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RESEARCH ARTICLE

Geophysical Monitoring at Laboratory Scale of Aerobic Degradation of Diesel Oil

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ABSTRACT: The study is aimed to monitor bioremediation of hydrocarbon-polluted soils by measuring geophysical electromagnetic parameters. A previous study at lab scale showed that biostimulated indigenous microorganisms can remove diesel oil from soil successfully. Herein, we focused on the result of a laboratory test using Time Domain Reflectometry (TDR) probes to measure electrical conductivity and dielectric permittivity in a column of sandy soil artificially contaminated with diesel oil. To simulate aerobic degradation of hydrocarbons, mesocosms were set-up in two Plexiglas columns (inner diameter = 13.8 cm) with 3.4 kg of soil (layer height = 14 cm) spiked with 0.24 kg of diesel oil and hydrated with 0.4 kg of Mineral Salt Medium for Bacteria. One mesocosm was aerated by air injection from the bottom of the column, while the other had only natural aeration due to air diffusion through the soil itself. In each column, electrical conductivity and dielectric permittivity were monitored by TDR probes for 105 days. TDR measurements were supported by microbiological and gas chromatographic analyses, along with SEM images. The findings showed that air injection heavily influenced the TDR monitoring, probably due to generation of air bubbles around the probe that interfered with probe-soil coupling. Therefore, the measurement accuracy was reduced in an irreversible way. In the non-aerated system, a slight (2%) and linear decrease of dielectric permittivity was observed over time, meanwhile electrical conductivity decreased by about 30%.

KEYWORDS: Time Domain Reflectometry, Monitoring, Aerobic degradation, Diesel oil.

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1. INTRODUCTION

Industrial activities have often caused environmental damages to soil and water, with dramatic consequences due to pollutants diffusion along the food chain [1]. Therefore, remediation is one of the pillars to recover the polluted areas and give them back to the local communities for a safe use. Whatever the remediation method, the process monitoring should be efficient, simple, fast, and possibly low-cost. Nowadays, there is growing attention towards bioremediation [2], a technique which takes advantage of activity of microorganisms or plants to degrade or accumulate pollutants. During biodegradation process, generation of by-products/co-metabolites and biofilms can change geophysical properties of the soil, such as electrical conductivity and dielectric permittivity; therefore, geophysical monitoring can be a useful tool to check the timeline of biodegradation [3]. Especially when a large contaminated area is considered, geophysical monitoring is less onerous and cheaper than coring campaigns. Therefore, monitoring of physical parameters seems a promising tool to check the process, even if the correlation between electromagnetic parameters and efficiency of pollutant removal is far than being fully understood [4]. This work fits in this research topic: TDR measurements can provide long-term monitoring of electromagnetic parameters of a contaminated soil in easy way, and our aim is validating their use as support instrument for bioremediation applications. The geophysical monitoring was supported by soil sampling, microbial counts and fluorescein diacetate (FDA) analysis. Furthermore, we carried out the diesel oil extraction and gas chromatographic analysis of the extract.

2. MATERIALS AND METHODS

2.1 Set-up of mesocosms

Mesocosms were set-up in Plexiglas columns, with diameter 13.8 cm and filled with 14 cm of soil (soil weight = 3.4 kg). Soil had been sieved to obtain a particle size distribution between 0.15 and 2 mm.

Contamination was simulated adding 0.24 kg of diesel oil (7 g of diesel oil/100 g of soil). Moisture content was fixed at 12% w/w, using a Mineral Salt Medium for Bacteria (MSMB) [5], a solution rich in macro and micro-nutrients.

One of the two columns was connected to compressed air pipeline to get upward column aeration. In the first month of the experiment, air was injected once a week, providing 2 dm^3 of air in 2 minutes. After 40 days, the second part of the experiment started, with daily aeration, namely 20 dm^3 per day. At last, at t = 50 days the forced aeration was stopped and just natural one worked

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until the test end (t = 105 days). Aerated column was also inoculated with microorganisms, in order to have initial concentration equal to $1.25 \cdot 10^3$ colony-forming units (CFU) per gram of soil. In the other column, only natural aeration due to air diffusion through the soil itself was done. This column was considered as control one.

2.2 TDR probes

Time domain reflectometry (TDR) probes were used to measure electromagnetic parameters of soil (electrical conductivityand dielectric permittivity). Multiparameter sensors were selected for their ability to provide temperature correction of measurements. The portability of these instruments makes them also adequate to be brought on site, with a view to scaling-up the research field. The probe, composed of two 12-cm long rods, was inserted vertically into the soil. The measurements gave average values of the sensing volume of the probe. Recording was performed every 10 minutes along the entire duration of the experiment (105 days).

2.3 Sampling

Sampling of soil matrix of mesocosms was made at the end of the experiment (t = 105 days). Samples were collected at 2 cm from the surface, in the middle (8 cm from the surface) and at the bottom of the columns (depth = 14 cm). To investigate microbial activity in mesocosms two methodologies were used: fluorescein diacetate (FDA) hydrolysis and microbial count. Samples were also observed with Scanning Electron Microscope (SEM).

2.4 Fluorescein Diacetate (FDA) Analysis

Fluorescein diacetate is a compound that can be hydrolysed by various enzymes, like protease, lipase and esterase. The product of this reaction is fluorescein, characterized by a strong yellow color and therefore easily detected by spectrophotometry. In this way, an overall estimation of microbial activity can be obtained. Methodology reported by Schnurer and Rosswall [6] and modified according to Adam and Duncan [7] was used.

Two solutions were used:

1. potassium phosphate buffer: 8.7 g/l of K_2HPO_4 and 1.3 g/l of KH_2PO_4. The pH of the solution is 7.57, which falls within the acceptable range to favor the hydrolysis reaction (7-8);
2. FDA stock solution in acetone at concentration of 2 g/l.

Samples of 2 g of soil (on a wet basis) were mixed to 15 ml of phosphate buffer and 100 µl of FDA stock solution in acetone. Each sample was agitated at 50 rpm for 1 h. Then, to stop the hydrolysis reaction, 15 ml of acetone were added. Subsequently, the samples were centrifuged at 6000 rpm for 5 minutes and then filtered through a 1.2 µm filter to remove any colloidal particles. Absorbance was measured by spectrophotometry at 490 nm. Two replicas were prepared for each sample and one blank containing only the potassium phosphate solution. A series of samples at known concentrations of pure fluorescein (dissolved in potassium phosphate buffer) was prepared to obtain a calibration line which correlated the absorbance measured with the spectrophotometer with the quantity of fluorescein diacetate hydrolysed by microorganisms.

2.5 Microbial Count

For each soil sample, three replicates were prepared. First, a MSMB liquid medium with Agar gelling agent (20 g/l) was sterilized in autoclave. Each sample was prepared by inserting 50 mg of soil into a Petri plate under a biological hood, adding and mixing 100 µl of sterile water. Then, 40 µl of diesel oil were filtered with 0.22 µm and dispersed on the opposite part of the plate. Eventually, the sterilized liquid medium was added.

Identification of microbial colonies was donewithout the use of a microscope as they were very well defined. The results are expressed in number of colony-forming units (CFU) per gram of soil.

2.6 Diesel Oil Extraction and Gas Chromatograph Analysis

For each sample, 2 g of contaminated soil were placed in a glass tube with 2 g of sodium sulphate, necessary to dehydration, and 30 ml of solvent (acetone and hexane in ratio of 1:1 v/v). The extraction was performed according to the EPA method 3546 (moisture 15-30%) [8]. At the end of the extraction, the sample was filtered through a sodium sulphate bed and then through a 0.45 µm nylon filter. A sample of 1 µl of the extract was analyzed in a gas chromatograph column by the EPA method 8015 [9]. To determine the quantity of diesel oil present in each sample, the area of each peak was estimated through “Valley" type integration. Results were compared to a calibration curve obtained with commercial diesel oil.

3. RESULTS AND DISCUSSION

3.1 Microbiological Activity

The mesocosm with aeration showed better results, but also had an evident stratification of biological parameters: near the surface there was more activity, that decreased going towards the bottom of the column. In the control mesocosm, biological parameters along the height of the column were more uniform.

The concentration of colony-forming units and the quantity of fluorescein produced by FDA hydrolysis reaction are showed in Figures 1 and 2, respectively.

3.2 SEM images

Images done with Scanning Electron Microscope confirmed the previous results. About aerated column, the multitude of microorganisms at the surface was higher than that of the samples taken in the middle and at the bottom of the column, also when compared to control column samples. Figure 3 shows a SEM image of a sample taken from the surface of the aerated column, in which microorganisms long up to 4 µm are visible. Besides, in all samples it was observed that soil grains were covered by an organic coating, vice versa absent in non-contaminated samples of the same soil. Figures 4 and 5 show the difference in soil structures.

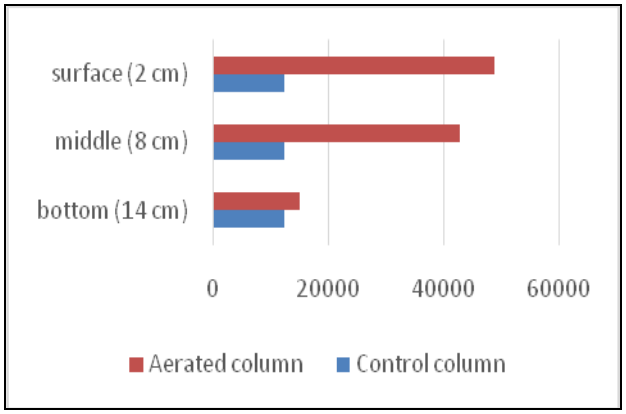

Figure 1. Microbial count in the mesocosms [CFU/g of soil]

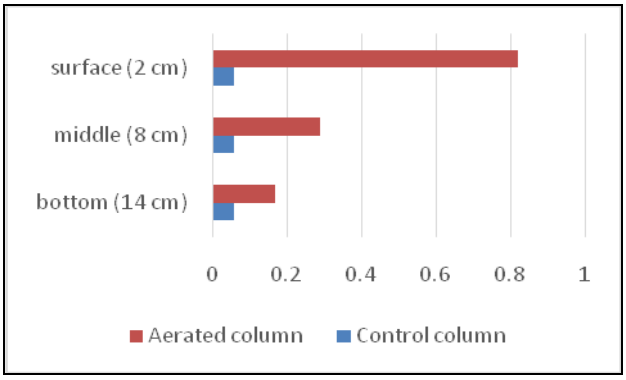

Figure 2. Concentration of fluorescein produced in the mesocosms [µg/g of soil]

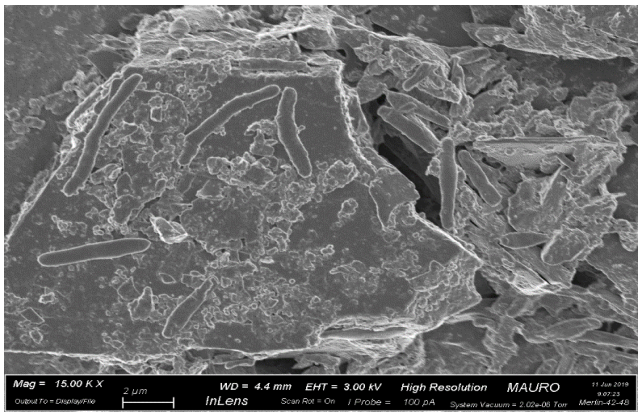

Figure 3. SEM image of soil from the surface of aerated mesocosm. Magnification: 15,000 X.

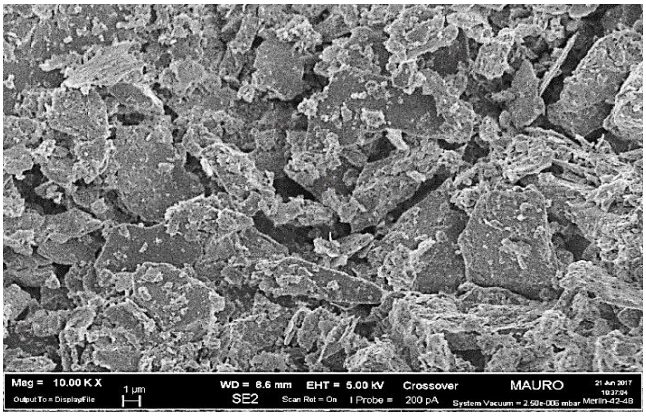

Figure 4. SEM image of non-contaminated soil. Magnification: 10,000 X.

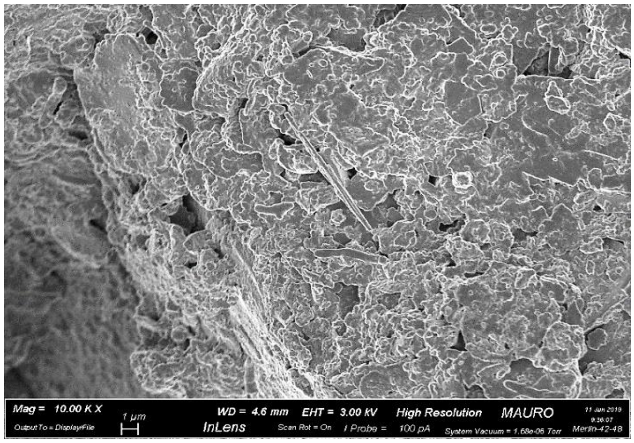

Figure 5. SEM image of aerated column sample (bottom). Magnification: 10,000 X.

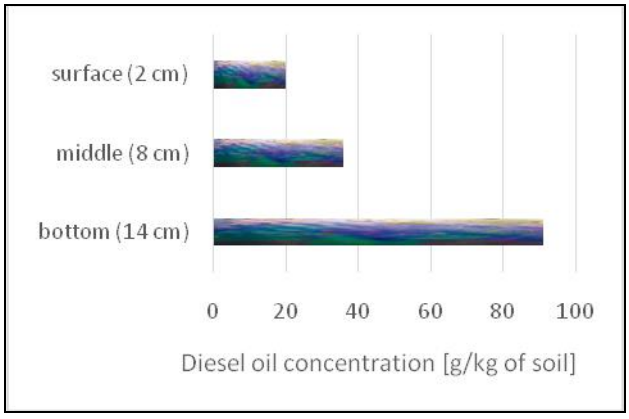

Figure 6. Diesel oil concentration in aerated column (t = 105 days)

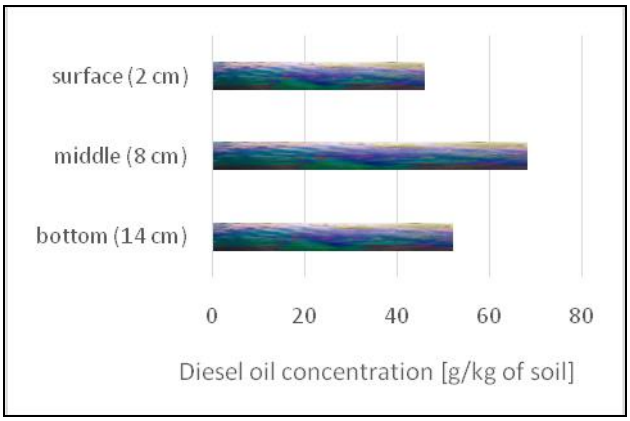

Figure 7. Diesel oil concentration in control column (t = 105 days)

3.3 Gas chromatographic Analysis

Extracts achieved with the EPA method 3546 were analysed with a gas chromatograph. The data of the diesel oil concentration at the top, middle and bottom of each column after 105 days are reported in Figures 6 and 7.

We noticed a different feature between the aerated system and the control one, considering that initial concentration of diesel oil in both columns was the same (70 g/kg of soil). In the control column, the diesel oil floated mainly in the middle of the column, while lower values of about 50 g/kg were observed at the surface and at the bottom. The number of samples did not cover the whole height of the column with adequate spatial discretization to estimate quantitatively the effectiveness of the removal of hydrocarbons, if any. Besides, microbiological analyses, especially FDA ones, did report much lower activity compared to aerated column. In the aerated column a greater displacement of oil is evident. At the surface, the diesel concentration dropped to about 20 g/kg, while the most part of the contaminant was collected at the bottom, with values above 90 g/kg. We explained this evidence assuming that: the air injection produced a great mixing of water and oil at the

bottom of the system and had the secondary effect of letting the bubbles of air escape towards the top. In the control column, a small amount of air bubbles remained trapped since the initial infiltration of water and oil until the end of the experiment and prevented the movement of liquid fluids towards the bottom. Again, the non-exhaustive discretization of sampling hindered an accurate estimation of diesel removal. Anyway, if we consider the results of the biological activity, it is realistic to assume that in the aerated system a more intense process of degradation occurred.

3.4 TDR measurements of Geoelectrical properties

Figures 8 and 9, show the monitoring data of dielectric permittivity and electrical conductivity for both columns, respectively. It is evident the dramatic change of the monitored parameters due to aeration. In the first month, when aeration was applied once a week, some perturbation in the trend of the monitored parameters was observed. Then, since t = 40 days the air was injected once a day and the air flux provided a stronger perturbation to the measurements. We assumed that locally around the probes the porosity increased due to the channelling of the air fluxes, creating additional pore volume that altered the coupling between the probe and the soil. For this reason, at t = 50 days we decided to stop the air injection. However, in the following days, the electrical conductivity and dielectric permittivity did not return to the initial values but kept constant. The system did not manage to refill the voids created by air injection and coupling was spoiled irreversibly.

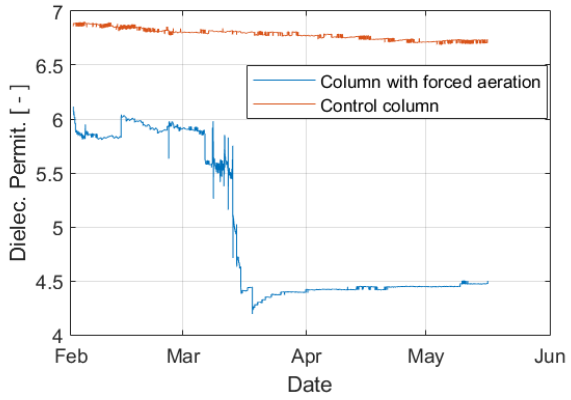

Figure 8. Dielectric permittivity in the two mesocosms.

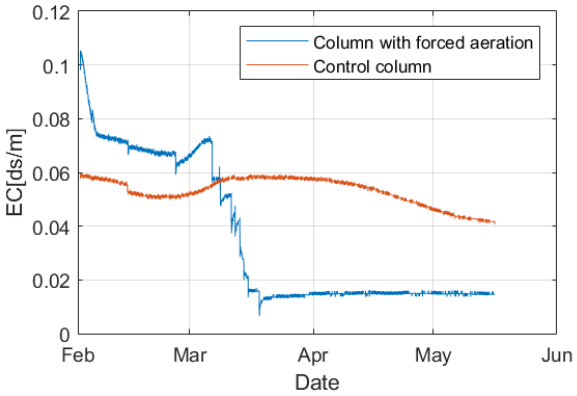

Figure 9. Electrical conductivity with temperature correction in the two mesocosms.

As far the control column is concerning, a slight and constant decrease in dielectric permittivity was observed. Instead, electrical conductivity has a more interesting trend: after an initial decrease, the electrical conductivity increases until the second half of March, then gradually decreases till the end of the experiment, passing from 0.06 dS/m to 0.04 dS/m. On one hand, it seems that the slow and constant decrease of the dielectric permittivity was caused by displacement of fluids, or the progressive pore clogging due to the coating of grain particles caused by organic matter. This latter phenomenon was also reported by Mori et al. [10]. On the other hand, the electrical conductivity variations are probably due to other phenomena. Salinity is one of the most important parameters that influence the electrical conductivity (but not dielectric permittivity). An increase of the bulk conductivity in similar conditions is reported by Atekwana et al. [11] and Masy et al. [12]. They linked this effect to an enhanced biological activity, which probably led to the production of acids as co-metabolites, or as consequence of the dissolution of CO_2 in water. A decrease of pH could dissolve some inorganic salts present in soil, causing an increase of salinity in the pore water and therefore in the electrical

conductivity. Studies at field scale showed that bacterial mineralization of organic compounds augments CO_2 in the aquifer and produces organic acids that increase mineral weathering [13].

The decrease in the bulk conductivity, observed after about 45 days, is noticed also in other studies [12]: after 50 days of geophysical monitoring of a similar system, Masy et al [12] observed an unexpected decrease in the bulk electrical conductivity. Authors assumed that a significant production of hydrophobic secondary metabolites occurred. To validate this assumption in our experiment, we performed an in-depth peak analysis of gas chromatograms; however, we did not observe any secondary production of organic compounds in our systems. Besides, also the study by Mori et al. [10] found a similar electrical conductivity trend, with a peak at about 30 days and then a decrease, by doing measurements in the leachate produced in the process. The evolution of electrical conductivity in soil is strictly connected to the modifications in the chemistry of the pore-water. The observed decrease of this parameter could be due to the progressive depletion of nutrient salts present in MSMB solution used to stimulate bacterial activity.

4. CONCLUSION

This research put in evidence that geophysical monitoring of bioremediation processes by TDR probes is feasible, with the concern that many phenomena can produce variations of electrical parameters of soil and it is not an easy task to clarify them. Movement and displacement of fluids during the process can be an explanation of variations in electrical parameters, but also changes in water chemistry and progressive clogging of pores. About aeration, the injection of air directly in the soil alters the coupling between the probe and the soil in an irreversible way. A future experiment will scale-up the mesocosms to bigger ones, introducing both long (30 cm) and short (12 cm) TDR probes, to show the differences between the conditions at the top and in the middle of the columns. Moisture content will be changed to have natural aeration through the porosity of the soil. Besides, a system with addition of nutrient salts will be compared to a system with addition of only distilled water, to better understand the role of salinity in the evolution of the bioremediation. Eventually, a more in-depth research will be done about variations in electromagnetic parameters of water due to its electrochemical adhesion to the surface of soil grains.

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