Biodegradation of Oil Sorbed on Peat after its Use as a Spill Cleanup Sorbent

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Abstract: **Thermally treated peat is known to be a prospective sorbent for oil removal. The used oil spill clean-up sorbents could be further reused for a few cycles, if an appropriate treatment is undertaken. In this study, a 36 days biodegradation batch experiment was performed with oil-degrading bacteria consortium MDK.EKO-7 and a peat sorbent (PeatOS) contaminated with diesel or raw oil. FDA hydrolysis, urease and dehydrogenase activity, as well as the concentration of hydrocarbons were measured in a peat-slurry system. Biodegradation of hydrocarbons up to 90 % was detected in the set with 2% (w/w) diesel-oil contaminated peat. The measured enzymes behaved differently over time. The peat sorbent (PeatOS) contaminated by raw oil with concentration 5mg/g dw, inhibited growth of bacteria consortium. Addition of nitrogen and plant extract to a peat-slurry with 2% diesel resulted in a significant ($p<0.05$) increase of FDA hydrolysis and urease activity after 36 days' incubation. The results obtained in this study, indicate that a recovery of the used oil-spilled peat sorbent is possible. Further experiments will be performed in order to optimise biodegradation conditions, using nutrients and surfactants.**

Keywords: **peat sorbent; hydrocarbons; biodegradation; enzymatic activity**

INTRODUCTION

Oil spillages result in environmental damage and economic loss. Technological approaches are aimed at reduction of the potential risk. For example, diesel spillages on highways traditionally have been treated with sand because it is cheap and generally available. However, this approach has some drawbacks, such as leaching out and contamination of surrounding land and watercourses; lesser adsorption capacity as compared to other sorbent products; not suitable in wet conditions [1].

Another group of sorbents represents natural materials, in particular, peat. Peat sorbent is an organic oil adsorbent produced from modified peat. Among the benefits of the use of peat sorbent are the effectiveness on land and water; it is non-toxic, non-leaching, lightweight, non-abrasive and vapour suppressive [1]. Thermally treated peat was shown to be a prospective sorbent for oil removal during spills [2,3].

In addition to peat's chemical and physical characteristics, its biological properties should be taken into consideration when analysing its effect in pollution control. Peat could play the role of an active agent for biological degradation [4]. That is an important issue for the treatment of the used oil spill clean-up sorbents and for their further reuse for several cycles [5,6] or their utilization.

Various types of microorganisms can degrade hydrocarbons, e.g., bacteria, yeasts, filamentous fungi. Almost all petroleum hydrocarbons can be oxidized to mainly water and carbon dioxide, but none of microorganisms degrade all of the possible hydrocarbon molecules at the same rate [7]. Due to different hydrophobicity and low solubility in water of the hydrocarbons, the process should be intensified by enhancing physical contact between microorganism and oil [7,8].

Bioavailability plays an important role in biodegradation of oil. In two-phase systems (water–oil), the rate of dissolution from a NAPL (non-aqueous phase liquid) determines the rate of microbial uptake. Dissolution is facilitated by microorganisms producing external biosurfactants and bioemulsifiers [5,9,10]. Direct uptake of oil from the oil–water interface by microorganisms that attach to the interface, was reported by [11,12]. However, in three-phase systems when oil is sorbed on a sorbent, its accessibility to microorganisms may decrease further. Clean-up sorbents have greater bioavailability limitations compared to soils and this is linked to their significantly higher loading capacity and internal porosity [5]. Microbial cell immobilization onto a hybrid support of peat moss for diesel biodegradation was reported by [13].

Two groups of factors can be identified that determine the success of bioremediation process: (1) the nature and character of contaminant, e.g., concentration, aggregation state, redox potential, presence of halogens, bonds type in the structure; (2) environmental conditions, e.g., temperature, pH, oxygen, water/air/soil characteristics, presence of inhibiting substances, sources of energy, carbon, nitrogen, etc [7]. However, if the oil is sequestered within the sorbent such that microbial uptake occurs at a very slow rate even under favourable conditions, reuse of sorbents is not practically feasible [5].

In this study, biodegradation batch experiments were performed with oil-degrading bacteria consortium MDK.EKO-7 and a peat sorbent contaminated with diesel and raw oil. The aim of these experiments was to determine the capacity of bacteria consortium MDK.EKO-7 to clean up the spent peat sorbent. The process occurred in a slurry model system and was evaluated by microbial enzymatic activity, as well as by a decrease of hydrocarbons concentration.

MATERIALS AND METHODS

Peat sorbent

The peat was obtained in Dzelves-Kroņu raised bog in Latvia. Mixed sample of peat from upper bog layer (*Sphagnum fuscum* peat, decomposition degree: 12%) was used in this study. Peat was modified to increase its

hydrophobicity by low-temperature pyrolysis. Thermal treatment of peat was carried out in an oven: in a closed steel cylinder with 5 cm diameter and 40 cm length. Before heating, samples were moistened with water, 1:1 by volume, to prevent the material from ignition. Heating was done at the temperature 240-250°C, for 6 hours. The obtained sorbent has the following characteristics: C 64.8 %, H 4.3 %, N 0.4 %, specific surface area 34 m^2/g, oil sorption capacity 7 g oil/g sorbent. The modified peat (Peat-based oil sorbent - PeatOS) was further used in biodegradation experiments. PeatOs porosity is considered to be one of the main factors that determine high sorption of hydrocarbons and formation of biofilm (Fig.1).

Scanning electron microscopy

Scanning electron microscopy (SEM) was done by fetching up of the peat samples to the SEM sample holder and covering with gold. Prepared samples were investigated using the JEOL ISM T–200 scanning electron microscope with 200-times magnification.

Microorganisms and growth conditions

A consortium of bacteria MDK-EKO-7 consists of 5 strains of *Stenotrophomonas maltophilia* and 2 strains of *Pseudomonas* spp. It had been previously isolated from hydrocarbon-contaminated soils and had exhibited the ability to degrade hydrocarbons. The inoculum with concentration 3.1 x 10^8 cfu/ml was prepared by 24 h cultivation in the liquid medium at 28 °C under aerobic conditions with agitation 140 rpm. The medium composition was as follows, g/l: Na_2HPO_4 x $12H_2O$ – 6.0; KH_2PO_4 – 3.0; NaCl – 0.5; molasses – 5.0; yeast extract – 2.0.

Cell concentration was expressed as colony-forming units (CFU) per ml and determined by making serial decimal dilutions and plating on Tryptone Glucose Yeast Extract Agar (TGA) (Sifin, Germany). CFU were counted after 72 h plate incubation at 28 °C.

Biodegradation experiment

10 g modified peat (PeatOS) was spiked with 50 mg of raw oil or 200 mg of diesel oil according to the following procedure: oil was dissolved in hexane, to obtain homogenous dispersion, and peat was treated with obtained solution in equivalent volume. Then samples were dried at room temperature till constant weight (24 hours). Biodegradation study was conducted in a batch slurry microcosms with oiled peat sorbent and bacteria consortium MDK-EKO-7. Experiment with different concentrations of nutrients and inoculum was performed in duplicate, according to the Table 1. 50 ml liquid were added to 10 g peat (dw), i.e., 5 ml 10x stock medium (with composition as described in 2.2), 5 ml inoculum (1.9 x 10^9 cfu/ml) and 40 ml sterile distilled water. Cabbage leaf extract was prepared according to [14].

Analytical methods

Dehydrogenase activity (DHA) was determined by reduction of 2-p-iodo-3-nitrophenyl-5-phenyltetrazolium chloride (INT) to iodonitrophenylformazan, in triplicate. 50 µl of (40 mg INT, 1 ml 1% glucose, 20 ml 0.25 M TRIS) were added to 50 µl slurry sample. Mixture was incubated at +28°C for 48 h. Afterwards, 300 µl of the extraction solution (ethanol and dimethylformamide 1:1) were added, vortexed and after 30 min centrifuged at 5000 rpm. Optical density was measured at 485 nm [15].

TABLE 1.

EXPERIMENT SCHEME

Set No.	Medium 10x stock, ml	Ammonium oxalate monohydrate, 4%, ml	Cabbage leaf extract, ml	Inoculum, ml	Water, ml	Total volume, ml
1	2,5	0	0	5	42,5	50
2	5	5	2,5	5	32,5	50
3	5	5	2,5	10	27,5	50
4	5	5	2,5	15	22,5	50

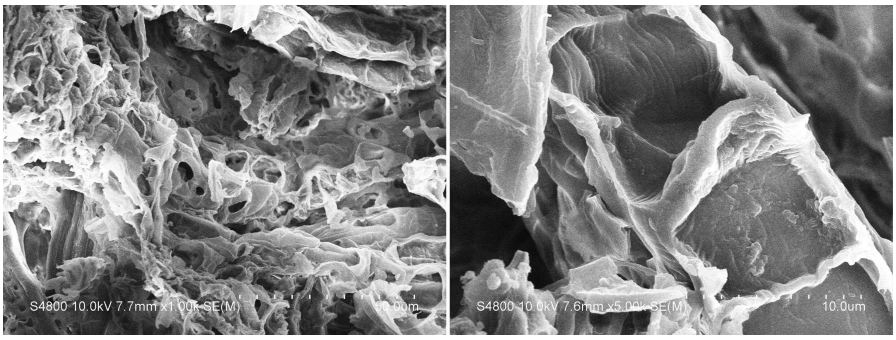

Fig.1. SEM micrographs of a modified peat sorbent (PeatOS) used in the experiment. Porosity of PeatOS is shown at different magnification.

Urease activity was determined after 24 h incubation at 37°C, in fourplicate. 100 µl of slurry were placed into 500 µl 0.2 M K_2HPO_4 x $3H_2O$ / KH_2PO_4 buffer (pH 7.1) with 0.1% urea. Reaction was stopped with 100 µl 1M KCl. Concentration of $N\text{-}NH_4^+$ ions was determined by spectrophotometry at 425nm after addition of 50 µl Nessler reagent into each epi.

To determine fluorescein diacetate (FDA) hydrolysis, 50 µl of slurry were added to 500 µl 0.06 M phosphate buffer (pH 7.6) with 50 µl FDA solution (0.002 gFDA/1ml acetone), in triplicate. After 60 min incubation at 37 °C, 500 µl of acetone were added to stop the reaction. FDA hydrolysis was determined by spectrophotometry at 490nm [16].

The standard method of gas-liquid chromatography EN ISO (9377-2) was used to determine changes of hydrocarbon concentration in samples.

Results and discussion

The appropriateness of peat sorbent as a carrier for microbial attachment is one of the most important criteria to predict biodegradation activity onto peat surface. Considering that peat is an organic material containing microorganisms and is in the process of fossilization, it is possible to establish the presence of native colonies capable of biodegrading complex organic molecules [17].

As stated earlier by [18], bacteria isolated from a core collected at different depths of the peat bog, were identified as *Pseudomonas chlororaphis*, *P. fluorescens*, *Bacillus mycoides* and *Alcaligenes denitrificans*. *Burkholderia* genus was found to be dominant in *Sphagnum magellanicum* peat [19]. Structure and activity of the microbial community is strongly dependent on the type of peatland [17].

In this study, the tested peat was treated with the aim to enhance oil sorption capacity. Therefore, attachment of microorganisms onto a modified peat was not obvious, for example, due to increased hydrophobicity of peat.

Enzymatic and degradation activity of the consortium MDK-EKO-7 during incubation with oiled peat sorbent

The changes of microbial enzymatic activity in soil/water/slurry during biodegradation serve as important criteria for evaluation of the process. A response of different enzymatic groups of microorganisms is specific in dependence on the environmental conditions and, in particular, on the type of contaminant. Therefore, the use of a battery of different enzymatic groups is of great importance for estimation of microbial activity.

It is important to note, that enzymatic activity does not correlate with the number of sessile microorganisms. It is not known how long enzymes are active after their mother cells may have died [20]. Nevertheless, enzymatic activity of microorganisms, in particular, hydrolysis of FDA has been suggested as an appropriate method in integrated bioecosystem studies because the ubiquitous lipase, protease, and esterase enzymes are involved in the hydrolysis of FDA [21].

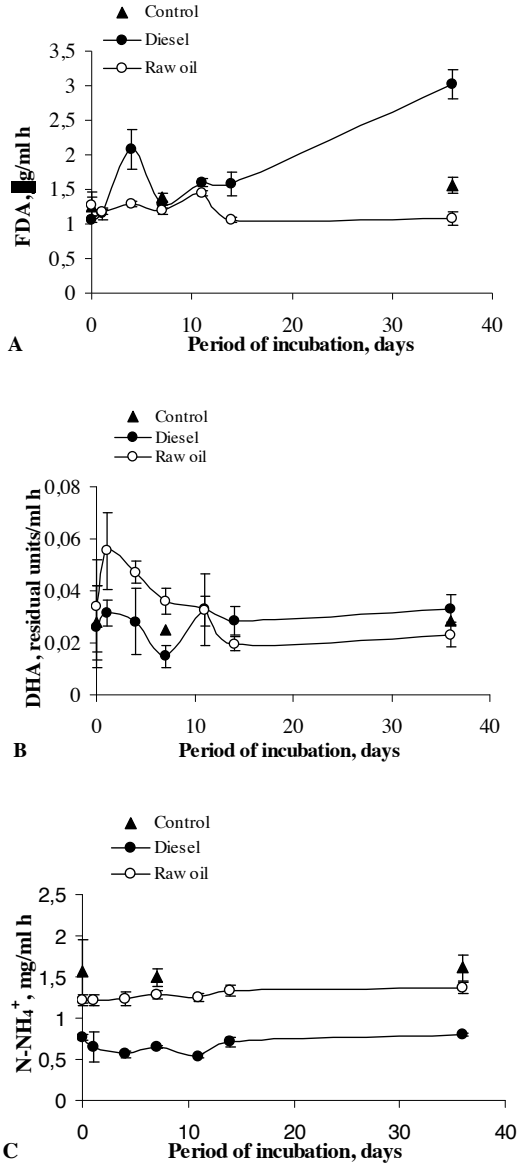

Fig.2. Changes of microbial enzymatic activity during 36 day incubation with hydrocarbons-contaminated and non-contaminated peat. (a) – FDA hydrolysis activity; (b) – dehydrogenase activity (DHA); (c) – urease activity. Control – non-contaminated peat. Error bars represent the standard deviation.

In this study, three groups of enzymes, i.e., FDA hydrolysis, urease and dehydrogenase were measured in a peat-slurry system inoculated with bacteria consortium MDK.EKO-7. The measured enzymes behaved differently over time. The highest FDA hydrolysis activity of microorganisms was found to be in the sets with diesel oil, as compared to those with raw oil and non-contaminated sets. Thus, during 36 day incubation in the presence of diesel, FDA hydrolysis activity was increased on average from 1.06 to 3.02 μg/ml h. Besides, one more peak of the FDA hydrolysis activity was shown at the beginning of the experiment, i.e., at the 7th day of incubation (Fig.2(a)). The same effect was shown also for DHA activity. As reported earlier by [22], oil contamination causes a significant initial increase of soil biological parameters. At the beginning of incubation, the DHA activity of microorganisms, incubated

with raw oil, was higher than that incubated with diesel (Fig.2(b)). Also urease activity was higher in the presence of raw oil, than in the sets with diesel (Fig.2(c)). However, other parameters pointed to the inhibition effect of raw oil to bacteria consortium. A decrease of the heterotrophic counts in the presence of 5mg raw oil/g dw peat during 36 day incubation was found. The number of CFU in the sets with diesel-contaminated and non-contaminated peat remained constant during incubation (Fig.3).

Assessment of microbial activity in a peat-slurry system with hydrocarbons was accompanied with the measurement of hydrocarbon concentration in dynamics. In the set with diesel-contaminated peat, a decrease of hydrocarbons concentration from 7.54 to 0.67 mg HC/g dw peat during 36 day incubation was discovered (Fig.4). The ratio between (n-C_8 to n-C_{23}) and (n-C_{24} to n-C_{40}) did not change considerably and varied in the range from 82% to 98 %, and from 2% to 18 %, respectively. This fact indicates that the biodegradation process occurred in the presence of bacteria consortium. Conversely, the concentration of hydrocarbons in the set with raw oil was not statistically relevant because of high fluctuation among tested samples (results not shown).

Effect of nutrients on microbial activity in the presence of oiled peat sorbent

Additional experimental sets were performed with the aim to examine the effects of nutrient amendments and initial concentration of inoculum on the degradation of petroleum hydrocarbons. Previous studies on hydrocarbons biodegradation have shown that addition of nitrogen and other growth factors can noticeably stimulate microbial activity [23,24,25]. The nutrient concentration should be maintained at a high enough level to support maximum oil biodegradation based on the kinetics of nutrient consumption. Higher concentrations may lead to ecological and toxicological impacts [24].

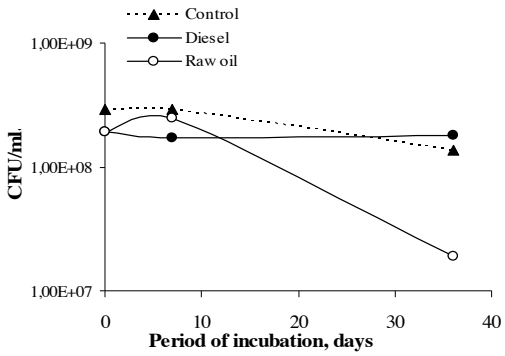

Fig.3. The number of colony forming units in a peat-slurry with hydrocarbons-contaminated and non-contaminated peat. Period of incubation 36 days. Control – non-contaminated peat.

In this study, addition of ammonium oxalate monohydrate as a nitrogen source, and leaf cabbage extract as a complex source of growth factors, resulted in an increase of FDA hydrolysis and urease activity of bacteria consortium in a peat-slurry system after 34 days of incubation (Fig.5a, b, variants 1 and 2).

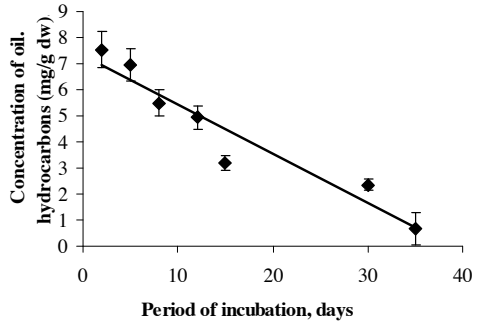

Fig.4. Hydrocarbons destruction in a peat-slurry with diesel oil during 36 days' incubation. Error bars represent the standard deviation.

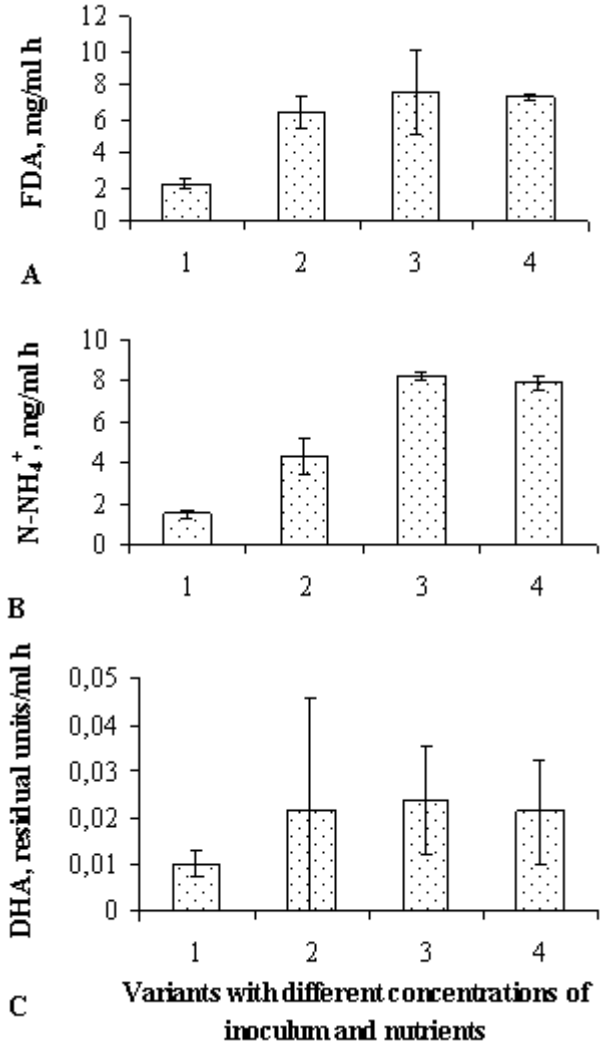

Fig.5. Microbial enzymatic activity after 36 days' incubation in a peat-slurry with 2% diesel oil in dependence on the concentration of inoculum and nutrients added. (a) – FDA hydrolysis activity; (b) – dehydrogenase activity (DHA); (c) – urease activity. Error bars represent the standard deviation. Description of variants see in Table 1.

The variants No. 2, 3 and 4 differed by the amount of inoculum added to the slurry (Table 1). As shown in Figure 5, no significant difference in FDA hydrolysis activity was shown between these sets, while urease activity was higher in the sets with 20% and 30% inoculum, as compared to 10%.

Dehydrogenase activity demonstrated a tendency to increase in the sets with additional nutrients, however these differences cannot be taken into consideration due to rather large standard deviation (Fig.5c).

Thus, the addition of nitrogen and other growth factors considerably stimulated bacteria consortium in a peat-slurry system. According to the literature data, concentrations approximately 5 to 10 mg/L of available nitrogen in the interstitial pore water is sufficient to meet the minimum nutrient requirement of the oil degrading microorganisms [26]. Jackson and Pardue [27] found that oil degradation rates could be increased by increasing concentrations of ammonia in the range of 10 – 670 mgN/L, with most of the consistent rate increases occurring between 100 – 670 mgN/L. In our experiment, all nitrogen originated from molasses; yeast extract; cabbage leaf extract and ammonium oxalate monohydrate contributed 1.58 g/L (0.20; 0.20; 0.38; and 0.80, respectively). This concentration of nitrogen is higher than mentioned above. However, it is still not clear what are the proportions of added nitrogen, which is (i) sorbed by peat and (ii) remained available in water phase.

Taking into consideration the sorption properties of peat, in further studies it is necessary to clarify the process of sorption of biogenic elements from the nutrient medium added to a slurry. Besides, estimation of microbial degrading activity by GC measurement of hydrocarbons will be done.

CONCLUSIONS

1. Degradation of the diesel fuel sorbed onto peat sorbent (PetaOS) (diesel concentration 20mg/g dw) was shown to be possible in a slurry system (diesel oil : modified peat : nutrient amendments : bacteria consortium). A decrease of hydrocarbons concentration up to 90 % was detected after 36 days incubation. Taking into consideration the effect of the diesel loading rate to biodegradation activity, testing of higher concentrations of diesel is required in future.
2. The peat sorbent (PeatOS) contaminated by raw oil with concentration 5mg/g dw, inhibited growth of bacteria consortium MDK.EKO-7 during 36 days' incubation. Chromatography test results have not revealed any significant decrease of hydrocarbon concentration in the slurry. Further experiments with raw-oil-contaminated peat should be accompanied by addition of surfactants.
3. Enzymes of bacteria in the sets with raw-oil- and diesel-oil-contaminated peat behaved differently over time. FDA hydrolysis activity in the set with diesel oil was notably increased during 36 days' incubation. Conversely, DHA and urease activity did not demonstrate considerable changes during the experiment, except for at the initial stage of incubation, which was accompanied by DHA increase.
4. The activity of bacteria able to oxidize hydrocarbons could be improved by supplementation with nutrients. Addition of nitrogen and plant extract to a peat-slurry with 2% diesel resulted in a significant ($p<0.05$) increase of FDA hydrolysis and urease activity after 36 days' incubation. Addition of 2- and 3-fold higher concentrations of inoculum resulted in an increase of urease activity.

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Olga Muter, Katrīna Potapova, Dmitrijs Prošņovs, Māris Kļaviņš. Ogļūdeņražu biodegradācija uz kūdras sorbenta pēc tā izmantošanas piesārņojuma sorbcijai
Termiski apstrādāta kūdra ir zināma kā perspektīvs sorbents vides attīrīšanai no naftas piesārņojuma. Tomēr izlietotā kūdras sorbenta utilizācija ir nopietna ekoloģiska problēma, kuras risināšana ir iespējama, izmantojot gan fizikāli ķīmiskās, gan bioloģiskās metodes. Uz kūdras sorbēto ogļūdeņražu biodegradācija ir iespējama, ja biodegradācijas procesa apstākļi ir atbilstoši mikroorganismu aktivitātei. 36-dienu eksperimentu veica ar naftas produktu degradējošo baktēriju konsorciju MDK-EKO-7 un kūdras sorbentu (PeatOS), kurš bija piesārņots ar dīzeļdegvielu (20 mg/g) vai jēlnaftu (5 mg/g). Mikroorganismu aktivitāti raksturoja dinamikā, kā vērtēšanas kritērijus izmantojot koloniju veidojošo vienību skaitu, enzimātisko aktivitāti un ogļūdeņražu koncentrāciju. Fluoresceīna diacetāta (FDA) hidrolīzes aktivitāte dīzeļdegvielas klātbūtnē 36 dienu laikā pieauga no 1.06 līdz 3.02 mgFDA/mL stundā. Ievērojamas izmaiņas mikroorganismu ureāzes un dehidrogenāzes aktivitātei eksperimenta laikā nebija konstatētas. Dīzeļdegvielas ogļūdeņražu koncentrācijas samazināšanās par 90 % pēc 36-dienu inkubēšanas liecina par dīzeļdegvielas degradācijas procesu. Proporcija starp (n-C8 līdz n-C23) un (n-C24 līdz n-C40) mainījās nedaudz un variēja diapazonā attiecīgi no 82% līdz 98% un 2% līdz 18%. Savukārt variantos ar jēlnaftu bija novērota mikroorganismu aktivitātes inhibēšana. Papildus eksperimentos bija pierādīts, ka slāpekļa un augu ekstrakta pievienošana ar dīzeļdegvielu piesārņotai kūdrai ievērojami stimulē mikroorganismu aktivitāti. Iegūtie rezultāti liecina par to, ka piesārņotā kūdras sorbenta attīrīšana ir iespējama dotajos apstākļos. Turpmākos pētījumos plānots optimizēt inkubēšanas apstākļus, lai paaugstinātu ogļūdeņražu biodegradācijas efektivitāti.

Ольга Мутер, Катрина Потапова, Дмитрий Поршнёв, Марис Клявиньш. Биодеградация углеводородов на торфе после его использования в качестве нефтепоглощающего сорбента.
Термически обработанный торф известен как перспективный сорбент для очистки окружающей среды от нефтесодержащих загрязнений. Однако, утилизация использованного сорбента является серьёзной экологической проблемой, решение которой возможно с применением как физико-химических, так и биологических методов. Биодеградация углеводородов на поверхности торфяного сорбента возможна при создании оптимальных условий для активности микроорганизмов. 36-дневный эксперимент проводили с торфяным сорбентом (PeatOS), предварительно загрязнённым дизельным топливом (20 мг/г) или сырой нефтью (5 мг/г), в присутствие консорция нефтеокисляющих бактерий MDK-EKO-7. Активность микроорганизмов оценивали в динамике по следующим критериям: число колонии образующих единиц, ферментативная активность, концентрация углеводородов. Активность

гидролиза флуоресцеина диацетата (ФДА) в присутствие дизеля после 36 дней эксперимента повысилась с 1.06 до 3.02 мгФДА/мл ч. Уреазная и дегидрогеназная активность вариировала незначительно. Снижение концентрации углеводородов дизеля на 90 % после 36-дневной инкубирования указывает на процесс биодеградации. Пропорция между (n-C8 до n-C23) и (n-C24 до n-C40) менялась незначительно и вариировала в пределах от 82% до 98% и 2% до 18%, соответственно. В свою очередь, в вариантах с сырой нефтью было отмечено ингибирование активности микроорганизмов. В дополнительных экспериментах было доказано, что добавление азота и экстракта растений к загрязнённому торфяному сорбенту оказывает стимулирующий эффект на микроорганизмы. Полученные результаты свидетельствуют о том, что очистка загрязнённого торфяного сорбента в данных условиях возможна. В дальнейших исследованиях планируется оптимизировать условия инкубирования в целях повышения эффективности процесса биодеградации.