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Effect of modified Fenton's reaction on microbial activity and removal of PAHs in creosote oil contaminated soil

M.R.T. Palmroth[1,3], J.H. Langwaldt[1], T.A. Aunola[1], A. Goi[2], U. Münster[1], J.A. Puhakka[1] & T.A. Tuhkanen[1]
[1]*Environmental Engineering and Biotechnology, Tampere University of Technology, P.O. Box 541, Tampere, 33101, Finland;* [2]*Department of Chemical Engineering, Tallinn Technical University, Ehitajate tee 5, Tallinn, 19086, Estonia;* [3]*Department of Biotechnology, Lund University, P.O. Box 124, Lund 221 00, Sweden (*author for correspondence: e-mail: marja.palmroth@tut.fi)*

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Abstract

This study describes the removal of polycyclic aromatic hydrocarbons (PAHs) from creosote oil contaminated soil by modified Fenton's reaction in laboratory-scale column experiments and subsequent aerobic biodegradation of PAHs by indigenous bacteria during incubation of the soil. The effect of hydrogen peroxide addition for 4 and 10 days and saturation of soil with H_2O_2 on was studied. In both experiments the H_2O_2 dosage was 0.4 g H_2O_2/g soil. In completely H_2O_2–saturated soil the removal of PAHs (44% within 4 days) by modified Fenton reaction was uniform over the entire soil column. In non-uniformly saturated soil, PAH removal was higher in completely saturated soil (52% in 10 days) compared to partially saturated soil, with only 25% in 10 days. The effect of the modified Fenton's reaction on the microbial activity in the soil was assessed based on toxicity tests towards *Vibrio fischeri*, enumeration of viable and dead cells, microbial extracellular enzyme activity, and oxygen consumption and carbon dioxide production during soil incubation. During the laboratory-scale column experiments, the toxicity of column leachate towards *Vibrio fischeri* increased as a result of the modified Fenton's reaction. The activities of the microbial extracellular enzymes acetate- and acidic phosphomono-esterase were lower in the incubated modified Fenton's treated soil compared to extracellular enzyme activities in untreated soil. Abundance of viable cells was lower in incubated modified Fenton treated soil than in untreated soil. Incubation of soil in serum bottles at 20 °C resulted in consumption of oxygen and formation of carbon dioxide, indicating aerobic biodegradation of organic compounds. In untreated soil 20–30% of the PAHs were biodegraded during 2 months of incubation. Incubation of chemically treated soil slightly increased PAH-removal compared to PAH-removal in untreated soil.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants of soils and sediments. Clean-up processes for soils contaminated with PAHs have, so far, been limited to high-cost on-site stabilization or excavation followed by subsequent off-site incineration. *In situ* remediation of contaminated soils is considered more cost-efficient than on-site and off-site treatment (EPA 1998). Advanced oxidation processes, such as modified Fenton's reaction, and biodegradation are promising *in situ* remediation techniques. The *in situ* biodegradation of high molecular weight PAHs occurs slowly (Eriksson et al. 2003).

In advanced oxidation processes highly reactive radicals, i.e. hydroxyl radicals ($^{\bullet}OH$), capable of

oxidizing persistent organic contaminants are formed. In the original Fenton's reaction, the radicals are formed during the reaction of dilute H_2O_2 solution with Fe(II), the so-called Fenton's reagents. In Fenton-like reactions Fe(III) instead of Fe(II) is used, whereas modified Fenton's reactions apply concentrated H_2O_2 solutions, iron chelating agents or iron containing minerals at circum neutral pH. Modified Fenton's reactions have been used in the clean-up of soils contaminated with e.g. PAHs (Aunola et al. 2002; Lee & Hosomi 2001; Nam et al. 2001) and pentachlorophenol (Watts et al. 1990). A short-term chemical oxidation process combined with biodegradation may enhance PAH removal more than natural processes, because partially oxidized metabolites of the PAH are more water-soluble and thus more bioavailable (Matscheko et al. 2002).

The H_2O_2 used and the radicals formed in the Fenton's reaction are biocides (Büyüksönmez et al. 1998) and, therefore, reduce the abundance of viable cells. Earlier studies focused on the combination of Fenton's reaction with addition of microbial enrichment as sediment or mixed culture (Lee & Hosomi 2001; Nam et al. 2001). If the number of viable and PAH-degrading bacteria could be maintained during the Fenton's reaction, subsequent addition of bacteria would not be necessary. The low pH of 2–3 required for a standard Fenton's reaction can disturb the soil ecosystem and thus, may be incompatible with biological treatment (Nam et al. 2001). Thus, in this study with modified Fenton's reaction, the soil pH was not acidified prior to H_2O_2 addition to minimize stress on the soil bacteria. Two different intensities of H_2O_2 addition, i.e. applying the H_2O_2 at two different periods of time, were tested in laboratory-scale simulation of *in situ* remediation of soil contaminated with creosote oil, i.e. mainly PAHs.

The oxidized metabolites of PAHs have been shown to be more toxic to *Vibrio fischeri* than the parent compounds (El-Alawi et al. 2002) and thus, the chemically treated soil may be more toxic to bacteria than untreated soil. *Vibrio fischeri* is very sensitive to organic compounds (Vaajasaari et al. 2002). In this study, possible toxic and inhibitory effects of modified Fenton's reaction to soil bacteria were monitored with analyses of soil and leachate toxicity to *Vibrio fischeri* and indigenous microbial extracellular enzymatic activity in soil. The removal of PAHs from soil was determined immediately after modified Fenton's reaction and after incubation. In this study microbial activity after oxidation and following incubation was determined with analyses of bacterial abundance and activity of microbial extracellular enzymes as well as monitoring of concentrations of O_2, CO_2 and CH_4 during incubation.

Materials and methods

Soil column experiments

In current full-scale applications of modified Fenton reaction, the Fenton reagents are delivered to contaminated soil via injection wells. Therefore, in this study experiments were carried out in packed soil columns with an H_2O_2 delivery system simulating *in situ* injection. Experiments were performed at room temperature. The effect of a higher experimental temperature compared to *in situ* temperature was considered negligible, since the Fenton reaction is exothermic, thus soil temperature will increase during *in situ* treatment. Treatment of creosote oil contaminated soil was studied in laboratory-scale down-flow soil columns simulating field applications. Sandy, creosote oil contaminated soil (Table 1) was sampled from a wood impregnation site, in which industrial activities started in 1956. The site is located in an esker and the bedrock in depth of 5 m is overlaid by sand and gravel. Contamination of the soil occurred approximately in the 1960s, due to a leak in a storage tank and uncontrolled operating procedures. The highest contaminant concentrations are located directly above the bedrock surface. Sieved (<6 mm) contaminated soil was packed to a density of 1.25 kg/dm^3 into duplicate acryl columns, inner diameter 8 cm. The studied soil was rich in iron (Table 1) and thus iron was not added in the experiments. A H_2O_2 dosage of 0.4 g as 100% H_2O_2/g soil were applied within 4 (500 g soil) and 10 (700 g soil) days treatment times, respectively (Table 2). The 30% H_2O_2 solution was injected about 4 cm below soil surface into the columns via perforated Teflon tubes three times per day (Table 2). In the longer treatment experiment upper soil remained unsaturated due to smaller injection volumes (Table 2). The flushing of contaminants out of the column due to

Table 1. Soil content of C, N, P, potential Fenton's reaction catalysts, i. e. Fe and Mn, and PAHs

Compound	Concentration (mg/kg)	Uncertainty of measurement (mg/kg)
Total C	21400	Not given
Total N	Below quantification limit of 500	–
Total P	260	Not given
Total Fe	16400	3280 (95% confidence level)
Total Mn	140	40 (95% confidence level)
Concentrations are mean values of five determinations		SD (mg/kg)
Napthalene	Below detection limit	–
Acenaphthene	336	15
Fluorene	328	15
Phenanthrene	612	42
Anthracene	173	24
Fluoranthene	1313	85
Pyrene	785	55
Benz(a)anthracene	205	22
Chrysene	209	19
Total PAHs	3960	254

Table 2. Set-up of the column experiments. Aimed H_2O_2 dose as 100% hydrogen was 0.4 g H_2O_2 /g soil, but applied doses varied slightly

Experiment	Treatment time (d)	3 Injections per day (ml/injection)	Total volume injected to each column (ml)	Soil content (g)	H_2O_2 dose as 100% H_2O_2 (g H_2O_2/g soil)
Shorter treatment time	4	50 (H_2O_2)	600	500	0.40
Longer treatment time	10	25 (H_2O_2)	750	700	0.37
Control with deionised water	10	25 (H_2O)	750	700	0 (injection of H_2O)

liquid injection was studied in a control experiment in which deionised water (Milli-Q®, Millipore) instead of H_2O_2 was added to the soil for 10 days. Column leachates were collected after each injection of H_2O_2. In leachate, residual H_2O_2 was removed by addition of catalase or 10% Na_2SO_3 in excess to terminate the oxidation reactions and to prevent inhibition of bacteria by high H_2O_2 concentrations. Untreated soil and soil from the columns after the modified Fenton's reaction, i.e. from the upper and lower part of the columns, were collected for analyses. Residual H_2O_2 was not removed from the soil.

Incubation experiments

Biodegradation of PAHs in the soil during incubation was studied in serum bottles. Control soil was prepared by autoclaving the soil (121 °C, 1 h) twice with three days in between interval in order to kill spore-forming bacteria. Soil samples, including control, were incubated in sealed serum bottles at 20 °C for 5–16 weeks.

Chemical analyses

Dry matter content of soil samples was determined by oven-drying at 105 °C overnight. C- and N-concentrations in the sieved soil were determined with a CN-analyser and metals as well as P and S in the soil were extracted in HCl–HNO_3 mixture, 3:1 ratio, at 90 °C. The extract was analysed with inductively coupled plasma atomic emission spectrometer (ICP-AES). Soil pH was determined with a pH meter from soil mixed with deionised water according to the ASTM standard method D 4972 (ASTM 2001).

From the gas phase of the sealed serum bottles O_2, CO_2 and CH_4 were determined with gas chromatograph equipped with thermal conductivity detector (GC-TCD) (Perkin Elmer Sigma 300). Before gas measurements excess pressure was

removed with a gas syringe and the volume was recorded. Thereafter, the soil samples were taken and the bottles were resealed for further incubation.

The PAHs were extracted from soil samples (5–7 g) dried with anhydrous Na_2SO_4 with dichloromethane (60 ml) in a Soxhlet apparatus for 16 h at 105 °C. The extracts were diluted with dichloromethane and filtered (0.2 μm) prior to analysis. Internal standard (d10-phenanthrene) was added into the vials. A solution containing 16 PAHs (Z-0313-17, Accustandard) was used as external standard. The extracts were analysed with Hewlett Packard 6890 gas chromatograph equipped with a mass-selective detector (GC-MS) using selected ion monitoring. Further, from the filtered (0.45 μm) leachate samples the PAHs were extracted with dichloromethane. Dichloromethane extracts were concentrated under nitrogen stream to final volume and the PAHs were analysed, as described above.

Bioluminescence toxicity assay

Toxicity of samples to *Vibrio fischeri* was determined using a short term incubation assay (flash kinetics) modified from Lappalainen et al. (2001). The toxicity of leachates was determined to follow the formation of toxic metabolites during the modified Fenton's reaction. Catalase treated column leachates were combined for the toxicity analysis. The toxicity of soil was also determined to investigate the effect of residual H_2O_2 and incubation. The assay accounts for the color and turbidity caused by soil particulate matter. Prior to measurement, 6 and 8 g of 2% NaCl solution were added to 2 g of soil to obtain ratios of 1:3 and 1:4, respectively. Serial dilutions were prepared from these solutions by addition of 2% NaCl solution in a 1:1 ratio. Leachate samples were first diluted with 2% NaCl solution 1:2 and 1:3 and then serially diluted. The pH of the final solutions was adjusted to 6–8 with 1 M NaOH. The assay was performed in 96-well plates and 150 μl of each sample dilution were added to duplicate wells. NaCl solution (2%, 150 μl) was added to 4 control wells. Lyophilised *Vibrio fischeri* was rehydrated according to Biotox™ kit instructions (BO1243-500, Aboatox). Luminometer (Fluoroskan Ascent FL, Thermo Labsystems) was used to dispense 150 μl of *Vibrio fischeri* solution to a well and to measure simultaneously the luminescence intensity in the well. The kinetic measurement, at 40 ms intervals up to 1000 measurements, was continued for 40 s and each well was measured, again, after 30 min for 1 min. Inhibition percentages (INH%) were calculated using following formula: $\mathrm{INH\%} = 100 - 100 \times \frac{IT_t \times IC_0}{IT_0 \times IC_t}$, where IC_t is the luminescence intensity of the control sample after contact time t, IC_0 the maximum luminescence intensity of control sample, IT_t the luminescence intensity of the test sample after contact time t and IT_0 the maximum luminescence intensity of the test sample. EC_{50} values were calculated by plotting inhibition percentages against dilution.

Enumeration of dead and viable bacteria

BacLight™live/dead assay with SYTO®9 and propidium iodide stains (Viability Kit L-7012, Molecular Probes) was used to determine the viability based on cell wall integrity of bacteria in treated and untreated soil. Soil (1 g) was mixed with 50 ml sterile deionised water (Milli-Q®) and the suspension was sonicated (5 × 1 min). The suspension (50 or 100 μl) was mixed with 0.01 M tetra sodium pyrophosphate to achieve final volume of 1 ml in an Eppendorf tube. BacLight™ reagent (5 μl) was added to the mixture, vortexed for 10 s and incubated for 15 min in the dark. The final suspension and Eppendorf tube rinsing liquid (0.1 M tetra sodium pyrophosphate) was filtered with polycarbonate membrane filter (0.2 μm), supported by cellulose acetate filter (0.45 μm). At least 130 cells per filter were enumerated with an epifluorescence microscope (Zeiss Axioskop 2).

Microbial extracellular enzymatic activity in soil

The activity of microbial extracellular enzymes was followed in soil samples to determine the effect of the modified Fenton's reaction. The stock and working solutions of enzyme substrates and the model fluorogenic molecules, 7-amino-4-methylcoumarin (AMC) and 4-methylumbelliferone (MUF), and were prepared in dimethyl sulfoxide, due to the low water solubility of the compounds. The concentrations of model substrates in the assay were 1–500 μmol/l for L-leucine-7-AMC,

L-alanine-7-AMC and L-Serine-AMC hydrochloride, from 0.5 to 200 μmol/l for 4-MUF-phosphate potassium salt and from 2 to 1000 μmol/l for 4-MUF acetate. Standards of MUF and AMC (0.001–50 μmol/l) were prepared in duplicates in soil solutions i.e. standard curves were prepared separately for each sample to take into account possible quenching of the fluorescence by soil particles. The soil samples from untreated and treated (0.4 g as 100% H_2O_2/g soil) were first suspended 1:50 in sterile deionised water (Milli-Q®) and sonicated (5 × 1 min). The soil suspension (90 μl) was transferred to each of the wells in a 96-well microtiter plate. The soil suspension was not buffered to investigate the potential enzyme activity at field conditions.

The fluorescence of the hydrolysed model substrates and standards was measured at excitation 355 nm and emission 460 nm with a fluorometer (Fluoroskan Ascent FL, Thermo Labsystems), immediately after addition of soil suspension soil, 1 and 3 h. The rates of hydrolysed enzyme substrate $[\frac{\mu\text{mol MUF}}{\text{l}\times\text{g soil}\times\text{h}}]$ or $[\frac{\mu\text{mol AMC}}{\text{l}\times\text{g soil}\times\text{h}}]$ were calculated for dry soil to enable comparison of soil samples with different moisture content. The potential maximum rate of microbial extracellular enzyme hydrolysis is referred in text as v^*_{max}. The term potential is used in this context due to the interference of the soil matrix to the assay. The v^*_{max} values were calculated from Michaelis–Menten plots (SigmaPlot 2001™ Enzyme Kinetics Module 1.1, SPSS Inc.).

Results and discussion

Removal of PAHs during column and incubation experiments

Naphthalene was not detected in the creosote oil contaminated soil (Table 1), although in creosote oil, naphthalene and phenanthrene are the most abundant PAHs of the 16 PAHs identified as priority pollutants by EU and US EPA (Mueller et al. 1989). This indicates that naphthalene had either volatilised or degraded prior to soil sampling and/or during storage of the samples. The soil was rich in iron (16.4 g/kg soil) (Table 1), thus eliminating the need for iron supplementation. Due to injection of acidic 30% H_2O_2 solution (pH of 2–3), the pH of the column leachate decreased to 2, thus optimal for Fenton's reaction (Watts et al. 1990). The soil pH was slightly acidic (5–6) and the pH remained stable throughout the modified Fenton's reaction. Also other studies showed that soil pH remained stable during addition of hydrogen peroxide (Yeh et al. 2002; Qi et al. 2004), likely due to buffering capacity of soils (Scheffer et al. 2002). Therefore, additional adjustment of the soil pH was not carried out. In the 4-day experiment, the soil was saturated with H_2O_2 solution throughout the entire study, while only the lower part of the soil was saturated with H_2O_2 in the 10-day experiment. In the 10 days treatment, the removal in the lower part of the soil columns was higher (52%) than in the upper part of the column (25%). In the completely saturated soil, PAH-removal was uniformly 44% within 4 days (Figure 1). This PAH removal is lower compared to the results obtained by Nam et al. (2001) of a degradation of 80–99% of naphthalene, fluorene and phenanthrene in a slurry of coal-tar contaminated soil. This could be due to an improved mass transfer in stirred soil slurry (Nam et al. 2001). For higher molecular weight PAHs removal was 20–40% (Nam et al. 2001).

Approximately 10% of the PAHs, mainly 2- and 3-ring PAHs, were removed from the contaminated soil by injection of deionised water into the control column for 10 days. Lundstedt (2003) found a decrease of PAH-concentration in control treatment with sole water of 20% within a study on Fenton oxidation of PAHs. The removal of mainly 2- and 3-ring PAHs may be due to their higher water solubility compared to PAHs with more aromatic rings. However, the water solubility accounts only partially for the measured PAH removal. Dissolved organic matter increases the water solubility of hydrophobic pollutants (e.g. Chiou et al. 1986; Raber et al. 1998; Kögel-Knabner et al. 2000), whereas bioavailability of PAHs decreases by sorption to organic matter (Manilal & Alexander 1991; Alexander 2000). Therefore, it is likely that most of the PAHs were removed from the column being adsorbed onto the suspended solids of the leachate.

On average, 31% of PAHs were removed during 2 months of incubation of untreated contaminated soil (Figure 1, untreated soil). Thus, approximately 31% of the PAHs were available for aerobic biodegradation at 20 °C. The removal of PAHs during incubation of soil from the control

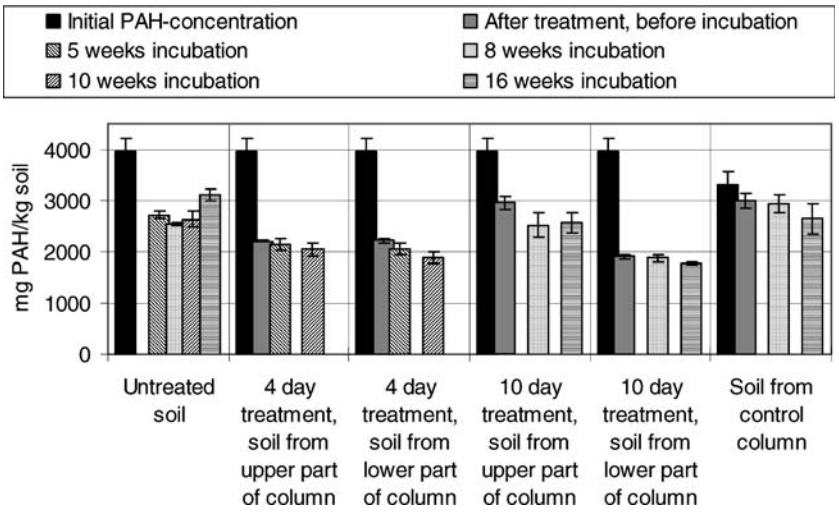

Figure 1. PAH concentrations in soil after modified Fenton's reaction and during incubation of untreated soil, soil from control column (10 days treatment) and H_2O_2 treated (4 and 10 days treatment) soil.

column was 12% (Figure 1, soil from control column). The removal of PAHs from soil treated with modified Fenton's reaction was up to 15% (increase from 25 to 36%) during incubation, as observed in unsaturated soil from upper part of column after the (Figure 1, 10-day treatment). The combination of modified Fenton's reaction and incubation slightly increased PAH-degradation and removed at most 54% of the PAHs (Figure 1, 10-day treatment, soil from lower part of column). Earlier, biodegradation studies in combination with Fenton's reaction have been conducted as slurry phase treatment for clean-up of PAH contaminated soil (Lee & Hosomi 2001; Nam et al. 2001). Increasing soil water content has been shown to increase the biodegradation of phenanthrene (Liu et al. 2001). In this study, the water content of incubated soil was up to 13%, the effect of water content on PAH biodegradation could be studied more in detail.

Effect of modified Fenton's reaction on toxicity to Vibrio fischeri

The toxicity of leachates and soil to *Vibrio fischeri* was determined to show the effect of modified Fenton's reaction on overall toxicity (Table 3). After application of H_2O_2, bioluminescence was below detection in all assay wells after 30 min of incubation of the microtiter plates, and, therefore, EC_{50} values of the 30 min assay could not be determined. Leachate toxicity was higher in the experiment with 4 days treatment time. This could be due to increased bioavailability of the PAHs or presence of formed toxic contaminant metabolites (Lundstedt 2003), since the oxidized PAH metabolites have been shown to be more toxic to *Vibrio fischeri* than the parent compounds (El-Alawi et al. 2002). Further, the oxidised PAH metabolites can accumulate in soil (Lundstedt 2003), which would indicate limited biodegradability. Whereas,

Table 3. Toxicity of column leachate to *Vibrio fischeri* in flash and 30 min toxicity test. EC_{50} values are given as percentage of the leachate after dilution and as dilution factor

Experiment	Flash test EC_{50} value		30 min test EC_{50} value	
	(%)	Dilution factor	(%)	Dilution factor
4 day modified Fenton's reaction treatment	< 0.3	> 333.3	< 0.3	> 333.3
10 day modified Fenton's reaction treatment	6.4	15.6	< 0.3	> 333.3
10 day control treatment, H_2O addition	11.9	8.4	5.8	17.2

Meulenberg et al. (1997) and Lee & Hosomi (2001) showed that PAH metabolites could be more biodegradable than the parent compounds, thus the soil and leachate toxicity may decrease due to the biodegradation of the metabolites. In the control experiment, addition of deionised water, the flash assay toxicity of was lower than the 30 min assay toxicity. Directly after the modified Fenton's reaction, the soil toxicity was similar for all soil samples, although soil heterogeneity caused higher variation than in for leachate toxicity measurements. After incubation, the toxicity in flash and 30 min assay was similar, but even after 16 weeks of incubation, treated soil was 5–10 times more toxic than untreated soil. The toxicity of soil from control column remained unchanged during incubation and was similar to that of untreated soil.

Effect of modified Fenton's reaction on survival of intact, viable cells

The effect of the modified Fenton's reaction and subsequent incubation on abundance and viability of cells was studied by enumerating viable (intact cell membrane) and dead (disrupted cell wall) cells. Cell wall disintegration is caused by H_2O_2 as well as radicals produced during the Fenton's reaction (Mead 1976). Thus, with respect to this study, disruption of the cellular membrane is a reasonable indicator for cell death. Cells were more abundant in untreated soil than in the incubated Fenton's treated soil (Figure 2). Right after modified Fenton's reaction, the cells were not enumerated because at that stage the enumeration method was still under development. The lowest cell counts were found in incubated soil, which was intensively pretreated for 4 days. Cells with intact cell membrane were more abundant in untreated soil than in incubated Fenton treated soil (Figure 2). In control with deionised water, the percentage of intact cells was much higher after incubation than in incubated oxidised soil (Figure 2), indicating that addition of H_2O_2 was more aggressive treatment than addition of deionised water.

The numbers of viable cells might be smaller than the numbers of intact cells, because it was shown that bacteria with intact cell walls can be nucleoid-free, thus non-viable (Zweifel & Hagström 1995). Thus, the BacLight^{TM}live/dead assay should be combined with functional tests such as incubation and substrate utilisation assays. In the 10-day experiment, the number of intact cells increased with longer incubation (Figure 2), although the percentage of viable cells was lower than in untreated soil. Since bacteria do persist in soil treated with modified Fenton's reaction, bioaugmentation of soil with PAH-degrading bacteria may not be required. Furthermore, successful inoculation of soil with contaminant degrading bacteria is limited by the contaminant bioavailability (Smith et al. 1999; Schwartz & Scow, 2001; Allard et al. 2000; Wick et al. 2001), especially in soils contaminated with aged contaminants.

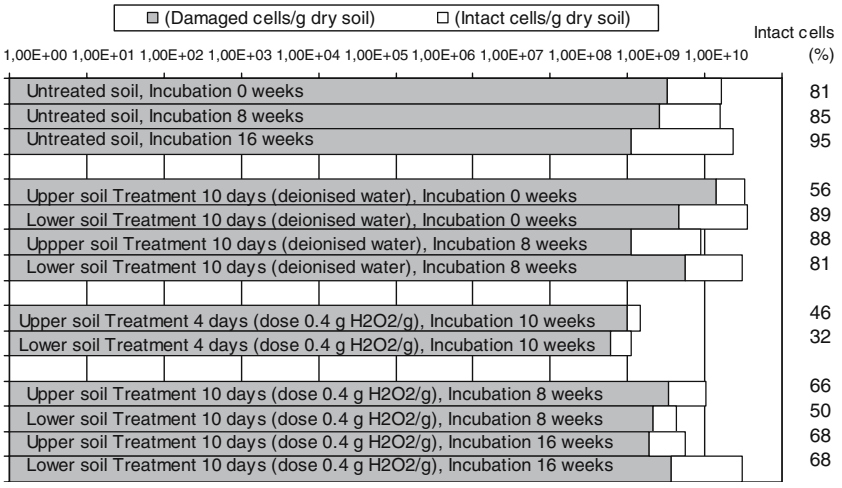

Figure 2. BacLight^{TM}live/dead cell counts and percentage of intact cells during incubation of untreated soil and soil with application of deionised water and modified Fenton's reaction.

National regulations may not allow addition of substances (inocula) to the environment, which might cause deterioration of quality, endanger or harm human health or the environment (e.g. Finnish Environment Protection Act 2000).

Microbial extracellular enzymatic activity in soil

At the end of the 4-day experiment, the effect of the modified Fenton's reaction on microbial extracellular enzyme activities was determined. Esterase activity was monitored with 4-MUF-acetate to assess general microbial activity. The activities of aminopeptidase and acidic phosphomonoesterase, measured as v^*_{max}, were studied to show if the hydrolysis of the respective enzyme substrates was affected by possible release of N- and P-containing compounds during the chemical treatment.

Aminopeptidase activities in soil from the control column receiving deionised water remained below detection limit. Acetate esterase activities were lower in chemically treated soil than in untreated soil (Table 4). Furthermore, esterase activities in soil taken from the lower part of columns were lower than in soil taken from the upper part of soil columns. Acetate esterase and aminopeptidase activities correlate with active biomass (Kähkönen et al. 1999) and can therefore be used to assess general metabolic activity of soil bacteria. Serine aminopeptidase (Table 4) activities were highest in untreated incubated soil, while leucine aminopeptidase activities were higher in Fenton treated soil. Aminopeptidase activities either increased or decreased due to the modified Fenton's reaction (Table 4). The increase may be due to the increased availability of polymers containing amino acid groups after cell walls and natural organic matter structures have been degraded by the oxidants. The phosphomonoesterase activities (Table 4) were high in the untreated samples, indicating phosphorus deficiency of the soil bacteria (Alef 1991). Therefore phosphate addition to the soil might support microbial growth and PAH-biodegradation.

Gas monitoring during soil incubation

Concentrations of O_2, CO_2 and CH_4 were monitored during soil incubation to determine microbial activity in the soil as well as biodegradation of organic compounds (Figure 3). Methane was not detected from the headspace of any serum bottle. Increase of oxygen concentrations above atmospheric concentration was observed in serum bottles with soil treated by modified Fenton's reaction during the first weeks of incubation (Figure 3). Although residual H_2O_2 was not determined from the soil itself, residual H_2O_2 was detected in the column leachate and formation of oxygen due to decomposition of residual H_2O_2 indicate presence of H_2O_2 in the soil incubated in serum bottles. In the serum bottles, aerobic conditions prevailed (Figure 3), also indicated by the absence of methane.

Decomposition of H_2O_2 to O_2 and H_2O competes with the Fenton's reaction (Watts & Dilly 1996) and causes an increase of H_2O_2 consumption

Table 4. Microbial extracellular enzyme activities in untreated and treated (0.4 g H_2O_2/ g soil) soil as indicated by potential maximum rate of microbial extracellular enzyme hydrolysis v^*_{max} values of substrate hydrolysis $\left(\frac{\mu\text{mol MUF}}{1\times\text{g soil}\times\text{h}}\right)$ or $\left(\frac{\mu\text{mol AMC}}{1\times\text{g soil}\times\text{h}}\right)$

Incubation time	Experiment		Serine	Leucine	Alanine	Acetate	Acidic Phosphomono-
(Weeks)			Aminopeptidase $\left(\frac{\mu\text{mol AMC}}{1\times\text{g soil}\times\text{h}}\right)$			esterase $\left(\frac{\mu\text{mol MUF}}{1\times\text{g soil}\times\text{h}}\right)$	
0	Untreated soil		70	60	150	5400	1300
5	Untreated soil		60	30	50	2300	1800
10	Untreated soil		230	20	110	5300	1400
		Sample taken from column part					
5	10 days oxidation	Upper	50	60	50	2400	410
5	10 days oxidation	Lower	130	100	180	510	260
10	10 days oxidation	Upper	[a]	[a]	30	1060	[a]
10	10 days oxidation	Lower	[a]	[a]	[a]	1620	[a]

[a]No enzyme saturation or no enzymatic activity observed.

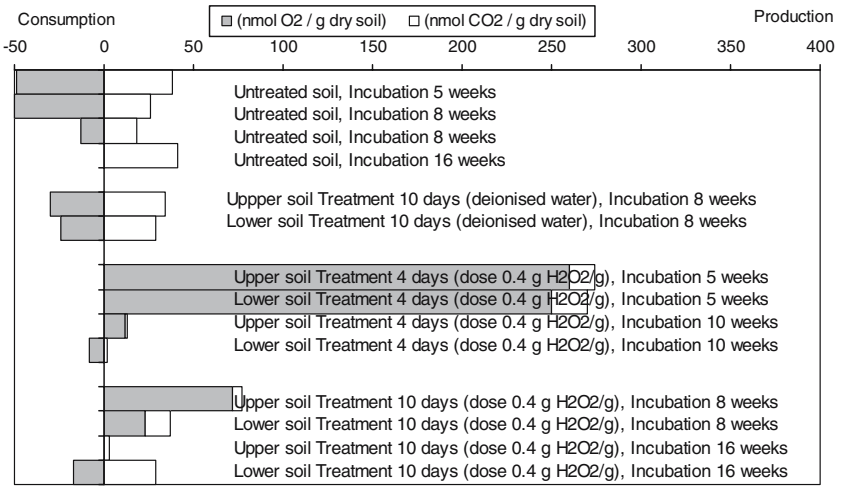

Figure 3. Production and consumption of O_2 and CO_2 during incubation of untreated soil and soil with application of deionised water and modified Fenton's reaction.

and is, therefore, not beneficial for the chemical oxidation of the contaminants. However, an increased oxygen concentration may facilitate biodegradation of PAHs, since aerobic conditions have been shown to improve the biodegradation of PAHs (Eriksson et al. 2003). H_2O_2 is decomposed to O_2 and H_2O abiotically by iron minerals as well as biotically by the catalase enzyme (Howsawkeng et al. 2001). So far, methods to measure the activity of the H_2O_2 decomposing enzyme catalase are based on monitoring residual H_2O_2 or O_2 formation after H_2O_2 addition. Due to the abiotic processes applied in this study, these methods were neither applicable nor used.

During the incubation, some of the PAH removal (Figure 1) may be attributed to chemical oxidation, by both the radicals formed in Fenton reaction and residual H_2O_2. The H_2O_2 dose could be reduced to minimize adverse effects on the soil bacteria, based on the similar PAH-removal regardless of the applied H_2O_2 doses (Figure 1) and initial O_2 formation during incubation (Figure 3).

Consumption of O_2 was detected in untreated soil and soil treated by Fenton's reaction, although in the 4-day experiment O_2 consumption was lower in soil samples from the upper part of soil column. Highest O_2 consumption was found in untreated soil. In all the serum bottles with untreated soil and Fenton treated soil, formation of CO_2 was observed. The CO_2 formation was highest in the 10-day experiment. Biodegradation of PAHs as well as natural organic carbon could have contributed to the CO_2 formation, since Miller et al. (1996) showed that Fenton's reaction dissolves organic carbon into soil solution and increases the biochemical oxygen demand of soil solution. The released and oxidized low molecular weight compounds may act as secondary substrates for the growth of the PAH-degrading bacteria. In control bottles with twice autoclaved soil, untreated as well as treated soil, the concentrations of O_2 and CO_2 remained close to atmospheric concentrations throughout the incubation. Thus, the microbial activity in the killed controls was low.

Conclusions

Polycyclic aromatic hydrocarbons can be partially removed, up to 52%, by Fenton's reaction modified by addition of 30% H_2O_2 solution to iron-rich soil without pre-acidification of the soil or ferrous iron supplementation. The modified Fenton's reaction causes increase of toxicity of leachate and soil to *Vibrio fischeri*, and inhibition to extracellular esterase activity of soil. The percentage of intact bacterial cells after incubation is lower in soil treated with modified Fenton's reaction compared to that of untreated soil. The combination of modified Fenton's reaction with subsequent aerobic biodegradation yields a slightly higher removal of PAHs than the sole processes.

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