Biodegradation combined with ozone for the remediation of contaminated soils

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1. Introduction

Among the widespread remediation technologies, the bioremediation process is one of the easiest and cost-effective methods to treat polluted soils [6]. It exploits the bacteria capability for the degradation of organic pollutants even if they are persistent and low-bioavailable, such as PAHs and phenols [9]. In particular, PAHs are characterized by polycondensation of benzene rings, which confers them high chemical stability and low water solubility. Moreover, their bioavailability is limited by strong bonds, which they could create with the natural soil organic matter [8] during the time (aging) elapsed between the soil pollution event and the beginning of its remediation. The SS-SBR process allows maintaining optimal microorganism conditions inside the bio-slurry reactor enhancing both the growth and the degradation activity of the biomass [2].

Several researches have been carried out in order to study the feasibility of bio-slurry treatments for the remediation of contaminated soils [3,8], while other studies investigated the effect of different parameters as the

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soil/water ratio [10] or the soil aging [1] on the degradation effectiveness. Moreover, biological treatments could be easily combined with a different remediation technology, such as a chemical oxidation [4,5].

The aim of this work was to evaluate the efficiency of the SS-SBR process applied on spiked and industrial soils both contaminated mainly by PAHs and phenols. Afterwards, a coupled biological and chemical treatment, using ozone as oxidizing agent, has been studied in order to lower the process duration and to improve the contaminants removal.

2. Materials and methods

The effectiveness of the bioremediation applied both on the spiked soil and the industrial aged soil has been evaluated. The SS-SBR process could be summarized in three phases: fill, the addition of the polluted slurry inside the biological reactor; react, the degradation of contaminants by means of microorganisms; draw, the renewal of 80% of the slurry. The residual 20% has been used as a bacteria inoculum for the following run, so SS-SBR process allows selecting the most capable bacteria for the degradation of the specific organic substrate adsorbed onto soil and, as a consequence, their acclimation period has been considerably reduced. The slurry, a suspension of soil in water, has been always prepared at a given soil/water ratio; in particular, it was set at about 40%, with a view to increase the amount of soil remediated for each run without affecting the biological treatment effectiveness.

The examined soils have been preliminarily sieved (mesh size: 2 mm) to remove coarse materials that are difficult to be suspended and usually considered unpolluted [8]; this way prevented also damages to the reactor stirrer. The industrial aged soil has been dried for 2 h at about 313 K, while the spiked soil has been dried for 1 h at about 383 K to remove water and the autochthonous biomass, that, in this case, could modify the performances of the selected inoculum. Then, the industrial aged soil has been mixed to a proper amount of water in order to constitute the slurry phase. Concentrations of aromatic pollutants contained in the industrial aged soil are reported in Table 1. On the other hand, the spiked soil has been artificially polluted with several contaminants, dissolved into a mixture of two organic solvents, methanol and acetone with a ratio of about 1/5, trying to obtain a homogeneous pollution in terms of contaminants distribution and concentration. In this case, the degradation of a wide range of organic compounds (listed in Table 2), such as PAHs, phenols and organic acids, has been investigated. Since the pollutants concentrations could be varied in each experimental run, their total amount added to the spiked soil has been always kept at about 3 g kg^{-1} of dry soil. Furthermore, after 5 weeks with a view to simulate the aging of the industrial soil, the slurry has been prepared and added to the bioreactor.

Table 1
Pollutants concentrations in the industrial aged soil

Class	Concentration [mg kg^{-1} dried soil]
Total aromatic solvents	0.54
Total nitrobenzenes	10.7
Total halogenated aromatics	1.13
Total aromatic amines	2.81
Total phenols	2.63
Total PAHs	10.2

A schematization of the experimental apparatus is sketched in Fig. 1. Two reactors have been loaded with 1.5 and 0.75 kg of slurry to perform removal tests on spiked soil and industrial aged soil, respectively. Inlet air stream, which is necessary for the bacteria lifecycle, has been regulated by a flow meter (about 50 ml min^{-1} per liter of slurry) and humidified to compensate the water losses from the reactor caused by evaporation and stripping due to the air flow rate. It was fed at the bottom of the reactor and a gas sparger was used to reduce the air bubbles mean size, thus improving the oxygen dissolution and bioavailability within the slurry. An activated carbon trap, located on the gas outlet port allows verifying the possible loss of the most volatile pollutants. Macronutrients containing Nitrogen (N) and Phosphorus (P), have been added to the slurry to support the bacteria growth at the beginning of each biological treatment. In particular, the amount of these

Table 2
Pollutants in the spiked soil

Class	Compound
Phenols	Phenol (PhOH)
	Acenaphthenol (ACE-OH)
	2-Naphtol
PAHs	Napthalene (NAP)
	Acenaphthene (ACE)
	Phenanthrene (PHE)
	Anthracene (ANT)
	Fluoranthene (FLA)
	Pyrene (PYR)
	Chrysene (CHR)
	Perylene (PER)
	Benzo[a]pyrene (BaP)
Organic acids	Para toluic acid (p-TA)

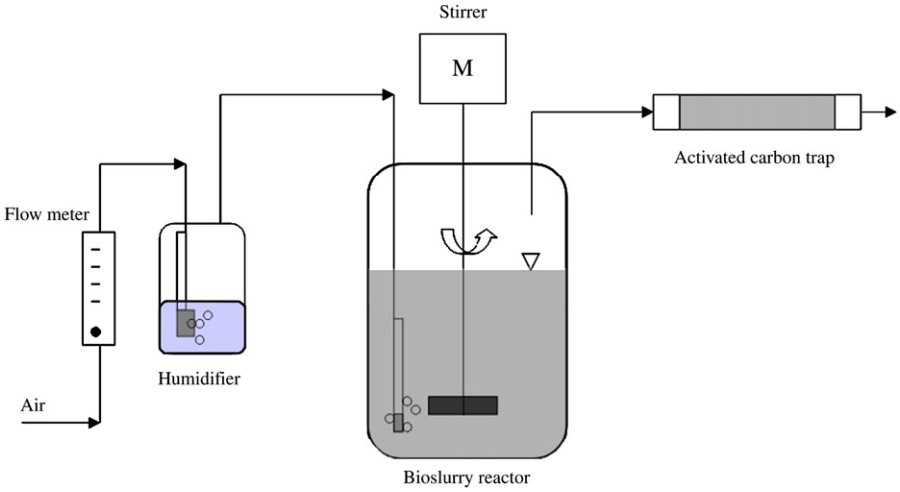

Fig. 1. Sketch of the experimental SS-SBR apparatus (M = stirrer engine).

macronutrients has been related to the theoretical contaminants COD (Chemical Oxygen Demand) according to the relationship COD:N:P = 100:5:1. Their bioavailability has been ensured by using a water-soluble salt, di-ammonium hydrogen phosphate ($[NH_4]_2HPO_4$). Slurry acidity has been periodically monitored and the pH was kept in the range 6.5−8 to provide an optimal microbial habitat [7]. The bioreactor was continuously stirred at about 300 rpm to have homogenous conditions within the slurry, in terms of pollutants, macronutrients and oxygen concentrations, as well as the suspension of soil in water.

Chemical oxidations have been always 16 h long and the amount of ozone was about 2 g h^{-1}. They were performed on 300 ml of slurry sampled from the bio-slurry reactor at different times of the biological treatment. These tests have been performed with an experimental equipment similar to that of Fig. 1 but, in this case, a flow meter regulated the oxygen flow rate at the ozone generator inlet. The gaseous stream was then fed at the bottom of the ozonation reactor through a gas sparger to enhance the dispersion and water solubility of the oxidizer.

To check the contaminants content of the soils, several slurry samples have been taken from the slurry reactor at the beginning of each treatment and an average concentration value is computed to minimize mainly the slurry heterogeneity. Then, they were sampled biweekly and every 3 h for bioremediation and ozonation runs respectively, so that their degradation effectiveness could be monitored. Both phases constituting the slurry have been separated by filtration on a hydrophilic acrylic copolymer membrane disc filter (Pall Life Sciences, Versapor 3000®; pore size: 3 μm). Water samples have been analyzed with HPLC (Jasco PU-1580 High Performance Liquid Chromatography) without further preparations, while a solvent extraction of pollutants (acetonitrile and an acetonitrile/acetone mixture with a ratio 6/4 for the industrial and the spiked soil samples, respectively) adsorbed onto soil has been carried out by 3 h mechanical mixing of the samples, completed by a 45 min sonication. After a further filtration on a 25 mm syringe filter, provided with a 0.45 μm GHP membrane (Pall Life Sciences), the contaminants concentrations have been measured with HPLC. Details concerning the HPLC procedures adopted are summarized in Table 3.

3. Results

3.1. Biological treatment

The industrial aged soil was characterized by a great heterogeneity of the pollution, in terms of kind, number and distribution of the contaminants adsorbed onto soil; although multiple slurry samples, at least four, have been regularly taken from the reactor to check the pollutants removal during the biological process, the aforementioned heterogeneity of soil properties always affected experimental measures performed for the industrial soil; this suggested to evaluate the overall removal efficiency of the process rather than the concentration trends of each pollutant in order to ensure accurate and useful results. As a consequence, the degradation effectiveness of the bioremediation has been evaluated monitoring a global normalized concentration C/Co. It

Table 3
Summary of adopted HPLC procedures

	Industrial aged soil	Spiked soil
Injection volume	10 μl	20 μl
Temperature	Ambient	Ambient
Mobile phase	Acetonitrile/water gradient	Acetonitrile/water solution (H_3PO_4 0.5% by weight) gradient
Flow rate	1 ml min^{-1}	1 ml min^{-1}
Column	250 × 4 mm, reversed phase, RP-18 (5 μm)	250 × 4 mm, reversed phase, RP-18 (5 μm)
Detector	UV @ 254 nm	UV @ 220−240−254 nm
Elution program	1. Linear gradient from 60% acetonitrile/40% deionized water to 100% acetonitrile over 20 min 2. Hold at 100% acetonitrile for 15 min 3. Linear gradient to initial conditions, 5 min	1. Hold at 20% acetonitrile/80% water solution for 4 min 2. Linear gradient to 35% acetonitrile over 11 min 3. Linear gradient to 40% acetonitrile over 5 min 4. Linear gradient to 70% acetonitrile over 10 min 5. Linear gradient to 80% acetonitrile over 10 min 6. Hold at 80% acetonitrile for 20 min 7. Linear gradient to initial conditions, 5 min
Analysis time	40 min	65 min

is computed as the ratio between the average amount of total pollutants detected in soil at the sampling day (C) and at the beginning of the SS-SBR treatment (Co). No aromatic pollutant has been found inside water samples of the industrial soil, probably due to the aging effect. The trend of the normalized concentration C/Co as a function of the elapsed time from the beginning of the bioremediation, namely weeks, is shown for the sake of example in Fig. 2, where the vertical lines describe the renewal of the slurry which occurs during the draw phase of the SS-SBR process and, consequently, at the beginning of a new biological run. It was found that bacteria were not able to completely metabolize the contaminants amount present in the industrial soil. For this reason, a residual fraction of total pollutants has been always detected at the end of the bioremediation tests, after about 7−9 weeks. Removal efficiencies higher than 80% have never been achieved for the industrial aged soil.

The degradation effectiveness of the SS-SBR process on the spiked soil has been evaluated by monitoring the pollutant concentration, in terms of mg kg^{-1} DS (Dried Soil) and mg l^{-1} for the soil samples and the water samples, respectively, as a function of the elapsed time, measured in weeks. Bioremediation did usually need up to 13 weeks to reach high contaminants removals. Several pollutants were found even in water, such as phenols, organic acids and only NAP and ACE among the PAHs, due to their very low water-solubility. However, their results concerning the water-phase have been omitted because of concentration levels that are practically negligible. For the sake of example, one representative compound belonging to each PAHs class has been chosen: NAP, PHE, PYR and BaP for two-, three-, four-, and five-benzene rings PAHs, respectively. Experimental results of the spiked soil bioremediation were summarized in Figs. 3 and 4. The meaning of vertical lines has been previously discussed. Good removal effectiveness has been achieved for all the examined contaminants added to the soil. Phenols and organic acids were rapidly and completely degraded in both phases constituting the slurry in 4 to 6 weeks while PAHs were almost completely removed in the whole process, except for five-rings PAHs, which were partly degraded by bacteria.

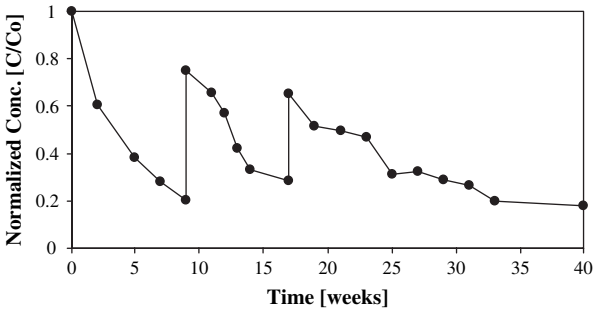

Fig. 2. Overall removal trend for a cycle of bioremediation treatments.

3.2. Combined treatment

Ozonation used as an independent remediation technology did not achieve significant degradation results on the industrial aged soil despite ozone is a very strong oxidant. Moreover, as shown by error bars in Fig. 5, results were scattered. The same treatment, applied to the spiked soil, evidenced low removals for high-molecular-weight and more recalcitrant compounds, while quite good results have been observed for more

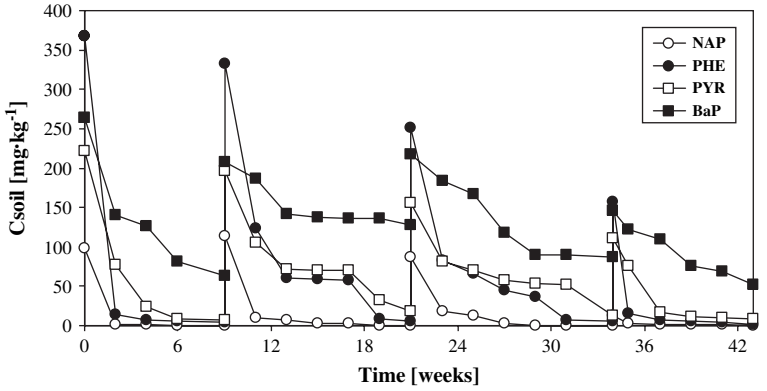

Fig. 3. Concentrations of several representative PAHs as a function of time for the SS-SBR process: NAP (2-rings), ANT (3-rings), PYR (4-rings) and BaP (5-rings).

volatile pollutants, such as 2-Naphtol, and for low-molecular-weight PAHs (Fig. 6). This behavior could be ascribed to the weak aging of the contamination which characterizes the spiked soil.

On the other hand, combined processes have been also performed using the chemical oxidation as a post-treatment of the bioremediation. Concerning the industrial aged soil, several tests were carried out on slurry samples drawn from the bioreactor at different times of the SS-SBR process, as illustrated in Fig. 5. After about a week of bioremediation, the overall amount of the contaminants adsorbed into soil has been almost completely degraded, while ozonation tests performed before this period did not allow to improve markedly the degradation effectiveness.

On the spiked soil, the efficiency of the combined process for the pollutants removal has been evaluated after few days of bio-slurry treatment, precisely after 3 and 7 days. As shown in Fig. 6, which compares results of several representative polyaromatic compounds for different treatments, best issues have been achieved in the last experimental test, where an almost complete degradation of the investigated compounds have been obtained. Furthermore, results obtained after 3 h of ozonation are also reported; it is possible to notice that removal efficiencies larger than 90% were evidenced for low-molecular-weight or most bioavailable pollutants after the short ozonation. However the most recalcitrant contaminants, such as four- and five-rings PAHs, have been massively removed, for the combined process, only after a 16 h ozonation.

4. Discussion

The application of the SS-SBR technology to soils polluted by several aromatic contaminants allows achieving good removal results providing an effective contact between microorganisms and pollutants, which

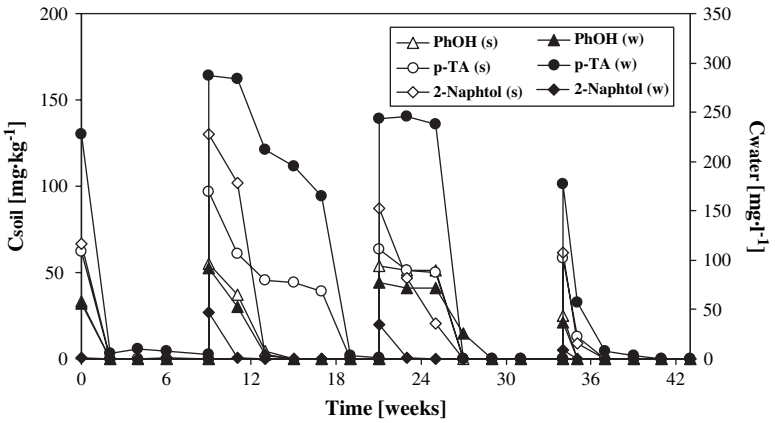

Fig. 4. Phenols and organic acid concentrations into soil (s) and water phase (w) for the SS-SBR process.

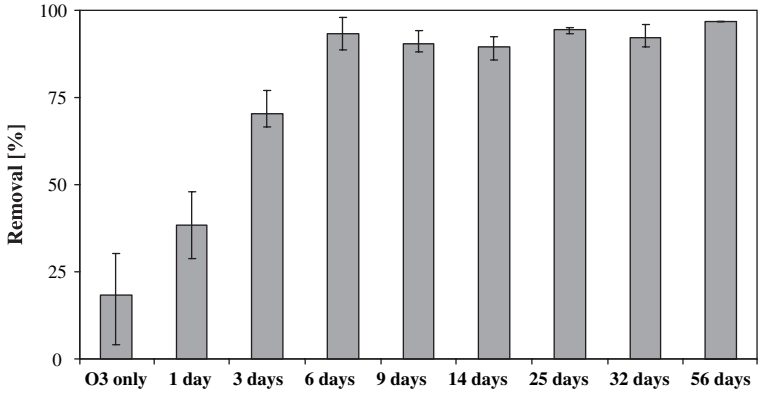

Fig. 5. Comparison between degradation efficiencies found applying ozonation both as the only process and after various periods of biological treatment on the industrial aged soil. Error bars show the maximum deviation from the average value of the measures performed for each experiment.

usually adhere onto soil, and keeping inside the slurry an optimal bacteria habitat enhancing, run by run, the selection of bacteria well-adapted for the degradation of this specific organic substrate.

In the spiked soil, bacteria have fast metabolized water-soluble compounds, such as phenols and organic acids, even if they could inhibit the biomass degradation activity modifying the slurry conditions. As experimentally verified, a long treatment was necessary to degrade them completely, when their concentrations were too high. Among the polyaromatic pollutants, low-molecular-weight compounds were degraded faster than the highest ones because an extended polycondensation of benzene rings confers them high chemical stability and low water solubility limiting their bioavailability and removal rates. Consequently, the five-rings PAHs were the most difficult to be degraded. It is worth to notice that these issues, highlighted by bioremediation tests, are independently confirmed by data obtained in ozonation runs, carried out as the only remediation process (Fig. 6).

In the industrial soil, a residual fraction of the total pollutants amount has been always detected at the end of the biological run probably due to the creation of strong bonds between the pollutants and the soil natural organic matter during the aging. It was not possible to overcome this residual pollution even increasing the treatment duration as highlighted by the last run shown in Fig. 2.

However, a combined treatment, involving slurry phase biodegradation followed by ozonation, has markedly shortened the soil remediation for both investigated soils, namely from 2−3 months to about 1 week. This treatment always improved the degradation

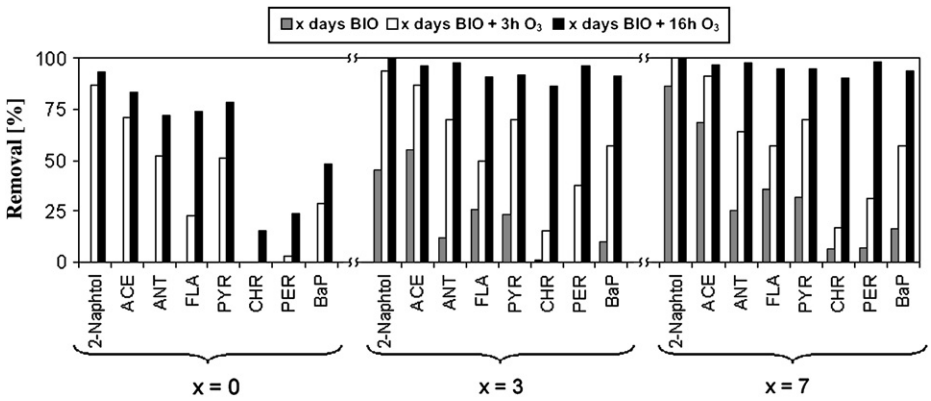

Fig. 6. Removal efficiencies found at different stages of the combined process for representative pollutants, on the spiked soil; results obtained in tests performed after 3 and 7 days of biological treatment are compared with those obtained applying ozonation as the only remediation treatment.

performances. It could be also noticed that a 3 h ozonation allows the complete removal of phenolic pollutants, while a 16 h treatment was necessary to degrade satisfactorily p-TA and PAHs, including five-rings compounds which were the most recalcitrant towards the biological process. The pollutant reactivity with ozone could be affected by their partitioning into the soil organic matter; for the sake of the example the degradation of CHR was slower than expected. Although a combined process, rather than biodegradation (or ozonation) as the only treatment, allows foreseeing the possibility to easily fulfill environmental standards and regulations, in terms of threshold concentrations of pollutants within the soil, to have a comprehensive understanding of sustainability and overall effects of this technology deeper investigations, especially concerning the evolution of biomass and the fate of pollutants during the process, are needed.

5. Conclusions

Laboratory-scale tests underlined that the SS-SBR process allows achieving good removal effectiveness for the degradation of recalcitrant compounds such as PAHs and phenols, also enhancing the growth and the activity of bacteria suitable to metabolize these specific organic substrates. It could be improved, in terms of efficiency and duration, by the combination between the biological SS-SBR process and the ozonation, using the chemical oxidation as a post-treatment. Experimental findings suggest that a combined treatment of a few days, this time strongly depending on the properties of contaminants and soil, may be a promising bioremediation technology for soils polluted by mixtures of polycyclic aromatic hydrocarbons or recalcitrant pollutants.

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