

In Situ Aerobic Biostimulation of Groundwater at a National Priority Site in Italy

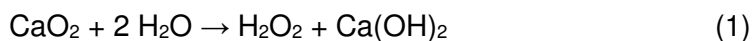
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ABSTRACT: Sarroch plant (CA, Italy) is an industrial area listed in the Italian Priority List of polluted sites. Pollution in groundwater has been addressed thanks to the design of a remediation action based on the aerobic biodegradation of petroleum hydrocarbons (predominantly mono-aromatic and short-chain aliphatic hydrocarbons) promoted by the injection of oxygen-releasing compounds. The feasibility of biostimulation at the site was preliminary assessed by means of laboratory tests. The direct push injection of the product has been foreseen at 2800 points, distributed along multiple lines perpendicular to the groundwater flow direction, over a total length of approximately 8 km and a total area of 90 hectares. According to the pollutant concentration measured in the different zones of the site, a different number of injection campaigns and injection frequency has been scheduled (3 to 10 campaigns, every 5 to 12 months). The estimated cost for the bioremediation action is 23 million Euros. Compared to the previous project approved in 2010, including a seafront physical barrier and groundwater circulation wells – in situ well stripping, the in situ injection of the oxygen-releasing compounds is an improvement toward a quicker, more effective and sustainable remediation of groundwater at the site. In view of all this, in 2017 the public authorities approved the variant of the project.

INTRODUCTION

Biological methods for the remediation of petroleum hydrocarbons in groundwater is considered as economical, efficient and environmental-friendly over other methods. Bioremediation of these pollutants is often limited by dissolved oxygen, as many hydrocarbons degrade very slowly or not at all under anaerobic conditions. Therefore, remediation techniques require supplying oxygen to aquifers to speed up their recovery from this kind of pollutants (Varjani, 2017).

Oxygen-releasing compounds (ORC) are among the most promising materials that slowly decompose in water and continually supply oxygen. Calcium peroxide (CaO_2) has been widely used for remediation of petroleum hydrocarbon-contaminated groundwater. By water exposure of CaO_2 particles, hydrogen peroxide (H_2O_2) is released (Eq. (1)). Hydrogen peroxide is stable only for minutes to several hours and, depending on the chemical and physical conditions of water, it decomposes to hydroxyl radical (Eq. (2)) or, by disproportionation, to oxygen (Eq. (3)) (Mosmeri et al., 2017):



The dissolution of calcium peroxide in water leads to the formation of Ca(OH)_2 , which raises the pH in groundwater. The rate of CaO_2 dissolution decreases as pH increases.

The persistence of H_2O_2 decreases with increasing pH, resulting in very rapid transformation and disproportionation prevailing over hydroxyl radical production at pH 12-13 (Northup and Cassidy, 2008).

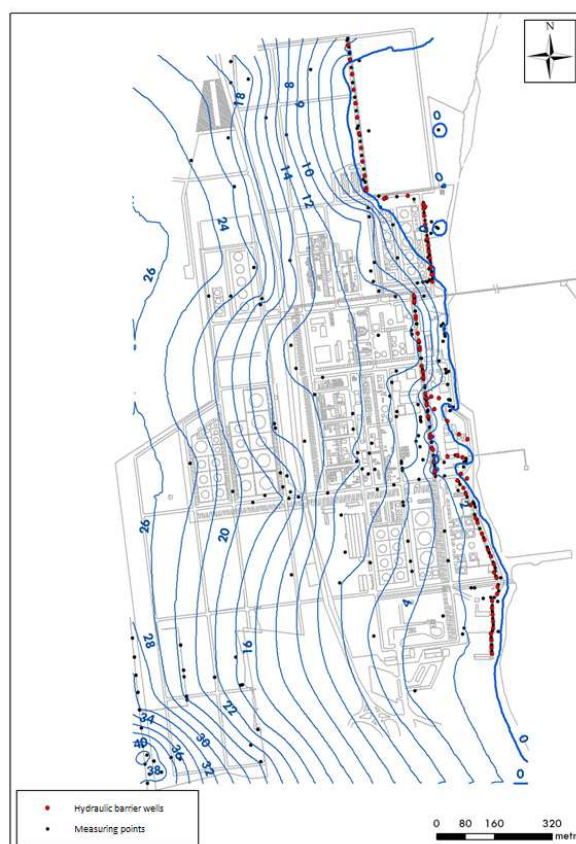
Site description. Sarroch plant (CA, Italy) is an industrial area listed in the Italian Priority List of polluted sites. In an area of about 90 hectares (Figure 1), monoaromatic solvents and short-chain aliphatic hydrocarbons affect groundwater. A hydraulic barrier is active close to the seafront at the eastern boundary, to confine pollution inside the area. Light Non Aqueous Phase Liquid (LNAPL) has also been widely reported in the past and, to a lesser extent, is actually present in some monitoring wells, which have been equipped with recovery systems, according to the approved project. Groundwater is almost everywhere under anoxic conditions, with dissolved oxygen below 3 mg/L.

The area consists over a phreatic aquifer composed of fluvial sandy deposits, which extend to a maximum depth of 45 m below ground surface (b.g.s.), in the western part of the area, to a minimum depth of 5 m b.g.s., in the eastern part. The hydraulic conductivity of the saturated portion of the aquifer varies between 10^{-6} and 10^{-4} m/s. The groundwater level is between 1 m b.g.s. (in the South West) to 12 m b.g.s. (in the North). The general flow direction is oriented from West to East.

Groundwater contamination has been addressed thanks to the design of a remediation action, in charge of Versalis SpA (ENI group), based on the aerobic biodegradation promoted by the injection of oxygen-releasing compounds containing CaO_2 . The approach was proposed in 2016 in a variant of a previous remediation project of 2010, based, among other things, on the installation of a seafront physical barrier, equipped with several upgradient groundwater pumping wells, and groundwater circulation wells – in situ well stripping (GCW-IWS), which have been discarded.

MATERIALS AND METHODS

Feasibility tests. The feasibility of biostimulation at the site was preliminary assessed by means of laboratory tests, performed at Politecnico di Milano. The bioreactors were set up using polluted soil and groundwater from the site (solid to liquid ratio of 9% on weight basis). Three of them (generically named B, C and D) were added with different commercial products containing calcium peroxide, which were dosed at 0.35% of soil weight. A control reactor (named A) without CaO_2 was set up dosing sodium azide as



**FIGURE 1. Piezometric lines (m a.s.l.)
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the biocide. All reactors were spiked with a buffer solution (1.4 mol $\text{KH}_2\text{PO}_4/\text{mol}$ K_2HPO_2) to keep the pH value at neutrality. Calcium peroxide, sodium azide and the buffer solution were added twice (at day 0 and 27) during the experiments, which lasted approximately two months. Unfortunately, the reactor D went broken at day 33, without chance to collect samples. The tests were performed at 21 ± 2 °C (average groundwater temperature at the site) without mixing, in order to exclude advection affecting oxygen release and transport in the reactors, and better simulate the aquifer's conditions.

Soil and water used in the reactors were analyzed before the tests in order to quantify the pollutants (benzene, toluene, ethylbenzene, xylenes, styrene - BTEXS, isopropylbenzene, trimethylbenzenes, naphthalene, total petroleum hydrocarbons - TPHs), ammonia and nitrates. During incubation (24 h, 6 d, 20 d and 63 d), small aliquots of water were taken from each reactor for the measurement of dissolved pollutants, ammonia and nitrates, as well as pH, dissolved oxygen and temperature. To keep the reactors completely full, without headspace, at the end of each sampling operation, the reactors were topped up with an aliquot of uncontaminated groundwater from the site. At the final sampling (63 d), soil samples were also collected to quantify the residual contamination on the solid matrix. An aliquot of these samples was used for the microbiological analyses of total heterotrophic bacteria and BTEX-degrading bacteria.

Solid phase micro-extraction and solid-liquid extraction were used to extract hydrocarbons from water and soil samples, respectively. Quantification was carried out by gas chromatography–mass spectrometry. The values returned for the TPHs did not include the individually quantified target compounds (BTEX, styrene, isopropylbenzene, trimethylbenzenes and naphthalene).

For the total heterotrophic bacteria plate count, 0.5 g of soil from each sample were suspended in 4 ml of buffer solution, vigorously shaking the vortex for 2 min. The suspension obtained was subsequently used to make 1:10 serial dilutions. 100 μL of the suspension were plated on Luria-Bertani agar medium. The plates were then incubated at 25 °C for 48-72 h. At the end of the incubation period, Colony Forming Units (CFUs) were counted.

For the count of BTEX-degrading bacteria with the three-replication Most Probable Number (MPN) method, 0.5 g of soil were suspended in 4 ml of buffer solution, vigorously shaking the vortex for 2 min. The resulting suspension was used to make 1:10 serial dilutions in M9 mineral medium. The test was set up in multi-well (96) plates. In each well 180 μL of M9 mineral medium, 20 μL of the previously made 1:10 dilution and 5 μL of a BTEX solution in heptamethylnonane were dosed, in order to reach the desired BTEX concentration in each well (2.5 mg/L to 45 mg/L, depending on the pollutant). The plates were then incubated for 7 d at 25 °C. At the end of the incubation, 50 μL of a 3 g/L solution of indophenyl nitrophenyl tetrazolium was added to each well and the dye was left to act for 1 h. Positive wells (purple staining) were identified and the MPN was calculated using the McCrady tables.

Full-scale design. The design of the full-scale treatment kept into consideration that production plants, structures and infrastructures cover a significant part of the site and interferences to the productive activities have to be limited as much as possible, as well as safety guaranteed. Pumping well locations were also taken into account; in fact, although it is not possible for the hydraulic barrier to pump out the solid oxygen-releasing compound, pumps do not have to capture water enriched in oxygen.

RESULTS AND DISCUSSION

Feasibility tests. A clear decrease (at least one order of magnitude) of the concentrations of all compounds in soil in all the microcosms (A, B, C) was observed between the beginning and the end of the tests (Figure 2). TPHs decreased less in the control reactor (A) than in the ORC-added reactors. For the microcosm D, no information is available, as the reactor went broken before the end.

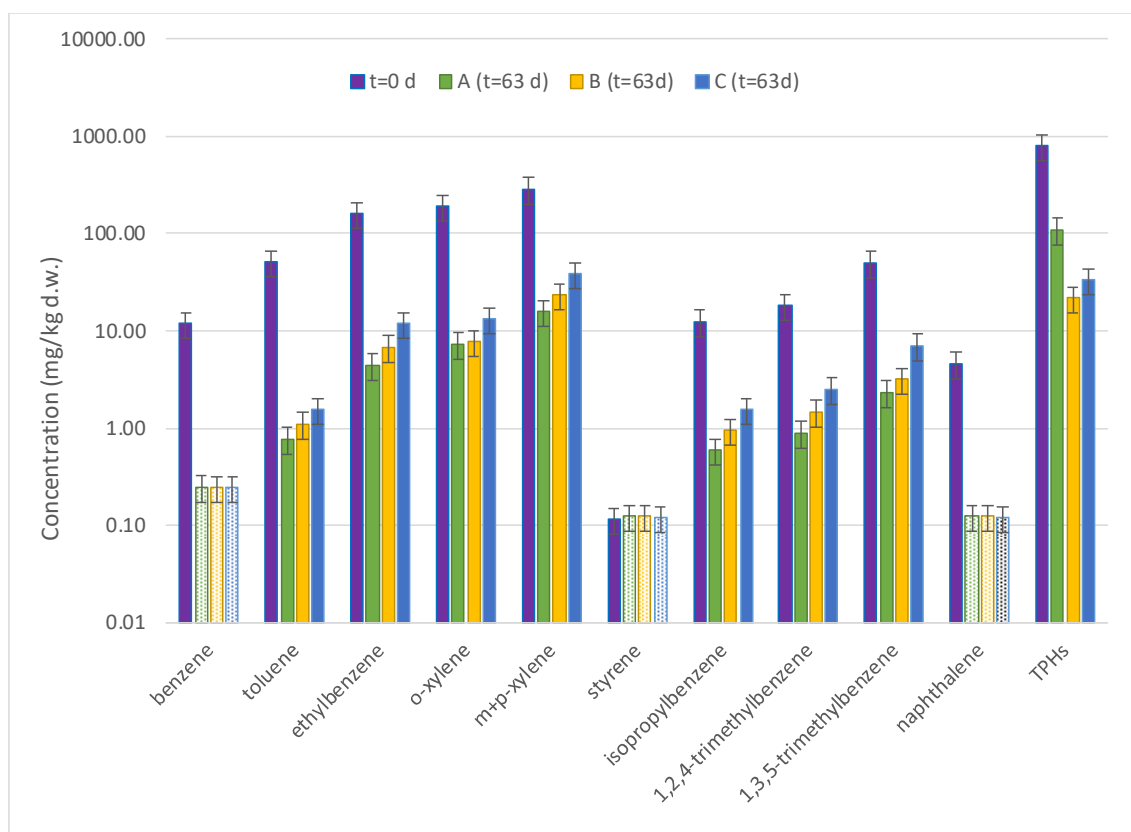


FIGURE 2. Pollutant concentrations in soil at the beginning and at the end of the tests. The dotted bars indicate values below the analytical detection limit. No information available for the reactor D, as it went broken.

At the end of the tests, a significant increase in heterotrophic biomass was observed in the soil samples from the ORC-added reactors, while inhibition due to sodium azide was observed in the reactor A. A significant growth of BTEX-degrading microorganisms was observed in the microcosm B only (from $1.3E+03$ to $3.5E+05$ MPN/g), while the concentration remained unchanged in C. The reason for the different growth of biomass in the microcosms B and C was ascribed to the different availability of macronutrients in the reactors; the product dosed in B, in fact, contains macronutrients, differently from the product dosed in C.

From the initial value of about 4.5 mg/L, the oxygen dissolved in water rapidly increased in all the batches added with the ORC, without significant difference between the microcosms. In reactors B, C and D, the measurements showed an oxygen content of about 11 mg/L after 24 h and 19 mg/L after 6 d. In the following two weeks (monitoring at 20 d) the oxygen was rapidly consumed in all microcosms with the ORC, down to a concentration <1.2 mg/L and again, following the second ORC dosage,

down to a final concentration <1.5 mg/L. In the control reactor A, oxygen showed a more gradual and slow decrease over time, starting from the initial value of about 4.5 mg/L down to a minimum of about 1.8 mg/L at the end of the test. Based on the variation of dissolved oxygen in the first 27 d of testing, an oxygen consumption rate exceeding 1.2 mg/L/d was calculated for the ORC-added reactors. In the second month of testing, following the further ORC dosage, the consumption rate was lower, but still higher than 0.5 mg/L/d, and at least one order of magnitude higher than that in the control microcosm (0.05 mg/L/d).

As for all the organic pollutants, the dissolved concentration (Figure 3) increased significantly in all reactors after 24 h from the setup, due to release from the soil (in fact, the initial dissolved concentrations were negligible). The release from the soil was not instantaneous (especially in the microcosms B, C and D), in particular for the less soluble compounds, whose dissolved concentration show further increase at day 6 and 20. Only in the final sampling, after two months of incubation, a decrease in the dissolved concentrations in the batches A, B and C was observed.

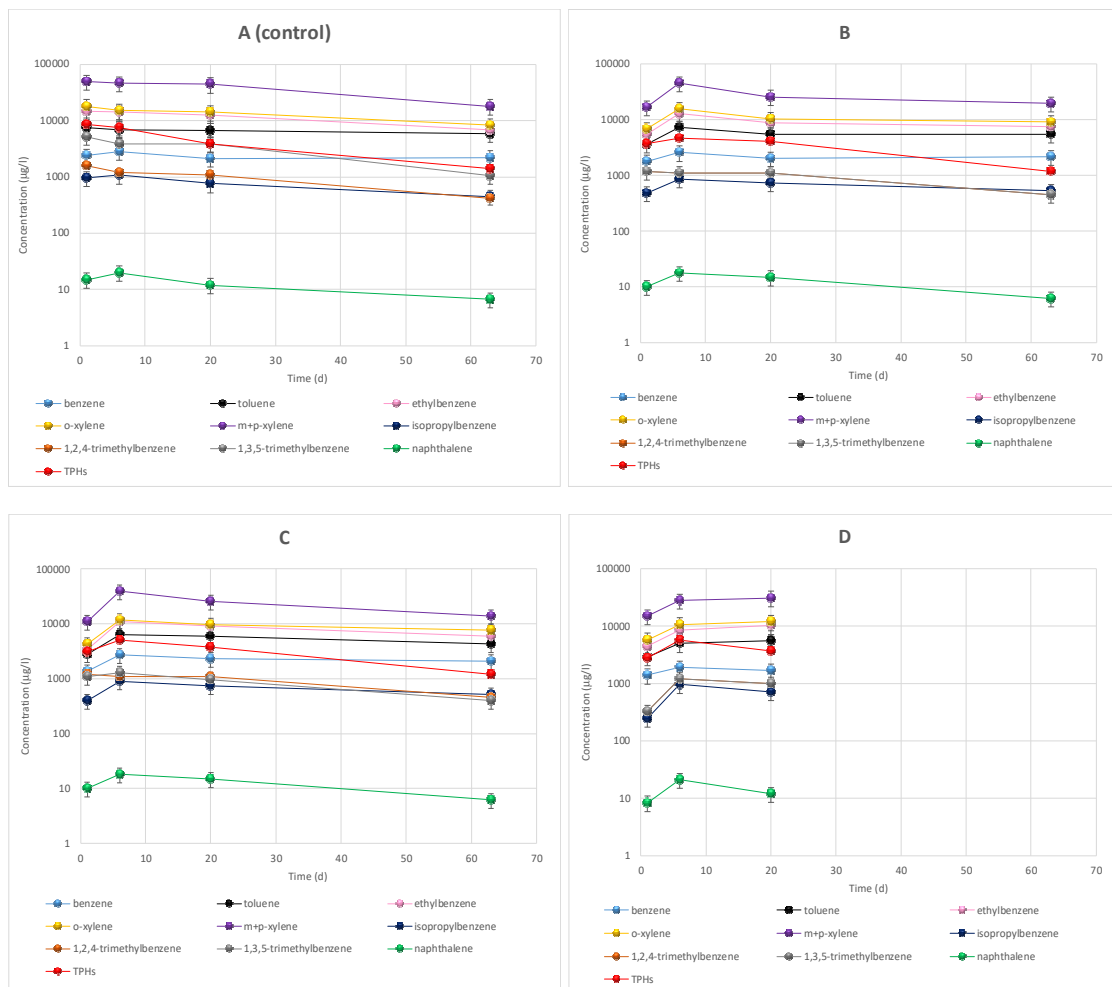


FIGURE 3. Pollutant concentrations dissolved in water during the tests. No information available for the reactor D at 63 d, as the reactor went broken in advance.

Starting from an initial dissolved concentration of 0.45 mg/L, ammonia initially showed an increase in all microcosms (up to a maximum value of about 2.5 mg/L after 6 d), due to release from the soil. The concentration further increased in the control reactor as time passed, while in the microcosms with the ORC (B, C, D), a gradual decrease was observed, down to values lower than 0.8 mg/L at the end of the tests (without significant differences among the reactors). Ammonia removal in the ORC reactors was probably due to nitrogen uptake for bacterial synthesis and to nitrifying activity, as suggested by the microbiological results and the increase in the nitrate concentration dissolved in water in the microcosms B and C with compared to the microcosm A.

On the basis of mass balance for the different organic compounds, the most significant mass reduction after 63 d was observed for TPHs, with removals of about 95% in the ORC-added reactors (95.7% in B, 94.5% in C) and 84.6% in the control reactor A (Figure 4). For the target aromatic compounds, in light of the analytical uncertainties, the residual mass at the end of the test was similar to the initial amount (for BTEX and isopropylbenzene) or not significantly different from those in the control microcosm (for 1,2,4-trimethylbenzene, 1,3,5-trimethylbenzene and naphthalene).

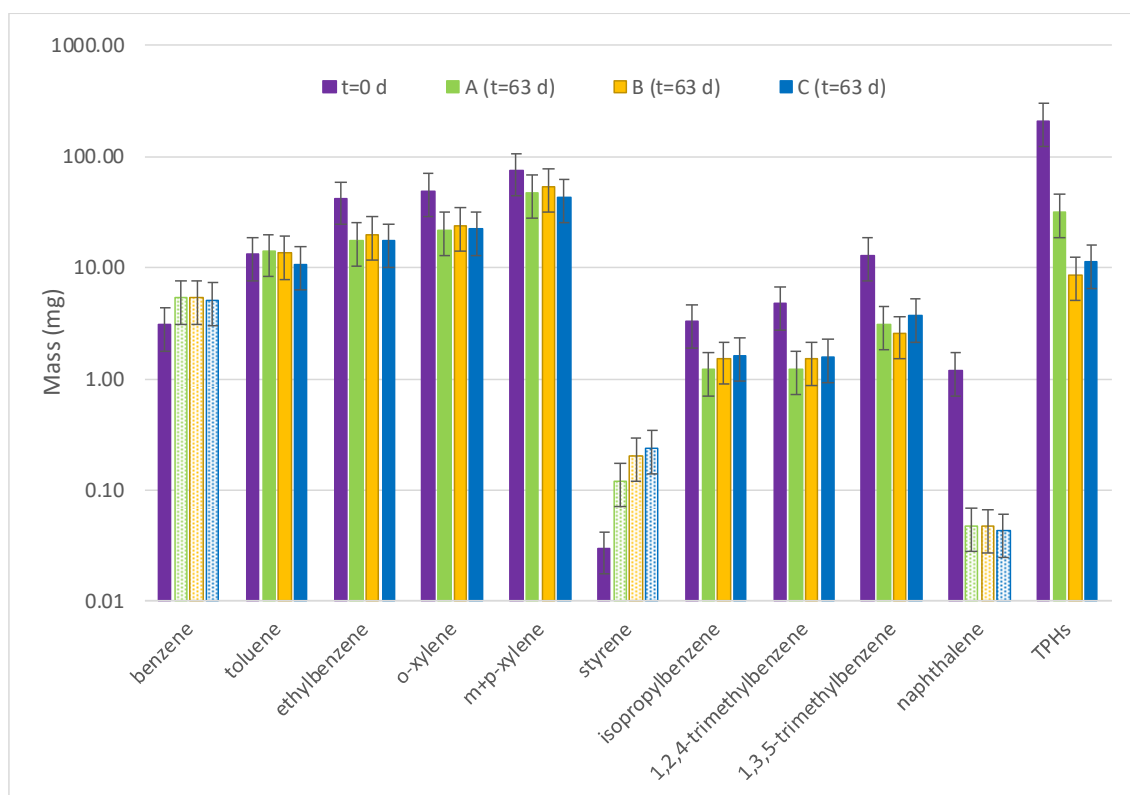


FIGURE 4. Pollutant mass at the beginning and at the end of the tests. The dotted bars indicate values based on data below the analytical detection limit. No information available for D at 63 d, as the reactor went broken in advance.

As highlighted by the microbiological analyses, despite the dosed biocide in the control reactor A an active heterotrophic biomass was found at the end of the test, probably responsible for the marked decrease in the total mass of TPHs measured in

this reactor. In contrast, the amount of BTEX-degrading bacteria in the control microcosm was low; therefore, any reduction in the overall mass between the beginning and the end of the test for the target monoaromatic compounds (BTEX, isopropylbenzene and trimethylbenzenes) in A was related to abiotic losses (eg., volatilization). The higher percentage of TPH mass removal compared to the target aromatics was probably due to the abundance and ease of biodegradation of some species present in the hydrocarbon mixture with respect to the aromatic compounds, and the greater presence of heterotrophic microorganisms (able to degrade aliphatic compounds) in the soil. As reported in the literature, in fact, in the biodegradation of hydrocarbon mixtures, susceptibility to degradation follows the order: linear alkanes > branched alkanes > low molecular weight aromatics > cycloalkanes > high molecular weight aromatics (Leahy and Colwell, 1990; Olajire and Essien, 2014). Therefore, it sounds reasonable that, in a test of 63 d, the effects of biodegradation have been more significant on low to medium molecular weight aliphatic compounds, compared to aromatic fractions.

Finally, taken into consideration the oxygen requirements for the oxidation of the various hydrocarbon compounds (on average 3.2 g O₂/g hydrocarbons), about 25% of the total oxygen releasable by the ORC dosed in the reactors was consumed for the pollutant degradation over the two months of testing. An additional 10% was used for nitrification (theoretically about 4.2 g O₂/g NH₃) and to satisfy the soil and water chemical demand.

The oxygen consumption rate obtained at laboratory scale (0.5÷1.2 mg O₂/L H₂O/d) was used for to estimate the oxygen consumption rate expected in the field, based on the following assumptions: a) the biodegradation of pollutants takes place exclusively in the liquid phase; b) the soil under treatment has concentrations dissolved in groundwater similar to those obtained at the beginning of the laboratory tests (about 100 mg/L in total); c) the average effective porosity in the saturated zone is 0.15. A range of values between 75 and 180 mg O₂/m³ of soil treated in situ/d was obtained, i.e. 27 ÷ 66 g O₂/m³ of soil treated in situ/year, corresponding to 171 ÷ 411 g ORC/m³ of soil to treat/year.

Full-scale design. The results of the laboratory tests confirmed that the aerobic biodegradation of the pollutants at the site can be promoted, starting from the aliphatic compounds. The treatment can be immediately applied where the pollutant concentration in groundwater is up to 100 mg/L, while at the most polluted areas of the site, likely affected by LNAPL, the application has to be postponed to free-phase recovery. In fact, besides in situ bioremediation, the variant of the project includes actions devoted to LNAPL recovery and to groundwater hydraulic confinement at the seafront.

The solid oxygen-releasing product will be injected as a slurry (with a solid to water ratio of 20% on weight basis) by direct-push technique. The injection system will be equipped with suitable rods infixed into the soil down to the established maximum injection depth, in order to proceed with a bottom-up approach. The direct-push injection of the product is foreseen at 2800 points, distributed along lines perpendicular to the groundwater flow direction, over a total length of approximately 8 km and a total area of 90 hectares (Figure 5). According to the pollutant concentration measured in the different zones of the site, a different number of injection campaigns and injection frequency has been scheduled (3 to 10 campaigns, every 5-12 months). In some specific areas, with significant logistical problems, the use of socks dropped in piezometers has been envisaged.

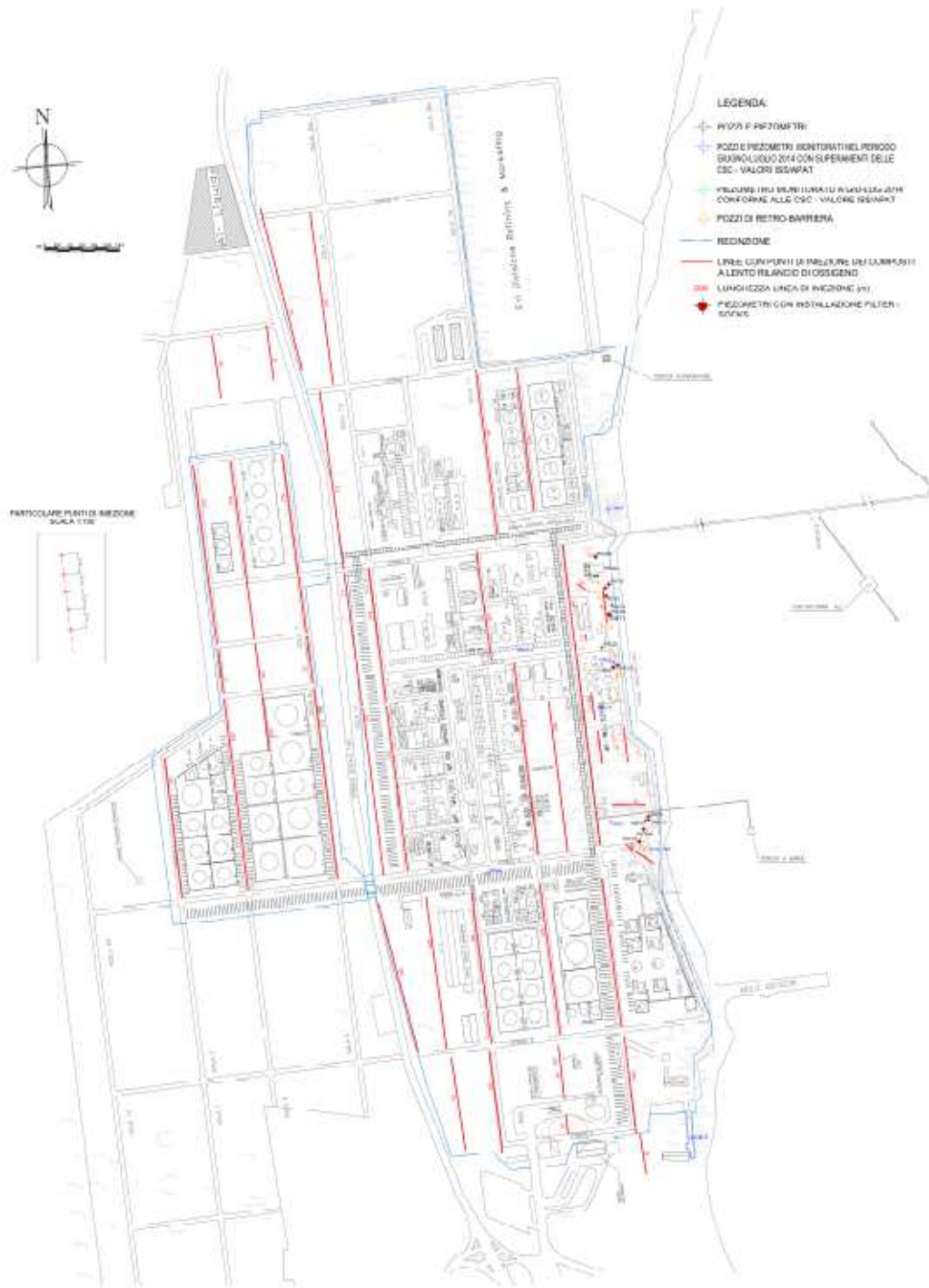


FIGURE 5. Distribution of the injection points along lines

CONCLUSIONS

The results obtained in the lab tests highlighted the possibility of using aerobic bioremediation for the treatment of groundwater, with the removal of pollution starting from TPHs. The treatment is immediately applicable in areas with dissolved organic contamination lower than or equal to 100 mg/L. The benefits on concentrations in

groundwater are expected after significant removal of contaminants sorbed on the solid phase of the soil. In terms of dosage to be carried out in situ to support the biodegradation of the organic compounds of concern, values between approximately 170 and 410 g ORC/m³ of treated soil a year were estimated for areas with dissolved contamination of the order of 100 mg /L.

The estimated cost for the full-scale bioremediation action is 23 million Euros, involving about 90 hectares. Compared to the previous project, and specifically referring to the physical barrier and the GCW-IWS, the in situ injection of the oxygen-releasing compound is an improvement toward a more effective, quicker and more sustainable remediation of groundwater at the site. In view of all this, the public authorities in 2017 approved the variant of the project.

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