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REMEDIATION OF CONTAMINATED AQUIFERS BY BIOLOGICAL AND ADVANCED OXYDATION PROCESSES: THE AQUATEC PROJECT

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INTRODUCTION

The increasing production and use of oil-derived fuels have led to the widespread release of these compounds into the environment. This contamination is particularly important for groundwaters because of accidental gasoline releases from underground storage tanks and from pipelines in petrochemical industry sites (DEEB *et al.*, 2003). To restore the groundwater quality to the required standards activated carbon is often used (QUINLIVAN *et al.*, 2005; SHIH *et al.*, 2003), typically used in combination with the groundwater circulation and air stripping technology (KHAN *et al.*, 2004). These methods, however, do not represent an environmentally-sustainable solution because they merely transfer the organic pollutants from one phase to another. To achieve this biological and physicochemical methods can be employed.

Among chemical remediation methods UV-based advanced oxidation processes (AOPs) can be effective in removing several compounds contained in gasoline such as methyl *ter*-butyl ether (MTBE) and aromatic hydrocarbons (ZANG & FARNOOD, 2005; BAUS *et al.*, 2005). However, data concerning the comparative effectiveness of UV based AOPs on real polluted groundwater are scarce. The chemical composition of polluted groundwater (concentrations of metals, inorganic species, pH and organic substances) can dramatically affect the efficiency of these oxidation processes compared to their performance in synthetic solutions.

Biological oxidation for clean-up of hydrocarbon-contaminated sites has gained increasing interest in recent years as a cost-effective remediation technology. Biodegradation of petroleum hydrocarbons has been demonstrated both in natural (CHAPELLE, 1999) and in engineered systems (NORRIS, 1994) and full scale *in situ* applications are currently increasing. The synthetic additive MTBE is particularly recalcitrant to biodegradation, because it contains an ether bond and a tertiary carbon. Despite its seemingly low biodegradation rate, a variety of microbial species have been shown to be capable of metabolising MTBE, mostly in aerobic conditions (DEEB *et al.*, 2000; FAYOLLE *et al.*, 2001). Cometabolic degradation also occurs in some bacteria grown on alkanes (SMITH *et al.*, 2003). Complete mineralization of MTBE occurs in most cases (SCHMIDT *et al.*, 2004). Field-scale bioremediation studies and applications have already given promising results, as demonstrated by several projects carried out in the USA (HICKS *et al*., 2001; ESTCP, 2003; US EPA, 2004; SALANITRO *et al.*, 2000; SALANITRO *et al.*, 2001), where a long MTBE contamination history (since the early 80s) first prompted MTBE research.

The specific approach to bioremediation depends on the conditions of the field site. Therefore, feasibility studies are required to determine whether microbial species able to degrade MTBE exist at the site and how the growth of such bacteria can be adequately stimulated to accelerate biodegradation. In the absence of suitable native bacteria, bioaugmentation should be evaluated. In Italy, a field study showed a remarkable MTBE decrease (from 14 to 0.6 mg/L in nine months) by ORC© in four months (AGLIETTO & DI GENNARO, 2003).

In the present work remediation of groundwater at a petrochemical industrial site was investigated by both UV based AOPs and by bioremediation. The main objective of the study was to compare the effectiveness of some UV-based AOPs (medium pressure UV, UV/H_2O_2 and UV/TiO_2), to verify the effectiveness of bioremediation and to identify the microbial species responsible for MTBE degradation.

The work was carried out in the framework of the Project "AQUATEC, Control, Treatment and Maintenance Innovative of Technologies for Water Emergency Solution" supported by the Italian Ministry of University and Research (MIUR), by the Funding Action P.O.N.(National Operative Plan) 2000-2006, for objective 1 Regions. The goal of the work was to compare biological and AOPs for treating a contaminated groundwater at a former petroleum refinery, in an area of National Interest Site (Napoli Orientale).

KEY-WORDS: *groundwater; advanced oxidation processes; bioremediation; MTBE; BTEX; UV; TiO_2*

MATERIALS AND METHODS

Chemicals. MTBE and aromatics compounds were purchased from Aldrich and were used as received. Standard stock solutions (between 10 and 250 g/L) were prepared in methanol. Working standard solutions were prepared fresh daily using groundwater from a local well. All solvents were pesticide grade and purchased from Baker. H_2O_2 (30% solution) was used as received from Baker.

Parameter	Concentration
Conductivity (20°C)	060 µS/cm
pH	8.3
Total alkalinity	880 mg as $CaCO_3$/L
Total hardness	245 mg as $CaCO_3$/L
Settleable solids	17 mL/L
MTBE	23 mg/L
Hydrocarbons C_{10}-C_{40}	2.3 mg/L
Fe	0.58 mg/L
Mn	1.9 mg/L

Table 1 - Average chemical composition of the investigated groundwater

Commercial TiO_2 was ''Degussa P25'' TiO_2 (nonporous anatase; surface area, 50 m^2/g; mean diameter, approximately 30 nm). Pure propane gas, manufactured in a pressure tin vessel, was purchased from Fluka.

Sampling of aquifer material. Groundwater was sampled at an industrial site, located in southern Italy, in which for many years there were reservoirs used for oil-derived fuels. The sampling procedure included the use of an immersion pump equipped with a Teflon tube. Initially, the water already present in the piezometer was pumped out and discharged. Then the aqueous phase was allowed to refill the piezometer. Finally, 30 L of sample was withdrawn at the desired flow rate (usually 0.5-2.0 L/min) into a stainless steel container equipped with Teflon sealing. The container was completely filled leaving as small as possible a head place (less than 50 mL) and immediately transported to the laboratory. The sample was refrigerated overnight to allow the suspended material to settle, and then the supernatant liquid was analyzed for hydrocarbons. One L amber-glass bottles equipped with Teflon septa were completely filled with the aqueous phase. The average chemical composition of the investigated groundwater is reported in Tables 1 and 2.

Soil samples were obtained by drilling close to the piezometer used for water sampling. Soils were collected in glass jars, filled with groundwater, closed with plastic lid and stored at 4°C until used.

Degradation experiments. AOP experiments were performed in a 1 L, cylindrical, glass, four-necked reactor. The light sources were either a 17 W low-pressure mercury arc lamp fixed at the central axis of the reactor or a 125 W medium-pressure mercury arc lamp (Helios Italquartz, Italy) at the inner jacket of the reactor. The irradiation was carried out on 500 mL of magnetically stirred groundwater solution. For the UV/H_2O_2 and UV/TiO_2 experiments several concentrations of H_2O_2 (0.13, 0.33, 0.66, 1.33 and 2 g/L) and TiO_2 (0.02 and 0.2 g/L) were tested. All experiments were carried out at room temperature. The progress of the reactions was monitored by analysis of small aliquots (0.8 mL) of the reaction mixture periodically withdrawn from the reactor.

Compound	Retention time (min)	Average concentration (µg/L)	Detection limit (µg/L)	Permit limit (µg/L)
MTBE	5.18	28700	1	10
Benzene	7.01	2670	0.1	1
Toluene	9.75	18	0.02	15
Ethybenzene	12.23	9	0.05	50
p-Xylene	12.45	14	0.05	10
Nonane	12.98	+	0.1	-
Cumene	13.63	67	0.05	-
Propylbenzene	14.28	132	0.1	-
Ethyltoluene	14.82	33	0.1	-
Decane	15.05	+	0.05	-
Trimethylbenzene	15.14	+	0.1	-
Indane	16.00	854	0.1	-
Diethylbenzene 1,3	16.08	161	0.1	-
Indene	16.18	++	0.1	-
Diethylbenzene 1,2	16.25	74	0.1	-
Butylbenzene	16.26	+	0.1	-
Diethylbenzene 1,4	16.31	19	0.1	-
1,2-Dimethyl-4-Ethylbenzene[a]	16.78	352	0.1	-
Undecane	16.85	+	0.05	-
1,2,3,5-Tetramethylbenzene	16.85	+++	-	-
C_{11}-paraffins	16.87	+++	-	-
Dimethylstyrene	16.95	+	0.1	-
1,2,4,5-Tetramethylbenzene	17.34	136	0.1	-
C_{11}-paraffins	17.81	++++	-	-
C_{11}-paraffins	17.97	++++	-	-
Naphthalene	18.70	+++	0.1	-
2-Metylnaphthalene	20.35	31	0.2	-
1-Methylnaphthalene[b]	20.60	70	0.2	-
1,4-Dimethylnaphthalene	22.28	6	0.2	-
Methylnaphthalenes[c] (Σ 8 isomers)	21.5-22.5	65	0.5	-
Acenapthene	22.75	+	0.05	-
Phenanthrene	25.64	+	1	-

Quantified as: (a) tethramethylbenzene, (b) 2-methylnaphthalene, (c) 1,4-dimethylnaphthalene. Unidentified compounds (GC/MS peak area): +: < 100 kcounts, ++ > 0.5, Mcounts, +++ > 2 Mcounts

Table 2 - Principal organic compounds identified in the polluted groundwater and their average concentrations, detection limits and Italian discharge limits (Italian Ministry Decree, 1999)

Microcosms set-up. Batch reactors were prepared in previously-autoclaved 250 mL serum bottles. Each microcosm received 100 g of wet soil (51 g dry weight). Groundwater containing an initial 2 mg MTBE /L was added up to a total volume of 180 mL. The bottles were sealed with Teflon faced butyl rubber stoppers and aluminum crimp caps. Aerobic reactors were amended weekly with 10 mL of pure oxygen, to maintain the dissolved oxygen concentration near saturation

(about 8 mg/L). Anaerobic reactors were fluxed with a N_2/CO_2 mixture (30% v/v CO_2: 70% v/v N_2) after addition of 1 mg/L resazurin as a redox indicator. 2 mM $Na_2S \cdot 9H_2O$ was also added to establish reducing conditions, *i.e.*, when the mixture turned from purple to colourless.

Six treatments, five aerobic and one anaerobic, were prepared in triplicate sets of reactors. Autoclaved control microcosms (blanks) were also prepared to evaluate abiotic loss of contaminant. Table 3 summarizes the experimental conditions for each treatment. All microcosms, with the exception of the biotic control set (CC), were amended with 5 mgP/L (as NaH_2PO_4) and 10 mgN/L (as NH_4Cl). Nutrients concentrations were kept constant by periodical additions. Three aerobic sets (DD, EE, FF) were also amended with primary substrates (respectively, pentane, hexane and propane) to test for cometabolic degradation.

After preparation, all microcosms were statically incubated in the dark at 20°C. The composition of the liquid phase (pH, concentrations of MTBE, dissolved oxygen and nitrogen species) was monitored

Treatment	Microcosm Properties	Composition
V	Anaerobic	Soil + GW + N
Z	Aerobic	Soil + GW + N + O_2
AA	Anaerobic autoclaved control	Soil + GW + N
BB	Aerobic autoclaved control	Soil + GW + N + O_2
CC	Aerobic no amendants (biotic control)	Soil + GW + O_2
DD	Aerobic with pentane (cometabolic set)	Soil + GW + N + O_2 + 300 µM pentane
EE	Aerobic with hexane (cometabolic set)	Soil + GW + N + O_2 + 300 µM hexane
FF	Aerobic with propane (cometabolic set)	Soil + GW + N + O_2 + 110 µM propane
II	Second generation (MTBE 40 mg/L)	GW + N + O_2 + inoculum from set Z

GW: groundwater; P = 5 mg/L (as NaH_2PO_4); N = 10 mg/L (as NH_4Cl)

Table 3 - Experimental conditions for batch microcosms

weekly according to the methods described below.

Analytical determinations. Groundwater organic pollutant characterization and their disappearance during UV treatment was performed by solid phase micro extraction/gas chromatography/mass spectrometry (SPME/GC/MS) using a Saturn 2200 GC/MS system (electron impact ion source) equipped with a 8200 autosampler (Varian) and a SPME syringe (Supelco) with a 100 µm (non-bonded) polydimethylsiloxane fiber. Aqueous samples (0.8 mL) were placed into 2 mL vials equipped with silicone/Teflon septa and the SPME fiber was exposed to the vapor phase for 30 min. to allow adsorption of the volatile organics. The SPME syringe was then automatically introduced into the injector of the GC/MS system to desorb and analyze the compounds. The column was a Factor Four VF-5ms (60m length, 0.25 mm i.d. and 0.25 µm film thickness) from Chrompack. The column exit (at 180°C) was connected directly to the ion source, through a transfer line heated to 220°C. The operating conditions were: He carrier gas flow = 1.0 mL min^{-1}; injector temperature = 250°C; desorption time = 5 min, initial column temperature = 40°C (5 min); temperature ramp rate = 10°C/min up to 200°C then 20°C/min up to 280°C. Electron impact mass spectra (electron energy 70 eV), were recorded by scanning the MS from 40 to 350 Dalton at 0.6 s/scan. Quantitative determinations were obtained from calibration curves. Dissolved metal concentrations were performed by inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis using an Optima 3000 instrumentation (Perkin-Elmer).

MTBE decay in microcosms was monitored by headspace gas-chromatography with flame ionization detector (FID). Samples (0.5 mL) withdrawn from microcosms were placed in 2 mL vials and saturated with 300 mg of sodium sulfate. After equilibration at 40°C, 100 µL of the vial headspace were withdrawn using a 250 µL gas-tight Hamilton Sample Lock syringe and injected into a Varian CP 3800 gas-cromatograph equipped with a Chrompack Porabond Q column (length: 50 m, i.d.: 0.32 mm, f.t.: 5 µm), in splitless mode. Injection parameters were: injector temperature = 250°C, detector temperature = 250°C, He carrier gas flow rate = 1.9 mL/min, oven temperature = 100°C for 1 min. then a ramp rate = 15°C/min[1] to 250°C and a final hold = 5 min. Nominal concentrations (i.e., the ratio between the total amount of MTBE and the volume of the liquid phase) were used for quantitative analysis.

Nitrate and nitrite in the filtered samples were determined by a Perkin Elmer Series 10 Liquid Cromatograph equipped with an IonPac AS14A column (4↔250 mm) and an UV detector. Injection parameters were the following: eluent = 8.0 mM Na_2CO_3 + 1.0 mM $NaHCO_3$, flow rate = 1.0 mL/min, operating temperature = 30°C, injection volume = 50 µL. NH_4 –N was determined by the direct Nessler method with spectrophotometric detection. Dissolved oxygen was measured by an Aqualytic OX24 oxygen meter with a galvanic sensor.

Microbiological characterisation of biomass was made by the DAPI (4',6'-diamidino-2-phenylindole) and FISH (Fluorescent *In Situ* Hybridisation) techniques, after detaching bacterial aggregates from inorganic soil particles by a method developed in our laboratory. Hybridization was carried out with a mixture of the probes EUB338, EUB338-II and EUB338-III (EUBmix). Cy3-labeled oligonucleotide probes were purchased from MWG AG Biotech, Germany. To estimate the amount of Eubacteria as a fraction of the total cells, DAPI was directly added to the hybridization buffer at a final concentration of 1 mg/mL. Slides were mounted with a few drops of VectaShield (Vector Laboratories, USA). The preparations were examined with an Olympus BX51 epifluorescence microscope. The estimation of the cells binding the eubacterial probes was evaluated as a proportion of the total DAPI-positive cells in at least 20 different randomly selected fields. Images were captured with an Olympus F-View II digital camera using AnalySIS image analysis

software (Soft Imaging System GmbH, Münster, Germany).

RESULTS AND DISCUSSION

UV based AOP degradation. Initial characterization of the polluted groundwater revealed the presence of a large number (>70) of aliphatic and aromatic hydrocarbons, 32 of which were unequivocally identified by comparison with authentic standards. The other compounds were tentatively identified on the basis of matching background-subtracted mass spectra against those of the NIST mass spectra library. In addition to MTBE and benzene, the hydrocarbons identified can be divided into two main categories: the alkyl-benzenes and the alkyl-naphthalenes. The disappearance of these compounds was used to compare the performance of the UV based treatments. The % removal of the main organic pollutants after 30 min of reaction are reported in Table 4. From Table 4 it is possible to see that the removal efficiency is in the order: $UV/H_2O_2 > UV/TiO_2 > UV$. Previous experiments (results not shown) showed that H_2O_2 alone was ineffective at removing the organic compounds and low pressure UV alone only resulted in their partial removal. Since iron was present in the groundwater, experiments were performed with H_2O_2 alone at pH 3 to determine whether there was a Fenton reaction. Since no organics removal occurred it was possible to exclude the Fenton reaction component during the investigated treatments.

Table 4 shows for the UV/H_2O_2 processes, MTBE was the hardest compound to remove. It was removed quite poorly with an initial H_2O_2 concentration of 0.13 g/L and only removed efficiently (> 99.99%) at H_2O_2 concentrations of 2 g/L.

Even though the percent removals of MTBE and other test compounds, were >99.99% when using H_2O_2 concentratins between 0.33 and 2 g/L the residual concentrations were significantly different. At an initial H_2O_2 dose of 0.13 g/L, a contact time of 120 min was required to reduce the MTBE concentration to below the maximum admissible concentration of 10 µg/L set by current Italian legislation on groundwater remediation. By using an initial H_2O_2 concentration of 2 g/L a residual MTBE concentration of lower than 5 µg/L was obtained after 30 min of reaction. Figure 1 shows that benzene and MTBE concentrations after UV and UV/TiO_2 treatments were much higher than required by the aforementioned legislation (10 µg/L). The significantly higher efficiency, after 30 min reaction, of UV/H_2O_2 treatments compared to the UV and UV/TiO_2 treatments is evident from the SPME/GC/MS chromatograms in Figure 1.

The low pollutant removal efficiency of UV/TiO_2 treatment compared to UV/H_2O_2 treatment was unexpected because it has previously been shown that the effectiveness of UV/TiO_2 treatment of model solutions is equal or better than UV/H_2O_2 treatment (Huang *et al.*, 1993). Our results could have been due to the presence of particulate matter in the groundwater that blinded the catalyst surface and thereby reducing its effectiveness. In support of this hypothesis is the fact that in the UV/TiO_2 treatments the TiO_2 concentration did not influence pollutant degradation rate and indeeed for some compounds (MTBE, p-xylene, cumene, alkyl-naphthalenes), higher TiO_2 dosages resulted in lower removal efficiencies. This could have been due to both the aforementioned proposed effect of particulate matter as well as to the presence of inorganic species (carbonate/bicarbonate) that consume hydroxyl radicals produced at the catalyst surface.

Biological degradation. The MTBE degradation was only observed in the aerobic microcosms amended with phosphorus and nitrogen (Z

	UV/H_2O_2					UV/TiO_2		UV
$[H_2O_2]$ or $[TiO_2]$, g/L	0.13	0.33	0.66	1.33	2	0.02	0.2	-
MTBE	xxx	xxxx	xxxx	xxxx	xxxxx	xx	x	x
Benzene	xxxx	xxxxx	xxxxx	xxxxx	xxxxx	xx	xx	xx
Alkyl-benzenes								
Toluene	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	x	x	x
p-xylene	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xx	x	x
Cumene	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xx	x	x
Ethyl-benzene	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	x	x	x
Propyl-benzene	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	x	x	x
Ethyl-toluene	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	x	xx	xx
Indane	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xx	xx	xx
Di-ethyl-benzenes	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xx	xx	x
1,2-dimethyl-4-ethylbenzene	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xx	xx	xxx
1,2,4,5-tetramethyl-benzene	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxx	xx	xxx
Alkyl-naphthalenes								
Methyl-naphthalenes	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xx	x	x
Dimethyl-naphthalenes	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xx	x	x

Tab. 4 - Removal percentages of the main organic pollutants after 30 min of reaction with UV based AOPs. Removal efficiency (%): x,<1; 1<xx<50; 51<xxx<99; 99.1<xxxx<99.9; xxxxx>99.99

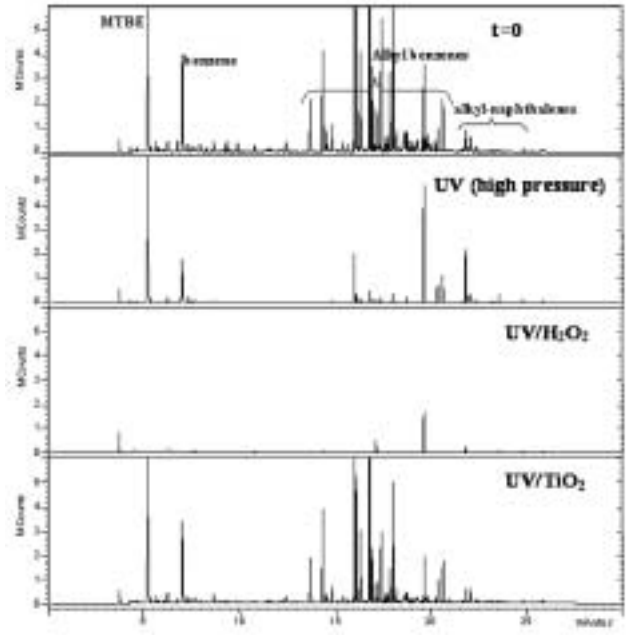

Fig. 1 - SPME-GC-MS chromatograms of groundwater before and after 30 min of treatment with UV (high pressure), UV/H_2O_2 and UV/TiO_2. The peaks present in the chromatograms related to the UV/H_2O_2 treatment are from the SPME-GC-MS system not the groundwater

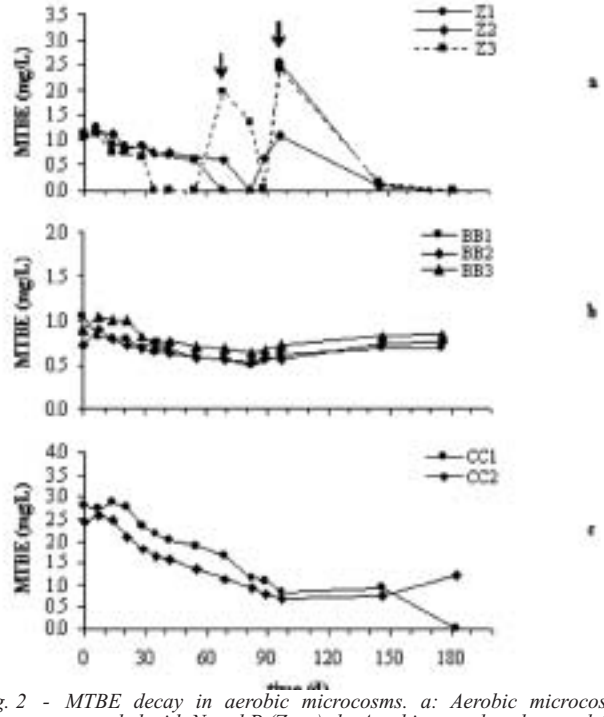

Fig. 2 - MTBE decay in aerobic microcosms. a: Aerobic microcosms amended with N and P (Z set); b: Aerobic autoclaved control (BB set); c: Aerobic microcosms with no amendants (biotic control, CC set). Arrows indicate MTBE additions

set, Figure 2a). Lag times of between 40 and 80 days occurred before degradation commenced. MTBE degradation occurred repeatedly after two subsequent MTBE additions. Neither nitrate nor nitrite accumulated during the experiment. There was no abiotic loss of MTBE (Figure 2b). In the absence of phosphorus and nitrogen the addition of oxygen resulted in only a very slow decay of MTBE (Figure 2c). These results indicate that, the nutrient addition is necessary to degrade MTBE in a time frame suitable for field remediation.

There was no appreciable MTBE degradation in any the anaerobic and cometabolic aerobic microcosms over a period between 120 to 190 days.

Microbiological characterisation of the consortia that developed in the active microcosms showed that the biomass was made up of 0.5-1.5 diameter cocci, and long (1.5 μm), thin(0.5 μm) bacilli that were positive to the DAPI and EUB mix probe (Figure 3).

CONCLUSIONS

Indigenous bacterial populations capable of degrading MTBE were found in the study site, and were developed over a 3-month period in aerobic microbial consortia amended with nutrients (N and P). Enhanced aerobic biodegradation was observed in laboratory scale reactors and led to very low MTBE concentrations that were below the limit established by the Italian Regulations. AOT using UV/H_2O_2 was able to achieve the same low MTBE concentrations with a 30 min contact time. Therefore, either an *in situ* aerobic bioremediation process (such as bioventing), or an *ex situ* AOT process appear suitable for the remediation of the contaminated site.

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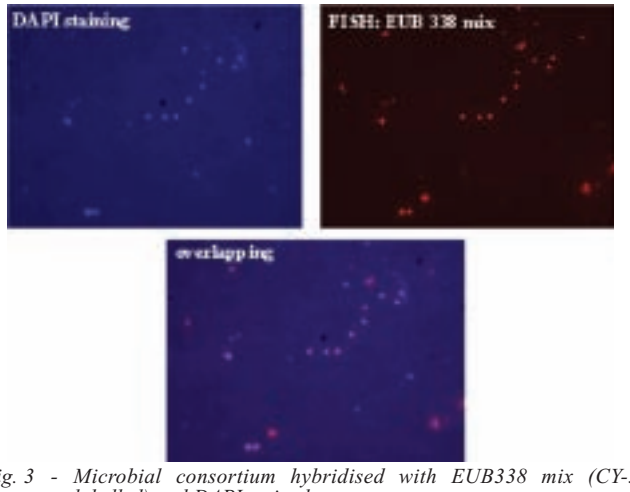

Fig. 3 - Microbial consortium hybridised with EUB338 mix (CY-3 labelled) and DAPI stained

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