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Impact of the Fenton-like treatment on the microbial community of a diesel-contaminated soil

Flavia Polli [a], Daniela Zingaretti [a], Silvia Crognale [b], Lorena Pesciaroli [b], Alessandro D'Annibale [b], Maurizio Petruccioli [b], Renato Baciocchi [a, *]

[a] Laboratory of Environmental Engineering, Department of Civil Engineering and Computer Science Engineering, University of Rome "Tor Vergata", Italy
[b] Department for Innovation in Biological, Agro-food and Forest Systems, University of Tuscia, Viterbo, Italy

HIGHLIGHTS

- The effect of a Fenton-like treatment on the soil microbial community was assessed.
- Adding H_2O_2 twice at lower amount is effective as adding it once at higher amount.
- Higher H_2O_2 concentration entails more severe impact on microbial community.
- Bioattenuation occurred after Fenton-like step despite the observed negative impacts.
- Fenton-like treatment can significantly shorten the bioremediation process.

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ABSTRACT

Fenton-like treatment (FLT) is an ISCO technique relying on the iron-induced H_2O_2 activation in the presence of additives aimed at increasing the oxidant lifetime and maximizing iron solubility under natural soil pH conditions. The efficacy of FLT in the clean-up of hydrocarbon-contaminated soils is well established at the field-scale. However, a better assessment of the impact of the FLT on density, diversity and activity of the indigenous soil microbiota, might provide further insights into an optimal combination between FLT and in-situ bioremediation (ISB). The aim of this work was to assess the impacts of FLT on the microbial community of a diesel-contaminated soil collected nearby a gasoline station. Different FLT conditions were tested by varying either the H_2O_2 concentrations (2 and 6%) or the oxidant application mode (single or double dosage). The impact of these treatments on the indigenous microbial community was assessed immediately after the Fenton-like treatment and after 30, 60 and 90 d and compared with enhanced natural attenuation (ENA). After FLT, a dramatic decrease in bacterial density, diversity and functionality was evident. Although in microcosms with double dosing at 2% H_2O_2 a delayed recovery of the indigenous microbiota was observed as compared to those subjected to single oxidant dose, after 60 d incubation the respiration rate increased from 0.036 to 0.256 μg $C–CO_2$ g^{-1}soil h^{-1}. Irrespective of the oxidant dose, best degradation results after 90 d incubation (around 80%) were observed with combined FLT, relying on double oxidant addition, and bioremediation.

1. Introduction

In-Situ Chemical Oxidation (ISCO) is an effective technology for the remediation of soil and groundwater contaminated by a wide range of organic pollutants (Pignatello et al., 2006; Baciocchi et al., 2014). An ISCO treatment involves the injection of an oxidant, such as permanganate (Chen et al., 2016), hydrogen peroxide (Laurent et al., 2012), persulfate (Sutton et al., 2014b) or ozone (Yu et al., 2007), into the subsurface to mineralize the contaminants of concern or, at least, transform them in less toxic products. Among the different formulations proposed so far, the Fenton process involves the reaction between H_2O_2 and ferrous iron yielding the hydroxyl radical (·OH) ferric iron and hydroxyl ions (OH^-) (Huling and Pivetz, 2006). Several approaches and methods involving the use of H_2O_2 and iron were investigated so far. Among these, the so called Fenton-like treatment (FLT) is a process relying on the iron-

* Corresponding author.
E-mail address: baciocchi@ing.uniroma2.it (R. Baciocchi).

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induced activation of hydrogen peroxide exerted by minerals naturally occurring in soils, in the presence of proper amendments (stabilizing and chelating agents) aimed at increasing both oxidant lifetime and iron solubility at natural soil pH conditions; its efficacy in the clean-up of soils contaminated by petroleum hydrocarbons has been already established (e.g. Watts and Dilly, 1996). The acceptance of Fenton treatments by regulators is often constrained by concerns on the effect of the process on soil microbial community. The Fenton process indeed affects negatively activity and vitality of indigenous microflora (Waddell and Mayer, 2003). Actually, hydrogen peroxide is described as an antiseptic reagent (Chapelle et al., 2005) able to either inhibit or kill microorganisms even at lower concentrations than those typically applied for other oxidants used in ISCO treatments (Waddell and Mayer, 2003; Huling and Pivetz, 2006; Pardieck et al., 1992; U.S. EPA, 2004). Hydroxyl radicals arising from Fenton reactions have been shown to enhance mutagenesis, cell death, cell membrane damage, and to reduce both microbial activity and acclimation ability of microbial populations (Waddell and Mayer, 2003; Sahl and Munakata-Marr, 2006; Sutton et al., 2011). The harmful effects of H_2O_2 on microbial community are concentration-dependent; as a result, the U.S. EPA recommends the use of aqueous solution of H_2O_2 with a concentration lower than 500 mg L^{-1} (U.S. EPA, 2004) to limit these phenomena.

Nevertheless, there is also evidence that the negative effect exerted by Fenton processes does not hinder a recovery of biological activity over time. For instance Sutton et al. (2014c), who investigated the effect of Fenton and modified Fenton processes on microbial diversity and activity during 8 weeks of incubation in two diesel-contaminated soils, observed that although microbial communities was adversely affected in the first 2 weeks, a rebound of microbial abundance and biodegradation activity was observed after 4 weeks. This behaviour opened the way for the development of a treatment train based on the sequential application of ISCO and in situ bioremediation (ISB), which was investigated in several studies. For instance, Chen et al. (2016) investigated the efficiency of diesel removal by in situ chemical oxidation evaluating the effects of different oxidants on indigenous microorganisms; they observed that the contaminant removal was highest in ISCO-treated microcosms enabling a rapid recovery of the microbiota. These studies indicated that the combination of chemical oxidation and in situ bioremediation could be more efficient than ISCO alone in achieving the clean-up goals, since chemical oxidation was capable of reducing the contaminant's concentration to a level suitable for the further biological degradation of the residual contamination. The optimal coupling of ISCO and ISB requires a careful selection of the operating conditions of the chemical oxidation step (e.g. oxidant dosage and number of sequential additions), aimed at limiting its negative effects on the microbial community, as also shown by Chen et al. (2016).

Despite the available literature, there is still need of further investigation in view of ISCO integration with ISB. This regards in particular Fenton-like processes since, although previous studies have analysed the combination of bioremediation and other ISCO treatment, such as Fenton, modified Fenton, persulfate and permanganate and other oxidants (e.g. Chen et al., 2016; Sutton et al., 2014b, 2014c), the combination of ISB with Fenton-like processes has not been fully investigated yet. Thus, this study was aimed to investigate this combination of processes, specifically assessing the response of the bacterial community of a diesel-contaminated soil to the disturbance of oxidative stress caused by the application of FLT treatment. In particular, the soil underwent different treatment conditions, differing for either H_2O_2 concentrations (2 and 6%) or application mode (single injection or double after depletion of the first dose). Short-term and long-term effects on the bacterial community of the different tested treatments were evaluated using different molecular tools (i.e., Real Time PCR and DGGE) and biochemical tests (respirometry and dehydrogenase activity). The obtained results were then compared, both in terms of degradation outcomes and impact on the microbial community with those obtained by enhanced natural attenuation (ENA).

2. Materials and methods

2.1. Reagents

Hydrogen peroxide (H_2O_2, 30% w/w), titanium (IV) oxysulfate, sulphuric acid (96%), monobasic potassium phosphate (KH_2PO_4), used as stabilizing agent, ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) (99%), used as chelating agent, were purchased by Sigma Aldrich. 5-alfa-androstane (99.9%, 2.00 μg mL^{-1} in methylene chloride, Sigma Aldrich) and 1-chlorooctadecane (99.6%, Sigma Aldrich) were used as internal standard and the surrogate, respectively. Anhydrous sodium sulphate (Sigma Aldrich > 99%), previously thermally activated at 500 °C for 4 h, was used for drying the soil samples during hydrocarbons extraction. All aqueous solutions employed were prepared with deionized water produced by reverse osmosis (Zeneer Power System).

2.2. Soil characterization

All experiments were performed using a Diesel-contaminated soil collected in a gasoline station site and sampled from the capillary fringe at a depth of 1–2 m. The soil of this contaminated site proved to be characterized by a significant heterogeneity and presented a concentration of hydrocarbon C > 12 in the range of 700–2600 mg/kg which was mainly due to the fraction C8–C28 with the fraction C28–C40 representing around 6–10% of the total concentration. Before use, the contaminated soil was sieved to remove the coarser fraction (d > 1.0 cm), mechanically homogenized in a stainless steel vessel and its main chemical and biological properties were evaluated. The particle size distribution was determined applying the ASTM D422 (2007) procedure. The total organic carbon (TOC) content was determined as difference between the total carbon (TC) and inorganic carbon (IC) data measured using a Shimadzu TOC VCPH analyser equipped with a SSM-5000A solid sampler (EN 15936, 2012). The soil oxidant demand (SOD) and total oxidant demand (TOD) were measured by potassium permanganate consumption (Crimi et al., 2003). The pH was measured in the supernatant of a suspension made by 10 g of soil and 25 mL of a $CaCl_2$ solution 0.01 M. The total Fe and Mn content was determined by acid extraction (US EPA 3050B, 1996) followed by ICP-OES analysis (Agilent 710-ES) of the solution (ISO 11885, 2011). The initial Diesel Range Organics (DRO) content of the soil was also analysed measuring the concentration of hydrocarbons C8–C28 by gas chromatography (see Subsection 2.4).

2.3. Experimental set up

Two types of static batch microcosms were performed using the diesel-contaminated soil, i.e. Fenton-like treatment (FLT) coupled with bioremediation (coded as FLT-BR) and enhanced natural attenuation (coded as ENA) alone. Table 1 reports a synoptic scheme of the remediation microcosms and main parameters of both the chemical oxidation and the biological attenuation step (i.e., liquid/solid ratio, oxidant dose and application mode, endpoint for each Fenton-like treatment, mineral solution, water content and bioremediation treatment times).

In the FLT-BR tests, the chemical oxidation step was followed by bioremediation; the ENA tests were incubated in parallel with the