DISSERTATIONES TECHNOLOGIAE CIRCUMIECTORUM
UNIVERSITATIS TARTUENSIS
10

DISSERTATIONES TECHNOLOGIAE CIRCUMIECTORUM
UNIVERSITATIS TARTUENSIS
10

JAANIS JUHANSON

Impact of phytoremediation and bioaugmentation on the microbial community in oil shale chemical industry solid waste

TARTU UNIVERSITY
PRESS

Department of Genetics, Institute of Molecular and Cell Biology, Faculty of Science and Technology, University of Tartu, Estonia.

Dissertation is accepted for the commencement of the degree of *Doctor philosophiae* (in environmental technology) at the University of Tartu on May 7th, 2010 by the Scientific Council on Environmental Technology, Faculty of Science and Technology, University of Tartu.

Supervisor: Jaak Truu, PhD (Institute of Molecular and Cell Biology, University of Tartu).

Opponent: Kim Yrjälä, PhD (Department of Biological and Environmental Sciences, University of Helsinki).

Commencement: Room No. 204, 18 Ülikooli str., Tartu, on June 21th in 2010, at 14.15.

Publication of this dissertation is granted by the Graduate School of Biomedicine and Biotechnology, University of Tartu.

ISSN 1736–3349
ISBN 978–9949–19–386–8 (trükis)
ISBN 978–9949–19–387–5 (PDF)

Tartu Ülikooli Kirjastus
www.tyk.ee
Tellimus nr 275

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I **Truu J, Heinaru E, Vedler E, Juhanson J, Viirmäe M, Heinaru A**: Formation of microbial communities in oil shale chemical industry solid wastes during phytoremediation and bioaugmentation. In *Bioremediation of Soils Contaminated with Aromatic Compounds*, H. J. Heipieper (ed.). 2007, 76, p 57–66.

II **Juhanson J, Truu J, Heinaru E, Heinaru A**: Temporal dynamics of microbial community in soil during phytoremediation field experiment. *J Environ Eng Landsc Manag* 2007, 15, 4:213–220.

III **Juhanson J, Truu J, Heinaru E, Heinaru A**: Survival and catabolic performance of introduced *Pseudomonas* strains during phytoremediation and bioaugmentation field experiment. *FEMS Microbiol Ecol* 2009, 70, 3:446–455.

My contribution to articles of current dissertation is following:
Ref. I – participation in soil sampling, laboratory experiments and data analyses.
Ref. II – participation in soil sampling, laboratory experiments, data analyses and article preparation.
Ref. III – participation in soil sampling, laboratory experiments, data analyses and article preparation.

ABBREVIATIONS

PAH	Polyaromatic hydrocarbon
MTBE	Methyl tert-butyl ether
TCE	Trichloroethylene
PCB	Polychlorinated biphenyl
2,4-D	2,4-Dichlorophenoxyacetic acid
PCP	Pentachlorophenol
BTEX	Acronym that stands for benzene, toluene, ethylbenzene, and xylenes
TNT	2,4,6-trinitrotoluene
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazene
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine
GC	Gas-liquid chromatography
GC-MS	Gas chromatography-mass spectrometry
HPLC	High-performance liquid chromatography/High pressure liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
D/TGGE	Temperature/Denaturing gradient gel electrophoresis
(T-)RFPL	(Terminal) restriction fragment length polymorphism
SSCP	Single-strand conformation polymorphism
SSU rRNA	Small subunit ribosomal ribonucleic acid
CFU	Colony forming unit
(Q)PCR	(Quantitative) polymerase chain reaction
AHB	Aerobic heterotrophic bacteria
PDB	Phenol degrading bacteria
PCA	Principal component analyses

I. INTRODUCTION

Oil shale mining, chemical processing (thermal treatment) and energetic use produce majority of solid waste in Estonia. Mining of oil shale in Estonia started in 1916 and reached its peak in 1980 when 31 million tons of oil shale were excavated per year (Kattai and Lokk, 1998). Nowadays the government allows to excavate up to 20 million tons of oil shale annually. The mining and processing of 1,000 million tons of oil shale up to now has been accompanied by the deposition of about 400 million tons of solid waste: more than 90 million tons of mining waste, ca 100 million tons of semi-coke (oil and chemical industry waste) and 200 million tons of combustion ashes (power generation waste). Currently ca 1.7 million tons of oil shale is treated thermally annually producing more than 200,000 tons of oil and oil shale chemicals. In addition, approximately 700,000 tons of semi-coke solid waste is disposed every year. As a result of oil shale thermal treatment during 85 years, semi-coke mounds cover an area about 180–200 ha in the north-eastern part of Estonia. Although semi-coke is produced in much smaller amounts than combustion ashes, semi-coke mounds are the most severe environmental concern in Estonia since semi-coke consists of high amount of different form of organic carbon that may pose hazard to the environment due to leaching of toxic compounds to both the surface water and the underlying aquifers, as well as the possibility of self-ignition. In addition, depositories have been historically used for dumping different wastes (e.g. oil pitch, waste sludge) therefore leachates from the depository area contain high concentration of oil products, phenol, cresols, dimethylphenols and resorcinols (Truu et al., 2002). According to the chemical properties and ecotoxicological tests fresh semi-coke is classified as hazardous waste while several years old semi-coke is practically neutral and considerably less toxic due to being rain-washed (Kahru and Põllumaa, 2006).

The storage of semi-coke in open dumps, as the present situation has developed, is not in accordance with European Union regulations and these landfills must be closed by 2013. A standard approach to cover the landfill involves the utilization of different substrate layers like isolation, drainage and cover layer. As a result, the diffusion of pollution to the ground and surface waters and air is prevented. The problem with the standard solution is that only the isolation of the source of pollution is performed without further remediation of the pollutants. The number of alternative solutions is however limited due to the strict regulations on toxic wastes. Since only fresh semi-coke solid waste is classified as toxic waste, different management options for remediation can actually be considered for certain parts of the depository to truly achieve the degradation of the contaminants. Because of the enormous amount of the solid waste, the only feasible ways for the remediation include different technologies, which can be performed on site or with minimal need to move the waste.

A variety of remediation technologies are available for on-site remediation. Soil vapour extraction, landfarming, bioventing, thermal desorption, biopiles are *in situ* remediation technologies, which have been used as real life appli-

cations for soil (reviewed by Khan et al., 2004). However, no single technology is appropriate for all contaminant types and the variety of site-specific conditions which exist at different contaminated sites. Site conditions, contaminant type, contaminant source, source control measures, and the potential impact of the possible remedial measure determine the choice of a remediation strategy and technology. Often more than one remediation technology is needed to effectively address contaminated site problems (Khan et al., 2004). In recent decades, phytoremediation as a cost effective and environmentally friendly technology has been used successfully for the remediation of soils contaminated with various pollutants.

2. THE AIM OF THE STUDY

The general aim of this thesis is to assess the feasibility of combination of phytoremediation and bioaugmentation as an alternative option for the remediation of semi-coke solid waste. Together with the use of vegetation, our purpose was to determine and target the kind of microorganisms in sampled habitats which are likely to suit specific conditions and remedial requirements. Among those temporally and spatially prevalent microbial populations, strains which can degrade a specific contaminant are selected and identified for the development of a collection of biodegradative microbes that can be used together with vegetation and fertilizers for the remediation of oil shale solid wastes (Figure 1). As a result of the remediation approach based on simultaneous use of plants (phytoremediation) and specific consortium of bacteria (bioaugmentation) concentration of pollutants will be reduced and semi-coke heaps will be covered with vegetation that prevents soil erosion and decreases amount of leachate.

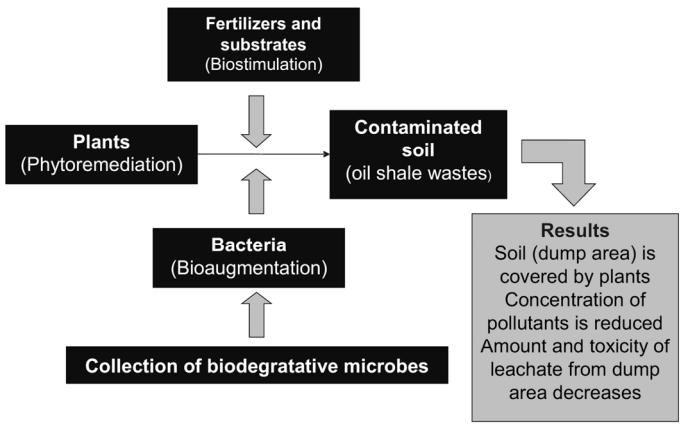

Figure 1. Principal scheme of technological approach for remediation of oil shale chemical industry solid waste dump area.

The specific aims were:

- to study the impact of vegetation and introduced bacterial strains on the concentrations of pollutants as well as on the metabolic activity of indigenous microbial community in soil.
- to study the impact of vegetation and introduced bacterial strains on the dynamics of bacterial numbers and bacterial community composition in soil.
- to study the survival and catabolic performance of the introduced bacterial strains in soil for a prolonged period.

3. LITERATURE REVIEW

3.1. Phytoremediation

According to Cunningham and Berti (1993) phytoremediation is defined as the use of green plants to remove, contain, or render harmless environmental contaminants. In this process specially selected or genetically engineered plants are used which are capable of direct uptake of pollutants from the environment (Macek et al., 2000). Generally, phytoremediation of contaminants by a plant involves steps like uptake, translocation, transformation, compartmentalization, and sometimes mineralization (Schnoor et al., 1995). Factors affecting the uptake, distribution and transformation of organic compounds by a plant are mainly related to the physical and chemical properties of the compound (water solubility, molecular weight, octanol-water partition coefficient), as well as environmental conditions (e.g. temperature, pH, organic matter, and soil moisture content) and plant characteristics (e.g. root system, enzymes) (Suresh and Ravishankar, 2004; Susarla et al., 2002). Phytoremediation can be applied to both inorganic and organic pollutants present in solid and liquid substrate (Salt et al., 1998). Although the designations of different phytoremediation strategies vary in literature, the principal scheme is given in Figure 2.

Inorganic contaminants (heavy metals and radionuclides) can be either taken up from the soil and immobilized by the roots (**phytoimmobilization**), or transported to the plant shoot (**phytoextraction**) (Reichenauer and Germida, 2008). Since under most circumstances there is rather low bioavailability of metals in soil, including some metals that are essential to life, plants possess highly effective metal uptake system using transporter molecules such as zinc-regulated transporter protein, copper transporter protein etc. (Krämer et al., 2007). In addition, plants are capable of secreting metal-chelating molecules like siderophores and organic acids (malate, citrate), and biosurfactants such as rhamnolipids to the surrounding soil, but also extruding protons from the roots to acidify the soil and mobilize soil bound metals (Fig. 2) (Eapen and D'Souza, 2005; Garbisu and Alkorta, 2001). Inside the plant, heavy metals cannot be biodegraded but are only transformed from one oxidation state or organic complex to another (Garbisu and Alkorta, 2001). As a result, metals tend to accumulate in the plant. Nearly 450 hyperaccumulator plants ranging from annual herbs to perennial shrubs and trees (e.g. tobacco, sunflower, mustard, maize, pennycress, brake fern, Russian thistle, rattlebush, python tree, willow, poplar) have been described to accumulate and detoxify extraordinary high levels of metal ions, such as Ni, Co, Pb, Zn, Mn, Cd, etc. in their above ground tissues (Meagher, 2000; Padmavathiamma and Li, 2007; Shah and Nongkynrih, 2007; Sheoran et al., 2009). It has been suggested, that the prevention of herbivory and disease may be the main function of hyperaccumulation for the plant (Fattorini et al., 2010; Shah and Nongkynrih, 2007). Still, in this case it is possible to harvest and remove plants from the site after remediation for disposal or recovery of the contaminants (Susarla et al., 2002). For some

inorganic elements (Hg, As, Se) uptake by roots followed by transport to the shoot and transpiration to the atmosphere through the leaf stomata (**phyto-volatilization**) have been observed (Padmavathiamma and Li, 2007; Pilon-Smits, 2005). Since the volatile forms of Hg and Se are also toxic, it is questionable whether the volatilization of these elements into the atmosphere is desirable or safe (Padmavathiamma and Li, 2007; Watanabe, 1997).

Organic pollutants in soil like chlorinated solvents and polyaromatic hydrocarbons (PAHs) can be taken up and immobilized by plant roots (Gao and Zhu, 2004) as well as transpired from the shoot (methyl tert-butyl ether – MTBE, trichloroethylene – TCE, ethyl-benzene, xylene) (Ma and Burken, 2003; Ma et al., 2004). In addition, plants are capable of metabolizing organic contaminants (**phytodegradation**). The metabolism of contaminants by a plant can be divided into three phases: transformation, conjugation and compartmentalization (Fig. 2). In the transformation phase, contaminant is chemically modified (oxidation, reduction, and hydrolysis) and transformed into more polar, water soluble form by enzymes such as cytochrome P450 or carboxylesterases. By conjugation with endogenous molecules like sugars or peptides, the transformed contaminant is made less phytotoxic by glycosyltransferases and glutathione S-transferases, followed by compartmentalization phase where contaminant is transferred to the various compartments of the cell (storage in the vacuole or integration into cell wall) or in some cases excreted from the cell (Eapen et al., 2007; Ma and Burken, 2003; Reichenauer and Germida, 2008). However, there is a principle difference between metabolism of contaminants by a plant and by microorganisms – most contaminants are not utilized as a source of C, N and energy by plant since plants do not possess complete catabolic pathways for degradation and mineralization of pollutants (Eapen et al., 2007; Schroder and Collins, 2002). Frequently, during the degradation process even more toxic by-products (from the human point of view) may be produced compared to the initial pollutant. For instance, the transformation of TCE into trichloroethane, or the release of some metabolites from volatile pollutants into the environment by evapotranspiration have been detected (Burken and Schnoor, 1998; Ma and Burken, 2003). Only a few contaminants, for example PCBs, PAHs, nitro-aromatics and linear halogenated hydrocarbons can be completely mineralized by plants such as poplar, willow, alfalfa and different grass varieties (Kuiper et al., 2004; Meagher, 2000).

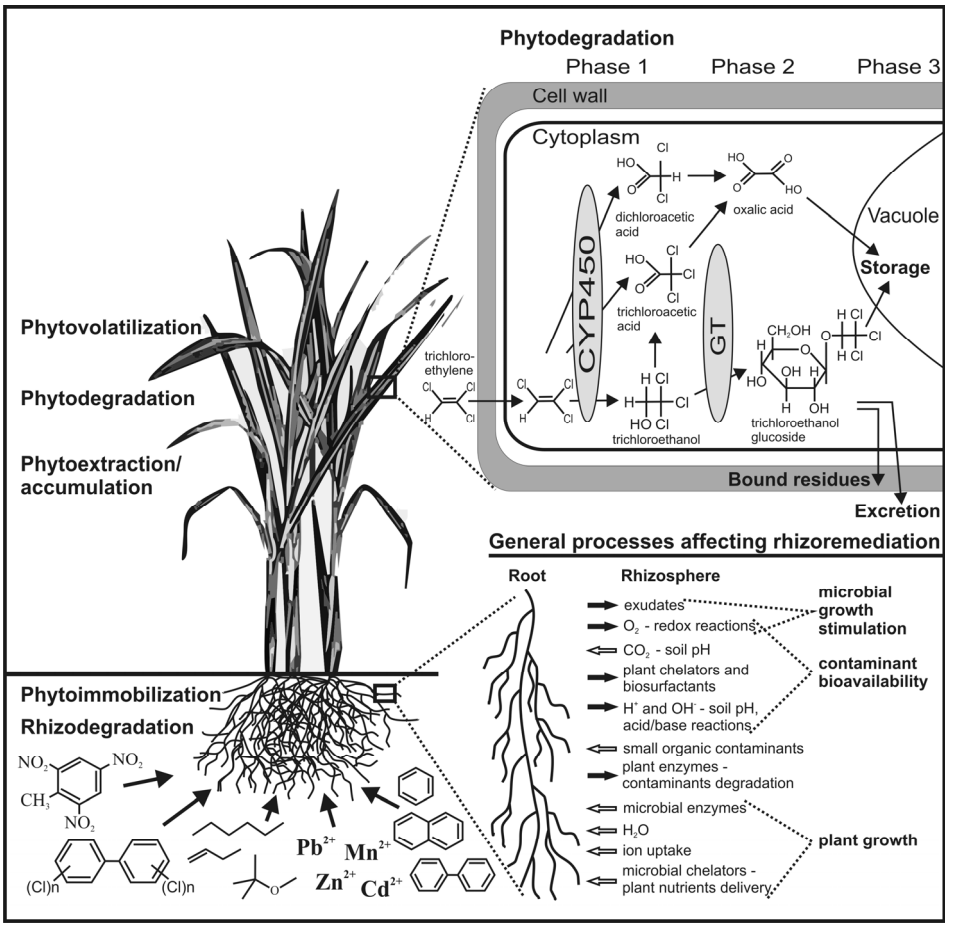

Figure 2. Phytoremediation of various organic and inorganic pollutants in soil. Plants are capable of removing organic and inorganic contaminants from soil by roots (**phyto-immobilization**), but also transporting and concentrating them in the harvestable part of the plant (**phytoextraction/accumulation**). In some cases transpiration to the atmosphere through leaf stomata may follow (**phytovolatilization**). Organic contaminants can be metabolized inside the plant (**phytodegradation**) in three sequential steps (phase 1 – transformation, phase 2 – conjugation, phase 3 – compartmentalization) using enzymes, such as CYP450 – cytochrome P450; GT – glycosyltransferase, resulting in the storage of the contaminant in the vacuole, integration into the cell wall, or excretion from the cell. In addition, organic contaminants can be degraded by plant-associated microorganisms in the rhizosphere (**rhizodegradation**). Plants facilitate the biodegradation of contaminants by releasing root exudates and other compounds to the surrounding soil as well as providing surface for the colonization of microbes, contributing in this way to the increased number and metabolic activity of microorganisms (rhizosphere effect) and enhanced bioavailability of the contaminant. Figure 2 is modified from Gerhardt et al., 2009; Reichenauer and Germida, 2008; Sheoran et al., 2009.

Transgenic plants can be developed by transferring genes from organisms which have the potential for degradation/mineralization of xenobiotic pollutants to candidate plants to improve the ability of plants to degrade/metabolize xenobiotic pollutants. Genes involved in the degradation of xenobiotic pollutants can be isolated from bacteria/fungi/animals/plants and introduced into candidate plants using *Agrobacterium* mediated or direct DNA methods of gene transfer (Eapen et al., 2007). Specific catabolic genes essential for the degradation of a contaminant are overexpressed in a plant, resulting in enhanced phytoremediation. For example, transgenic tobacco, arabidopsis, mustard, poplar, rice, potato have been reported to be able to improve phytoextraction, phytovolatilization and phytodegradation of heavy metals and organic contaminants like explosives, chlorinated solvents, PAHs, polychlorinated biphenols, various herbicides, and atrazine (Cherian and Oliveira, 2005; Eapen et al., 2007; Rylott and Bruce, 2009). The most recent and very promising approach to improve phytoremediation ability is the construction of plants with enhanced secretion of enzymes capable of degrading xenobiotics into the rhizosphere (Abhilash et al., 2009; Gerhardt et al., 2009). The advantage of this method is that the plants do not need to take up the pollutants in order to detoxify them; instead, the secreted enzymes can degrade the pollutants in the rhizospheric zone (Kawahigashi, 2009). However, there are strict regulatory restrictions for *in situ* applications of genetically modified organisms in the European Union and promising results have been obtained only in the laboratory and greenhouse experiments.

3.1.1. Rhizosphere effect and rhizodegradation

In addition to the plant metabolic capacity for the degradation of contaminants, plants have another important function in phytoremediation – plant roots establish favourable conditions for the microbes in rhizosphere, in this way facilitating the biodegradation of the contaminants (**rhizodegradation**). The term "rhizosphere" describes the portion of soil where microorganism-mediated processes are under the influence of the root system. In comparison to the bulk soil, increased metabolic activity, number and in certain cases, phylogenetic diversity of species of microorganisms can be found on the surface and in the vicinity of roots (Kuiper et al., 2004). This "rhizosphere effect" is mainly caused by the chemical impact (root exudates), supported by the physical (i.e. gas exchange, soil moisture) impact of plant roots on the soil (Fig. 2) (McCully, 1999). Root exudates contain organic acids (lactate, acetate, oxalate, succinate, fumarate, malate, and citrate), sugars and amino acids as main components but also secondary metabolites (isoprenoids, alkaloids, and flavonoids) which are released to the soil as rhizodeposits (Jones, 1998; Singer et al., 2003; Singh et al., 2004). The amount and composition of root exudates is specific to plant family or species. It has been suggested that 10–44% of the photosynthetically fixed carbon is excreted by rhizodeposition (Bais et al., 2006; Kumar et al.,

2006). As a result, nutrient rich environment in the vicinity of roots is created. Root exudates can be used as an energy source by microorganisms but since the structure of many secondary metabolites resembles those of contaminants, root exudates can also induce the expression of specific catabolic genes in microorganisms necessary for the degradation of the contaminant (Singer et al., 2003). This is important under many circumstances where microorganism cannot rely on energy gain from the contaminant and cometabolism is the only route for the degradation of contaminant. For instance, plant secondary metabolite salicylate has been linked to the microbial degradation of PAHs (naphthalene, fluoranthene, pyrene, chrysene) and PCB (Chen and Aitken, 1999; Master and Mohn, 2001; Singer et al., 2000), while terpenes can induce the microbial degradation of toluene, phenol, TCE (Kim et al., 2002). Therefore, roots can regulate the soil microbial community by producing and releasing root exudates and exoenzymes to the surrounding soil as well as providing surface for the colonization of microbes resulting in enhanced biodegradation of the contaminants. In turn, rhizosphere microorganisms provide plant with nitrogen, phosphorus and other minerals through decomposition of soil organic matter (Fig. 2). Rhizodegradation applies to a wide range of contaminants including those which due to their physicochemical properties are taken up by plants only in very small amount (higher-ring PAHs as an example; Reichenauer and Germida, 2008). In many cases, rhizosphere microbes are the main contributors to the degradation process.

A recent strategy to improve phytoremediation and detoxification of contaminants is the use of endophytic bacteria. Endophytic bacteria are described as non-pathogenic bacteria and they seem to have a ubiquitous existence in most if not all higher plant species. They often belong to genera commonly found in soil, including *Pseudomonas*, *Burkholderia*, *Bacillus* and *Azospirillum* (Lodewyckx et al., 2002; Moore et al., 2006; Yrjala et al., 2010). Endophytic bacteria are also known to have plant growth promoting and pathogen control activities (Berg et al., 2005; Ryan et al., 2008). A major advantage of using endophytic bacteria over rhizospheric bacteria in phytoremediation is that while a rhizospheric bacterial population is difficult to control, and competition between rhizospheric bacterial strains often reduces the number of the desired strains (unless metabolism of the pollutant is selective), the use of endophytes that naturally inhabit the internal tissues of plants reduces the problem of competition between bacterial strains (Doty, 2008; McGuinness and Dowling, 2009). Studies suggest that these bacteria can be used to complement the metabolic potential of their host plant through direct degradation (Barac et al., 2004; Germaine et al., 2006; Phillips et al., 2008; Phillips et al., 2009) as well as transfer of degradative plasmids to other endophytes (Taghavi et al., 2005; Wang et al., 2007). To date, many successful cases of phytoremediation of various contaminants using rhizospheric or endophytic bacteria have been reported (Table 1).

Table 1. Examples of successful phytoremediation cases of different contaminants using rhizospheric bacteria (RH) or endophytic bacteria (EN).

Microbes	Plants	Contaminant	Strategy	Reference
Pseudomonas sp.	Sugar beet	PCBs	RH	(Villacieros et al., 2005)
	Rockcress	PCBs	RH	(Narasimhan et al., 2003)
	Alfalfa	PCBs	RH	(Brazil et al., 1995)
	Wheat	TCE	RH	(Yee et al., 1998)
	Wild rye	Chlorobenzoic acid	EN	(Siciliano et al., 1998)
	Pea	2,4-D	EN	(Germaine et al., 2006)
	Poplar	MTBE, TCE, BTEX	EN	(Germaine et al., 2004; Moore et al., 2006)
	Pea	Naphthalene	EN	(Germaine et al., 2009)
	Barmultra grass	Naphthalene	RH	(Kuiper et al., 2001; Kuiper et al., 2004)
	Barley	Phenanthrene	RH	(Anokhina et al., 2004)
Sinorhizobium meliloti P221	Common reed	Phenanthrene	RH	(Golubev et al., 2009)
Indigenous degraders	Switchgrass	PCB	RH	(Chekol et al., 2004)
	Red clover; Ryegrass	2,4-D	RH	(Shaw and Burns, 2004)
	Ryegrass	PCP	RH	(He et al., 2005)
	White mustard	Petroleum hydrocarbons	RH	(Liste and Prutz, 2006)
	Hybrid poplar	BTEX, toluene	RH, EN	(Barac et al., 2009)
	English oak; common ash	TCE, toluene	RH, EN	(Weyens et al., 2009)
	Birch	PAHs	RH	(Sipila et al., 2008)
	Altai wild rye; tall wheat grass	Petroleum hydrocarbons	EN	(Phillips et al., 2009)
Burkholderia cepacia	Yellow lupine	Toluene	EN	(Barac et al., 2004)
	Poplar	Toluene	EN	(Taghavi et al., 2005)
	Barley	2,4-D	RH	(Jacobsen, 1997)
Azospirillum lipoferum spp.	Wheat	Crude oil	RH	(Muratova et al., 2005; Shaw and Burns, 2004)
Azospirillum brasilense Cd; *Enterobacter cloacae* CAL2; *P.putida* UW3	Tall fescue grass	PAHs	RH	(Huang et al., 2004)
Methylobacterium populi BJ001	Poplar	TNT, RDX, HMX	EN	(Van Aken et al., 2004a; Van Aken et al., 2004b)
Herbaspirillum sp. K1	Wheat	PCBs, TCP	EN	(Mannisto et al., 2001)

To summarise, phytoremediation is technology that is based on the combined action of plants and their associated microbial communities to degrade, remove, transform, or immobilize toxic compounds located in soils, sediments, ground water and surface water. Phytoremediation has been used to treat many classes of contaminants including petroleum hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and radionuclides, and landfill leachates (Joner and Leyval, 2003; Susarla et al., 2002). There are several advantages of phytoremediation compared to conventional techniques, such as low cost, low disruptiveness to the environment, public acceptance, and potentiality to remediate various pollutants (Macek et al., 2000; Pilon-Smits, 2005; Susarla et al., 2002). In addition, plants as autotrophic systems with large biomass require only a modest nutrient input, and they also prevent the spread of contaminants through water and wind erosion (Cherian and Oliveira, 2005). Candidate plant for phytoremediation should have the characteristics such as high biomass production, extensive root system, and ability to tolerate high concentration of pollutant and withstand environmental stress. Like other methods, phytoremediation has its disadvantages e.g. climatic and geological limitations, potential phytotoxicity of contaminant, potential for the contaminant or its metabolites to enter the food chain, and potentially longer timescale compared to other technologies (Macek et al., 2000). Although some success has been reported using plants alone in bioremediation (Gerhardt et al., 2009; Pilon-Smits, 2005), the use of plants in conjunction with plant associated (rhizosphere or endophytic) bacteria offers more potential for bioremediation (McGuinness and Dowling, 2009).

3.2. Bioaugmentation

As pointed out above, plants frequently lack the metabolic capacity for the degradation of many contaminants. Unlike plants, microorganisms are commonly able to degrade and mineralize vast variety of different pollutants. The problem is that usually we deal with the pollutant which is a very complex compound or is a mixture of different compounds and is therefore degradable or mineralizable by very specific set of microorganisms (consortium). It is suggested that several microbial populations together degrade pollutants more efficiently than a single strain due to the presence of partners which use the various intermediates of the degradation pathway more efficiently (Heinaru et al., 2005; Pelz et al., 1999). During rhizodegradation, the degradation of a pollutant, in many cases, is also the result of the action of a microbial consortium. But even when the appropriate catabolic traits are present in the local microbial community, the abundance and activity of the microorganisms at the site may be too low for successful bioremediation. In this case bioaugmentation strategy is used. Bioaugmentation is a method to improve degradation and enhance the transformation rate of xenobiotics by the inoculation of specific microbes, able to degrade the xenobiotics of interest. Introduction of one single

strain with the complete degradation pathway as well as a consortium of bacteria, each with different parts of the catabolic degradation route involved in the degradation of a certain pollutant/intermediate can be applied for bioaugmentation (Kuiper et al., 2004). Bioaugmentation is performed either directly by introduced microorganisms, or via the transfer of catabolic genes to the local microbial community (plasmid mediated bioaugmentation). Regardless of the approach chosen, the isolation and characterization of the appropriate microorganisms as well as their survival and catabolic activity in the contaminated environment are the key factors for successful bioaugmentation (Thompson et al., 2005). According to Dejonghe et al. (2001), bioaugmentation should aim for the rearrangement of the group of organisms dominantly involved in the overall energy flux, so that specific catabolic traits necessary for the cleanup of pollutants are part of that active group.

The advantage of bioaugmentation relies on its ability to accelerate the removal rate of pollutant several fold over a relatively short time scale. However, studies frequently observe that improvement of the bioremediation activity is temporary and the number of exogenous microorganisms decreases shortly after the addition of the biomass to the site (Bouchez et al., 2000a; Bouchez et al., 2000b; Ruberto et al., 2003). Several possible reasons for the failure of inocula, such as abiotic (extremes in temperature, water content, pH, nutrient availability, low availability or potentially toxic level on pollutants) and biotic factors (antibiotic production, antagonistic interactions) have been reported (Bouchez et al., 2000a; Gentry et al., 2004). According to Thompson and coauthors (2005), the most important factor determining the result of bioaugmentation comes first of all from the initial strain selection step. It has been suggested that the best way to increase the survival of the inoculum is to look for candidate microorganisms from the same ecological niche as the polluted area (El Fantroussi and Agathos, 2005). Such microorganisms are more adapted to the biotic and abiotic conditions in the polluted environments. It is also easier to incorporate them to the local microbial community. In addition, factors like inoculum density, physiological state and modes of introduction are known to considerably influence the survival and performance of the introduced microorganisms (Cunliffe et al., 2006; Thompson et al., 2005). Despite of the difficulties, successful bioaugmentation has been applied to both nonvegetated and vegetated soils (Jacques et al., 2008; Jézéquel and Lebeau, 2008; Ruberto et al., 2003; Siciliano et al., 1998; Singer et al., 2003), as well as activated sludge bioreactor systems (Boon et al., 2000; Cordova-Rosa et al., 2009) treating contaminants, such as phenols, clorophenols, pesticides and oil products. Also, biostimulation approach based on the addition of nutrients (carbon, nitrogen, phosphorus, potassium) or electron acceptor/donors (acetate, nitrate, sulfate, glutamate) can be used in combination with bioaugmentation to improve the survival and catabolic activity of introduced microorganisms.

Plasmid-mediated bioaugmentation has been suggested as an alternative strategy where the survival of the introduced donor strain is no longer needed once catabolic genes are transferred and expressed in indigenous bacteria (Top

et al., 2002). It is known that genetic information encoding the degradation of xenobiotic compounds is often found on plasmids or other mobile elements, and this genetic information can be potentially transferred to the local microbial community also from the dead inoculum. Therefore, the idea behind this strategy is that more important than the survival of introduced bacteria is the survival of their catabolic traits. Successful plasmid-mediated bioaugmentation of organic contaminants and transfer of catabolic genes to the indigenous bacteria has been demonstrated in soils and in activated sludge systems (Bathe et al., 2004; Bathe et al., 2005; Mohan et al., 2009; Top et al., 1999; Top et al., 1998). *Pseudomonas* sp., *Alcaligenes* sp., *Achromobacter* sp., *Comamonas* sp., *Burkholderia* sp., *Ralstonia* sp., have been used as donor strains for the transfer of catabolic genes. Plasmid-mediated bioaugmentation has been suggested particularly in the context of rhizoremediation, as the rhizosphere may be a habitat that allows a higher frequency of catabolic gene transfer as well as higher metabolic activity compared with bulk soils, both of which are necessary for a successful plasmid-mediated bioaugmentation (Top et al., 2002).

3.3. Microbial community structure in the vicinity of roots

A multitude of biotic and abiotic factors, for example climate and season, grazers and animals, pesticide treatment, soil type, structure and history, plant health and developmental stage are assumed to influence the structural and functional diversity of bacterial communities in the rhizosphere (Garbeva et al., 2004; Germida and Siciliano, 2001; Graner et al., 2003; Jousset et al., 2006; Siciliano et al., 2001). However, plant species (root morphology, plant age and health) and soil type (soil as the main reservoir for rhizosphere microorganisms) are considered to be factors that most substantially influence the structure and function of rhizosphere associated microbial community (Berg and Smalla, 2009; Kuiper et al., 2004). Although root exudates and the response of microorganisms to the latter as well as root morphology shapes the rhizosphere microbial communities (Berg and Smalla, 2009), it is not conclusively known whether plants are capable of actively select beneficial soil microbial communities in their rhizosphere through rhizodeposition process. It is also suggested, that there is actually no convincing evidence for direct stimulation of degrader microorganisms by plant roots through signalling, since increased abundance and activities of degrader populations in the rhizosphere could not be separated from other ecological interactions such as the effect of contamination (Siciliano and Germida, 1998; Wenzel, 2009). At least in theory, microbial strains or populations with degradation capabilities could be selected by a plant by microbial induced root exudation of compounds that can only be utilized by selected microorganisms (Dzantor, 2007; Siciliano and Germida, 1998). The evolutionary significance of a plant nourishing microbes in the rhizosphere is at least partially based on the protective value of the microbes in the root zone (Arthur

et al., 2005; Siciliano and Germida, 1998). Some data also suggest that beneficial symbioses and protective associations for the plant are encouraged in the rhizosphere, ensuring the supply of vital nutrients and changing the chemical and physical properties of the soil (Bais et al., 2004; Singh et al., 2004). Many microbes isolated from the rhizosphere are described to have plant/root growth-stimulating or growth-inhibiting properties. Beneficial microorganisms can benefit plant health by phytostimulatory and biofertilizing properties, inducing systemic response in the plant, resulting in the activation of plant defense mechanisms against various types of phytopathogens, and in terms of phytotoxicity of the contaminant, degrading the contaminant before it can negatively impact the plant (Chaudhry et al., 2005; Dams et al., 2007; Hontzeas et al., 2004; Liu et al., 2007; Raaijmakers et al., 2009). Also, the role of root-associated microbes in maintaining soil structure (i.e. aggregate stability) has been documented (Sen, 2003). Many of these beneficial rhizobacteria (mostly *Pseudomonas* spp.) can act as plant growth promoters as well as contaminant degraders (Cherian and Oliveira, 2005; Hontzeas et al., 2004; Kuiper et al., 2004) and this rhizodegradation efficiency may be a factor determining the selection of appropriate plant-contaminant degrader microorganisms pairs (Siciliano and Germida, 1998). Some findings suggest that instead of increasing the overall number of microorganisms, plants indeed select for taxonomic and functional groups in the rhizosphere regions (Bremer et al., 2007; Briones et al., 2002; Grayston et al., 2001), which are necessary for the degradation of specific contaminant (Leigh et al., 2006; Phillips et al., 2009; Siciliano et al., 2003).

3.4. Monitoring bioremediation

The majority of *in situ* bioremediation experiments have attempted to explain the efficiency of the process only on the basis of the kinetics of pollutant removal using direct (GC, GC-MS, HPLC, LC-MS, ion chromatography, proton nuclear magnetic resonance) or indirect (growth response of the pollutant degrading strain, appearance of degradation metabolites, consumption of O_2, or evolution of CO_2) methods (Baroja et al., 2005; Cledera-Castro et al., 2004; Combourieu et al., 2004; Esteve-Nunez et al., 2005; Gea et al., 2004; Korenkova et al., 2006; Pandey et al., 2009; Pieper et al., 2002). However, the differentiating metabolic degradation of the pollutant from the nonbiological removal is complicated and effective monitoring of microbial degradation under *in situ* conditions is rather poor because in many cases the decrease in the pollutant concentration may be observed as an outcome of the adsorbance of the pollutant to the environmental matrix (Pandey et al., 2009).

Currently it is generally acknowledged that in addition to the monitoring of pollutant removal, the environmental fate of degradative organism (i.e. the survival and activity) has to be monitored as well to maximize sustained bioremediation under natural conditions. Several cultivation based (microbial enumeration, soil enzyme activity analysis) and cultivation independent methods are

used for this purpose. However, because of the limitations of culture-dependent methods (for example the well known fact, that less than 1% of the microorganisms are culturable in standard laboratory conditions), molecular methods are mostly used nowadays for the study of microbial community structure and function. It is suggested, that DNA (extracted directly from the soil) based molecular techniques permit the most detailed determination of microbial community structural diversity and evaluation of the presence of certain functional genes in soil (Little et al., 2008). The majority of these techniques have been based on the sequencing/fingerprinting analysis of some phylogenetically relevant genes (such as 16S rRNA gene) amplified from the total community DNA. Among the most common fingerprinting methods, D/TGGE, T-RFLP, RFLP, SSCP are used to characterise of microbial community structure in soil (Nicomrat et al., 2006; Nicorarat et al., 2008; Sundberg et al., 2007; Truu et al., 2005). In addition, SSU rRNA clone libraries have provided fundamental information about the composition as well as the diversity of complex microbial communities. However, it is very complicated to reliably assess the number of microbial species, compare microbial communities and relate community composition to the environmental parameters in soil because of the estimation that one gram of soil can contain up to 10 billion of microorganisms from a thousand of different species (Torsvik and Ovreas, 2002). In order to assess full taxonomic diversity of microbes in environmental samples, high throughput DNA pyrosequencing (large 16S rDNA libraries) can be applied to obtain sufficient number of 16S rRNA gene sequences (Roesch et al., 2007). However, DNA based molecular techniques do not reveal information about the relationship between the identity and the function of microorganisms. Although metagenomic approaches and microarrays (GeoChip) have been developed in recent years for the direct monitoring of microbial genomic content in the environment, still only the genomic/degradation potential of the community can be described by these methods instead of realized activities (Truu et al., 2009). In order to get more than a functional prediction, gene transcripts (catabolic genes or the amount of 16S rRNA gene) and translated proteins must be obtained from environmental samples for the direct examination. In addition, stable isotope probing (DNA and RNA-SIP in combination with the previously mentioned techniques) has been successfully applied in bioremediation studies enabling the linkage between microbial community structure and function (Uhlik et al., 2009).

4. MATERIAL AND METHODS

4.1. Site description

Phytoremediation and bioaugmentation field experiment was performed at the semi-coke depository area in Kohtla-Järve. The depository area consists of semi-coke mounds that have formed from solid waste of oil shale thermal treatment. These mounds have a shape of excentric cones, dark-gray or black in colour and with specific smell (Pae et al., 2005). Natural vegetation and undergrowth are absent in the experimental area. In addition, there are no distinctive layers or horizons (including the humic layer) in the solid waste profile characteristic of the regular soils. According to Truu and coauthors (2003), semi-coke solid waste is characterized by a high initial pH value, a high concentration of sulphides, Ca^{2+} and Mg^{2+} ions and high amount of organic carbon (Table 2). The organic carbon in semi-coke is not similar to the organic carbon in regular soils because half of it is in the form of asphaltenic and bitumoid compounds which are very recalcitrant to biodegradation. Semi-coke has a granular texture, and the composition of the mineral part of semi-coke consist mainly of calcite, dolomite and ettringite (Motlep et al., 2007).

Test plots were established at the flat and older part (10–15 years) of the depository area in July 2001–2006 on the principle that the plots would have no influence on each other.

Table 2. Chemical properties of the solid waste at experimental area (Truu et al., 2003).

Variable	Measured value
pH	8.0–11
Total nitrogen (%)	0.08
P-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

4.2. Phytoremediation experiment

In phytoremediation experiment, two different vegetation approaches were applied (Table 3):

I) inoculation of mixture of grass seeds onto the semi-coke (50 m^2 test plots). The mixture of grass seeds was based on the four species: *Lolium perenne* – perennial ryegrass, *Poa pratensis* – Kentucky bluegrass, *Festuca rubra* – red fescue, and *Festuca ovina* – blue fescue. In addition to grass seeds, addi-

tional treatments were applied: sand treatment (grass seeds in semi-coke were covered by sand layer of 1–2 cm), peat treatment (grass seeds in semi-coke were covered by peat layer of 1–2 cm), no treatment (grass seeds in semi-coke). Part of the phytoremediation plots (10 m^2) was used for bioaugmentation.

II) utilization of previously planted birches (*Betula bendula*; planted at the depository area in 1998; distance between birches is ca 1 m; 10 m^2 test plots) in semi-coke.

4.3. Bioaugmentation experiment

The previously characterized *Pseudomonas* strains isolated from the nearby polluted area were selected for the bioaugmentation experiment (characterization of the strains in detail in Heinaru et al., 2000; Merimaa et al., 2006; Table 3 in Reference III). The strains were *Pseudomonas mendocina* (PC1), *P. fluorescens* biotype F (PC17 and PC20), *P. fluorescens* biotype B (PC18) and *P. fluorescens biotype* C (PC24). Microbial strains used in current study are deposited in the Collection of Environmental and Laboratory Strains of Tartu University (CELMS, http://www.miccol.ut.ee). Various mixtures of *Pseudomonas* strains were applied simultaneously with vegetation (Table 3):

I) in combination with grass species, a mixture of three bacterial strains (PC1, PC24 and PC18; strains in ratio 3:1:1, respectively).

II) in combination with birches, a) a mixture of four bacterial strains (PC1, PC18, PC20 and PC24; strains in equal ratios), and b) a mixture of five bacterial strains (PC1, PC17, PC18, PC20 and PC24; strains in equal ratios).

Before the introduction, the strains were cultivated in Luria–Bertani (LB) medium. Cells from the stationary growth phase were mixed with 0.9% NaCl solution and inoculated onto the surface of experimental plots. Inoculation was performed by spreading the mixture (20 L; final concentration of each strain approximately 10^8 CFU ml^{-1}; the total amount of bacteria introduced into the plots was ca 10^{12} CFU m^{-2}) onto the surface of experimental plots. Inoculation was performed in July 2002–2006 only to the plots established in the same year.

4.4. Chemical analyses

Oil products (extracted with pentane) were measured by gas chromatography. Volatile phenols were determined spectrophotometrically. Total orgacic carbon was determined using an infrared spectrophotometer. All analyses were carried out by Tartu Environmental Research, Ltd. (Tartu, Estonia).

Table 3. Principal scheme of the phytoremediation and bioaugmentation field experiments at the semi-coke depository area.

Phytoremediation	grass seeds	previously planted birches		
Bioaugmentation	PC1, PC18, PC24	PC1, PC18, PC20, PC24		PC1, PC17, PC18, PC20, PC24
Plots establishment	2001	2004	2006	2005
Bacterial biomass inoculation in July	2002	2004	2006	2005
Additional treatments	no treatment, sand layer; peat layer	–	–	mineral fertilizers
Soil sampling in October	2001–2003	2004–2007	2006–2007	2005–2007

4.5. Soil sampling and bacteria cultivation dependent analyses

Soil sampling was performed during 2001–2007 at the beginning of October. Bulk soil and rhizosphere sampling was performed as described in Reference III. Bulk soil and rhizosphere samples were used for serial dilutions and plating selective media. The numbers of aerobic heterotrophic and phenol degrading bacteria were determined as described in References I and II. In addition, obtained bacterial colonies from selective media were characterized by different analyses (Gram test, oxidase test, growth on King's B medium, presence of catabolic genes, rep-PCR) as described in Reference III for the detection of introduced strains from semi-coke.

Potential metabolic activity of the microbial communities in bulk soil and rhizosphere samples were characterized using Biolog EcoPlates and substrate-induced soil respiration tests as described in References I and III, respectively. Substrate utilization dynamics of microbial communities obtained from Biolog EcoPlates were used for Principal Component Analyses (PCA) to assess the changes in culturable microbial community composition due to vegetation and bioaugmentation.

4.6. Molecular analyses

Microbial DNA was extracted from the samples using UltraClean Soil DNA kit (Mo Bio Laboratories) according to the manufacturer´s instructions. In addition, protocol by Peršoh and coworkers (2008) was used for the isolation of total nucleic acids (DNA and RNA) from the samples (description of the isolation and subsequent analyses are given below).

Microbial communities in semi-coke were characterized based on the sequences of total bacterial 16S rRNA gene and 16S rRNA specific cDNA, genus *Pseudomonas* specific 16S rRNA gene and 16S rRNA specific cDNA, and large subunit of multicomponent phenol hydroxylase gene (*lmPH*). PCR amplification was performed, using following primers: PRBA338f, PRUN518r, PRBA338f-GC for universal bacterial 16S rRNA gene and 16S rRNA specific cDNA; pseF, pseR, pseF2 (5′-GGTCTTCGGATTGTAAGCAC-3′) for genus *Pseudomonas* specific 16S rRNA gene and 16S rRNA specific cDNA; phe00, phe212, pheGC for *lmPH* gene (nucleotide sequences of used primers are given in tabel 4 in Reference III). Amplification of total bacterial 16S rRNA gene is described in Reference II; amplification of genus *Pseudomonas* specific 16S rRNA gene is described in Reference III; amplification of *lmPH* gene fragments is described in Reference III.

Amplified products were applied for DGGE analyses, resulting in the fingerprints of the samples. Comparison of the fingerprints were perfomed to assess the changes in the microbial community composition or the diversity of catabolic genes caused by vegetation and bacterial strains as well as detection of the gene fragments corresponding to the introduced bacterial strains. Similarity values based on densitometric curves of the gel tracks were calculated using the Pearson correlation coefficient. Dendrogram based on cluster analyses of the DGGE profiles was performed. Also PCA was used for the analysis of DGGE banding patterns. DGGE analyses was performed as described in Referencess II and III, subsequent cloning and nucleotide sequencing was performed as described in Reference III in order to verify, whether the detected 16S rRNA ja *lmPH* gene fragments from soil samples show similarities to sequences from the introduced bacterial strains.

4.6.1. Isolation of total nucleic acids from the soil and subsequent analyses

Protocol by Peršoh et al. (2008) was used for the isolation of nucleic acids from the soil samples in order to assess the impact of vegetation and introduced bacterial strains on the dynamics of microbial community composition in the levels of both, DNA and RNA. Nucleic acids isolation was performed in three replicates per sample. Obtained DNA was then used for the DGGE and quantitative PCR (QPCR), targeting the total bacterial and genus *Pseudomonas* specific 16S rRNA gene (Fig. 3). Part of the obtained samples from nucleic acids extraction were treated with the Dnase I (Fermentas), followed by the cDNA synthesis (RevertAid M-MuLV Reverse Transcriptase; Fermentas) according to the protocols provided by the manufacturer. cDNA synthesis was performed with 16S rRNA gene specific primer: primer PRBA338f for the total bacterial 16S rRNA gene, and primer PseF for the genus *Pseudomonas* specific 16S rRNA gene. The obtained total bacterial 16S rRNA specific cDNA was

used for QPCR and DGGE, obtained genus *Pseudomonas* 16S rRNA specific cDNA was used for DGGE (Fig. 3).

QPCR was performed using Platinum SYBR Green qPCR Supermix-UDG kit (Invitrogen) according to the manufacturer´s instructions. Universal bacterial primers PRBA338f and PRUN518r were used for the total bacterial 16S rRNA gene and 16S rRNA specific cDNA, primers PseF2 and PseR were used for the genus *Pseudomonas* specific 16S rRNA gene and 16S rRNA specific cDNA. The reaction conditions for the QPCR were as follows: 50ºC for 2 min, 95ºC for 2 min, 40 cycles of 95ºC for 15 s, 60ºC for 30 s, 72ºC for 30 s, followed by melting curve analysis to confirm the fluorescence signal resulted from specific PCR products and not primer-dimers or other artifacts. QPCR was performed in triplicate for each sample, including standards and negative control. Reactions were carried out with an ABI Prism 7900HT machine (Applied Biosystems, Foster City, CA, USA) and data were analysed using S.D.S 2.2.2 software (Applied Biosystems). In addition, the abundance of *Pseudomonas* sp. group was calculated as the ratio between the measured copy numbers for *Pseudomonas* sp. specific 16S rRNA gene and cDNA of 16S rRNA, and the total bacterial 16S rRNA gene and cDNA of 16S rRNA, respectively.

For standard curves, both targeted sequences were amplified from positive control strain *Pseudomonas mendocina* PC1. Amplified products were run on 2% agarose gel to confirm the specificity of the amplification, and cloned into vector pTZ57R using InsT/Aclone™ PCR Product Cloning kit (Fermentas). Plasmids were isolated using QIAprep Spin Miniprep kit (Qiagen, CA, USA). In case of using DNA as template, plasmid DNA concentration were determined with spectrophotometer (Nanodrop 1000) and standard curves were obtained with serial plasmid dilutions of a known amount of plasmid DNA containing the targeted fragment. In case of using cDNA as template, the plasmids were linearized with EcoRI and purified with UltraClean 15 DNA Purification Kit From Gels and Solutions (MoBio Laboratories Inc) followed by *in vitro* transcription with T7 RNA polymerase (Fermentas) according to the protocol provided by manufacturer. Obtained sample was treated with the Dnase I (Fermentas) according to the protocol and purified with UltraClean 15 DNA Purification Kit From Gels and Solutions kit (MoBio Laboratories Inc). RNA concentration was determined spectrophotometrically (Nanodrop 1000). Ten-fold serial dilutions (20 pg μl^{-1}–0.0002 pg μl^{-1}) of the RNA was used for the cDNA synthesis using total bacterial 16S rRNA gene specific primer PRBA338f according to the protocol (Fermentas) followed by the QPCR. Contamination with DNA was tested with the control PCR. Copy numbers were calculated from the standard curves, assuming that the average molecular mass of a double-stranded DNA molecule is 660 g mol^{-1}. Copy numbers of the samples were quantified by comparing the cycle at which fluorescence crossed a threshold to a standard curve.

DGGE fingerprinting of both, the total bacterial and genus *Pseudomonas* specific 16S rRNA gene and 16S rRNA specific cDNA was performed. Total bacterial 16S rRNA gene was first amplified with primers PRBA338f and

PRUN518r followed by amplification with primers PRBA338fGC and PRUN518r, while the genus *Pseudomonas* specific 16S rRNA gene was first amplified with primers PseF and PseR followed by amplification with primers PRBA338fGC and PRUN518r (Fig. 3). Total bacterial and genus *Pseudomonas* specific 16S rRNA cDNA were amplified with primers PRBA338fGC and PRUN518r (Fig. 3). Primer PRBA338fGC contains a GC clamp (40 bp) at the end of 5′ end to enable DGGE analyses. The PCR mixture included 1 × PCR buffer [with $(NH_4)_2SO_4$], 200 μM concentrations of each dNTP, 2.5 mM $MgCl_2$, 20 pmol of both primers, 60 ng μl^{-1} of bovine serum albumin and 0.5 U of Taq DNA polymerase (Fermentas). The reaction conditions of the PCR were as follows: 95°C for 5 min, 30 cycles of 95°C for 1 min, 57°C for 1 min, 72°C for 1.5 min, and a final extension of 72°C for 5 min. The PCR product were quantified in 2% (w/v) agarose gel by comparison with the standard (100 bp DNA size marker, Fermentas) using EASY WIN32 software (Herolab GmbH). Approximately 500 ng of the PCR products were applied for the DGGE analysis as described in Reference III. A linear denaturing gradient of 35–62% was used for the total bacterial community, and 38–49% for the genus *Pseudomonas* community. Subsequent cloning and nucleotide sequencing of the fragments specific from genus *Pseudomonas* community was performed as described in Reference III.

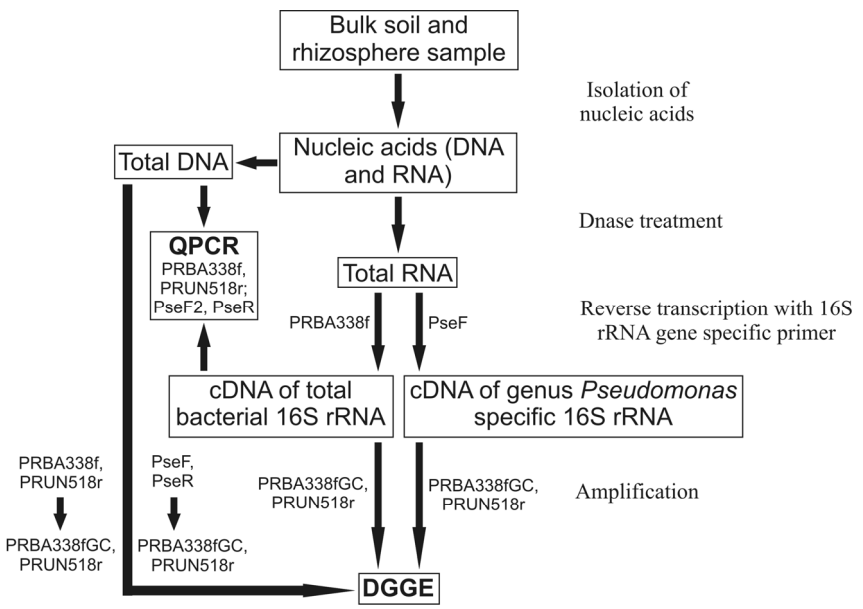

Figure 3. Schematic representation of steps of analyses followed by DNA and RNA based QPCR and DGGE. Nucleotide sequences of the used primers are given in table 4 in Reference III.

5. RESULTS AND DISCUSSION

5.1. Impact of vegetation and bioaugmentation on the concentration of organic pollutants in semi-coke

Chemical analyses were performed to assess the impact of vegetation and bioaugmentation on the concentration of different pollutants in semi-coke. As mentioned earlier, semi-coke consists of various contaminants some of which are recalcitrant to biodegradation (PAHs and asphaltenic compounds for example). However, chemical analyses revealed that vegetation and introduced bacterial strains had substantial impact on the removal of the contaminants. Within a two and a half year period the concentration of oil products decreased more than three times in the plots with vegetation and added bacterial biomass (from 340 mg kg^{-1} to 100 mg kg^{-1}, Reference I). In addition, the concentration of volatile phenols reduced up to 100% and the total content of organic carbon decreased by 10 to 30 g kg^{-1} (from 15% to 12%) on average in plots with grass species compared to the control plot without vegetation (Reference I). In case of phenols removal it is known, that in addition to the biodegradation, phenolic compounds are easily to be removed from the soil by leaching. In case of some other pollutants adsorption of the contaminant to the soil particles may occur reducing the mobility and bioavailability of the contaminant instead of biodegradation of the pollutant. However, in our experiment, the reduction of the concentrations of oil products and phenols was found to be exceptionally high in upper soil layer with the highest root density referring to the biodegradation by microorganisms. It is generally recognized that enhanced biodegradation activity in the rhizosphere is due to rhizodeposition consisting of root exudates and root debris. Also, release of glutathione conjugates into the rhizosphere by plants during detoxification process has been shown, where they could be metabolized by microorganisms (Schroder et al., 2007). Since the degradation rates of pollutants did not differ significantly between plots with vegetation we may suggest that establishment of vegetation on semi-coke was the key factor for the acceleration of pollutants degradation (Reference I).

5.2. Impact of vegetation and bioaugmentation on the microbial abundance

Different mechanisms for the facilitation of phytoremediation have been shown for different plant species. Kirk and coauthors (2005) found, that perennial ryegrass supported general increase in microbial activity and numbers in the rhizosphere, some of which had catabolic activity towards petroleum hydrocarbons in petroleum-contaminated soil, while alfalfa specifically increased the number of microorganisms capable of degrading more complex hydrocarbons.

We also studied the impact of vegetation and bioaugmentation on the microbial abundace. Results from earlier study showed, that vegetation increased the number of biodegradative bacteria as well as the activity and diversity of microbial community in semi-coke, while the number of aerobic heterotrophic bacteria remained at the same level compared to the plot without vegetation (Truu et al., 2003). However, these results were obtained only four months after the inoculation of the grass seeds into the semi-coke. By assessing microbial counts over longer time period we may suggest, that neither vegetation nor introduced bacterial strains had influence on the number of aerobic heterotrophic bacteria (AHB) in semi-coke, but had short term effect on the number of phenol degrading bacteria (PDB). The numbers of AHB were in the range of 10^6–10^7 CFU g^{-1} soil (dw) in bulk soil of both planted samples, unplanted control, and bioaugmented samples (Table 2 in Reference II; Table 4). The numbers of PDB were one order of magnitude higher in bulk soil samples from plots with vegetation and added bacterial biomass on the first year of the experiment (four months after the addition of bacterial strains to the semi-coke) compared to the unplanted/planted control (ca 10^5 and 10^4 CFU g^{-1} soil dw, respectively), but these numbers decreased to the level of control sample next year (Table 2 in Reference II; Table 4). The dynamics of bacterial counts were similar between two vegetation approaches. Rhizosphere samples from different plots demonstrated generally one to two orders of magnitude higher numbers of both bacteria compared to the numbers obtained in bulk soil and these numbers remained stable during the experiments. Similar dynamics could be obtain when assessing the proportion of PDB in total bacterial abundance during the experiment – the proportion of PDB in bulk soil from plots with birches and added bacterial strains decreased within four months of bioaugmentation to the level of 20%, and to the level of control sample in following months (Table 4). The proportion of PDB in rhizosphere of birches followed similar dynamics as in bulk soil, although in smaller scale.

The problem with these results is that bacteria enumeration depends largely on the ability of bacteria to grow at the laboratory conditions. To obtain cultivation independent data about the abundance of bacteria, isolation of nucleic acids directly from semi-coke was performed for the quantitative PCR. Estimation of the copy numbers of total bacteria and genus *Pseudomonas* specific 16S rRNA gene (DNA based analyses) and cDNA of 16S rRNA (16S rRNA gene transcript – RNA based analyses) was performed. 16S rRNA gene as commonly used marker is present in all bacterial species, and 16S rRNA gene transcript is even more abundant in RNA extracts because its high transcription rate in metabolically active cells.

Table 4. The impact of birches and added bacterial strains (combination of four strains) on the numbers of aerobic heterotrophic bacteria (AHB) and phenol degrading bacteria (PDB), and on the copy numbers of total bacterial and genus *Pseudomonas* specific 16S rRNA gene and cDNA of 16S rRNA. Mean values of copy numbers are presented (n = 9; stdev < 15% of the mean value).

Sample	AHB (CFU g^{-1} soil dw)	PDB (CFU g^{-1} soil dw)	PDB/AHB	16S rRNA gene		cDNA of 16S rRNA		16S rRNA gene copy ratio Pse[a]/total	16S rRNA cDNA copy ratio Pse[a]/total
				total bacteria	genus Pse[a]	total bacteria	genus Pse[a]		
				No. copy g^{-1} soil dw					
R4	8.22E+06	5.19E+06	0.63	9.47E+08	7.92E+08	1.04E+07	6.21E+06	0.8365	0.5971
BC	1.58E+06	3.49E+04	0.02	7.64E+06	1.37E+05	2.29E+07	1.06E+05	0.0179	0.0046
BC*	1.58E+07	1.46E+05	0.01	1.69E+09	1.12E+07	6.79E+10	1.72E+09	0.0066	0.0253
4	1.37E+06	2.75E+05	0.20	5.60E+06	1.49E+05	7.47E+07	5.23E+05	0.0265	0.0070
4*	1.24E+07	8.57E+05	0.07	2.45E+08	1.59E+06	3.85E+10	1.12E+09	0.0065	0.0291
16	2.50E+06	1.60E+04	0.01	2.16E+06	1.86E+05	2.58E+07	1.32E+05	0.0859	0.0051
16*	2.09E+07	5.86E+05	0.03	7.68E+08	1.00E+06	4.53E+10	1.07E+09	0.0013	0.0236
28	3.03E+06	2.22E+04	0.01	6.41E+06	6.28E+05	1.20E+07	8.47E+04	0.0979	0.0071
28*	1.92E+07	3.65E+05	0.02	4.65E+08	1.85E+06	6.37E+10	1.33E+09	0.0040	0.0209

AHB – aerobic heterotrophic bacteria; PDB – phenol degrading bacteria; R4 – soil sample was obtained 2h after the introduction of the bacterial strains onto the semi-coke; BC – control with birches; * – rhizosphere sample; sample numbers indicate bioaugmentation age in months; [a] – *Pseudomonas* sp.

Bacteria from the genus *Pseudomonas* were targeted because of the utilization of *Pseudomonas* sp. strains in bioaugmentation experiment as well as the fact that species from genus *Pseudomonas* were found to be abundant within indigenous bacterial community in semi-coke. In our experiment, within four months period of bioaugmentation, the copy numbers of 16S rRNA gene and cDNA of 16S rRNA of both, total bacteria and bacteria from genus *Pseudomonas* in bulk soil samples decreased to the level of control sample (Table 4). Similarly to the bacterial counts, these copy numbers were higher in rhizosphere samples compared to the bulk soil (Table 4). In addition to the overall higher copy numbers of 16S rRNA gene and cDNA of 16S rRNA, rhizosphere samples demonstrated one to several orders of magnitude higher transcription rate of 16S rRNA compared to the bulk soil, indicating higher metabolic activity in rhizosphere. Results also revealed that *Pseudomonas* sp. specific 16S rRNA gene constitute up to 9.7% of the total bacterial 16S rRNA gene in bulk soil, while rhizosphere samples demonstrated exceptionally low abundance of *Pseudomonas* sp. specific 16S rRNA gene (< 0.7% of the total bacterial community 16S rRNA gene). In contrary to the results obtained in rDNA level, *Pseudomonas* sp. specific 16S rRNA cDNA was found to constitute ca 3% of the total bacterial ribosomal cDNA in birches rhizospheres, and the abundance of *Pseudomonas* sp. specific cDNA in bulk soil was very low (Table 4). Higher proportion of *Pseudomonas* sp. in rhizosphere samples was somehow expected, since it is known, that *Pseudomonas* sp. are good root colonizers and they often possess plant growth promoting properties. In addition, genus *Pseudomonas* have been found to prevail in the total microbial community as well as in the hydrocarbon degrading microbial community in soil (Fierer et al., 2005; Fulthorpe et al., 2008; Ruberto et al., 2003). However, results obtained from our experiments do not show the overall domination of *Pseudomonas* sp. in the birches rhizosphere, but rather suggest the existence of diverse bacterial community.

5.3. Impact of vegetation and bioaugmentation on the potential metabolic activity of microbial community in semi-coke

Activity of microbial communities in semi-coke were compared using kinetic model based on either summed well color development obtained from Biolog EcoPlates, or the time course of phenol removal obtained from the substrate induced soil respiration analysis. The model provides two kinetic parameters (K and r from the Biolog EcoPlates data, and C and k from the substrate induced soil respiration analysis data) that are invariant with respect to introduced bacterial biomass density, and reflect the composition of cultivable microbial communities. Results obtained from these analyses indicated changes in the microbial community metabolic activity due to bioaugmentation.

In case of the results from the Biolog EcoPlates, microbial community kinetic parameters K and r indicated higher potential metabolic activity in bioaugmented

samples compared to the samples without additional bacterial strains (Table 3 in Reference I; Table 5). When mixture of grass species was used, also vegetation was found to increase the microbial community metabolic activity in semi-coke (Table 3 in Reference I). However, higher metabolic activity of the microbial communities were obtained only in the year of bacterial biomass application, and these values decreased in following year (Table 3 in Reference I; Table 5). Bulk soil sample with mixture of four strains demonstrated higher potential metabolic activity in the year of bacterial biomass application compared to the sample with mixture of five strains. During the field experiment, rhizosphere samples from both, bioaugmented and control plots demonstrated higher potential metabolic activity compared to the bulk soil (Table 5).

When comparing soil samples of different bioaugmentation age using substrate-induced soil respiration test, the results revealed, that phenol biodegradation in semi-coke samples remained higher even 40 months after the inoculation of the bacterial strains into the semi-coke compared to the control (Table 6 in Reference III). The kinetic parameters showed that although soil samples with different bioaugmentation age demonstrated similar pseudo-first-order constants of degradation of phenol, the rate of degradation was > 30% higher than in the planted control samples and three times higher than in unplanted soil (Table 6 in Reference III). Also the degree of biodegradation of phenol (BOD/ThOD %) was higher, and the length of lag phase (parameter S) was shorter in bioaugmented samples of different age compared to the control and unplanted control in particular (Table 6 in Reference III).

Table 5. Comparison of kinetic model parameters from summed well color development of Biolog EcoPlates in bulk soil and rhizosphere samples from semi-coke plots planted with birches. Bioaugmentation was performed in July 2005; year indicates soil sampling in October. Means and standard deviations are presented.

Sample	Year	K	r	s (h)	R^2
C	2005	11.5±0.3	0.027±0.004	136.3±6.2	99.9
	2006	11.9±0.9	0.033±0.006	139.6±7.0	99.7
BC	2005	12.8±0.8	0.030±0.001	140.3±3.2	99.9
	2006	7.5±0.9	0.023±0.003	137.5±12.9	98.8
B4	2005	25.0±1.0	0.067±0.016	56.3±4.2	96.4
	2006	11.1±0.7	0.034±0.006	90.8±7.0	97.2
B5	2005	16.5±0.6	0.053±0.010	70.2±3.9	97.8
	2006	11.5±0.6	0.033±0.010	90.2±3.9	97.8
BC*	2005	33.8±1.2	0.047±0.006	77.4±3.7	98.6
	2006	32.9±1.8	0.040±0.008	71.3±6.0	99.6
S4*	2005	36.8±1.7	0.045±0.009	68.5±5.0	97.1
	2006	38.3±1.9	0.039±0.007	75.8±5.2	97.5
S5*	2005	31.8±1.8	0.038±0.007	77.4±6.2	96.8
	2006	37.8±1.8	0.040±0.007	73.4±6.2	96.8

C – control; BC – control with birches; B4 – bulk soil sample with added bacterial strains (mixture of strains PC1, PC18, PC20, PC24); B5 – bulk soil sample with added bacterial strains (mixture of strains PC1, PC17, PC18, PC20, PC24); * – rhizosphere sample.

5.4. Microbial community composition and survival of the introduced strains in semi-coke

Based on the results so far, the impact of vegetation and bioaugmentation on the abundance of bacteria was not observed based on bacterial counts or copy numbers of 16S rRNA gene and 16S rRNA gene transcript, except the short term effect on the number of phenol degrading bacteria. However, the potential metabolic activity in semi-coke and phenol biodegradation in particular remained higher for a prolonged period of time. Most likely this enhanced microbial activity is caused by either changes in the structure of microbial community and catabolic genes, or the survival and catabolic activity of the introduced bacterial strains, or both. Siciliano and coworkers (2001) have stated that 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. We used data from substrate utilization dynamics on Biolog EcoPlates to assess the changes in culturable microbial community composition due to vegetation and bioaugmentation. In order to reveal the impact of vegetation and bioaugmentation on the microbial community composition as well as the survival of the introduced bacterial strains in semi-coke, the microbial communities were characterized based on total bacterial 16S rRNA gene and 16S rRNA specific cDNA, genus *Pseudomonas* specific 16S rRNA gene and 16S rRNA specific cDNA, and the diversity of *lmPH* gene sequences.

5.4.1. Dynamics in microbial community composition in semi-coke planted with grass species

Substrate utilization pattern of microbial communities obtained from Biolog EcoPlates showed that although vegetation and bioaugmentation enhanced the microbial community potential metabolic activity only for short period of time, changes in microbial community composition were continuous (Fig. 4 a). In addition to the vegetation and added bacterial strains, properties of the covering material were found to have substantial influence on the microbial community composition and development. During the experiment, the temporal dynamics of bacterial communities in semi-coke was less significant compared to the treatment effect, and similar trend in bacterial community succession was not found. Similar results were obtained from the DGGE analyses of bacterial 16S rRNA gene fingerprint (Fig. 3 in Reference II). In detail, major variation in bacterial community structure between samples were obtained in the year of bioaugmentation application, when impact of the different covering materials on the microbial community structure was greatest (Fig. 4 a; Paper II). Bacterial community structure from different treatments distinguished from the control sample also at the end of the experiment two and a half years later, but the

samples were more similar to each other mostly due to the impact of vegetation. Also the values of Shannon diversity indexes of microbial community based on metabolic profiles obtained with Biolog EcoPlates were generally higher in planted plots compared to the control, but these values decreased in all plots during the experiment (Table 2 in Reference II). Shannon diversity indexes based on the DGGE fingerprints did not demonstrate substantial differences between untreated and treated plots and these values remained relatively stable during the experiment (Table 2 in Reference II). These results may indicate that vegetation rather influenced the rearrangement of some specific groups of microorganisms within microbial community than the total microbial diversity.

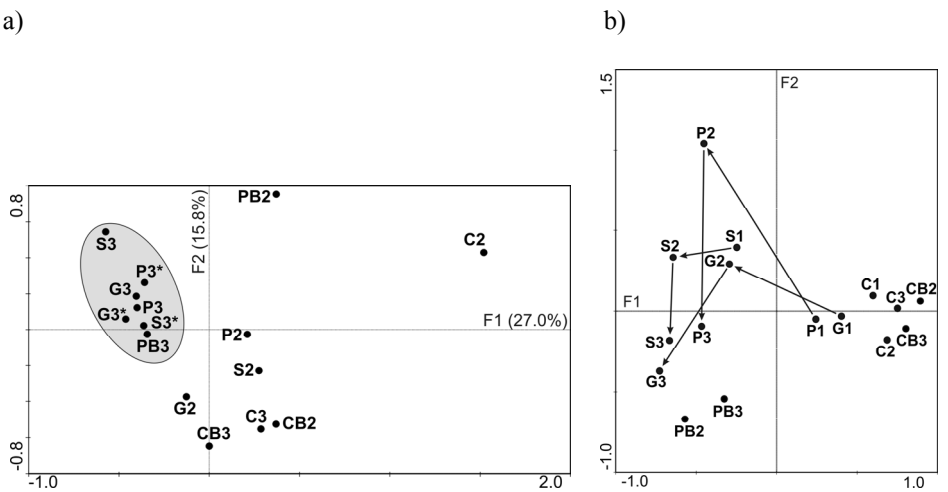

Figure 4. Principal component analyses representing the temporal dynamics in microbial community structure in semi-coke samples with mixture of grass species and added bacterial strains. a) PCA plot based on substrate utilization patterns of culturable bacterial communities estimated using Biolog EcoPlate data; first and second principal components (F1 and F2) describe 27.0 and 15.8% of the overall data variation, respectively; b) PCA plot of the species-specific composition of bacteria from genus *Pseudomonas*; PCA is based on the densitometric curves of DGGE fingerprint of genus *Pseudomonas* specific 16S rRNA gene; first and second principal components (F1 and F2) describe 34.2 and 15.1% of the overall data variation, respectively. * – rhizosphere sample; sample codes are same as in Fig. 3 in Reference II

We also found, that although microbial community potential metabolic activity was constantly higher in rhizosphere samples compared to the bulk soil, PCA analyses of metabolic profiles from the Biolog EcoPlated and DGGE fingerprint of 16S rRNA gene did not distinguish the bacterial communities between rhizosphere and bulk soil (Fig. 4 a; Fig. 1 in Reference I). It is known, that bulk soil and rhizosphere microbial community structure is determined first of all by the local native microbial community in soil, followed by impact of soil effects and vegetation

(Buyer et al., 2002). But in addition to that, these results suggest, that the impact of vegetation on the microbial community composition was not merely associated to the rhizospheric effect, but to the use of vegetation in general.

Species-specific composition of bacteria from genus *Pseudomonas* in semi-coke was mainly influenced by vegetation (Fig. 4 b). Effect of the covering material was secondary and influence of introduced bacterial strains on the species-specific composition of bacteria from genus *Pseudomonas* was significant only in case of planted plots. In addition, semi-coke plots with vegetation demonstrated similar temporal dynamics in the composition of *Pseudomonas* sp. bacteria (Fig. 4 b). Based on the data from the nucleotide sequencing of the 16S rRNA gene fragments from the DGGE gel, vegetation favors the species of genus *Pseudomonas* related to the plant growth promotion and degradation of contaminants.

5.4.2. Dynamics in microbial community composition in semi-coke planted with birches

Although bioaugmentation increased the metabolic activity of microbial communities in semi-coke for short period of time, the changes in microbial community composition were continuous like in the case of vegetation approach with mixture of grass species. In addition to the DNA based fingerprinting, we also assessed the changes in the active microbial community structure with RNA-based DGGE analyses. Culture-independent techniques generally use the 16S rRNA gene to profile phylogeny and community structure. However, DNA based analysis may not effectively discriminate between functionally active and dormant populations. DNA is known to persist extracellularly in environments after cell death, leaving a residue of legacy DNA; as RNA is more labile, it is probably a more representative measure of active populations (Mengoni et al., 2005). Previous studies have also found significant RNA- and DNA-based differences in bacterial community composition (Lillis et al., 2009; Mengoni et al., 2005) as well as demonstrated little variation in DNA based profiles compared to the RNA based profiles (Duineveld et al., 2001; Hoshino and Matsumoto, 2007).

In our study, analyses of DGGE fingerprints of total bacterial community and species from genus *Pseudomonas* indicated significant differences between structure of active communities (RNA-based) and total communities (DNA-based) (Fig. 5). In addition, differences in microbial community composition (both, total bacteria and species from genus *Pseudomonas*) between bulk soil and rhizosphere were found (Fig. 5). In case of bacteria from genus *Pseudomonas*, greater variation in RNA-based profiles was found compared to the DNA-based profiles (Fig. 5 b). Despite of the differences in RNA and DNA level, the changes in the total bacterial and active bacterial community in rhizosphere samples follow similar temporal trend (Fig. 5 a). Similar pattern, although different from the pattern of rhizosphere samples, may be observed in bulk soil samples. PCA plot shows, that major changes in the bacterial commu-

nity composition in rhizosphere occur within four months after the addition of bacterial strains to the semi-coke. Changes in the community composition during following months were continuous although in smaller scale. In case of bulk soil samples, higher temporal variation in the bacterial community composition compared to the rhizosphere samples was found. In addition, changes in bacterial community structure in bulk soil between months 16 and 28 were almost towards opposite direction compared to the changes observed in rhizosphere samples (Fig. 5 a).

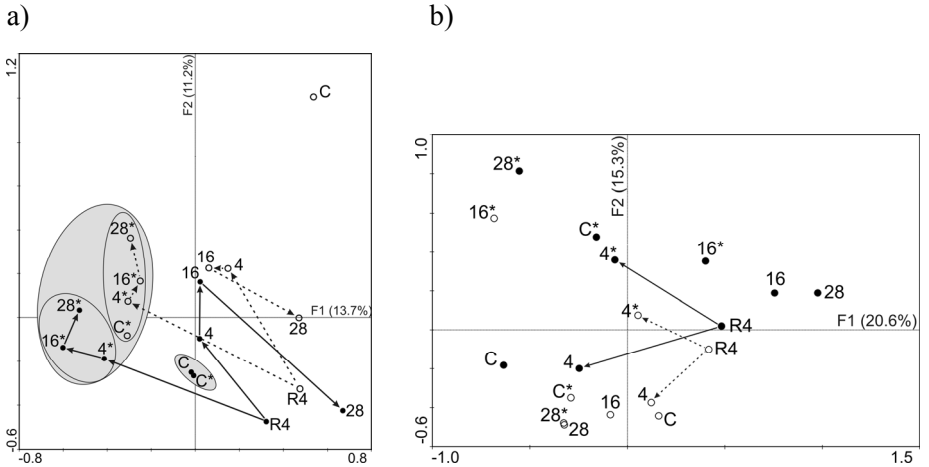

Figure 5. Principal component analyses representing the temporal dynamics in microbial community structure in semi-coke samples with birches and added bacterial strains. PCA is based on the densitometric curves of DGGE fingerprints. a) PCA plot of the total bacterial community composition; first and second principal components (F1 and F2) describe 13.7 and 11.2% of the overall data variation, respectively; b) PCA plot of the species-specific composition of bacteria from genus *Pseudomonas*; first and second principal components (F1 and F2) describe 20.6 and 15.3% of the overall data variation, respectively. ○ – 16S rRNA gene was used as template in the amplification; ● – cDNA of 16S rRNA was used as template in the amplification; C – control with birches; R4 – sample was obtained 2h after the introduction of bacterial strains on the semi-coke; * – rhizosphere sample; sample numbers indicate bioaugmentation age in months.

In case of the species-specific composition of bacteria from genus *Pseudomonas*, similar temporal dynamics were found only within four months after addition of bacterial strains to the semi-coke (Fig. 5 b). Changes during following months were even bigger in some cases, but similar temporal trend was not observed. The differences in species-specific composition of bacteria from genus *Pseudomonas* between bulk soil and rhizosphere samples were not as significant as in the case of total bacterial community composition. However, semi-coke samples demonstrated bigger differences in the community structure of *Pseudomonas* sp. compared to the differences in the total microbial community structure.

5.4.3. Changes in the diversity of *mPH* genes

In addition to the changes in microbial community composition in taxonomic level, vegetation, added bacterial strains and covering material grass seeds were covered also influenced the diversity of *mPH* genes in the semi-coke. Multi-component phenol hydroxylase gene was used since it has been reported that this gene is predominant in bacteria isolated from phenol-polluted area (Peters et al., 1997). Result revealed that the greatest temporal changes in the diversity of *mPH* genes occurred in the control plot and plot with sand treatment, and the temporal dynamics were similar in these cases (Fig. 6). The *mPH* gene diversity in plots with no treatment, peat, and added bacterial strains was relatively stable in time compared to the control and sand treatment. Previous study showed that two different multicomponent phenol hydroxylases belonging to low- and moderate *Ks* groups dominated in semi-coke plots with vegetation (Truu et al., 2003). Also in this study, during the experiment mostly *mPH* gene sequences from low- and moderate *Ks* groups were found in vegetated plots indicating more efficient degradation of aromatics at these plots.

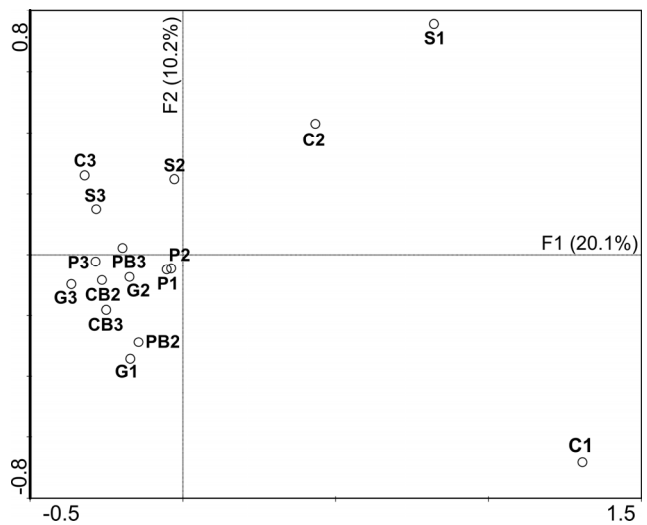

Figure 6. Principal component analyses representing the changes in the diversity of *mPH* genes in semi-coke plots with grass species and different treatments. PCA is based on the densitometric curves of DGGE fingerprint. The first and second principal components (F1 and F2) describe 20.1 and 10.2% of the overall data variation, respectively. Sample codes are same as in Fig. 3 in Reference II.

5.4.4. Survival and catabolic performance of introduced strains

16S rRNA and *lmPH* gene fragments and cDNA of 16S rRNA were targeted to study the presence of the introduced *Pseudomonas* strains and their catabolic traits in semi-coke. According to the results obtained from the DGGE and subsequent nucleotide sequencing, gene fragments corresponding to the inoculated bacterial strains were found in both the bulk soil and rhizosphere samples of the birches (Fig. 3 and Fig. 4 in Reference III). In addition, the survival of the strains mainly in the rhizosphere samples was confirmed by cultivation based methods (Table 5 and Fig. 2 in Reference III). The best survival among introduced bacteria was demonstrated by strain PC20, whereas strain PC17 survived only for a very short period of time. Based on these results it is obvious that vegetation contributed very effectively to the survival of introduced strains. In addition to the vegetation, the origin of the strains may be the second reason for their long-term survival, as the set of bacteria was isolated from a nearby area with similar conditions concerning pollutants. It has been suggested, that the best way to increase the survival of the inoculum is to look for the candidate microorganisms from the same ecological niche as the polluted area (El Fantroussi and Agathos, 2005).

In addition to the presence of introduced strains, substrate induced soil respiration test revealed that bioaugmented samples demonstrated an elevated phenol biodegradation as long as 40 months after the introduction of bacterial biomass to the site (Table 6 in Reference III). However, it is complicated to assess the contribution of the introduced bacteria to that biodegradation activity. In respirometric tests with varying inoculum density, the obtained pseudo-first-order constant values positively correlated with the density of the bacterial mixture in suspension but this relationship cannot be directly transferred to the data obtained with field samples as contact time between the soil and introduced bacteria are too different in these two cases. Still, the RNA based microbial community fingerprint of the samples detected and confirmed the presence of 16S rRNA fragments corresponding to the introduced strains in both, bulk soil and rhizosphere at least in the short period of time.

5.5. General discussion

The degradation of organic contaminants in soil during phytoremediation relies mostly on the rhizodegradation by plant-associated microbes. Some contaminants, such as phenols are easily biodegradable by microorganisms, also oil products, although crude oil consists of a mixture of different compounds some of which are more recalcitrant to biodegradation. However, because of the chemical properties of the semi-coke, the rate of biodegradation in semi-coke is hardly comparable with the rate of biodegradation in natural soils. Estonian oil shale is rich in sulphur and in the retorting process more than half of it remains in the solid residue. In addition to the sulphuric compounds, high initial pH

value as well as high concentration of Ca^{2+} and Mg^{2+} ions may limit microbial activity in semi-coke. Also, the inhibition to the germination and radicle growth of timothy (*Phleum pratense*) caused by calcium and other ions in semi-coke water extracts have been found (Raave et al., 2007). Nevertheless, it has been shown that seeds can easily germinate and grasses grow on semi-coke of several years of age, and mixing semi-coke with acidic sphagnum peat and weathering decrease the inhibition effect of semi-coke (Raave et al., 2004). Another group of factors affecting the biodegradation activity is the susceptibility of organic pollutants to microbial attack. Among organic compounds found in semi-coke, asphaltenes are the most resistant fraction for bioremediation (Capelli et al., 2001; Peressutti et al., 2003). Asphaltenes are also known for the inhibition of some physiological groups of microorganisms in soil like actinomycetes as well as microbes participating in the processes of nitrogen and phosphorus transformations (Muratova et al., 2005).

We found in our experiments that vegetation and bioaugmentation enhanced the removal of contaminants from the soil. Semi-coke plots with vegetation and bacterial strains demonstrated higher microbial metabolic activity, and phenol biodegradation for example remained higher even 40 months after the inoculation of the bacterial strains into the semi-coke. Rhizosphere samples demonstrated generally higher values of microbial counts as well as microbial activity. Introduced bacterial strains were found to survive, and their catabolic traits persisted in semi-coke for a long period of time, especially in rhizosphere zone. In addition, vegetation and bioaugmentation was found to influence the microbial community composition and the structure of catabolic genes. In the light of these results, it is also tempting to speculate over the possibility of catabolic gene transfer from the introduced bacterial strains to the indigenous microbial community in semi-coke. We know that four *Pseudomonas* strains used in these experiments (PC17, PC18, PC20, and PC24) contain plasmids that carry catabolic genes. Strain PC20 has two large plasmid replicons, a conjugative naphthalene plasmid and a smaller plasmid bearing the *pheBA* operon encoding the enzymes for phenol degradation via *ortho* pathway of catechol degradation. The transfer of the genetic information between bacteria has been shown in soils and it is suggested that rhizosphere may be a habitat which allows a higher frequency of catabolic gene transfer. In our experiment, semi-coke plots with a mixture of grass species possessed a very intense root area compared to the plots covered birches. Therefore, the transfer of catabolic genes is possible but it needs extensive study.

However, vegetation and bioaugmentation facilitated the development of a functional and stable microbial community with an elevated capacity for the degradation of pollutants in semi-coke.

CONCLUSIONS

Phytoremediation and bioaugmentation field experiment was applied at the semi-coke depository area to study the impact of vegetation and introduced bacterial strains on the degradation of pollutants, microbial activity, composition of microbial community and structure of catabolic genes in semi-coke. In addition, survival and catabolic performance of used *Pseudomonas* sp. strains was studied in this thesis. Based on the results following conclusions can be made:

- Vegetation was the key factor for the acceleration of pollutants degradation in semi-coke. Vegetation and bioaugmentation had short term effect on the numbers of phenol degrading bacteria and on the microbial potential metabolic activity. Microbial counts and metabolic activity in rhizosphere were higher compared to bulk soil and these values remained stable during the experiment. Also, the biodegradation of pollutants was exceptionally high in rhizosphere.
- Vegetation, added bacterial strains and covering material had substantial influence on the bacterial community composition and development in semi-coke. Although bacterial community composition in rhizosphere was different compared to that in bulk soil, the effect of vegetation on the removal of pollutants was not merely associated with the rhizosphere effect but the utilization of vegetation in general (particularly in case of grass species). Bacterial community composition in rhizosphere was relatively stable over the time compared to the dynamics in bulk soil. The microbial diversity was similar in vegetated samples and samples without vegetation showing that instead of increasing the overall bacterial diversity only specific bacterial populations and their abundance were influenced by the plants resulting in the enhanced metabolic activity.
- Used *Pseudomonas* sp. strains both survived and their metabolic traits persisted in the contaminated site over a long period of time due to vegetation. The strains were found to survive particularly well in rhizosphere. Vegetated soil samples with added bacterial strains also demonstrated a higher rate and efficiency of biodegradation of pollutants for long period of time. Therefore, in addition to the survival of the strains vegetation and added bacterial strains facilitated the development of a functional and stable microbial community with an elevated capacity for the degradation of pollutants in semi-coke.

On the basis of our findings it can be concluded that phytoremediation and bioaugmentation can be considered as an alternative management option for the remediation of oil shale solid waste.

REFERENCES

Abhilash PC, Jamil S, Singh N. Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics. Biotechnol. Adv. 2009; 27: 474–488.

Anokhina TO, Kochetkov VV, Zelenkova NF, Balakshina VV, Boronin AM. Biodegradation of phenanthrene by *Pseudomonas* bacteria bearing rhizospheric plasmids in model plant-microbial associations. Appl. Biochem. Microbiol. 2004; 40: 568–572.

Arthur EL, Rice PJ, Rice PJ, Anderson TA, Baladi SM, Henderson KLD, Coats JR. Phytoremediation – an overview. Crit. Rev. Plant Sci. 2005; 24: 109–122.

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM. How plants communicate using the underground information superhighway. Trends Plant Sci. 2004; 9: 26–32.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu. Rev. Plant. Biol. 2006; 57: 233–266.

Barac T, Taghavi S, Borremans B, Provoost A, Oeyen L, Colpaert JV, Vangronsveld J, van der Lelie D. Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nat. Biotechnol. 2004; 22: 583–588.

Barac T, Weyens N, Oeyen L, Taghavi S, van der Lelie D, Dubin D, Spliet M, Vangronsveld J. Field note: Hydraulic containment of a BTEX plume using poplar trees. Int. J. Phytoremediat. 2009; 11: 416–424.

Baroja O, Rodriguez E, de Balugera ZG, Goicolea A, Unceta N, Sampedro C, Alonso A, Barrio RJ. Speciation of volatile aromatic and chlorinated hydrocarbons in an urban atmosphere using tct-gc/ms. J. Environ. Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng. 2005; 40: 343–367.

Bathe S, Mohan TV, Wuertz S, Hausner M. Bioaugmentation of a sequencing batch biofilm reactor by horizontal gene transfer. Water Sci. Technol. 2004; 49: 337–344.

Bathe S, Schwarzenbeck N, Hausner M. Plasmid-mediated bioaugmentation of activated sludge bacteria in a sequencing batch moving bed reactor using pnb2. Lett. Appl. Microbiol. 2005; 41: 242–247.

Berg G, Krechel A, Ditz M, Sikora RA, Ulrich A, Hallmann J. Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiol. Ecol. 2005; 51: 215–229.

Berg G, Smalla K. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 2009; 68: 1–13.

Boon N, Goris J, De Vos P, Verstraete W, Top EM. Bioaugmentation of activated sludge by an indigenous 3-chloroaniline-degrading *Comamonas testosteroni* strain, i2gfp. Appl. Environ. Microbiol. 2000; 66: 2906–2913.

Bouchez T, Patureau D, Dabert P, Juretschko S, Dore J, Delgenes P, Moletta R, Wagner M. Ecological study of a bioaugmentation failure. Environ. Microbiol. 2000a; 2: 179–190.

Bouchez T, Patureau D, Dabert P, Wagner M, Delgenes JP, Moletta R. Successful and unsuccessful bioaugmentation experiments monitored by fluorescent in situ hybridization. 4th International Symposium of Environmental Biotechnology (ISEB 4). I W a Publishing, Noordwijkerhout, Netherlands, 2000b, pp. 61–68.

Brazil GM, Kenefick L, Callanan M, Haro A, Delorenzo V, Dowling DN, Ogara F. Construction of a rhizosphere pseudomonad with potential to degrade poly-

chlorinated-biphenyls and detection of bph gene-expression in the rhizosphere. Appl. Environ. Microbiol. 1995; 61: 1946–1952.
Bremer C, Braker G, Matthies D, Reuter A, Engels C, Conrad R. Impact of plant functional group, plant species, and sampling time on the composition of nirK-type denitrifier communities in soil. Appl. Environ. Microbiol. 2007; 73: 6876–6884.
Briones AM, Okabe S, Umemiya Y, Ramsing NB, Reichardt W, Okuyama H. Influence of different cultivars on populations of ammonia-oxidizing bacteria in the root environment of rice. Appl. Environ. Microbiol. 2002; 68: 3067–3075.
Burken JG, Schnoor JL. Predictive relationships for uptake of organic contaminants by hybrid poplar trees. Environ. Sci. Technol. 1998; 32: 3379–3385.
Buyer JS, Roberts DP, Russek-Cohen E. Soil and plant effects on microbial community structure. Can. J. Microbiol. 2002; 48: 955–964.
Capelli SM, Busalmen JP, de Sanchez SR. Hydrocarbon bioremediation of a mineral-base contaminated waste from crude oil extraction by indigenous bacteria. Int. Biodeterior. Biodegrad. 2001; 47: 233–238.
Chaudhry Q, Blom-Zandstra M, Gupta S, Joner EJ. Utilising the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. Environ. Sci. Pollut. Res. Int. 2005; 12: 34–48.
Chekol T, Vough LR, Chaney RL. Phytoremediation of polychlorinated biphenyl-contaminated soils: The rhizosphere effect. Environ. Int. 2004; 30: 799–804.
Chen SH, Aitken MD. Salicylate stimulates the degradation of high molecular weight polycyclic aromatic hydrocarbons by *Pseudomonas saccharophila* p15. Environ. Sci. Technol. 1999; 33: 435–439.
Cherian S, Oliveira MM. Transgenic plants in phytoremediation: Recent advances and new possibilities. Environ. Sci. Technol. 2005; 39: 9377–9390.
Cledera-Castro M, Santos-Montes A, Izquierdo-Hornillos R. Comparison of the performance of conventional microparticulates and monolithic reversed-phase columns for liquid chromatography separation of eleven pollutant phenols. 25th International Symposium on Chromatography. Elsevier Science Bv, Paris, FRANCE, 2004, pp. 57–63.
Combourieu B, Besse P, Sancelme M, Maser E, Delort AM. Evidence of metyrapone reduction by two *Mycobacterium* strains shown by h-1 nmr. Biodegradation 2004; 15: 125–132.
Cordova-Rosa SM, Dams RI, Cordova-Rosa EV, Radetski MR, Correa AX, Radetski CM. Remediation of phenol-contaminated soil by a bacterial consortium and *Acinetobacter calcoaceticus* isolated from an industrial wastewater treatment plant. J. Hazard. Mater. 2009; 164: 61–66.
Cunliffe M, Kawasaki A, Fellows E, Kertesz MA. Effect of inoculum pretreatment on survival, activity and catabolic gene expression of *Sphingobium yanoikuyae* b1 in an aged polycyclic aromatic hydrocarbon-contaminated soil. FEMS Microbiol. Ecol. 2006; 58: 364–372.
Cunningham SD, Berti WR. Remediation of contaminated soils with green plants: An overview. In Vitro Cell. Dev. Biol. 1993; 29: 207–212.
Dams RI, Paton GI, Killham K. Rhizoremediation of pentachlorophenol by *Sphingo-bium chlorophenolicum* atcc 39723. Chemosphere 2007; 68: 864–870.
Dejonghe W, Boon N, Seghers D, Top EM, Verstraete W. Bioaugmentation of soils by increasing microbial richness: Missing links. Environ. Microbiol. 2001; 3: 649–657.
Doty SL. Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol. 2008; 179: 318–333.

Dzantor EK. Phytoremediation: The state of rhizosphere 'engineering' for accelerated rhizodegradation of xenobiotic contaminants. J. Chem. Technol. Biotechnol. 2007; 82: 228–232.

Duineveld BM, Kowalchuk GA, Keijzer A, van Elsas JD, van Veen JA. Analysis of bacterial communities in the rhizosphere of Chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. Appl. Environ. Microbiol. 2001; 67: 172–178.

Eapen S, D'Souza SF. Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotechnol. Adv. 2005; 23: 97–114.

Eapen S, Singh S, D'Souza SF. Advances in development of transgenic plants for remediation of xenobiotic pollutants. Biotechnol. Adv. 2007; 25: 442–451.

El Fantroussi S, Agathos SN. Is bioaugmentation a feasible strategy for pollutant removal and site remediation? Curr. Opin. Microbiol. 2005; 8: 268–275.

Esteve-Nunez A, Rothermich M, Sharma M, Lovley D. Growth of *Geobacter sulfurreducens* under nutrient-limiting conditions in continuous culture. Environ. Microbiol. 2005; 7: 641–648.

Fattorini D, Notti A, Nigro M, Regoli F. Hyperaccumulation of vanadium in the antarctic polychaete perkinsiana littoralis as a natural chemical defense against predation. Environ. Sci. Pollut. Res. 2010; 17: 220–228.

Fierer N, Jackson JA, Vilgalys R, Jackson RB. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 2005; 71: 4117–4120.

Fulthorpe RR, Roesch LFW, Riva A, Triplett EW. Distantly sampled soils carry few species in common. Isme J. 2008; 2: 901–910.

Gao Y, Zhu L. Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere 2004; 55: 1169–1178.

Garbeva P, van Veen JA, van Elsas JD. Microbial diversity in soil: Selection microbial populations by plant and soil type and implications for disease suppressiveness. Annu. Rev. Phytopathol. 2004; 42: 243–270.

Garbisu C, Alkorta I. Phytoextraction: A cost-effective plant-based technology for the removal of metals from the environment. Bioresour. Technol. 2001; 77: 229–236.

Gea T, Barrena R, Artola A, Sanchez A. Monitoring the biological activity of the composting process: Oxygen uptake rate (our), respirometric index (ri), and respiratory quotient (rq). Biotechnol. Bioeng. 2004; 88: 520–527.

Gentry TJ, Rensing C, Pepper IL. New approaches for bioaugmentation as a remediation technology. Crit. Rev. Env. Sci. Technol. 2004; 34: 447–494.

Gerhardt KE, Huang XD, Glick BR, Greenberg BM. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. Plant Sci. 2009; 176: 20–30.

Germaine K, Keogh E, Garcia-Cabellos G, Borremans B, van der Lelie D, Barac T, Oeyen L, Vangronsveld J, Moore FP, Moore ERB, Campbell CD, Ryan D, Dowling DN. Colonisation of poplar trees by gfp expressing bacterial endophytes. FEMS Microbiol. Ecol. 2004; 48: 109–118.

Germaine KJ, Keogh E, Ryan D, Dowling DN. Bacterial endophyte-mediated naphthalene phytoprotection and phytoremediation. FEMS Microbiol. Lett. 2009; 296: 226–234.

Germaine KJ, Liu X, Cabellos GG, Hogan JP, Ryan D, Dowling DN. Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. FEMS Microbiol. Ecol. 2006; 57: 302–310.

Germida JJ, Siciliano SD. Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars. Biol. Fertil. Soils 2001; 33: 410–415.

Golubev S, Schelud'ko A, Muratova A, Makarov O, Turkovskaya O. Assessing the potential of rhizobacteria to survive under phenanthrene pollution. Water Air Soil Pollut. 2009; 198: 5–16.

Graner G, Persson P, Meijer J, Alstrom S. A study on microbial diversity in different cultivars of *Brassica napus* in relation to its wilt pathogen, *Verticillium longisporum*. FEMS Microbiol. Lett. 2003; 224: 269–276.

Grayston SJ, Griffith GS, Mawdsley JL, Campbell CD, Bardgett RD. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biol. Biochem. 2001; 33: 533–551.

He Y, Xu JM, Tang CX, Wu YP. Facilitation of pentachlorophenol degradation in the rhizosphere of ryegrass (*Lolium perenne l.*). Soil Biol. Biochem. 2005; 37: 2017–2024.

Heinaru E, Merimaa M, Viggor S, Lehiste M, Leito I, Truu J, Heinaru A. Biodegradation efficiency of functionally important populations selected for bioaugmentation in phenol- and oil-polluted area. FEMS Microbiol. Ecol. 2005; 51: 363–373.

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 Microbiol. Ecol. 2000; 31: 195–205.

Hontzeas N, Zoidakis J, Glick BR, Abu-Omar MM. Expression and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the rhizobacterium *Pseudomonas putida* uw4: A key enzyme in bacterial plant growth promotion. Biochim. Biophys. Acta 2004; 1703: 11–19.

Hoshino YT, Matsumoto N. DNA- versus RNA-based denaturing gradient gel electrophoresis profiles of a bacterial community during replenishment after soil fumigation. Soil Biol. Biochem. 2007; 39: 434–444.

Huang XD, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM. A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ. Pollut. 2004; 130: 465–476.

Jacobsen CS. Plant protection and rhizosphere colonization of barley by seed inoculated herbicide degrading *Burkholderia* (*Pseudomonas*) *cepacia* dbo1(pro101) in 2,4-d contaminated soil. Plant Soil 1997; 189: 139–144.

Jacques RJS, Okeke BC, Bento FM, Teixeira AS, Peralba MCR, Camargo FAO. Microbial consortium bioaugmentation of a polycyclic aromatic hydrocarbons contaminated soil. Bioresour. Technol. 2008; 99: 2637–2643.

Jézéquel K, Lebeau T. Soil bioaugmentation by free and immobilized bacteria to reduce potentially phytoavailable cadmium. Bioresour. Technol. 2008; 99: 690–698.

Joner EJ, Leyval C. Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular mycorrhiza. Environ. Sci. Technol. 2003; 37: 2371–2375.

Jones DL. Organic acids in the rhizosphere – a critical review. Plant Soil 1998; 205: 25–44.

Jousset A, Lara E, Wall LG, Valverde C. Secondary metabolites help biocontrol strain *Pseudomonas fluorescens* cha0 to escape protozoan grazing. Appl. Environ. Microbiol. 2006; 72: 7083–7090.

Kahru A, Põllumaa L. Environmental hazard of the waste streams of Estonian oil shale industry: An ecotoxicological review. Oil Shale 2006; 23: 53–93.

Kattai V, Lokk U. Historical review of the kukersite oil shale exploration in Estonia Oil Shale 1998; 15: 102–110.
Kawahigashi H. Transgenic plants for phytoremediation of herbicides. Curr. Opin. Biotechnol. 2009; 20: 225–230.
Khan FI, Husain T, Hejazi R. An overview and analysis of site remediation technologies. J. Environ. Manage. 2004; 71: 95–122.
Kim D, Park MJ, Koh SC, So JS, Kim E. Three separate pathways for the initial oxidation of limonene, biphenyl, and phenol by *Rhodococcus* sp strain t104. J. Microbiol. 2002; 40: 86–89.
Kirk JL, Klironomos JN, Lee H, Trevors JT. The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil. Environ. Pollut. 2005; 133: 455–465.
Korenkova E, Matisova E, Slobodnik J. Application of large volume injection GC-MS to analysis of organic compounds in the extracts and leachates of municipal solid waste incineration fly ash. Waste Manage. 2006; 26: 1005–1016.
Krämer U, Talke IN, Hanikenne M. Transition metal transport. FEBS Letters 2007; 581: 2263–2272.
Kuiper I, Bloemberg GV, Lugtenberg BJJ. Selection of a plant-bacterium pair as a novel tool for rhizostimulation of polycyclic aromatic hydrocarbon-degrading bacteria. Mol. Plant-Microbe Interact. 2001; 14: 1197–1205.
Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJ. Rhizoremediation: A beneficial plant-microbe interaction. Mol. Plant-Microbe Interact. 2004; 17: 6–15.
Kumar R, Pandey S, Pandey A. Plant roots and carbon sequestration. Curr. Sci. 2006; 91: 885–890.
Leigh MB, Prouzova P, Mackova M, Macek T, Nagle DP, Fletcher JS. Polychlorinated biphenyl (PCB)-degrading bacteria associated with trees in a PCB-contaminated site. Appl. Environ. Microbiol. 2006; 72: 2331–2342.
Lillis L, Doyle E, Clipson N. Comparison of DNA- and RNA-based bacterial community structures in soil exposed to 2,4-dichlorophenol. J. Appl. Microbiol. 2009; 107: 1883–1893.
Liste HH, Prutz I. Plant performance, dioxygenase-expressing rhizosphere bacteria, and biodegradation of weathered hydrocarbons in contaminated soil. Chemosphere 2006; 62: 1411–1420.
Little AEF, Robinson CJ, Peterson SB, Raffa KE, Handelsman J. Rules of engagement: Interspecies interactions that regulate microbial communities. Annu. Rev. Microbiol. 2008; 62: 375–401.
Liu L, Jiang CY, Liu XY, Wu JF, Han JG, Liu SJ. Plant-microbe association for rhizoremediation of chloronitroaromatic pollutants with *Comamonas* sp. Strain cnb-1. Environ. Microbiol. 2007; 9: 465–473.
Lodewyckx C, Vangronsveld J, Porteous F, Moore ERB, Taghavi S, Mezgeay M, van der Lelie D. Endophytic bacteria and their potential applications. Crit. Rev. Plant Sci. 2002; 21: 583–606.
Ma X, Burken JG. TCE diffusion to the atmosphere in phytoremediation applications. Environ. Sci. Technol. 2003; 37: 2534–2539.
Ma X, Richter AR, Albers S, Burken JG. Phytoremediation of MTBE with hybrid poplar trees. Int. J. Phytoremediat. 2004; 6: 157–167.
Macek T, Mackova M, Kas J. Exploitation of plants for the removal of organics in environmental remediation. Biotechnol. Adv. 2000; 18: 23–34.

Mannisto MK, Tiirola MA, Puhakka JA. Degradation of 2,3,4,6-tetrachlorophenol at low temperature and low dioxygen concentrations by phylogenetically different groundwater and bioreactor bacteria. Biodegradation 2001; 12: 291–301.

Master ER, Mohn WW. Induction of bphA, encoding biphenyl dioxygenase, in two polychlorinated biphenyl-degrading bacteria, psychrotolerant *Pseudomonas* strain cam-1 and mesophilic *Burkholderia* strain lb400. Appl. Environ. Microbiol. 2001; 67: 2669–2676.

McCully ME. Roots in soil: Unearthing the complexities of roots and their rhizospheres. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 1999; 50: 695–718.

McGuinness M, Dowling D. Plant-associated bacterial degradation of toxic organic compounds in soil. Int. J. Environ. Res. Public Health 2009; 6: 2226–2247.

Meagher RB. Phytoremediation of toxic elemental and organic pollutants. Curr. Opin. Plant. Biol. 2000; 3: 153–162.

Mengoni A, Tatti E, Decorosi F, Viti C, Bazzicalupo M, Giovannetti L. Comparison of 16S rRNA and 16S rDNA T-RFLP approaches to study bacterial communities in soil microcosms treated with chromate as perturbing agent. Microb. Ecol. 2005; 50: 375–384.

Merimaa M, Heinaru E, Liivak M, Vedler E, Heinaru A. Grouping of phenol hydroxylase and catechol 2,3-dioxygenase genes among phenol- and *p*-cresol-degrading *Pseudomonas* species and biotypes. Arch. Microbiol. 2006; 186: 287–296.

Mohan SV, Falkentoft C, Nancharaiah YV, Sturm BSM, Wattiau P, Wilderer PA, Wuertz S, Hausner M. Bioaugmentation of microbial communities in laboratory and pilot scale sequencing batch biofilm reactors using the TOL plasmid. Bioresour. Technol. 2009; 100: 1746–1753.

Moore FP, Barac T, Borrernans B, Oeyen L, Vangronsveld J, van der Lelie D, Campbell CD, Moore ERB. Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: The characterisation of isolates with potential to enhance phytoremediation. Syst. Appl. Microbiol. 2006; 29: 539–556.

Motlep R, Kirsimae K, Talviste P, Puura E, Jurgenson J. Mineral composition of Estonian oil shale semi-coke sediments. Oil Shale 2007; 24: 405–422.

Muratova AY, Turkovskaya OV, Antonyuk LP, Makarov OE, Pozdnyakova LI, Ignatov VV. Oil-oxidizing potential of associative rhizobacteria of the genus azospirillum. Microbiology 2005; 74: 210–215.

Narasimhan K, Basheer C, Bajic VB, Swarup S. Enhancement of plant-microbe interactions using a rhizosphere metabolomics-driven approach and its application in the removal of polychlorinated biphenyls. Plant Physiol. 2003; 132: 146–153.

Nicomrat D, Dick WA, Tuovinen OH. Assessment of the microbial community in a constructed wetland that receives acid coal mine drainage. Microb. Ecol. 2006; 51: 83–89.

Nicorarat D, Dick WA, Dopson M, Tuovinen OH. Bacterial phylogenetic diversity in a constructed wetland system treating acid coal mine drainage. Soil Biol. Biochem. 2008; 40: 312–321.

Padmavathiamma PK, Li LY. Phytoremediation technology: Hyper-accumulation metals in plants. Water Air Soil Pollut. 2007; 184: 105–126.

Pae T, Luud A, Sepp M. Artificial mountains in north-east Estonia: Monumental dumps of ash and semi-coke. Oil Shale 2005; 22: 333–344.

Pandey J, Chauhan A, Jain RK. Integrative approaches for assessing the ecological sustainability of *in situ* bioremediation. FEMS Microbiol. Rev. 2009; 33: 324–375.

Pelz O, Tesar M, Wittich RM, Moore ER, Timmis KN, Abraham WR. Towards elucidation of microbial community metabolic pathways: Unravelling the network

of carbon sharing in a pollutant-degrading bacterial consortium by immunocapture and isotopic ratio mass spectrometry. Environ. Microbiol. 1999; 1: 167–174.
Peressutti SR, Alvarez HM, Pucci OH. Dynamics of hydrocarbon-degrading bacteriocenosis of an experimental oil pollution in patagonian soil. Int. Biodeterior. Biodegrad. 2003; 52: 21–30.
Persoh D, Theuerl S, Buscot F, Rambold G. Towards a universally adaptable method for quantitative extraction of high-purity nucleic acids from soil. J. Microbiol. Methods. 2008; 75: 19–24.
Peters M, Heinaru E, Talpsep E, Wand H, Stottmeister U, Heinaru A, Nurk A. Acquisition of a deliberately introduced phenol degradation operon, pheBA, by different indigenous *Pseudomonas* species. Appl. Environ. Microbiol. 1997; 63: 4899–4906.
Phillips LA, Germida JJ, Farrell RE, Greer CW. Hydrocarbon degradation potential and activity of endophytic bacteria associated with prairie plants. Soil Biol. Biochem. 2008; 40: 3054–3064.
Phillips LA, Greer CW, Farrell RE, Germida JJ. Field-scale assessment of weathered hydrocarbon degradation by mixed and single plant treatments. Appl. Soil Ecol. 2009; 42: 9–17.
Pieper DH, Pollmann K, Nikodem P, Gonzalez B, Wray V. Monitoring key reactions in degradation of chloroaromatics by *in situ* h-1 nuclear magnetic resonance: Solution structures of metabolites formed from cis-dienelactone. J. Bacteriol. 2002; 184: 1466–1470.
Pilon-Smits E. Phytoremediation. Annu. Rev. Plant. Biol. 2005; 56: 15–39.
Raaijmakers J, Paulitz T, Steinberg C, Alabouvette C, Moënne-Loccoz Y. The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 2009; 321: 341–361.
Raave H, Kapap S, Orupõld K. Phytotoxicity of oil shale semi-coke and its aqueous extracts: A study by seed germination bioassay. Oil Shale 2007; 24: 59–72.
Raave H, Kuldkepp P, Leedu E, Merivee A. Recultivation substance and composts produced from semi coke: The effect of soil characteristics, the yield of field crops and the environment. Oil Shale 2004; 21: 59–73.
Reichenauer TG, Germida JJ. Phytoremediation of organic contaminants in soil and groundwater. ChemSusChem 2008; 1: 708–717.
Roesch LF, Fulthorpe RR, Riva A, Casella G, Hadwin AKM, Kent AD, Daroub SH, Camargo FAO, Farmerie WG, Triplett EW. Pyrosequencing enumerates and contrasts soil microbial diversity. Isme J. 2007; 1: 283–290.
Ruberto L, Vazquez SC, Mac Cormack WP. Effectiveness of the natural bacterial flora, biostimulation and bioaugmentation on the bioremediation of a hydrocarbon contaminated antarctic soil. Int. Biodeterior. Biodegrad. 2003; 52: 115–125.
Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. Bacterial endophytes: Recent developments and applications. FEMS Microbiol. Lett. 2008; 278: 1–9.
Rylott EL, Bruce NC. Plants disarm soil: Engineering plants for the phytoremediation of explosives. Trends Biotechnol. 2009; 27: 73–81.
Salt DE, Smith RD, Raskin I. Phytoremediation. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 1998; 49: 643–668.
Schnoor JL, Licht LA, McCutcheon SC, Wolfe NL, Carreira LH. Phytoremediation of organic and nutrient contaminants. Environ. Sci. Technol. 1995; 29: 318–323.
Schroder P, Collins C. Conjugating enzymes involved in xenobiotic metabolism of organic xenobiotics in plants. Int. J. Phytoremediat. 2002; 4: 247–265.

Schroder P, Scheer CE, Diekmann F, Stampfl A. How plants cope with foreign compounds. Translocation of xenobiotic glutathione conjugates in roots of barley (*Hordeum vulgare*). Environ. Sci. Pollut. Res. Int. 2007; 14: 114–122.

Sen R. The root-microbe-soil interface: New tools for sustainable plant production. New Phytol. 2003; 157: 391–394.

Shah K, Nongkynrih JM. Metal hyperaccumulation and bioremediation. Biol. Plant. 2007; 51: 618–634.

Shaw LJ, Burns RG. Enhanced mineralization of [u-c-14]2,4-dichlorophenoxyacetic acid in soil from the rhizosphere of *Trifolium pratense*. Appl. Environ. Microbiol. 2004; 70: 4766–4774.

Sheoran V, Sheoran AS, Poonia P. Phytomining: A review. Miner. Eng. 2009; 22: 1007–1019.

Siciliano SD, Fortin N, Mihoc A, Wisse G, Labelle S, Beaumier D, Ouellette D, Roy R, Whyte LG, Banks MK, Schwab P, Lee K, Greer CW. Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. Appl. Environ. Microbiol. 2001; 67: 2469–2475.

Siciliano SD, Germida JJ. Biolog analysis and fatty acid methyl ester profiles indicate that pseudomonad inoculants that promote phytoremediation alter the root-associated microbial community of *Bromus biebersteinii*. Soil Biol. Biochem. 1998; 30: 1717–1723.

Siciliano SD, Germida JJ, Banks K, Greer CW. Changes in microbial community composition and function during a polyaromatic hydrocarbon phytoremediation field trial. Appl. Environ. Microbiol. 2003; 69: 483–489.

Siciliano SD, Goldie H, Germida JJ. Enzymatic activity in root exudates of dahurian wild rye (*Elymus dauricus*) that degrades 2-chlorobenzoic acid. J. Agric. Food Chem. 1998; 46: 5–7.

Singer AC, Crowley DE, Thompson IP. Secondary plant metabolites in phytoremediation and biotransformation. Trends Biotechnol. 2003; 21: 123–130.

Singer AC, Gilbert ES, Luepromchai E, Crowley DE. Bioremediation of polychlorinated biphenyl-contaminated soil using carvone and surfactant-grown bacteria. Appl. Microbiol. Biotechnol. 2000; 54: 838–843.

Singh BK, Millard P, Whiteley AS, Murrell JC. Unravelling rhizosphere-microbial interactions: Opportunities and limitations. Trends Microbiol. 2004; 12: 386–393.

Sipila TP, Keskinen AK, Akerman ML, Fortelius C, Haahtela K, Yrjala K. High aromatic ring-cleavage diversity in birch rhizosphere: PAH treatment-specific changes of ie3 group extradiol dioxygenases and 16S rRNA bacterial communities in soil. Isme J. 2008; 2: 968–981.

Sundberg C, Stendahl JSK, Tonderski K, Lindgren PE. Overland flow systems for treatment of landfill leachates – potential nitrification and structure of the ammonia-oxidising bacterial community during a growing season. Soil Biol. Biochem. 2007; 39: 127–138.

Suresh B, Ravishankar GA. Phytoremediation – a novel and promising approach for environmental clean-up. Crit. Rev. Biotechnol. 2004; 24: 97–124.

Susarla S, Medina VF, McCutcheon SC. Phytoremediation: An ecological solution to organic chemical contamination. Ecol. Eng. 2002; 18: 647–658.

Taghavi S, Barac T, Greenberg B, Borremans B, Vangronsveld J, van der Lelie D. Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene. Appl. Environ. Microbiol. 2005; 71: 8500–8505.

Thompson IP, van der Gast CJ, Ciric L, Singer AC. Bioaugmentation for bioremediation: The challenge of strain selection. Environ. Microbiol. 2005; 7: 909–915.

Top EM, Maila MP, Clerinx M, Goris J, De Vos P, Verstraete W. Methane oxidation as a method to evaluate the removal of 2,4-dichlorophenoxyacetic acid (2,4-d) from soil by plasmid-mediated bioaugmentation. FEMS Microbiol. Ecol. 1999; 28: 203–213.

Top EM, Springael D, Boon N. Catabolic mobile genetic elements and their potential use in bioaugmentation of polluted soils and waters. FEMS Microbiol Ecol 2002; 42: 199–208.

Top EM, Van Daele P, De Saeyer N, Forney LJ. Enhancement of 2,4-dichlorophenoxyacetic acid (2,4-d) degradation in soil by dissemination of catabolic plasmids. Anton. Leeuw. Int. J. G. 1998; 73: 87–94.

Torsvik V, Ovreas L. Microbial diversity and function in soil: From genes to ecosystems. Curr. Opin. Microbiol. 2002; 5: 240–245.

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. Environ. Sci. Pollut. Res. 2002: 8–14.

Truu J, Karme L, Talpsep E, Heinaru E, Vedler E, Heinaru A. Phytoremediation of solid oil shale waste from the chemical industry. Acta Biotechnol. 2003; 23: 301–307.

Truu J, Nurk K, Juhanson J, Mander U. Variation of microbiological parameters within planted soil filter for domestic wastewater treatment. J. Environ. Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng. 2005; 40: 1191–1200.

Truu M, Juhanson J, Truu J. Microbial biomass, activity and community composition in constructed wetlands. Sci. Total Environ. 2009; 407: 3958–3971.

Uhlik O, Jecna K, Leigh MB, Mackova M, Macek T. DNA-based stable isotope probing: A link between community structure and function. Sci. Total Environ. 2009; 407: 3611–3619.

Van Aken B, Peres CM, Doty SL, Yoon JM, Schnoor JL. *Methylobacterium populi* sp nov., a novel aerobic, pink-pigmented, facultatively methylotrophic, methane-utilizing bacterium isolated from poplar trees (*Populus deltoides* x nigra dn34). Int. J. Syst. Evol. Microbiol. 2004a; 54: 1191–1196.

Van Aken B, Yoon JM, Schnoor JL. Biodegradation of nitro-substituted explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, an octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine by a phytosymbiotic *Methylobacterium* sp associated with poplar tissues (*Populus deltoides* x nigra dn34). Appl. Environ. Microbiol. 2004b; 70: 508–517.

Wang Y, Xiao M, Geng X, Liu J, Chen J. Horizontal transfer of genetic determinants for degradation of phenol between the bacteria living in plant and its rhizosphere. Appl. Microbiol. Biotechnol. 2007; 77: 733–739.

Watanabe ME. Phytoremediation on the brink of commercialization. Environ. Sci. Technol. 1997; 31: 182–186.

Wenzel W. Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 2009; 321: 385–408.

Weyens N, Taghavi S, Barac T, van der Lelie D, Boulet J, Artois T, Carleer R, Vangronsveld J. Bacteria associated with oak and ash on a TCE-contaminated site: Characterization of isolates with potential to avoid evapotranspiration of TCE. Environ. Sci. Pollut. Res. 2009; 16: 830–843.

Villacieros M, Whelan C, Mackova M, Molgaard J, Sanchez-Contreras M, Lloret J, de Carcer DA, Oruezabal RI, Bolanos L, Macek T, Karlson U, Dowling DN, Martin M, Rivilla R. Polychlorinated biphenyl rhizoremediation by *Pseudomonas fluorescens*

f113 derivatives, using a *Sinorhizobium meliloti* nod system to drive *bph* gene expression. Appl. Environ. Microbiol. 2005; 71: 2687–2694.

Yee DC, Maynard JA, Wood TK. Rhizoremediation of trichloroethylene by a recombinant, root-colonizing *Pseudomonas fluorescens* strain expressing toluene ortho-monoooxygenase constitutively. Appl. Environ. Microbiol. 1998; 64: 112–118.

Yrjala K, Mancano G, Fortelius C, Akerman ML, Sipila TP. The incidence of *Burkholderia* in epiphytic and endophytic bacterial cenoses in hybrid aspen grown on sandy peat. Boreal Environ. Res. 2010; 15: 81–96.

SUMMARY IN ESTONIAN

Fütoremediatsiooni ja bioaugmentatsiooni mõju poolkoksi mikroobikooslusele

Eestis tekib enamik tahkete jäätmete kogusest põlevkivi kaevandamise ning kasutamise tulemusena. Põlevkivitööstuse erinevates harudes tekkivad tahked jäätmed võib jaotada kolmeks: kaevandamise aheraine (40% kogu põlevkivitööstuses tekkivast jäätmemassist), elektrijaamade tuhk (50%) ning keemiatööstuse poolkoks (10%). Poolkoks tekib keemiatööstuses põlevkivist termilise töötlemise, utmise abil põlevkiviõli tootmisel. Osaliselt meil esineva põlevkivi spetsiifilise keemilise koostise, kuid peamiselt põlevkiviõli tootmisel kasutatava tehnoloogia iseärasuste tõttu on protsessis tekkiv tahke jääde poolkoks neist keskkonnale kõige ohtlikum. Värske poolkoks on väga aluseline ning sisaldab suurtes kogustes sulfiide, Ca^{2+} ja Mg^{2+} ioone ning orgaanilist süsinikku. Nende omaduste tõttu on värske poolkoks Eestis kehtivas jäätmenimistus klassifitseeritud ohtlike jäätmete kategooriasse, kuid juba mõne aasta vanune poolkoks kuulub tavajäätmete hulka. Kuna poolkoksil puudub märkimisväärne taaskasutusväljund, on valdav osa sellest ladestatud prügilatesse. Käesoleval ajal kasutatakse põlevkivikeemiatööstuses umbes 1.7 miljonit tonni põlevkivi aastas, mille tagajärjel tekib ligikaudu 700 000 tonni poolkoksi. Kuna põlevkivi on Kirde-Eestis termiliselt töödeldud juba 85 aastat, on selle tulemusena tekkinud umbes 100 miljonit tonni poolkoksi, mis on ladestatud ligi 200 ha suurusele maa-alale. Suurema osa poolkoksiprügilatesse ladestatud jäätmetest hõlmab poolkoks, kuid prügilaid on ajaloo vältel kasutatud ka vedelate jäätmete, näiteks pigijäätmete (fuusid), väävlit sisaldavate setete, mineraalõlisid sisaldavate jäätmete, happetõrva (gudroon) ja reoveesette ladestamiseks. Igal juhul on poolkoksiprügilad oma praegusel kujul avatud erinevatele keskkonnamõjudele ning seega ohuks ümbritsevale keskkonnale. Poolkoksimägedelt pärit nõrgvesi ohustab eelkõige põhjavett ning ümberkaudseid veekogusid, sisaldades suurtes kogustes nii orgaanilisi (fenoolid, polüaromaatsed süsivesinikud, õliproduktid, bitumoidid) kui anorgaanilisi ühendeid (sulfiidid). Poolkoksimägedelt tulenev õhusaaste on ohtlik läheduses elavatele inimestele. Samuti on probleemiks poolkoksi iseeneslikud süttimised, mis on põhjustatud poolkoksis suures koguses sisalduvast orgaanilisest süsinikust.

Vastavalt Euroopa Liidu eeskirjadele peavad senini ladustamiseks kasutatud poolkoksiprügilad olema aastaks 2013 nõuetekohaselt suletud. Prügilad tuleb sulgeda selliselt, et ladestusala pindmine kiht muutuks ohutuks ja sellest ei lähtuks täiendavat põhja- ja pinnavee ning õhureostust, mis sisuliselt tähendab saasteallika isoleerimist keskkonnast. Kuna vanem poolkoks liigitatakse tavajäätme kategooriasse, siis oleks poolkoksiprügila teatud osades võimalik rakendada ka mõnd sellist saneerimise tehnoloogiat, mille eesmärgiks on saasteainete sisalduse vähendamine poolkoksis. Üheks selliseks tehnoloogiaks on taimede ja spetsiifiliste omadustega bakterite kasutamine. Käesoleva töö üldiseks eesmärgiks oli hinnata taimede ning spetsiifiliste omadustega bakterite kasutamise

võimalikkust poolkoksiprügila remediatsioonil. Töö kitsamateks eesmärkideks oli hinnata taimede ja spetsiifiliste omadustega bakteritüvede mõju saasteainete kontsentratsioonile, mikroobide aktiivsusele ning bakterikoosluse ja kataboolsete geenide struktuurile. Samuti oli eesmärgiks jälgida poolkoksi viidud bakteritüvede ellujäämist ja kataboolset aktiivsust uues keskkonnas.

Töö käigus saadud tulemused on kokkuvõtlikult järgmised:

- Taimestik ja lisatud bakteritüved kiirendasid saasteainete biodegradatsiooni poolkoksis. Samuti suurenes poolkoksis taimestiku ja poolkoksi viidud bakteritüvede mõjul lühiajaliselt fenooli lagundavate bakterite arvukus ning mikroobide potentsiaalne metaboolne aktiivsus. Taimede juurte vahetus läheduses, risosfääris, oli saasteainete biodegradatsiooni kiirus, aga ka bakterite arvukused ning metaboolne aktiivsus suurimad ning ajas stabiilsed.
- Taimestik, poolkoksi lisatud bakteritüved ning muruseemnete katmiseks kasutatud pinnas mõjutasid oluliselt bakterikoosluse struktuuri ja dünaamikat. Bakterikoosluse struktuur kaskede risosfääris erines juurevaba poolkoksi bakterikoosluse struktuurist ning oli ajas palju stabiilsem. Vaatamata bakterikoosluse struktuuri erinevustele olid risosfääri ja juurevaba poolkoksi bakterite liigilist mitmekesisust näitavate indeksite väärtused suhteliselt sarnased. Seega tulenes risosfääriproovide suurem metaboolne aktiivsus tõenäoliselt spetsiifiliste mikroobipopulatsioonide domineerimisest mikroobikoosluses. Rohttaimedega läbiviidud katses ei erinenud risosfääri mikroobikoosluse struktuur oluliselt juurevaba poolkoksi mikroobikoosluse struktuurist, mis näitab, et suurem metaboolne aktiivsus taimestatud poolkoksiproovides polnud ainuüksi seotud risosfääriefektiga, vaid taimede kasutamisega üldiselt.
- Bioaugmentatsioonil kasutatud bakteritüved ning neile iseloomulikud kataboolsete radade geenid leiti poolkoksist mitmeid kuid pärast bakteritüvede sisseviimist. Taimede osa bakteritüvede ellujäämisel oli oluline, sest bakteritüved leiti eelkõige risosfäärist. Lisaks bakterite ellujäämisele oli bakteritüvedega ja taimestikuga poolkoksiproovides veel 40 kuud pärast bakteritüvede poolkoksi viimist suurem fenooli lagundamise kiirus ning parem efektiivsus võrreldes bakteritüvedeta ja taimestikuta poolkoksiproovidega. Seega võib tulemustele tuginedes väita, et taimestiku ja sisseviidud bakteritüvede mõjul tekkis poolkoksis stabiilne ja funktsionaalne mikroobikooslus, mis oli võimeline pika aja jooksul näitama suuremat saasteainete lagundamise kiirust ja efektiivsust.

Kokkuvõtteks võib öelda, et taimede ja spetsiifiliste omadustega bakterite kooskasutamine on poolkoksiprügila saneerimise alternatiivse lahendusena sobivaks tehnoloogiaks.

ACKNOWLEDGEMENTS

I am most grateful to my supervisor Dr. Jaak Truu for guidance and encouragement he gave me during the entire course of my studies. I am also endlessly grateful to Eeva Heinaru for always having some free time to discuss details and problems related to field and lab experiments, writing manuscripts etc. I owe many thanks to the colleagues from room 105 (Eeva, Eve, Katja, Merike, Signe, Hiie, Mario) for creating and keeping a friendly working atmosphere during the time of studies. I also owe many thanks to prof. Ain Heinaru.

I would like to thank Ene Põldroos, Annely Kukk and Tiiu Rootslane for their support with technical and administrative work. I am thankful to Dr. Andres Mäe for taking continuous interest in my work performance and encouraging me when necessary.

Many thanks go to my greatest fans Liis, Paula and Signe. They were always present when I needed advice on any topic. Liis taught me the precision for lab experiments, carefulness and critical attitude when interpreting results; Paula showed me the importance of networking and socializing; and … well, the advice and support I received from Signe was just indescribable. These "extended discussions" I held with them at the dinette or in the office were usually the best part of my day.

I am very grateful to Stella for supporting me. Thanks to her I have tremendously expanded my horizon in other disciplines besides natural sciences. We held the most interesting discussions on religion, arts, philosophy, history, literature etc. I truly appreciate these times.

Finally, I am very grateful to my friends and family for all their support.

PUBLICATIONS

CURRICULUM VITAE

I. General data

Name:	Jaanis Juhanson
Date of birth:	30.09.1980, Pärnu
Citizenship:	Estonia
Aaddress:	Pikk 86–53, Tartu,
telephone:	+372 56 208 743,
e-mail:	jassj@ut.ee
Current position:	University of Tartu, Institute of Molecular and Cell Biology, extraordinary researcher

Education:

since 2005	University of Tartu, doctoral studies
2005	University of Tartu, MSc (environmental technology)
2003	University of Tartu, BSc (environmental technology)
1999	Pärnu Koidula Gymnasium

Language skills: Estonian, English

Working experience:

01.09.2009–	University of Tartu, Institute of Molecular and Cell Biology, extraordinary researcher
01.01.2007–31.12.2007	University of Tartu, Institute of Molecular and Cell Biology, extraordinary researcher
01.09.2005–31.12.2006	University of Tartu, Institute of Molecular and Cell Biology, specialist of technology

II. Scientific activities

1. Main research interests: Soil microbiology, bioremediation, phytoremediation, bioaugmentation.
2. List of publications:

Juhanson J, Truu J, Heinaru E, Heinaru A: Survival and catabolic performance of introduced *Pseudomonas* strains during phytoremediation and bioaugmentation field experiment. *FEMS Microbiol Ecol* 2009, 70 (3), 446–455.

Truu M, Juhanson J, Truu J: Microbial biomass, activity and community composition in constructed wetlands. *Sci Tot Environ* 2009, 407 (13), 3958–3971.

Limane B, Juhanson J, Truu J, Truu M, Muter O, Versilovskis A, Dubova L, Zarina Dz: Changes in microbial population affected by physicochemical conditions of soils contaminated by explosives. In *Current Re-*

search Topics in Applied Microbiology and Microbial Biotechnology, A. Mendez-Vilas (ed.). 2009, p 637–640.

Juhanson J, Truu J, Heinaru E, Heinaru A: Temporal dynamics of microbial community in soil during phytoremediation field experiment. *J Environ Eng Landsc Manag* 2007, 15 (4), 213–220.

Truu J, Heinaru E, Vedler E, Juhanson J, Viirmäe M, Heinaru A: Formation of microbial communities in oil shale chemical industry solid wastes during phytoremediation and bioaugmentation. In *Bioremediation of Soils Contaminated with Aromatic Compounds*, H. J. Heipieper (ed.). 2007, p 57–66.

Truu J, Nurk K, Juhanson J, Mander Ü: Variation of microbiological parameters within planted soil filter for domestic wastewater treatment. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 2005, 40 (6–7), 1191–1200.

3. Fellowships: FEMS Research Fellowship for studies in Helsinki University, Helsinki, Finland, March in 2005.
4. Other organizational and professional activities: Member of Estonian Society for Microbiologists

ELULOOKIRJELDUS

I. Üldandmed

Ees- ja perekonnanimi: Jaanis Juhanson
Sünniaeg ja koht: 30.09.1980, Pärnu
Kodakondsus: Eesti
Aadress: Pikk 86–53, Tartu,
telefon: +372 56 208 743,
e-post: jassj@ut.ee

Praegune töökoht, amet: Tartu Ülikool, erakorraline teadur

Haridus:

alates 2005	Tartu Ülikool, doktorantuur
2005	Tartu Ülikool, MSc (keskkonnatehnoloogia)
2003	Tartu Ülikool, BSc (keskkonnatehnoloogia)
1999	Pärnu Koidula Gümnaasium

Keelteoskus: Eesti, Inglise

Töökogemus:

alates 01.09.2009–	TÜ Molekulaar- ja Rakubioloogia Instituut, Geneetika õppetool; erakorraline teadur
01.01.2007–31.12.2007	TÜ Molekulaar- ja Rakubioloogia Instituut, Geneetika õppetool; erakorraline teadur (0.8)
01.09.2005–31.12.2006	TÜ Molekulaar- ja Rakubioloogia Instituut, Geneetika õppetool; tehnoloogiaspetsialist.

II. Teaduslik ja arendustegevus

1. Peamised uurimisvaldkonnad: Mulla mikrobioloogia, bioremediatsioon, fütoremediatsioon, bioaugmentatsioon.
2. Publikatsioonide loetelu

   Juhanson J, Truu J, Heinaru E, Heinaru A: Survival and catabolic performance of introduced *Pseudomonas* strains during phytoremediation and bioaugmentation field experiment. *FEMS Microbiol Ecol* 2009, 70 (3), 446–455.

   Truu M, Juhanson J, Truu J: Microbial biomass, activity and community composition in constructed wetlands. *Sci Tot Environ* 2009, 407 (13), 3958–3971.

   Limane B, Juhanson J, Truu J, Truu M, Muter O, Versilovskis A, Dubova L, Zarina Dz: Changes in microbial population affected by physico-chemical conditions of soils contaminated by explosives. In *Current Re-*

search Topics in Applied Microbiology and Microbial Biotechnology, A. Mendez-Vilas (Toim.). 2009, 637–640.

Juhanson J, Truu J, Heinaru E, Heinaru A: Temporal dynamics of microbial community in soil during phytoremediation field experiment. *J Environ Eng Landsc Manag* 2007, 15 (4), 213–220.

Truu J, Heinaru E, Vedler E, Juhanson J, Viirmäe M, Heinaru A: Formation of microbial communities in oil shale chemical industry solid wastes during phytoremediation and bioaugmentation. In *Bioremediation of Soils Contaminated with Aromatic Compounds*, H. J. Heipieper (Toim.). 2007, 57–66.

Truu J, Nurk K, Juhanson J, Mander Ü: Variation of microbiological parameters within planted soil filter for domestic wastewater treatment. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 2005, 40 (6–7), 1191–1200.

3. Saadud uurimistoetused ja stipendiumid: „FEMS Research Fellowship" stipendium uurimistöö läbiviimiseks Helsinki Ülikoolis Soomes (märts 2005).
4. Muu teaduslik organisatsiooniline ja erialane tegevus: Eesti Mikrobioloogide Ühenduse liige.

DISSERTATIONES TECHNOLOGIAE CIRCUMIECTORUM UNIVERSITATIS TARTUENSIS

1. **Sille Teiter.** Emission rates of N_2O, N_2, CH_4 and CO_2 in riparian grey alder forests and subsurface flow constructed wetlands. Tartu, 2005.
2. **Kaspar Nurk.** Relationships between microbial characteristics and environmental conditions in a horizontal subsurface flow constructed wetland for wastewater treatment. Tartu, 2005.
3. **Märt Öövel.** Performance of wastewater treatment wetlands in Estonia. Tartu, 2005.
4. **Alar Noorvee.** The applicability of hybrid subsurface flow constructed wetland systems with re-circulation for wastewater treatment in cold climates. Tartu, 2005.
5. **Christina Vohla.** Phosphorus removal by various filter materials in subsurface flow constructed wetlands. Tartu, 2008.
6. **Martin Maddison.** Dynamics of phytomass production and nutrient standing stock of cattail and its use for environment-friendly construction. Tartu, 2008.
7. **Marika Truu.** Impact of land use on microbial communities in Estonian soils. Tartu, 2008.
8. **Elar Põldvere.** Removal of organic material, nitrogen and phosphorus from wastewater in hybrid subsurface flow constructed wetlands. Tartu, 2009.
9. **Margit Kõiv.** Treatment of landfill leachate and municipal wastewater in subsurface flow filters using mineralized peat and hydrated oil shale ash. Tartu, 2010.