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RESEARCH ARTICLE

Remediation of phenanthrene-contaminated soil by simultaneous persulfate chemical oxidation and biodegradation processes

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Abstract Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds with carcinogenic and/or mutagenic potential. To address the limitations of individual remediation techniques and to achieve better PAH removal efficiencies, the combination of chemical and biological treatments can be used. The degradation of phenanthrene (chosen as a model of PAH) by persulfate in freshly contaminated soil microcosms was studied to assess its impact on the biodegradation process and on soil properties. Soil microcosms contaminated with 140 mg/kg$_{DRY\ SOIL}$ of phenanthrene were treated with different persulfate (PS) concentrations 0.86–41.7 g/kg$_{DRY\ SOIL}$ and incubated for 28 days. Analyses of phenanthrene and persulfate concentrations and soil pH were performed. Cultivable heterotrophic bacterial count was carried out after 28 days of treatment. Genetic diversity analysis of the soil microcosm bacterial community was performed by PCR amplification of bacterial 16S rDNA fragments followed by denaturing gradient gel electrophoresis (DGGE). The addition of PS in low concentrations could be an interesting biostimulatory strategy that managed to shorten the lag phase of the phenanthrene biological elimination, without negative effects on the physicochemical and biological soil properties, improving the remediation treatment.

Keywords Phenanthrene · Persulfate treatment · Contaminated soil · DGGE

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds of particular concern because of their widespread occurrence in the environment and their carcinogenic and/or mutagenic potential. Microbial degradation of PAH has received much attention as a possible strategy for bioremediation of PAH-contaminated soils. Bioremediation is a low-cost and low-disturbance solution for the cleaning of contaminated sites. However, a general problem of bioremediation of PAH-contaminated soil is the slow degradation rate (Coppotelli et al. 2008).

On the other hand, in situ chemical oxidation (ISCO) has been increasingly regarded as a relevant alternative to conventional treatment technologies for remediation of groundwater and soils contaminated by recalcitrant organic contaminants, including PAH (Sirguey et al. 2008). Chemical oxidation is able to degrade PAH and not merely transfer them to another compartment. However, the remediation of PAH-contaminated soil using chemical oxidation technology remains a big challenge (Lemaire et al. 2013); the efficiency of chemical oxidation of PAH in soils is dependent on soil characteristics and PAH properties (Gan et al. 2009).

To address the limitations of individual remediation techniques and to achieve better PAH removal efficiencies, the combination of chemical and biological treatments can be used (Gan et al. 2009). Although recent investigations have shown that a biphasic treatment consisting of chemical oxidation and bioremediation is a feasible in situ remediation

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technology (Sutton et al. 2011), the impact of type and concentration of chemical oxidant on the soil microbial community and contaminant dynamics still requires a thorough understanding.

The persulfate (PS) is the newest oxidant that is being used for ISCO in the remediation of soil and groundwater (Tsitonaki et al. 2008). Although PS is not an oxidant strong enough to degrade most hazardous organic contaminants, it could generate the very active sulfate free radical ($SO_4^{\cdot-}$), roughly equivalent to the hydroxyl free radical in reactivity (Osgerby 2006). Generation of these radicals, with a redox potential $E°$ ($SO_4^{\cdot-}/SO_4^{2-}$) of 2.6 V can significantly accelerate the kinetics of oxidation in a wide range of matrix conditions (Wardman 1989). Activated persulfate has the potential to in situ destruct many organic contaminants commonly encountered in soil and groundwater (House 1962; Huang et al. 2005; Liang et al. 2003).

PS can be chemically (Anipsitakis and Dionysiou 2004), photochemically (Rosso et al. 1999), or thermally activated (Mora et al. 2009) to generate sulfate radical. For in situ applications, Fe(II) and Fe(III) are the most commonly used metal activators due to their natural abundance in porous media and benign nature (Tsitonaki et al. 2010). Moreover, its addition is not always required for the remediation of contaminated environmental solids, due to the presence of natural iron in soils and sediments (Palmroth et al. 2006; Ferrarese et al. 2008). However, one of the most common problems is finding the optimum persulfate/iron ratio to avoid reaction of the sulfate radicals with excess iron instead of the target contaminants (Tsitonaki et al. 2010). The use of chelated ferrous ion to regulate and maintain Fe(II) activity as an activator for persulfate oxidation seems to be an effective strategy. Persulfate oxidation of trichloroethylene (TCE) in aqueous and soil slurry systems shows that higher chelated ferrous ion concentrations has resulted in faster TCE degradation and more persulfate decomposition. Moreover, the citric acid–Fe^{2+} acted as a most effective activator in comparison with others (Liang et al. 2003).

Although the use of persulfate has been widely studied for the treatment of contaminated soil, the information about its interaction with the soil components are quite limited (Tsitonaki et al. 2010). On the other hand, most of the information discussed in literature about soil chemical oxidation processes comes from experiments that required a significant amount of water. Few studies have been performed under unsaturated condition (Palmroth et al. 2006; Lee and Homosi 2001; Laurent et al. 2012). An important issue to be considered is the ecological cost of the utilization of water, which sometimes could be reused but in some cases could be the limiting "reactant."

The aim of this work was to evaluate the feasibility of using a surface application of persulfate, holding the natural moisture content of soil, looking on PAH elimination and soil physicochemical and biological parameters. Six different concentrations of persulfate were applied for the degradation of phenanthrene in soil microcosms freshly contaminated (unsaturated condition) under controlled conditions of temperature, soil moisture, and oxygen availability, allowing a simultaneous biological process. Analyses of phenanthrene and persulfate concentration, soil pH, soil electrical conductivity, bacterial density, and bacterial genetic diversity were performed with treated and control soils.

Materials and methods

Chemicals

Sodium persulfate ($Na_2S_2O_8$) and sodium citrate ($Na_3C_6H_5O_7 5½H_2O$) of analytical grade were obtained from Merck (Schuchardt, Germany); phenanthrene ($C_{14}H_{10}$) with a purity of more than 99.5 % was from Carlo Erba (Milano, Italy), and $FeSO_4 7H_2O$ of analytical grade was from Mallinckrodt.

Soil

The soil selected for the study was an uncontaminated soil from an area near La Plata City, Argentina (34°50′ S, 58°10′ O). It was analyzed in the Laboratory of Soil Science at the University of La Plata and showed the following physicochemical properties: a pH of 6.6, 4.67 % organic carbon (Walkley-Black method), 8.05 % soil organic matter (OM(%)=1.724×C (%)), 3,890 mg/kg total nitrogen (wet digestion and evaluation by Micro Kjeldahl method), 4.0 mg/kg of available phosphorus (Bray Kurtz no. 1 method), electrical conductivity of 1.1±0.2 dS/m (1:5), and 109.9±0.9 ppm of Fe (extracted with EDTA and determined according to EPA's method 7950). Posttreatment soil samples were analyzed applying the same methodology.

Decomposition of sodium persulfate in soil

Soil microcosms consisting of 80 g of sieved soil (2-mm mesh) were placed in a glass container of 200 g capacity. Sodium persulfate (PS) was added as an aqueous solution to arrive at $[PS]_0=20$ g/kg$_{DRY\ SOIL}$. This experiment was carried out in triplicate trays and incubated at 24±2°C (regional climate conditions) for 14 days. The residual concentration of PS was determined at the start of the incubation time and after 2 h and 1, 2, 4, 7, and 14 days of treatment.

Soil treatment systems

Soil microcosms consisting of 150 g of sieved soil (2-mm mesh) were placed in a glass container of 250 g capacity. They

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were contaminated with 140±10 mg of phenanthrene per kilogram of dry soil. Phenanthrene was delivered in an acetone solution and mixed manually into the soil with a spatula as reported (Kulik et al. 2006).

Nine treatments were carried out in triplicate trays and incubated at 24±2°C (regional climate conditions), in the dark for 28 days. Soil microcosm with no persulfate added was used for biological degradation (BioD) measurement. The chemical degradation (ChemD) was determined in sterilized microcosm by autoclaving the soil (Trevors, 1996) with initial persulfate concentration ($[PS]_0$) of 8.61 g/kg$_{DRY\ SOIL}$.

Degradation of phenanthrene by oxidation with PS without iron supplementation was investigated, due to the presence of natural iron in soil (109.9±0.9 ppm of Fe). Five different $[PS]_0$ (0.86, 4.34, 8.61, 21.0, 41.7 g/kg$_{DRY\ SOIL}$) were assayed, named PS1, PS2, PS3, PS4, and PS5, respectively. These concentrations were chosen around $[PS]_0$=6.54 g/kg$_{DRY\ SOIL}$ which was calculated from the soil oxidant demand (0.1 kg$_{PS}$/m^3$_{SOIL}$) (Osgerby, 2006) and the amount of PS needed to oxidize phenanthrene to CO_2 (33 mol PS per mol of phenanthrene).

Two other microcosms with PS and Fe(II)/citrate were carried out in order to improve the degradation of phenanthrene. In these treatments, the $[PS]_0$ was 8.61 g/kg$_{DRY\ SOIL}$ because this one was the highest PS concentration that did not cause dramatic changes on the soil microbial community. The molar ratio Fe(II)/citrate/contaminant proposed in aqueous solution was 5/25/1 (Liang et al. 2004), and then two conditions were tested: $[FeSO_4 7H_2O]_0/[Na_3C_6H_5O_7 5½H_2O]_0$= 1.17/7.46 and 2.30/14.96 g/kg$_{DRY\ SOIL}$, named PS3FeC(L) and PS3FeC(H), respectively.

PS and Fe(II)/citrate were added as aqueous solutions, 2 h after artificial contamination of soil with phenanthrene, and mixed manually with a spatula. Ultrapure water (>18 MΩ cm, <20 ppb of organic carbon) was obtained from a Millipore system.

To support the aerobic biodegradation process, the moisture content was standardized to 25±2 % *w/w*, corresponding to 44.5 % of soil water-holding capacity. The microcosms were aerated every week by manual mixing, and the moisture content of the soil was corrected when necessary by adding ultrapure water.

Physical and chemical analysis

pH values Soil sample (2.5 g) was mixed in a polycarbonate tube (15 ml volume) with 2.5 ml of ultrapure water and shaken for 4 h (Foth 1990). The pH was measured with a glass electrode (Phoenix Electrode Company).

Electrical conductivity (1:5) after the addition of oxidant Soil sample (5.0 g) was mixed in a polycarbonate tube (50 ml volume) with 25 ml of ultrapure water and shaken for 5 min. The soil suspension stands for 1 h for EC measurement.

Phenanthrene concentration in soil samples Soil sample (1.00 g) was mixed in a polycarbonate tube (15 ml volume) with 10 ml of ethyl acetate. Phenanthrene was extracted using the ultrasonic bath (Testlab Ultrasonic TB04TA, 40 kHz, 160 W) for 60 min (Luque-García and Luque de Castro 2003; Song et al. 2007). The mixture was centrifuged at 3,000 rpm for 10 min (Presvac model DCS-16 RV), and the solution was filtered (nylon filter 0.45-µm pore size). Then, 10 µl were injected into a Hewlett-Packard HPLC model 1050 (Ti series) chromatograph with multiwavelength detection, with a C18 Restek Pinacle II column (particle size 5 µm, 2.1 mm, id 250 mm) and using 80/20/0.1 (*v/v/v*) methanol/water/H_3PO_4 mixture as eluent at 0.5 mL/min constant flux.

Spectrophotometric determination of PS concentrations (Liang et al. 2008) Of soil, 4.5 g were mixed with 4.5 ml of H_2O, and the aqueous phase was filtered (nylon filter 0.45-µm pore size). An aliquot of 0.5 ml of this aqueous phase was mixed with 1 ml of H_2O and 1 ml $NaHCO_3$/KI (0.05 g $NaHCO_3$, 1.0 g KI, and ultrapure water in analytical flask of 10 mL). Absorbance at 350 nm was measured in a quartz cuvette (Shimadzu UV-1800 spectrophotometer). The detection limit was 0.05 g/kg$_{DRY\ SOIL}$.

Microbiological analysis

Cultivable heterotrophic bacterial count was carried out after 28 days of treatment (at the end of the incubation time). For this purpose, 10 g (wet weight) of soil sample suspended in 100 mL of 0.85 % NaCl were homogenized for 30 min on a rotary shaker (250 rpm). Samples (0.1 mL) of 10-fold dilution were spread on plates containing R2A agar (Reasoner and Geldreich, 1985). Agar plates were incubated at (24±2 °C) for 10 days.

Molecular analysis

Genetic diversity analysis of the soil microcosm bacterial community was performed by PCR amplification of bacterial 16S ribosomal DNA (rDNA) fragments followed by denaturing gradient gel electrophoresis (DGGE).

The total DNA was extracted from 1 g of soil aliquots from each soil microcosm after 14 and 28 days of treatment, using the E.Z.N.A.™ Soil DNA Isolation Kit (Omega Bio-tek, Inc., Norcross, GA, USA) according to the instructions of the manufacturer.

The 16S rDNA was amplified using eubacteria primers GC-341F (5′-CGCCCGCCGCGCCCCGCGCCCGGCCC GCCGCCCCCGCCCCCTCCTACGGGAGGCAGCAG-3′)

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and 907R (5′-CCGTCAATTCCTTTGAGTTT-3′) (Muyzer et al. 1998). The PCR reactions contained 1 µl of DNA, 1 U of AmpliTaq, the manufacturer's recommended buffer as supplied with the polymerase enzyme, 200 mM of BSA, 0.2 mM dNTPs, and 20 pM of each primer in a total reaction volume of 50 µl. Amplification was performed on a Mastercycler® Eppendorf thermocycler (Eppendorf, Wesseling-Berzdorf, Germany) using a step-down PCR. The program included an initial denaturation step for 4 min at 95 °C, the first cycle step at 94 °C for 30 s, 62 °C for 40 s, and 1 min at 72 °C (10 cycles), followed by a step down of 30 s at 94 °C, 40 s at 57 °C and 72 °C for 1 min (25 cycles). The final extension was carried out at 72 °C for 10 min. The PCR products were analyzed by agarose gel electrophoresis and purified using a QIAquick PCR Purification Kit (Qiagen Inc., Chatsworth, CA, USA).

DGGE was performed on a DGGE-2401 apparatus (C.B.S Scientific Co., Del mar, CA, USA). The purified PCR amplicons were directly applied onto 6 % (wt/vol) polyacrylamide gels (acrylamide-*N*,*N*′-methylen-bisacrylamide, 37.5:1). The gel contained a linear gradient of 40–75 % denaturant (100 % denaturant corresponds to 7 M urea and 40 % (vol/vol) formamide).

Electrophoresis was performed in 1X TAE buffer (40 mM Tris (pH 8.1), 20 mM acetic acid, 1 mM Na_2EDTA) at a temperature of 60 °C. A pre-run at 50 V for 30 min was followed by the main run at a constant voltage of 100 V for 16 h. The postelectrophoresis gel was stained for 30 min with SYBR Gold and documented with a Chemidoc gel documentation system (Bio-Rad, Hercules, CA, USA). Similar matrixes of the banding patterns were made with the dice equation, and the dendrogram was calculated by the unweighted pair group method with arithmetic mean (UPGMA) (Sokal and Michener, 1958).

DGGE-banding data were used to estimate the Shannon index of general diversity ($H' = -\Sigma(n_i/N)\log(n_i/N)$). For this analysis, each band was treated as an individual operational taxonomic unit (OTU). The relative surface intensity of each band, expressed as peak height in the densitometric curve (n_i), and the sum of all peak heights in the curve of a given sample (N) were used as estimates of species abundance (Fromin et al. 2002).

Statistics

All experiments in this study were performed by triplicates. The mean and standard deviations of triplicate independent experiments were calculated. The mean values were compared by a parametric two-way ANOVA test. All statistical analyses were performed using the SigmaPlot/SigmaStat software program (SPSS Inc., Chicago, IL, USA).

Results and discussion

Decomposition of sodium persulfate in soil

Persulfate decomposition to generate the sulfate radicals ($SO_4^{\cdot -}$) and the later kinetic mechanism were extensively reported for aqueous solutions (Rosso et al. 1999; Johnson et al. 2008). However, in soils, sulfate radicals are free to react with available contaminants, soil organic matter, and microorganisms; they can also form other reactive species such as the hydroxyl radical, peroxymonosulfate, and hydrogen peroxide (Richardson et al. 2011). In consequence, the time of life of PS in soils depend on a particular matrix. Experiments with soil and PS (but without phenanthrene) were performed to determine the persistence of PS. Figure 1 shows the concentration of PS in soil along 14 days of incubation at 24 °C.

During the first day of incubation, 64 % of the PS was decomposed, and after 7 days, the residual amount of PS was only about 8 %. After 14 days, no PS was detected. This result indicates that soil natural conditions were enough to decompose PS, without the addition of activators, and that the persistence of PS was around 7 days.

Effect of PS initial concentrations on phenanthrene elimination

The effect of $[PS]_0$ on the percentage of residual phenanthrene concentration ($[PHEN]_R$) in microcosms throughout 28 days of treatment is shown in Fig. 2a and b.

Biological degradation (BioD) control did not show any elimination of phenanthrene during the first 14 days of incubation (Fig. 2a). This lag phase, which shows the microbial adaptation time, is an interesting biodegradation parameter and is suggested to be a good measure of catabolic potential of the soil microbial community (Couling et al. 2010). After 28 days, the BioD control reached 80 % of degradation.

On the other hand, chemical degradation (ChemD) control did not show any significant phenanthrene elimination during

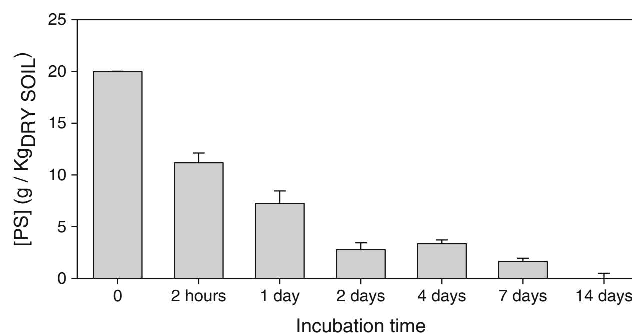

Fig. 1 Evolution of the persulfate concentration in soil (without phenanthrene), along 14 days. Results are means of three independent experiments. *Bars* represent standard deviations

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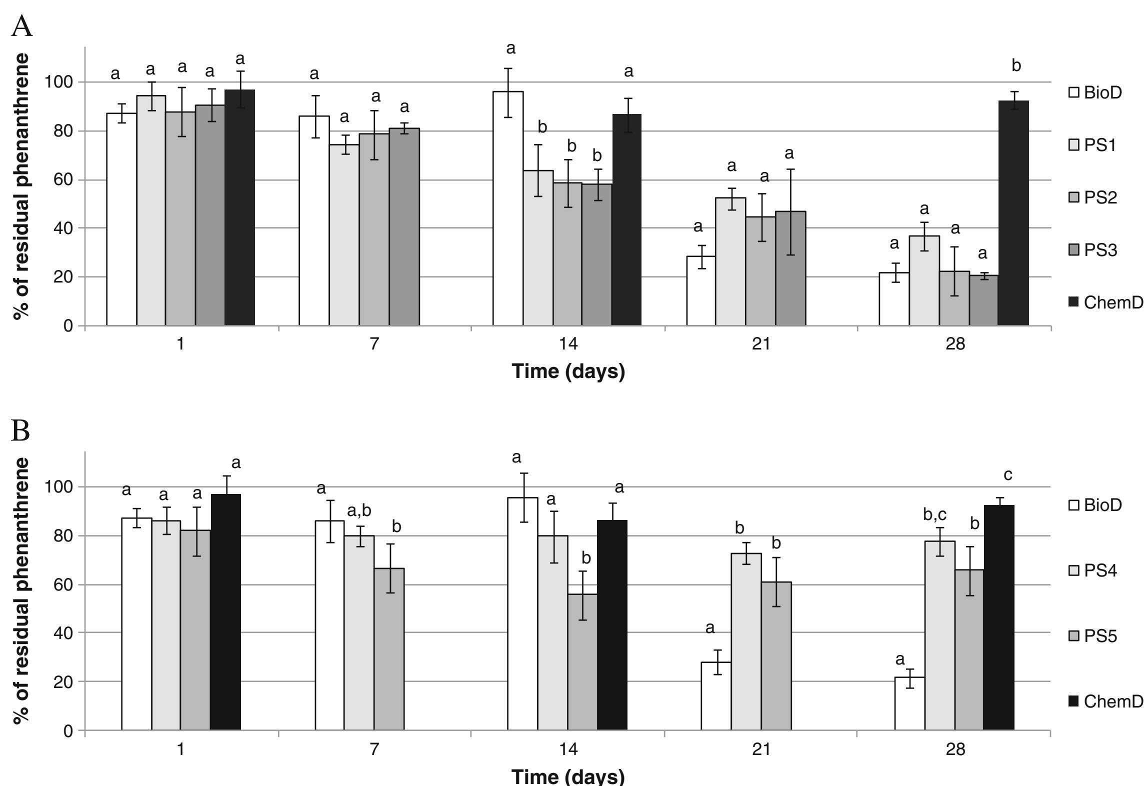

Fig. 2 Percentage of residual phenanthrene vs. time (days) in BioD and ChemD controls, PS1, PS2, and PS3 microcosms (**a**), and PS4 and PS5 microcosms (**b**), along 28 days of treatment. Results are means of three independent experiments. *Bars* represent standard deviations. Values followed by the same letter are not significantly different at the 5 % level (two-way ANOVA, Tukey test)

the whole experiment. On this microcosm, the phenanthrene elimination would be produced by an abiotic process. The oxidative effect resulting from an initial PS concentration of 8.61 $g/kg_{DRY\ SOIL}$ removed only a small amount of phenanthrene (10 % after 28 days of incubation). Based on the ability of the soil to decompose PS, this result could be assigned to the scavenging of sulfate radicals by soil components, avoiding the reaction with the pollutant.

Application of oxidative treatments did not produce an immediate change (24 h) in $[PHEN]_R$. Phenanthrene concentrations were not statistically different ($p>0.05$) to BioD after 1 day of treatment, in spite of the expected decomposition of PS. This observation indicates that sulfate radicals produced by PS decomposition were scavenged by soil components, in line with ChemD control behavior.

Moreover, at 7 days, a significant reduction ($p<0.05$) in phenanthrene concentration was only observed for microcosm PS5 (higher oxidant concentration studied) (Fig. 2b). Only PS5 microcosm had an amount of PS able to overcome the oxidation of soil components, providing oxidant for the phenanthrene degradation. Along the whole experiment (28 days), the $[PHEN]_R$ for PS5 was coincident (within the experimental error) with the value reached at 7 days ($[PHEN]_R=64\pm6$ %).

After 7 days, no more chemical oxidation could be expected, because the PS was totally decomposed. However, at 14 days of treatment, the $[PHEN]_R$ in PS1, PS2, and PS3 were significantly lower ($p<0.05$) than $[PHEN]_R$ in the BioD (Fig. 2a). This behavior could be assigned to biological processes, accelerated by the oxidative PS effect. Soil organic matter is composed by a high diversity of molecules (carbohydrates, amino acids, proteins, lipids, nucleic acids, humic compounds) more or less available and sensitive to oxidation (Sirguey et al. 2008), as reported for sulfate radical (David Gara et al. 2008). These reactions yielded low molecular weight compounds which could act as nutrient improving the activity of the microbial soil communities (Bosio et al. 2008). This biostimulatory effect managed to shorten the lag phase in PS2 and PS3 microcosms. However, at the end of the treatment, the $[PHEN]_R$ in PS1, PS2, and PS3 did not show significant differences with those determined in BioD microcosm.

The amount of PS added in PS4 microcosm was not enough to show direct oxidation of phenanthrene (as mentioned before) and this situation was maintained until the end of treatment. At 28 days of incubation, the $[PHEN]_R$ was not statistically different ($p>0.05$) from ChemD.

Table 1 shows the impact of the different PS concentrations on pH and the number of cultivable heterotrophic bacteria. In PS1, PS2, and PS3, the number of cultivable heterotrophic bacteria determined at the end of the treatment was similar to

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Table 1 Initial and final pH values, initial electrical conductivity (1:5), and number of cultivable heterotrophic bacteria after 28 days of incubation, in BioD, PS1, PS2, PS3, PS4, PS5, ChemD, PS3FeC(L), and PS3FeC(H) microcosms

Microcosms	pH after PS addition	EC (1:5) after PS addition (dS/m)	final pH	Log of CFU/g of dry soil (final)
BioD	7.0±0.1	Not determined	6.0±0.1	9.3±0.6
PS1	6.5±0.1	Not determined	6.4±0.1	9.44±0.05
PS2	5.3±0.1	Not determined	5.6±0.1	8.9±0.7
PS3	4.6±0.1	2.2±0.2	5.2±0.1	9.2±0.7
PS4	3.6±0.1	3.4±0.2	3.5±0.1	6.60±0.01
PS5	2.7±0.1	5.5±0.2	2.9±0.1	5.60±0.01
ChemD	4.7±0.1	Not determined	4.8±0.1	Not determined
PS3FeC(L)	5.4±0.1	2.7±0.2	6.0±0.1	8.68±0.05
PS3FeC(H)	6.0±0.1	3.4±0.2	7.1±0.1	9.40±0.04

BioD. However, the values for PS4 and PS5 were two orders of magnitude lower; this is in agreement with the inhibition of the phenanthrene biodegradation process observed in these microcosms, after 7 days of incubation.

A decrease of the initial soil pH was observed after the addition of PS (see Table 1). The PS used in the experiments contained water and acid (<0.3 %) as impurities (Rosso et al. 1999), and then, the pH of PS aqueous solutions was acid (for example, for a solution with concentration similar to PS3, the pH was 2.9). Decreases ranging from 2.8 to 6 pH units were observed under laboratory conditions using both aqueous (Huang et al. 2005; Liang et al. 2004) and soil slurry experiments (Tsitonaki et al. 2008). In our experiments the pH values determined in the soil microcosms were less acid than expected, possibly due to higher buffering capacity of our soil. However, the amount of PS added in PS4 and PS5 microcosms yielded an extremely acid pH along the whole experiment.

Our experiments showed a correlation between a decrease of pH values and the number of cultivable heterotrophic bacteria populations. This kind of behavior, reduction of soil pH and quantity of microorganisms, was already reported (Laurent et al. 2012) for a high dose of Fenton reagents (65 g H_2O_2 and 82 g $FeSO_4 7H_2O$ per kg of dry soil), in unsaturated conditions, to treat soil polluted by PAH.

We suggest that a low pH value may result in a significant environmental perturbation that would limit the survival and activity of the soil microorganisms and as a consequence, the biodegradation process. After 14 days of treatment, the behavior of PS1, PS2, and PS3 microcosms is coincident with BioD control because the small perturbation on pH allows the biodegradation process. On the other hand, PS4 and PS5 microcosms do not show any change after 7 days because the amount of PS added to these treatments produce an important reduction on pH value, which inhibits the biological activity.

Effect of addition of Fe(II) on phenanthrene elimination

The effect of PS activation with two concentrations of Fe(II)/citrate complex was studied. As shown in Fig. 3, no significant differences among PS3FeC(L), PS3FeC(H), and PS3 microcosms were observed at 7 days, indicating that the presence of Fe(II)/citrate was not able to improve the chemical process.

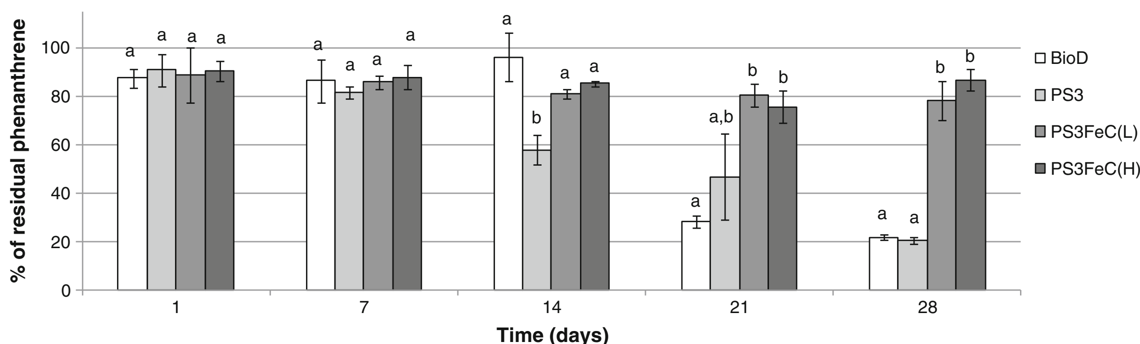

Fig. 3 Percentage of residual phenanthrene vs. time (days) in BioD, PS3, PS3FeC(L), and PS3FeC(H) microcosms, along 28 days of treatment for PS activated by Fe(II) and citrate. Results are means of three independent experiments. Bars represent standard deviations. Values followed by the *same letter* are not significantly different at the 5 % level (two-way ANOVA, Tukey test)

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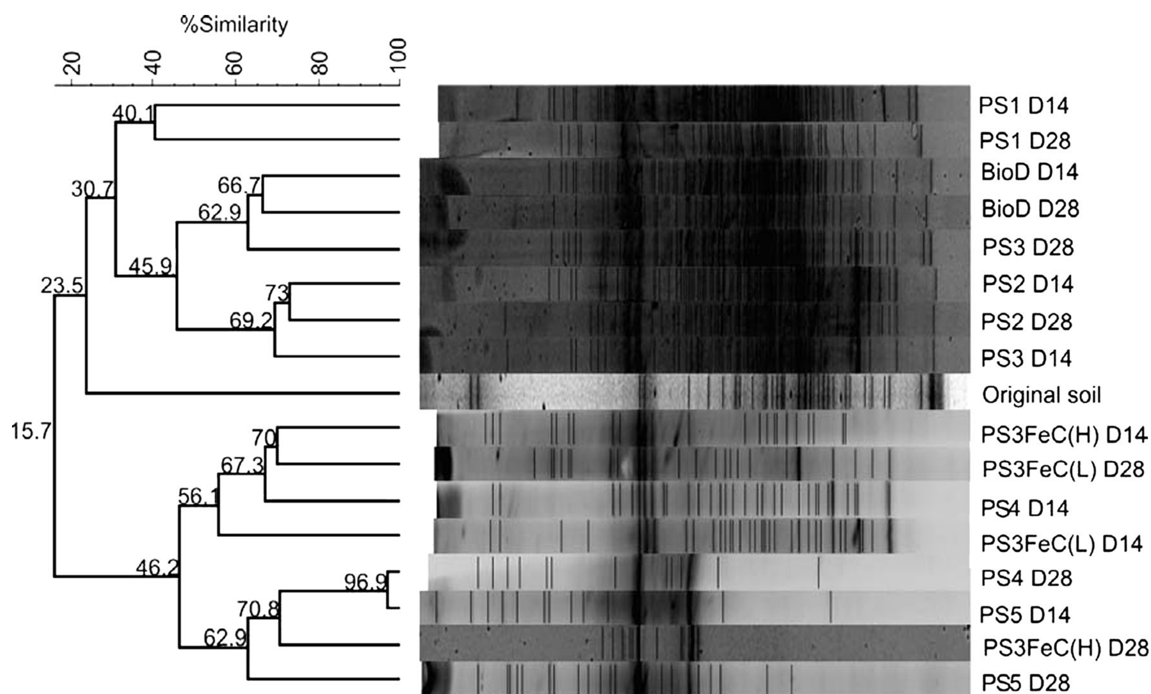

Fig. 4 Dendrogram of the UPGMA analysis of the DGGE patterns of the original soil and BioD, PS1, PS2, PS3, PS4, PS5, PS3FeC(L), and PS3FeC(H) microcosms, at 14 and 28 days of treatments. The differences between profiles are indicated by percentage similarity

After 14 days of incubation, PS3 showed biological degradation of phenanthrene while the systems with Fe(II)/citrate remained steady. The final percentage of $[PHEN]_R$ remained at around 85 % for both systems with Fe(II).

The global effect of the addition of Fe(II)/citrate complex was negative, in our experimental conditions. The incorporation of Fe(II)/citrate did not improve the chemical process and inhibited the biological process although the number of cultivable heterotrophic bacteria populations in PS3FeC(L) and PS3FeC(H) microcosms was not different from BioD at the end of the treatment (see Table 1).

We suggest that the presence of citrate, an easily assimilated carbon source, affects the activity of soil microbial community in detriment of the biodegradation processes. The microbial consumption of citrate releases Fe(II), which could act as scavenger of the sulfate radicals because of the reaction of these radicals with iron instead of the target contaminants (Tsitonaki et al. 2010; Liang et al. 2004).

Effect of chemical oxidation on the genetic diversity of soil bacterial community

To investigate the changes in the genetic diversity under the influence of different conditions, DGGE was performed for original soil (initial time) and all the systems at two different times of treatment (14 and 28 days).

The dendrogram of the UPGMA analysis of the DGGE patterns (Fig. 4) showed two main clusters markedly different (15.7 % of similarity). The first cluster included the DGGE profiles of the original soil and the BioD, PS1, PS2, and PS3 microcosms, at the two studied times. These profiles described the microbial community structure of the microcosms with the highest percentages of phenanthrene elimination (high-degradation cluster, HDC). The second cluster (PS4, PS5, PS3FeC(L), and PS3FeC(H)) corresponded to the microcosms with lower phenanthrene degradation (low-degradation cluster, LDC).

The bacterial diversity was compared based on the *H*′ index (Table 2). After 14 days of treatment, the *H*′ index for all

Table 2 Genetic diversity of soil bacterial community of BioD, PS1, PS2, PS3, PS4, PS5, ChemD, PS3FeC(L), and PS3FeC(H) microcosms, at 14 and 28 days of incubation

Microcosms	Shannon diversity index (*H*′)	
	Day 14	Day 28
BioD	3.41	4.84
PS1	4.76	5.15
PS2	4.96	4.41
PS3	4.84	5.32
PS4	4.97	3.84
PS5	4.14	3.96
PS3FeC(L)	4.70	4.70
PS3FeC(H)	4.36	2.76

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microcosms with PS ranged between 4.14 and 4.97, even though they belonged to different clusters (Fig. 3), while BioD showed the lowest *H′* index (3.41). As it was previously mentioned, PS attacks soil organic matter yielding a wide group of organic compounds and increasing the availability of P and N. The appearance of diverse and easily available carbon sources might support the growth and activity of soil bacteria and could explain partially the highest *H′* diversity index found in the microcosms with PS at the 14th day of treatment. This result was in line with observations reported for the pre-ozonation of crude oil-contaminated soil (Liang et al. 2004).

At the end of the incubation time, for PS4, PS5, and PS3FeC(H) (included in the same cluster), a decrease of *H′* index values (see Table 2) was observed. The low *H′* index value found in PS4 and PS5 was in line with the low cultivable heterotrophic bacterial counts (Table 1), but the case of the PS3FeC(H) could not be explained in the same way.

Although the PS activation with chelated ferrous ion did not produce significant changes at level of the number of cultivable bacteria (Table 1) and the soil pH was kept near neutrality, dramatic changes in DGGE profiles of the soil bacterial community were observed (Fig. 4). As shown in Table 1, the electrical conductivity of microcosms that forms the LDC is higher than PS3 due to the incorporation of higher PS concentration or additional ions (Fe^{+2}, SO_4^{2-}, Na^+, citrate), and consequently could be considered as saline soil (Setia et al. 2011). Microbial community composition can be affected by salinity as microbial genotypes differ in their tolerance to osmotic stress (Wichern et al. 2006). We hypothesized that in the LDC, the salinity might be the principal driving force for community changes. This hypothesis could explain the low *H′* index value found in PS4, PS5, and PS3FeC(H), whereas in the PS3FeC(L), the treatment that could have the lowest salinity inside the LDC and no drastic changes in *H′* index were observed (Table 2). Moreover, it has been established that the salt-resistance mechanisms of the adapted microorganisms are energy demanding and affect the efficiency of C utilization by soil microorganisms (Setia et al. 2011). This observation could explain the inhibition of phenanthrene degradation, not only in the PS4 and PS5 microcosms (where the inhibitory effect was evident at cultivable bacteria level) but also in the PS3FeC(L) and PS3FeC(H) microcosms.

Conclusions

In soils freshly contaminated with phenanthrene and treated with persulfate, residual concentration of PS decreased and disappeared after 7 days of treatment. Therefore, the chemical degradation of phenanthrene as such had to take place before a week. This process increased with the concentration of oxidant ($[PS]_0$), and for the highest concentration tested, a 30 % of phenanthrene elimination was reached, but with an important impact on soil pH, on heterotrophic bacteria counts, and bacterial community composition. Besides the negative impact of the extremely acid pH on microbial soil community, the increase in the salinity of microcosms by the incorporation of highest PS concentrations or additional ions (activation with Fe(II)/citrate) can affect microbial community composition, as shown by DGGE profiles and *H′* index values, inhibiting the biodegradation process.

The addition of PS in low concentrations could be an interesting strategy for shortening the microbial adaptation time, improving the biological treatment of soil contaminated with phenanthrene, without negative effects on the physicochemical and biological soil properties.

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