Oil Shale, 2003, Vol. 20, No. 3 SPECIAL
pp. 421-428
ISSN 0208-189X

ENHANCED BIODEGRADATION OF OIL SHALE CHEMICAL INDUSTRY SOLID WASTES BY PHYTOREMEDIATION AND BIOAUGMENTATION

J. TRUU*, E. TALPSEP, E. VEDLER
E. HEINARU, A. HEINARU

Institute of Molecular and Cell Biology,
University of Tartu
23 Riia St., Tartu 51010, Estonia

Field experiments were carried out in order to test the effect of phytoremediation and bioaugmentation for remediation of semi-coke. Four pilot test plots (each 50 m^2) were established at semi-coke depository in July 2001. The growth rate of plants was approximately twice higher in the case of soil amendment. The phytoremediation increased the number of bacteria and diversity of microbial community in semi-coke. Within one and a half year period starting from establishment of test plots, the concentration of phenolic compounds decreased up to 35 % and that of oil products up to three times compared to control. In bioaugmentation experiments the biomass of bacteria consisting of three strains isolated from nearby area was supplied in order to study the stability of microbial populations around root area favoring the biodegradation efficiency of phenolic and oil pollutants.

Introduction

More than 70 years of oil shale thermal processing has resulted in huge dump sites of the retorted shale (semi-coke) in the areas surrounding oil shale chemical industry plants in the northeastern part of Estonia. The semi-coke dumps cover an area about 200 ha and contain up to 100 million tons of solid waste. Although the production of oil shale energy and oil has decreased steadily during the last years with a corresponding decrease in wastes (12.7 million tons per year in 1995 to 9.4 million tons in 1999), the semi-coke dumps constitute one of the major adverse environmental challenges in Estonia [1].

Semi-coke solid wastes contain several organic and inorganic compounds (PAHs, oil products, sulfuric compounds), while liquid wastes (leachate) from the depository area are characterized by high concentration of phenol,

* Corresponding author: e-mail *jtruu@ebc.ee*

cresols, dimethylphenols and resorcinols. The liquid pollution from semi-coke dump area deteriorates surface water as well as the underlying aquifers [2].

Phytoremediation is an emerging technology based on the combined action of plants and their associated microbial communities to degrade, remove, inactivate or immobilize toxic compounds in soil. Plants may act directly accumulating (phytoaccumalution) and in some cases metabolizing (phytodegradation) pollutants [3], but one way to achieve actually *in situ* bioremediation is by utilizing plants to perform rhizosphere bioremediation (rhizodegradation or phytoremedition *explanta*) [4]. Vegetation facilitates bioremediation of contaminated soils due to root exudates that act as substrates for soil microorganisms, increasing in this way abundance and diversity of microbial community [5]. Phytoremediation has been proposed as an efficient, low-cost remediation technique to restore areas contaminated with chlorinated solvents, BTEX compounds, phenols and PAHs [6–9].

Another possibility to achieve higher biodegradation rate of pollutants in soil is by introducing specific strains or microbial consortia. This approach, called bioaugmentation delivers to contaminated environment specific catabolic traits necessary for the clean up of pollutants [10].

In the current study several grass species and different soil amendments were evaluated in order to test the effect of phytoremediation (use of plants to enhance microbial biodegradation) as well as bioaugmentation (the inoculation of a contaminated site with naturally occurring microorganisms) for remediation of semi-coke.

Material and Methods

Phytoremediation Experiment

Four pilot test plots (each 50 m^2) were established at semi-coke depository in July 2001. Plant treatment was based on a grass mixture of four species. In addition to plants, four different treatments were utilized. The following treatments were applied: 1st plot – no treatment (grass seeds in semi-coke), 2nd plot – seeds in semi-coke were covered by sand layer (1-2 cm), 3rd plot – seeds in semi-coke were covered by peat layer (1-2 cm), 4th plot – semi-coke was covered with the layer of pre-grown lawn. In October 2001 and 2002, the soil sampling was performed on plots and control area. We analyzed semi-coke samples collected from the test plots at the depository area for chemical and microbiological parameters.

Bioaugmentation Experiment

For the bioaugmentation experiment the set of bacteria consisting of three strains isolated from nearby 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 [11]. In bioaugmentation experiments the biomass of these bacteria was supplied to the part experimental plots (each 10 m^2) in July 2002. Each treatment received 20 L of bacterial suspension with concentration 10^8 CFU ml^{-1} (CFU – colony-forming units). The ratio of bacterial strains PC1, PC18 and PC24 was 3 : 1 : 1 in suspension.

Chemical Analysis

From each experimental plot pooled soil samples (20 subsamples) were collected with soil corer from two depths: 0–10 and 0–20 cm. Oil products (extracted with pentane) were measured by gas chromatography. Volatile phenols were measured spectrophotometrically and by high-pressure liquid chromatography (HPLC). Total organic carbon was determined with infrared spectrophotometer. Chemical analyses were performed by Tartu Environmental Research Ltd. and Estonian Environmental Research Center.

Microbiological Methods

The microbial communities were removed from semi-coke and plant root surface by vortexing in sterile tap water. 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). Agar plates were incubated at 22 °C for one week and the numbers of biodegradative bacteria were expressed as CFU per gram of dry soil. The heterotrophic activity and diversity of microbial community was measured using *Biolog EcoPlates* (Biolog, Inc.). Each well of the *Biolog EcoPlates* was inoculated by 150 μL of extract (10^{-2} dilution) and the plates were incubated at 25 ºC. Color development was measured every 24 h for 120 h as absorbance at 590 nm, with scanning spectrophotometer *Multiscan* 340C. Results of *Biolog* profiles were presented as total activity (summed absorbances of all 31 wells) and by Shannon diversity index.

Results and Discussion

Chemical properties of the semi-coke from the experimental area are shown in Table 1. After retorting at 500 ºC, processed oil shale is highly saline, alkaline, biologically sterile, nutrient deficient material with no structure. According to ecotoxicological tests the fresh semi-coke is classified as of "high-acute toxic hazard", whereas aged semi-coke is classified as of "acute toxic hazard" [12].

Table 1. **Chemical Properties of Semi-Coke from the Control Plot in 2001**

Parameter	Measured value
pH	8.0
Total nitrogen, %	0.08
$P\text{-}PO_4^{3-}$, mg kg^{-1}	12.27
K^+, mg kg^{-1}	799.1
Ca^{2+}, mg kg^{-1}	18,673
Mg^{2+}, mg kg^{-1}	826
Total carbon by loss on ignition at 550 °C, %	18.2
Total organic carbon, %	17.0
Oil products, mg kg^{-1}	310
Volatile phenols, mg kg^{-1}	5.3

The chemical analysis of soil samples showed impact of the plant treatment on degradation rate of pollutants. Within a 16-months period starting from the establishment of test plots in July 2001, the concentration of volatile phenols decreased up to 1.5 times (from 5.3 to 3.4 mg kg^{-1}), the concentration of oil products nearly 3 times (from 310 to 120 mg kg^{-1}), and the total content of organic carbon decreased by 10 to 30 g per kg (from 18 to 15 %). The best results were obtained on the plots with peat amendment and pregrown lawn with the highest root density in semi-coke. In upper layer samples (0–10 cm) the reduction of oil products and phenols was even more bigger being in the range from 83 to 98 %.

Bacterial biomass consisting of three bacterial strains was applied to three experimental plots in June 2002. Within a three-months period the concentration of residual shale oil in semi-coke decreased by 13.6 to 53.6 % at plots treated with bacterial biomass compared to untreated parts of experimental plots (Fig. 1).

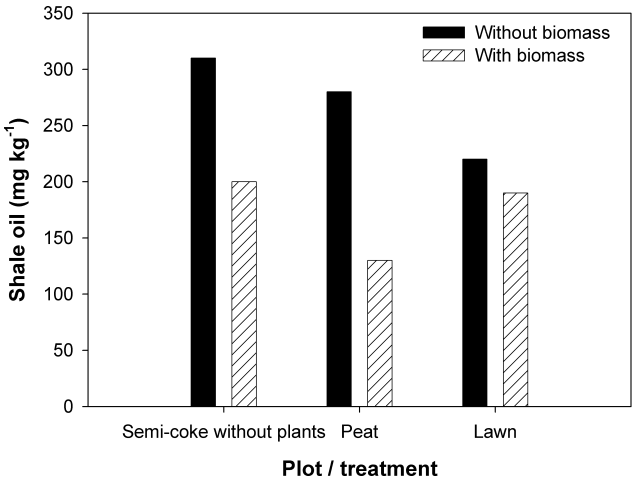

Fig. 1. Effect of bioaugmentation on concentration of shale oil in semi-coke

In the case of the plot without vegetation the decrease in concentration of volatile phenols was also remarkable (from 5.3 to 3.8 mg kg^{-1}). The effect of bioaugmentation could be attributed either to activity of introduced bacterial strains or to horizontal transfer of biodegradative genes. The genetic information encoding degradation of pollutants is transferred from inoculated strains to indigenous bacterial populations by catabolic genetic elements [13]. Introduced bacterial strains may also increase root biomass, length and/or exudation leading in this way to enhanced rhizosphere bioremediation. In our experiments we also observed the increase in root biomass and length on the plots amended with bacterial biomass, which in turn may lead to enhanced rhizodegradation.

From the semi-coke samples the number of aerobic heterotrophic and phenol-degrading bacteria was determined. The number of phenol-degrading bacteria increased by order of magnitude, while the number of heterotrophic aerobic bacteria remained on the same level compared to the untreated plot (Table 2). Samples from the second year (2002) showed lower values of aerobic heterotrophic bacteria, which could be due to extremely dry vegetation period. The general trend was the increase of proportion of biodegradable bacterial numbers within microbial community due to the treatment. Highest values for all measured microbiological parameters were found in rhizosphere samples. While bacterial total numbers increased by order of magnitude compared to control, the number of phenol-degrading bacteria was more than 100 times higher in the rhizospheric soil. Addition of bacterial biomass to semi-coke resulted in increase both in absolute number (up to 7.8×10^6 CFU g^{-1}) and relative abundance (up to 30 %) of phenol-degrading bacteria in the studied samples. The highest values for microbial activity and diversity measured with *Biolog EcoPlates* were recorded in rhizosphere samples (Fig. 2).

Table 2. **Effect of Treatment on Microbiological Parameters of Semi-Coke, CFU g^{-1}**

Treatment/sample	Aerobic heterotrophic bacteria		Phenol-degrading bacteria	
	2001	2002	2001	2002
Control	12×10^7	8.2×10^6	2.3×10^4	1.0×10^4
Control with biomass		6.9×10^6		1.3×10^4
Grass only	1.5×10^7	4.2×10^6	3.0×10^5	1.5×10^4
Peat + grass	1.1×10^7	5.7×10^6	4.5×10^4	6.5×10^5
Peat + grass/rhizosphere	5.3×10^7	1.3×10^8	4.3×10^6	2.2×10^6
Peat + grass with biomass		6.0×10^6		1.9×10^6
Peat + grass with biomass/rhizosphere		5.7×10^7		7.8×10^6
Sand + grass	1.1×10^7	1.5×10^6	3.3×10^5	3.7×10^3
Lawn	1.1×10^7	5.6×10^6	3.5×10^5	1.2×10^4
Lawn/rhizosphere	4.8×10^7	6.5×10^7	2.3×10^6	1.4×10^5
Lawn with biomass		1.0×10^7		3.2×10^5
Lawn with biomass/rhizosphere		1.0×10^8		2.6×10^6

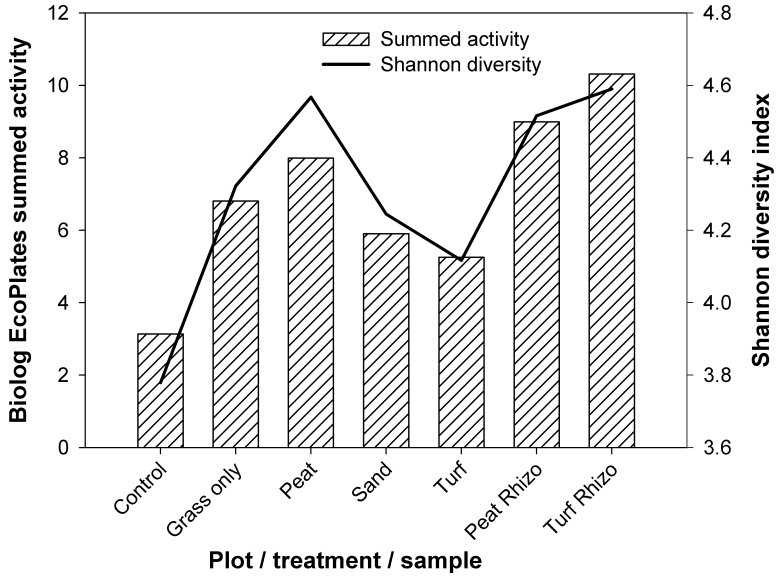

Fig. 2. Effect of treatment of microbial community metabolic activity and diversity of semi-coke. Rhizosphere samples are indicated by label Rhizo

From four tested grass species *Lolim perenne* (perennial ryegrass), *Poa pratensis* (Kentucky bluegrass) and *Festuca rubra* (red fescue) were found growing on plots. The growth rate of plants was approximately twice higher in the case of peat and sand amendments. The mechanism of phytoremediation systems, based on rhizodegradation, is increase in microbial numbers and activity due to nutrient release by plants. This scenario has been suggested by many researchers [14, 15]. Recently Joner and coworkers [16] showed that artificial root exudates increased bacterial numbers and promoted PAH dissipation in soil. There are also field evidence that PAH disappearance is related to plant root system and their microbially active rhizosphere [17]. Our data suggest that establishment of plants promoted increase of biodegradative bacterial number in semi-coke, especially in the vicinity of roots.

Conclusions

In summary, our results indicate that phytoremediation could be considered as an alternative management option for remediation of oil shale solid waste. Our preliminary field experiments show that presence of plants accelerates removal of oil products and phenols in oil shale chemical industry solid waste. There are several additional beneficial side effects of establishment of vegetation on semi-coke depository. Besides avoiding the surface erosion

and reducing the amount and toxicity of leachate, plant cover probably diminishes the dispersion of pollutants into adjacent areas by air. Our findings also indicate that biodegradation of residual shale oil as well as plant growth could be enhanced by adding mixture of selected bacterial strains to semicoke.

Acknowledgements

The study was funded by the Maj and Tor Nessling Foundation and partly by the Estonian Science Foundation Grant No. 4344.

References

1. Estonian Environment Information Center. State of Environment in Estonia – on the threshold of XXI century. – Tallinn, 2001.
2. *Truu, J., Heinaru, E., Talpsep, E., Heinaru, A*. Analysis of river pollution data from low-flow period by means of multivariate techniques: A case study from the oil-shale industry region, Northeastern Estonia // Environmental Science and Pollution Research. 2002. No. 1. P. 8–4.
3. *White, P*. Phytoremediation assisted by microorganisms // Trends Plant Sci. 2001. Vol. 6. P. 502.
4. *Alkorta, I., Garbisu, C.* Phytoremediation of organic contaminants in soil // Bioresource Technology. 2001. Vol. 79. P. 273–276.
5. *Romantschuk M., Sarand I., Petänen T., Peltola R., Jonsson-Vihanne M., Koivula T., Yrjälä K., Haahtela K*. Means to improve the effect of *in situ* bioremediation of contaminated soil: an overview of novel approaches // Environmental Pollution. 2000. No. 107. P. 179–185.
6. *Harvey P., Campanella B., Castro P., Harms H., Lichtfouse E., Schaffner A., Smrcek S., Werck-Reichhart D.* Phytoremediation of polyaromatic hydrocarbons, anilines, and phenols // Environ Sci. Pollut. Res. Int. 2002. Vol. 9. P. 29–47.
7. *Lin, Q., Mendelssohn, I.* The combined effects of phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands // Ecological Engineering. 1998. Vol. 10. P. 263–74.
8. *Sarand I., Timonen S., Koivula T., Peltola R., Haahtela K., Sen R. Romantschuk M*. Tolerance and biodegradation of *m*-toluate by Scots pine, a mycorrhizal fungus and fluorescent pseudomonads individually and under associative condition // J. Appl. Microbiol. 1999. Vol. 86. P. 817–826.
9. *Suominen L., Jussila M., Mäkeläinen K, Romantschuk M., Lindström K*. Evaluation of the Galega-Rhizobium galegae system for the bioremediation of oil-contaminated soil // Environmental Pollution. 2000. Vol. 107. P. 239–244.
10. *Dejonghe, W., Goris J., Dierickx, A., Dobbeleer, V., Crul, K., Vos, P., Verstraete, W., Top, E.* Diversity of 3-chloroaniline and 3,4-dichloro-aniline degrading bacteria isolated from three different soils and involvement of their plasmids in chloroaniline degradation // FEMS Microbiology Ecology. 2001. Vol. 42. P. 315–325.

11. *Heinaru, E, Truu, J., Stottmeister, U., Heinaru, A.* Three types of phenol and *p*-cresol catabolism in phenol- and *p*-cresol-degrading bacteria isolated from river water continuously polluted with phenolic compounds. // FEMS Microbiology Ecology. 2000. Vol. 31. P. 195–205.
12. *Põllumaa, L., Maloveryan, A., Trapido, M., Sillak, H., Kahru, A.* A study of the environmental hazard caused by the oil shale industry solid waste // ATLA. 2001. Vol. 29. P. 259–267.
13. *Top, E., Springael, D., Boon, N.* Catabolic mobile genetic elements and their potential use in bioaugmentation of polluted soils and waters. FEMS Microbiology Ecology. 2002. Vol. 42. P. 199–208.
14. *Anderson, T., Walton, B.* Comparative fate of 14C-trichloroethylene in the root zone of plants from a former solvent disposal site // Environ Toxicol Chem. 1995. Vol. 14. P. 2041–2047.
15. *Haby, P., Crowley, D.* Biodegradation of 3-chlorobenzoate as affected by rhizodeposition and selected carbon substrates // J. Environ. Qual. 1996. Vol. 25. P. 304–310.
16. *Joner, E., Corgie, S., Amellal, N, Leyval,L.* Nutritional constraints to degradation of polycyclic aromatic hydrocarbons in a simulated rhizosphere // Soil Biol. Biochem. 2002. Vol. 34. P. 859–864.
17. *Olson, P., Fletcher, J., Philo, P.* Natural attenuation/phytoremediation in the vadouse zone of a former industrial sludge basin // Environmental Science and Pollution Research. 2001. Vol. 8. P. 243–249.