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TEMPORAL DYNAMICS OF MICROBIAL COMMUNITY IN SOIL DURING PHYTOREMEDIATION FIELD EXPERIMENT

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Abstract. Oil-shale chemical industry creates approximately 600 000 tons of thermally processed oil shale solid wastes (semi-coke) every year in Estonia. A field phytoremediation and bioaugmentation experiment has been monitored for three years in the solid waste depository area of oil-shale chemical industry. We found enhanced degradation rates of pollutants in plots with vegetation and added bacterial biomass. The concentration of volatile phenols had decreased almost by 100 %, and the concentration of oil products had decreased approximately 3 times in planted plots compared to the control plots. The degradation rates were the highest in the upper soil layer which has the highest root density. Vegetation also changed the microbial community structure in comparison with the control plots. In addition to the vegetation, properties of the substrate had an essential effect on the microbial community.

Keywords: oil shale, chemical industry solid waste, bioaugmentation, phytoremediation, microbial community.

1. Introduction

Oil shale is fine-grained sedimentary rock containing relatively large amounts (10–65 %) of organic matter (kerogen). The main industrial activities using the oil shale resource in Estonia are electricity production and heat generation and conversion to other forms of fuels (shale-oil, shale-oil gas). In Estonia currently about 1.4 million tons of oil shale is treated thermally annually, and approximately 600 000 tons of processed semi-coke (spent shale) is disposed every year. 80 years of oil shale thermal treatment has accompanied semi-coke mounds in northeastern part of Estonia that covers an area of about 180–200 ha and contains about 100 million tons of semi-coke [1]. Semi-coke solid wastes contain several organic and inorganic compounds (oil products, asphaltenes, phenols, PAHs, sulfuric compounds). Composition of mineral part of fresh unhydrated semi-coke is mainly characterized by calcite, dolomite, quartz, K-feldspar and clay minerals. Liquid wastes (leachate) from depository area contain high concentration of oil products, phenol, cresols, dimethylphenols and resorcinols [2, 3]. Open deposition of semi-coke causes distribution of pollutants via air (dust) as well as via aqueous vectors (leaching by rainfall and snowmelt). Although oil shale mining and semi-coke is produced in much lower amounts in recent years, due to the organic component fresh semi-coke is classified as „toxic“, deposition and remediation of semi-coke is still the most severe environmental problem in Estonia.

Phytoremediation is technology that is based on the combined action of plants and their associated microbial communities to degrade, remove, inactivate or immobilize toxic compounds in the soil [4]. In situ phytoremediation strategy exploits natural or genetically engineered plant species to accumulate toxic substances (heavy metals, radioactive compounds, organic pollutants) directly from the soil. Partial or complete degradation of organic substances have been demonstrated in some cases [5, 6]. In addition to the ability of immediate remediation, plants may also enhance toxic compounds biodegradation by producing and releasing root exudates and exoenzymes to the surrounding environment and by providing surface for the colonization of microbes (phytoremediation *explanta* or rhizoremediation). Root exudates contain mostly organic acids, sugars and amino acids which promote microbial metabolic activity in root zone and enhance bioavailability of toxic compounds for microbes' thereby increasing microbial density and diversity in contaminated environment. [7]. One of recent strategies to improve phytoremediation and detoxification of contaminants is use of endophytic bacteria. Endophytic bacteria seem to have a ubiquitous existence in most, if not all, higher plant species, and these bacteria can be used to complement the metabolic potential of their host plant. Studies have revealed that endophytic bacteria, possessing the appropriate degradation pathway, may significantly improve *in planta* degradation of toluene in yellow lupine and in poplar, resulting in its reduced phytotoxicity and release [8, 9]. Germaine et al. also found that horizontal gene transfer of the toluene degradation plasmid pTOM-Bu61 had occurred to different species of poplar's endogenous endophytic community [9]. Phytoremediation has been successfully applied for remedia-

tion of different pollutants, including chlorinated and radioactive substances, explosives, pesticides, heavy metals, oil hydrocarbons and landfill leachates [10, 11].

Plants frequently do not possess complete metabolic degradation pathway for pollutants, and even more toxic byproducts may be produced. In addition to plant specific microorganisms (indigenous microorganisms or genetically modified microorganisms with degradative properties) are used for the contaminated environment to enhance biodegradation of pollutants (bioaugmentation) [12]. The idea of bioaugmentation is that the metabolic capacities of the local native microbial community will be increased by an exogenously enhanced genetic diversity in order to boost biodegradation processes [13]. Enhancement of pollutant degradation rate using bioaugmentation has been successfully applied in several cases with non-vegetated soil [14, 15] and planted soil [16, 17].

Currently the methods are sought to remediate the solid waste dump area of oil shale chemical industry. One of the options is to apply phytoremediation in certain parts of the dump area. Together with the use of vegetation, our purpose is to determine and target the kind of microorganisms in sampled habitats (e.g. resistance to pollutants and poor nutrient conditions or high salinity in our case) that are likely to suit specific conditions and remedial requirements (Fig 1). Strains derived from a population that is temporally and spatially prevalent in a specific type of habitat, are more likely to persist as an inoculum when reintroduced than those that are transient or even alien to such a habitat. Once abundant populations have been identified, the second phase of the selection procedure should be to identify strains which can degrade the target contaminant. This should then allow us to develop a collection of biodegratative microbes that can be used together with vegetation and fertilizers for remediation of oil shale solid wastes. As a result of the remediation approach based on simultaneous use of plants and specific consortium of bacteria, concentration of pollutants will be reduced and semi-coke heaps will be covered with vegetation that prevents soil erosion and decreases amount of leachate.

The aim of current study was to assess the temporal changes in soil microbial community during the phytoremediation and bioaugmentation field experiment.

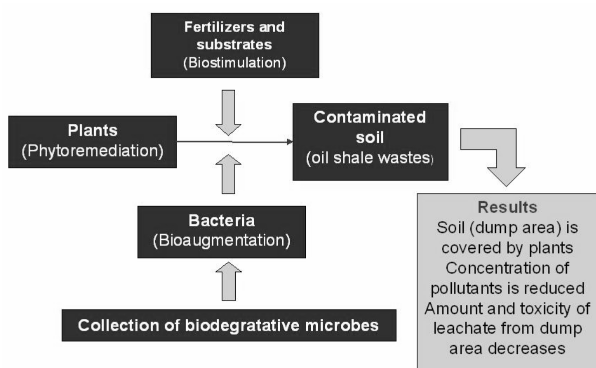

Fig 1. Principal scheme of technological approach for remediation of oil-shale chemical industry solid waste dump area

2. Material and methods

2.1. Phytoremediation experiment

Four test plots (each of 50 m^2) were established at semi-coke depository in July 2001. Plant treatment was based on a grass mixture of four species (*Lolium perenne* - perennial ryegrass, *Poa pratensis* - Kentucky bluegrass, *Festuca rubra* - red fescue, and *Festuca ovina* - blue fescue). In addition to plants, three different treatments were utilized. The following treatments were applied: plot 1 – no treatment (grass seeds in semi-coke); plot 2 – seeds in semi-coke were covered by sand layer (1–2 cm); plot 3 – seeds in semi-coke were covered by peat layer (1–2 cm). In October 2001, 2002 and 2003 soil sampling (depth of 0–20 cm) was performed on treatment plots and control area. We analysed semi-coke samples, collected from the test plots in the depository area, for chemical and microbiological parameters.

2.2. Bioaugmentation experiment

For the bioaugmentation experiment a set of bacteria consisting of three strains isolated from nearby polluted area was selected. These three bacterial strains *Pseudomonas mendocina* PC1, *P. fluorescens* PC24 and *P. fluorescens* PC18 degrade phenols via catechol meta, catechol or protocatechuate ortho or via the combination of catechol meta and protocatechuate ortho pathways, respectively [18]. In bioaugmentation experiments the biomass of these bacteria was supplied to the part of experimental plots (each of 10 m^2) in July 2002. Each treatment received 20 l of bacterial suspension with concentration of 10^8 CFU ml^{-1}. The ratio of bacterial strains PC1, PC18 and PC24 was in suspension 3:1:1.

2.3. Microbiological methods

The microbial communities were removed from semi-coke by vortexing in 0.9 % NaCl solution. Heterotrophic plate count was enumerated by the spread plate method in triplicate on R2A agar (Difco). The number of phenol-degrading bacteria was determined in triplicate sets on M9-salts agar plates supplemented with trace elements and phenol (2,5 mM). The heterotrophic activity and diversity of microbial community was measured using Biolog EcoPlates (Biolog, Inc.). Results of Biolog profiles are presented by Shannon diversity index.

Microbial DNA was extracted from soil samples with UltraClean Soil DNA kit (Mo Bio Laboratories, Inc.). Bacterial community structure was assessed with 16S rDNA sequence specific primer pair p338f-GC [19] and p518r [20]. The GC clamp (40 bp) was added to the 338f primer to enable denaturing gradient gel electrophoresis (DGGE). PCR amplification was performed in a total volume of 50 µl. Isolated DNA was added as a template to a 50 µl reaction mixture. The PCR mixture included 1 x PCR buffer (with $(NH_4)_2SO_4$), 200 µM concentrations of each deoxynucleoside triphosphate, 2.5 mM $MgCl_2$, 20 pmol of each primer and 0.5 U of *Taq* DNA polymerase (Fermentas). After 5 min. of denaturation at 95 °C and 30 thermal cycles of 1 min. at 95 °C, 1 min. at

53 °C and 2 min. at 72 °C PCR was finished by an extension step at 72 °C for 10 min. PCR mixes (5 μl) were subjected to agarose gel (2 %) electrophoresis for approximately 15 min. at 100 V in 1 x TAE buffer, pH 8.3. DNA fragments were stained for 20 min. in 1 x TAE buffer with ethidium bromide (final concentration 0.5 $\mu g l^{-1}$) and destained twice in MilliQ water for 20 min. prior to UV transilluminator. A molecular weight marker (100 bp DNA ladder, Fermentas) was included at both sides of gel, and PCR products were quantified by comparison with standard using E.A.S.Y Win32 Software (Herolab GmbH, Germany).

A denaturing gradient gel electrophoresis system DCode (Bio Rad, Inc.) was used to separate the amplified gene fragments as recommended by the manufacturer. Approximately 500 ng of PCR products was applied for the DGGE analysis and electrophoresis was performed as described by Muyzer *et al.* [21] with 10 % (vol/vol) polyacrylamide gel (acrylamide : bisacrylamide = 37.5:1 in 1x TAE buffer). Linear denaturing gradient of 30–55 % was used. DNA denaturing gradient was formed with deionized formamide and urea (100 % denaturant agent is 7 M urea and 40 % (vol/vol) deionized formamide). Gel was electrophorised in 1x TAE buffer for 11 hours at constant temperature of 60 °C and at constant voltage of 100 V. Gel was stained in MilliQ water containing 0.5 $\mu g\ l^{-1}$ ethidium bromide and de-stained twice in MilliQ water. DGGE gel was digitized and banding pattern analysed using cluster analysis based on Pearson correlation coefficient.

3. Results

Chemical properties of the semi-coke from experimental area are shown in Table 1.

Thermally processed oil shale is very alkaline and saline, biologically sterile, nutrient deficient material with no structure. Semi-coke contains high amount of organic carbon nearly half of which are asphaltenic compounds. Semi-coke also contains very low concentrations of n-alkanes and PAH compounds [22].

Table 1. Chemical properties of semi-coke from the control plot in July 2001

Variable	Measured value
pH	8.0–11
Total nitrogen (%)	0.08
$P\text{-}PO_4^{3-}$(mg kg^{-1})	12.3
K^+ (mg kg^{-1})	799
Ca^{2+} (mg kg^{-1})	18673
Mg^{2+} (mg kg^{-1})	826
Total organic carbon (%)	15.0–18.0
Oil products (mg kg^{-1})	340
Volatile phenols (mg kg^{-1})	0.30–0.34

Chemical analysis of soil samples showed impact of the plant treatment on the degradation of pollutants. By the autumn of 2003 concentration of volatile phenols had decreased almost by 100 %, and the concentration of oil products had decreased approximately 3 times in plots with vegetation compared to the control plot (Fig 2).

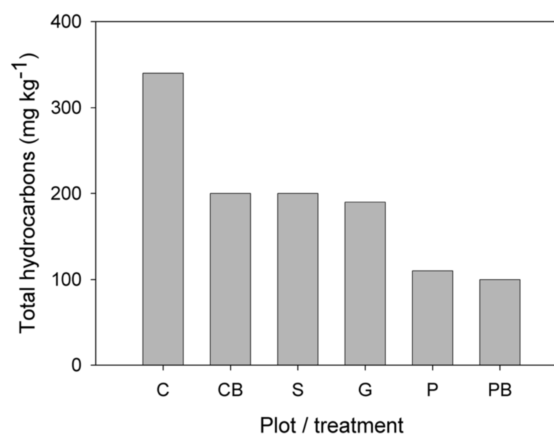

Fig 2. Effect of the treatment on the concentration of total hydrocarbons in semi-coke. Sample codes are given in Table 2

Table 2. Microbiological properties of semi-coke in different treatment plots

Sample	Symbol	Year	Aerobic heterotrophic bacteria (CFU g^{-1} dw)	Phenol degrading bacteria (CFU g^{-1} dw)	Shannon index (BIOLOG48h)	Shannon index (DGGE)
Control	C	2001	$1.2*10^7$	$2.3*10^4$	2.7	3.2
		2002	$8.2*10^6$	$1.0*10^4$	2.6	2.8
		2003	$3.7*10^6$	$4.9*10^5$	1.8	3.1
Control with biomass	CB	2002	$6.9*10^6$	$1.3*10^4$	1.9	3.0
		2003	$8.4*10^6$	$7.2*10^5$	2.3	2.9
Grass	G	2001	$1.5*10^7$	$3.0*10^5$	3.1	3.1
		2002	$4.2*10^6$	$1.5*10^4$	2.7	3.1
		2003	$1.8*10^7$	$1.8*10^6$	2.6	3.3
Grass and sand layer	S	2001	$1.1*10^7$	$3.3*10^5$	3.0	3.2
		2002	$1.5*10^6$	$3.7*10^3$	1.7	3.2
		2003	$7.4*10^6$	$2.1*10^5$	2.3	3.1
Grass and peat layer	P	2001	$1.1*10^7$	$4.5*10^4$	3.2	3.2
		2002	$5.7*10^6$	$6.5*10^5$	3.0	3.1
		2003	$9.4*10^6$	$5.6*10^4$	2.6	3.0
Grass and peat layer with biomass	PB	2002	$6.0*10^6$	$1.9*10^6$	3.1	3.2
		2003	$6.0*10^6$	$5.5*10^5$	2.6	3.1

Vegetation did not influence the numbers of aerobic heterotrophic bacteria in semi-coke compared to the control plot. The numbers of aerobic heterotrophic bacteria in semi-coke samples remained nearly the same during the experiment (Table 2). Vegetation and added bacterial biomass influenced the numbers of phenol-degrading bacteria in semi-coke samples. Planted plots demonstrated one order of magnitude higher numbers of phenol-degrading bacteria compared to the control plot. In the case of bioaugmentation, numbers of phenol-degrading bacteria were higher in plots with added bacterial biomass in the year of bioaugmentation treatment, but these numbers decreased next year.

Values of Shannon diversity indeces (Table 2) based on metabolic profiles obtained with Biolog EcoPlates demonstrate the impact of vegetation on the microbial community diversity in semi-coke samples.

Diversity indeces were higher in planted plots compared to the unplanted plot. During the experiment microbial community diversity index values decreased both in the control plot and plots with different treatments. According to the values of Shannon diversity indeces based on the DGGE fingerprints, there were no substantial differences between untreated and treated semi-coke plots and values remained constant during the experiment. These results may indicate the fact that vegetation influenced rather some specific degradative groups of microorganisms already present in the semi-coke than the total microbial diversity.

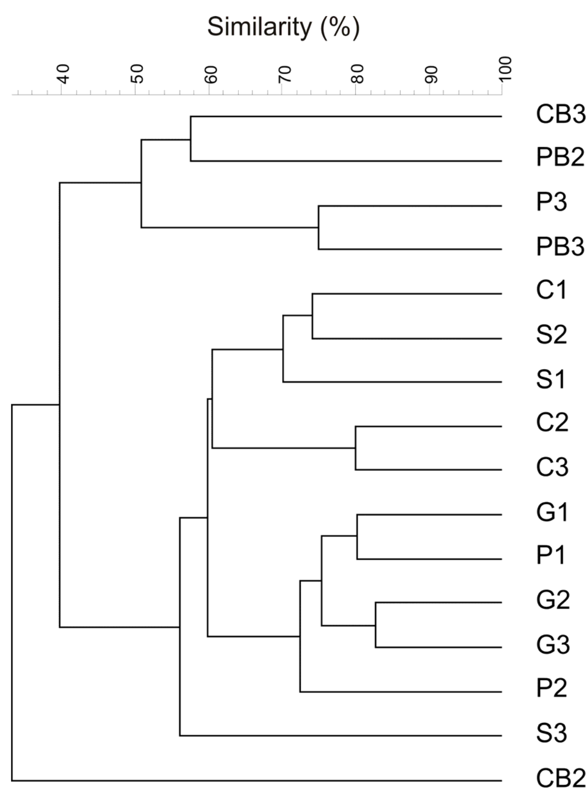

Fig 3. Dendrogram of soil samples based on cluster analysis of the DGGE profiles of microbial communities. Sample codes are given in Table 2. Numbers after sample name indicate the year soil samples were taken (1 – 2001, 2 – 2002, 3 – 2003)

Results of 16S rRNA gene based DGGE fingerprints of soil samples also refer to the impact of plant treatment on the soil microbial community structure in semi-coke. According to the dendrogram (Fig 3) based on the DGGE fingerprints, microbial communities in plots with vegetation and added bacterial biomass differ from the control plots. Microbial communities in semi-coke samples from bioaugmented plots, samples from control plots and sand treatment plots, and samples from peat treatment and no treatment plots generate three distinct groups. Dendrogram demonstrates that in addition to the effect of vegetation on the microbial communities in semi-coke, influence of the covering material or amendment type on the microbial community structure was also important. Based on the DGGE fingerprint analysis, the temporal dynamics of microbial community in semi-coke was less significant compared to treatment effect.

4. Discussion

It is very complicated to achieve biodegradation of organic pollutants in semi-coke compared to natural soils. Fresh semi-coke is classified as "very toxic", and old semi-coke of different age (after 10–50 years of deposition) are remarkably less toxic than fresh ones and are classified as "toxic" [23]. Estonian oil shale is rich in sulphur, and in the retorting process more than 50 % of it remains in the solid residue. In addition to sulphides, high initial pH value as well as high concentrations of Ca and Mg may also limit microbial activity in semi-coke.

Besides the inhibition of microbial activity in semi-coke by environmental conditions, some studies have revealed the toxicity of fresh semi-coke to plant seeds [24, 25]. Fresh semi-coke and water extracts from semi-coke were found to be very toxic to the germination and radicle growth of timothy (*Phleum pratense*) seeds. High electrical conductivity of semi-coke water extracts, mainly caused by calcium and other ions, were found to be the cause for the toxicity. Phytoremediation relies mostly on the survival of the plants because plants and rhizosphere play essential role in formation, metabolic activity and survival of microbial community and hereby rhizoremediation of organic pollutants in semi-coke. When excessive calcium ions are present in the rhizosphere solution, not only microbial activity is limited but also plants may suffer calcium toxicity which may prevent germination of seeds and reduce plant growth rates [26]. However, it has been also shown that mixing of semi-coke with acidic sphagnum peat and weathering decrease the inhibition effect of semi-coke. Trials on semi-coke heaps have revealed that seeds can easily germinate and grasses grow on semi-coke of several years of age as well as its weathered mixture with peat [24].

In addition to environmental conditions, bioavailability of contaminant to the microbial community is another factor influencing biodegradation of pollutants in semi-coke [27]. Plant roots may impact the desorption of xenobiotics by modifying soil properties in rhizosphere via changing pH or by release of phytosurfactants [28]. Microbial attack has been shown to occur primarily toward n- and iso-alkanes, then toward cycloalkanes and

1- to 3-ring aromatics, and finally towards polyaromatics, asphaltenes and resins [29, 30]. Among organic compounds found in semi-coke, asphaltenes are the most resistant fraction for bioremediation.

By using more than one method to study the microbial community, a more complete picture of the microbial community structure and change dynamics can be obtained. The result from plate counts, Biolog profiles and DGGE indicated that a shift in the microbial community in semi-coke occurred in the presence of plants. In our field experiment phytoremediation increased the number of phenol-degrading bacteria in semi-coke as well as metabolic diversity of microbial community. The degradation rates were the highest in the upper soil layer which has the highest root density [31]. Vegetation also increased 1.3–2.5 times the microbial biomass measured by fumigation-extraction methods by the end of the third year [32]. Since there were no significant differences in rates of degradation of pollutants among plots with vegetation, we may suggest that establishment of vegetation on semi-coke was a key factor for accelerating degradation of pollutants. Some studies have shown the different mechanisms of different plant species to facilitate phytoremediation. Kirk et al. [33] found that perennial ryegrass, for example, seems to support a general increase in microbial activity and numbers in the rhizosphere, some of which have catabolic activity towards petroleum hydrocarbons in petroleum-contaminated soil. Alfalfa, on the other hand, seems to specifically increase the number of microorganisms capable of degrading more complex hydrocarbons [33]. In our field experiment mixture of four plant species were used and during the three-year monitoring period the general trend was the increase of proportion of biodegradable bacterial numbers within microbial community due to the plant treatment. As a result of the bioaugmentation and phytoremediation, content of total organic carbon had decreased by up to 17 % compared to the control plots [32, 34]. It is generally recognized that enhanced biodegradation activity in the rhizosphere is due to rhizodeposition consisting of root exudates and root debris. However, recent studies indicate that plant may release glutathione conjugates formed during detoxification process into the rhizosphere where they could be metabolized by microbes [35].

Analysis of 16S rDNA DGGE fingerprints shows that vegetation also changed the microbial community structure in comparison with the control plots. In addition to the vegetation, properties of the substrate had an essential effect on the microbial community. It is generally accepted that bulk soil and rhizosphere microbial community structure is determined by the local native microbial community and, impacted by the soil effects and vegetation, affects only a small portion of the total bacteria [36]. As reported by Siciliano et al. some plants, when exposed to different contaminants, selectively enriched catabolic genotypes of microorganisms living within the rhizoplane, this mechanism would not only protect the plants from the toxic effect of the contaminant but also contribute to phytoremediation [37]. Although there was detectable temporal variation in microbial community composition in semi-coke samples in our field experiment, temporal dynamics of the microbial community in semi-coke was less important, and no similar trend in microbial community succession neither in plots with vegetation nor without plants were found.

Effective bioaugmentation has been shown in several studies based on wetlands and activated sludge systems [38, 39, 40]. Some studies have indicated that the best bioaugmentation performance can be achieved by the use of microorganisms that are already present in the soil, since indigenous microorganisms are well adjusted to their own environment [41, 42]. To enhance degradation of pollutants, fertilizers are sometimes used together with bioaugmentation [43]. Despite that it is still complicated to perform successful bioaugmentation and enhanced bioremediation activity, and increase in microbial counts has often been short-term [14].

In our field experiment the impact of bioaugmentation on the rate of the degradation of pollutants and on the functional microbial community occurred in the year of bioaugmentation application. Values of the biodegradation rates and microbial counts decreased a year later. The same temporal pattern was also detected when microbial community metabolic profile kinetic model parameters were compared between bioaugmented and non-treated plots. The difference in model parameters was bigger in the year of bacterial biomass application compared to the values obtained a year later [32].

Mode of action of bioaugmentation in soil may be either transfer of catabolic genes (plasmid mediated bioaugmentation) or survival of introduced bacterial strains. It is not clear whether and for how long period introduced bacterial strains survived in semi-coke. Also, it is not known what was the mechanism for the increased biodegradation rates in the year of biomass application. Two out of three introduced bacterial strains, used in our bioaugmentation experiment, contain plasmids which carry catabolic genes. Further research is needed to identify the mechanisms how introduced bacterial strains enhance degradation of pollutants in semi-coke. However, it is known that members of the same species do not all have equal fitness, and some are likely to be more competitive in a broader range of scenarios, while others may be more suited to specific conditions and habitats. Cunliffe et al. found that pretreatment of the inoculum had a dramatic impact on the survival, metabolic activity and PAH-catabolic gene expression of *Sphingomonas yanoikuyae* B1 in an aged PAH contaminated soil. The highest levels on *bph*C and *xyl*E expression were seen, and degradation of PAHs was significantly enhanced in soils which had been treated with inocula precultivated on complex medium. On the basis of results, they suggest using complex media instead of minimal media for cultivating bioaugmentation inocula, which may improve the subsequent efficiency of contaminant biodegradation in the soil [44].

Our previous studies have shown that in order to perform successful bioaugmentation it is important to select microbe or mixture of microbes, which possess necessary degradation pathway for contaminants. Biodegradation of the contaminant may result in the toxic

byproducts inhibiting complete mineralization of the contaminant. One solution is to use constructed mixture of microbes (microbial consortium), which contains bacteria with different catabolic properties based on the nature of the pollutant [45].

In addition to type and properties of inoculum used for bioaugmentation, soil abiotic and biotic factors may determine the survival and activity of the introduced microorganisms [46]. In the case of semi-coke the soil properties probably do not favor the survival of introduced bacteria, and exudates from plant roots alleviate this adverse effect.

5. Conclusions

Our results indicate that establishment of plants in the solid waste dump area soil of oil-shale chemical industry had pronounced impact on microbial community, shown by changes in taxonomic and metabolic diversity of bacterial community. Addition of specific biodegradative bacterial strains to soil resulted in enhanced biodegradation activity as well altered microbial community structure. On the basis of our findings, we conclude that phytoremediation and bioaugmentation could be considered as an alternative management option for remediation of oil shale solid waste.

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MIKROORGANIZMŲ DIRVOŽEMYJE KAITA VYKDANT FITOATKŪRIMO LAUKO EKSPERIMENTĄ

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S a n t r a u k a

Kasmet Estijoje naftos skalūnų chemijos pramonėje susidaro apytiksliai 600 000 t termiškai apdorotų naftos skalūnų kietųjų atliekų (pusiau kokso). Fitoatkūrimo ir biopapildymo lauko eksperimentas buvo vykdomas trejus metus naftos skalūnų chemijos pramonės kietųjų atliekų saugojimo zonoje. Pastebėta, kad padidėjo teršalų degradacijos greitis plotuose, kur yra augalijos, ir pridėta bakterinės biomasės. Lakiųjų fenolių koncentracija sumažėjo beveik 100 %, o naftos produktų koncentracija sumažėjo apytiksliai 3 kartus apsodintuose plotuose, palygti su kontroliniais plotais. Degradacijos greitis buvo didžiausias viršutinimame dirvožemio sluoksnyje, kuriame yra didžiausias šaknų tankis. Augalija taip pat pakeitė mikrobiologinės bendrijos struktūrą, palyginti su kontroliniais plotais. Be augalijos, dar ir substrato savybės turėjo didelės įtakos mikrobiologinei bendrijai.

Reikšminiai žodžiai: naftos skalūnai, chemijos pramonės kietosios atliekos, biopapildymas, fitoatkūrimas, mikrobiologinė bendrija.

СМЕНА МИКРООРГАНИЗМОВ В ПОЧВЕ ВО ВРЕМЯ ПОЛЕВОГО ЭКСПЕРИМЕНТА С ПРИМЕНЕНИЕМ ФИТОРЕМЕДИАЦИИ

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Р е з ю м е

В химической промышленности по обработке нефтяных сланцев Эстонии ежегодно примерно 600 000 т составляют термически обработанные твердые отходы нефтяных сланцев. Эксперимент по полевой фиторемедиации и биодополнению проводился в течение трех лет на территории хранения твердых отходов химической промышленности по обработке нефтяных сланцев. Установлено, что скорость деградации загрязнителей увеличивается на площадях, где имеется растительность и добавлена бактерицидная биомасса. На засаженных площадях концентрация летучих фенолей уменьшилась почти в два раза, а нефтяных продуктов – приблизительно в 3 раза по сравнению с контрольными площадями. Скорость деградации была наибольшей в верхнем слое почвы, в котором больше всего корней. Растительность также изменила структуру микробиологического сообщества по сравнению с контрольными площадями. Кроме растительности, существенное воздействие на микробиологическое сообщество оказали также свойства субстрата.

Ключевые слова: нефтяной сланец, твердые отходы химической промышленности, биодополнение, фиторемедиация, микробиологическое сообщество.

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