes place both at a level of interlayer cation and hydroxyl exchange. The oil adsorbed by glauconite will be further degraded by cells of microorganisms.

In addition, biogeosorbents from zeolites solve problems of not only utilization of sorbing materials, but also of replacement of freeze drying for immobilization of microorganisms, which increases preservation of their vitality and activity of extracellular enzymes. The freeze drying process of microorganisms has a negative impact on the structure of their surface proteins, activity of adhesion, and also leads to destruction of valuable metabolites [12]. In environmentally unfavorable conditions, zeolite sorbents serve as a base to transport bacteria, which allows them to remain suspended for up to 10 years [2].
The aim of this work was to study the change in the sorption, destructive and biochemical properties of GEOLEX biogeosorbent, depending on storage conditions and age since manufacture.

2. Materials and methods
In the experiments we used:
1. Glauconite of Bondarskoe deposit from Tambov region (Ionsorb™) – mineral ionite obtained from a complex of aluminosilicates according to the author's technology [11].
2. Strains of hydrocarbon-oxidizing microorganisms (HOM):
   - bacteria Pseudomonas yamanorum VKM B-3033D [9];
   - yeast Rhodotorula glutinis, VKM Y-2998D [4];
   - microalgae Chlorella vulgaris Beijer. f. globosa V. Andr. A1123 [6].
   BIOTRIN biopreparation comprises all these strains [3].
3. GEOLEX biogeosorbent, consisting of glauconite with immobilized strains of microorganisms of BIOTRIN biopreparation [5].
4. Oil contaminated water for experiment 1 as a Control: 0.5 g of oil added to 100 cm³ of sugarless Czapek medium was aerated in a shaker at 180 rpm during 4 days under normal conditions. Then water was filtered off.

Experiments were conducted as follows:
Experiment 1. In sterile tap water 50 cm³ we added oil (0.25 g), biogeosorbent or glauconite 0.5 g. The control was a sample of oil contaminated water. The experiment was performed in triplicate within 3 days in the shaker (180 rpm) under normal conditions. The intensity of oil destruction was assessed according to the residual total petroleum hydrocarbons (TPH) content.

Experiment 2. The contaminated water prepared according to the above was bottled in 250 ml flasks and 1 g of glauconite and GEOLEX biogeosorbent were added. The experiment was carried out during 4 days in the shaker (180 rpm) under normal conditions. The residual TPH content in water and sorbents were separately determined.

Experiment 3. 35 g of glauconite or GEOLEX biogeosorbent suspended in 1 dm³ of tap water were applied per 1 m² contaminated soil of the railroad track. Tilling to a depth of 15 and sampling after 30 days for residual TPH content were conducted. Untreated soil samples before glauconite and biogeosorbent application were analyzed as a control.

Experiment 4. The oil polluted sand-gravel mixture (SGM) that coated the concrete railway platform was treated with GEOLEX biogeosorbent until complete coating, then mixed and sampled for residual TPH content after 30 minutes and after 6 days. The SGM sampled before the biogeosorbent application was investigated as the control.

In the Experiment 5 biogeosorbent samples of different age from manufacture and the temperature conditions of storage were used. Biogeosorbents applied were: freshly prepared GEOLEX (G-fp), GEOLEX stored at room temperature for 1 year (G-rts), GEOLEX stored in frozen form (G-ffs). 55 mg of oil were suspended in 50 ml of sterile tap water, and then biogeosorbents (G-fp, G-rts, G-ffs) in the amount of 0.5 g were added. A sample of oil contaminated water was used as a control. The experiment was carried out under carefully controlled conditions such as room temperature, plant grow light illumination, aeration in the shaker for 3 days (180 rpm). The experiment was performed in triplicate. At the end of the experiment, the residual TPH content was determined.

We determined the dehydrogenase activity of the samples according to the procedure [10].

The TPH content in the samples was analyzed fluorometrically [8].

To assess the effectiveness of adsorptive immobilization, the samples were examined microscopically with a scanning electron microscope VEGA3 TESCAN, × 5000 (Fig. 1).
3. Results and discussions
The study's first phase identified the possibility of fixing microbial cells on a carrier, the sorption and destructive properties of raw glauconite and glauconite based biogeosorbent (GEOLEX).

The composition and structure of Ionsorb™ allow using it as an effective carrier for immobilization of specialized oil oxidizing microorganisms. The BIOTRIN biopreparation strains cultivated were immobilized on Ionsorb™ sorbent. Accumulation of cells in the body of the carrier was shown under the microscope (Fig. 1). In this case the carrier was not only a sorbent of hydrocarbons, but also a source of biogenic elements.

![Figure 1. Ionsorb™ with immobilized cells of BIOTRIN biopreparation.](image)

In the experimental water, when glauconite and GEOLEX biogeosorbent were applied, adhesion of petroleum hydrocarbons was observed. It should be noted that sorption properties of glauconite declined 1.2-2.0 times after immobilization of strains (Fig. 2, Table 1). This was the result of a partial decrease in sorption capacity because of decline of porous permeability and sorbent specific surface area by microorganisms adhered.

![Figure 2. TPH content in the experimental water after application of biogeosorbent and glauconite.](image)
When studying the destructive properties of the biogeosorbent in the experimental water, it was determined that 62 % of hydrocarbons sorbed had been oxidized by immobilized microorganisms (Table 1).

Table 1. Residual TPH content in the Experiment 2.

|       | GEOLEX                        | Glauconite       | Control          |
|-------|-------------------------------|------------------|------------------|
|       | 0.045 ± 0.016                 | 0.035 ± 0.012    | 0.12±0.041       |
|       | 34 ± 14                       | 90 ± 40          |                  |

*Note: the numerator – TPH content in the experimental water, mg/dm³, the denominator – TPH content in the sorbent and biogeosorbent after the experiment, mg/g.

In the bulk of the railroad ballast, the reduction of TPH content due to glauconite sorption was 8.3 % after 30 days (Fig. 3). GEOLEX biogeosorbent application reduced the pollutant content by twice in 30 days. The efficiency of soil clean up was 60 %.

![Figure 3. Effectiveness of TPH reduction in the contaminated soil.](image)

Evaluations of cleaning of SGM on a concrete site by GEOLEX biogeosorbent have found that sorption efficiency of hydrocarbons in 30 minutes was 14 % from the reference value. HOM immobilized on the geosorbent, activate metabolic processes and after 6 days the efficiency of purification at the concrete site was 45 % (Fig. 4).

![Figure 4. TPH Content in SGM.](image)

The study's second phase revealed sorption, destructive properties of GEOLEX biogeosorbent depending on the conditions of its storage and age since manufacture (Table 2).
Table 2. Storage conditions of GEOLEX biogeosorbent of various age since manufacture

| Sample | Storage temperature range, °C | Age since manufacture |
|--------|-------------------------------|-----------------------|
| G-ffs  | +25…−35                      | 12 months             |
| G-rts  | +23…+25                      | 12 months             |
| G-fp   | +23…+25                      | 7 days                |

There is evidence in the scientific literature [13] that biochemical methods, such as viability, metabolic and synthetic activity are used for the analysis of various functional parameters of immobilized cells. We chose a method of dehydrogenase activity determination, because the HOM cultural fluids have a high DA level. As a result of the immobilization on glauconite, the cells of microorganisms, as well as their various enzymes, stabilize and remain active for a long time. So DA of the biogeosorbents confirms the presence of viable cells in them.

The DA of the biogeosorbent G-fp is 1.5 to 4 times higher than that of the biosorbents G-rts and G-ffs, i.e. after 12 months of storage GEOLEX biogeosorbent reduces the activity by 2.5 times in average. Dehydrogenation processes do not depend on storage conditions. The microorganisms, immobilized on the biogeosorbent G-ffs in 60 °C temperature swing do not decrease their activity; moreover, DA was higher than in biogeosorbent stored at room temperature (Fig.5). Thus, immobilization on glauconite increases cell thermo resistance.

Despite decreasing dehydrogenase activity in the biogeosorbents stored for 12 months (G-ffs, G-rts) in comparison with freshly prepared one (G-fp), the microorganisms, immobilized on glauconite, remain viable and start multiplying in favorable conditions. Thus, during the test of GEOLEX biogeosorbent, the decrease in TPH content in water varied within the error margin, which proved that both the storage temperature factor and the age since manufacture of biogeosorbent practically do not influence the oxidizing properties of the microorganisms immobilized (Table 7). The effectiveness of hydrocarbon destruction using GEOLEX biosorbent amounted to: G-fp - 27.7 %, G-rts - 25.6 %, G-ffs - 17.7 %. Based on that glauconite is a prolonged action carrier increasing the thermal stability of the microorganisms.

![Figure 5](image-url)

**Figure 5.** The effect of different age since manufacture and temperature conditions of storage on residual TPH content an DA of biogeosorbents.

4. **Conclusion**

Our studies revealed that the sorption properties of glauconite after application of microorganism cells were reduced by 1.2-2 times.
We determined that strains of microorganisms immobilized oxidize oil hydrocarbons by 62% in water within 4 days and in the bulk of a railroad ballast to 50% within 30 days, in SGM of concrete site by 45% within 6 days.

The age since manufacture of the biogeosorbent affects its dehydrogenase activity. We observed decreasing dehydrogenation processes by 2.5 times in average.

The effectiveness of the TPH reduction by GEOLEX does not depend on the term and storage conditions investigated.

**Acknowledgment**
The research was carried out with the financial support of the State task “Development of biocatalytic systems based on enzymes, microorganisms and plant cells, their immobilized forms and associations for the processing of plant raw, production of biologically active substances, biofuels, remediation of contaminated soils and wastewater treatment” No. AAAA-A17-117121270025 -1. The work was carried out with a partial financial support of UB RAS Programs (project 18-5-544).

**References**
[1]. C Courbe, B Velde and A Meunier 1981 *Clay Miner* **16** (3) 231 [https://doi.org/10.1180/claymin.1981.016.3.02](https://doi.org/10.1180/claymin.1981.016.3.02)
[2]. T N Shchemelinina, O B Kotova, M Harja, E M Anchugova, Y Pelovski, I Cretescu 2017 *Vestnik IG Komi SC UB RAS* **36** 40 [https://doi.org/10.19110/2221-1381-2017-6-40-42](https://doi.org/10.19110/2221-1381-2017-6-40-42)
[3]. Conclusion on the toxicological and hygienic assessment of "BIOTRIN" consortium of strains of oil-oxidizing microorganisms (in Russian).
[4]. Application for a patent of the Russian Federation No. 2016126521
[5]. Application for a patent of the Russian Federation No. 2018120922
[6]. Application for a patent of the Russian Federation No. 2018120704
[7]. M L Levchenko, A M Gubaiedullina 2009 *Drilling and oil* **4** 56 (in Russian)
[8]. Federal environmental regulations PND F 16.1.21-98 Quantitative chemical analysis of soils. Methods of measurement of mass concentration of petroleum products in the soil samples using Fluorat-02 fluid analyzer 1998 15 (in Russian)
[9]. S M Meshkelo, T N Shchemelinina, E M Anchugova, M Yu Markarova, S V Zheludkova 2017 Strain of bacteria *Pseudomonas yamanorum* VKM B-3033D for activation of biodegradation of oil and oil products in water, as well as in oil soils on sections of the railway RU Patent 2 615 458 (in Russian)
[10].SNIIP 2.04.03-85 Water-supply and sewerage outdoor networks and structures. The USSR State Committee for Construction USSR 1986. (in Russian)
[11].Prize of the Russian Geological Society (ROSGEO) and the Federal Agency for mineral resources for merits in the field of science and innovative technologies in the geological study of the mineral resources of Russia 2010 (in Russian)
[12].I V Solovyeva, A G Tochilina, I V Belova, E I Efimov, N A Novikova, T P Ivanova 2012 *Bulletin of the Nizhny Novgorod University named after N.I. Lobachevsky* **2-3** 85 (in Russian)
[13]. N Mallick 2006 Immobilization of microalgae Immobilization of enzymes and cells Ed J M Guisan (Totowa, New Jersey: *Humana Press Inc.*) 373