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NATURAL ATTENUATION IN A FRACTURED CHALK SITE: A STRATEGY FOR SITE MANAGEMENT

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ABSTRACT: Intrinsic biodegradation was examined at a polluted gasoline storage site. The contamination occurred in 1962, and due to fractured bedrock, the oil phase was nearly not subjected to weathering. This ensured for over 40 years a constant release of hydrocarbons in groundwater. Major upwelling events of the groundwater table (more than 14 m) were due to a hilly topography. This led us to remove as much free oil phase as possible in order to reduce the risk of oil phase extension in the case of a very low groundwater event.

The mains characteristics of the aquifer have been determined (groundwater velocity, permeability, temperature…). About 5 tons of the mobile gasoline phase were pumped over a period of 5 years. Plume expansion (approximately 1.300 m length) and dissolved hydrocarbons have been monitored through a net of 30 wells settled mostly in lines perpendicular to the flow direction. Decreasing pollutant concentrations (from 30 mg/L to µg/L range) and redox zonation with increasing plume length were mostly attributed to biodegradation since only limited dilution and dispersion occurred. The oxygen concentration in the plume is very low, and only nitrate is present as alternative electron acceptor (maximal concentration of 15 mg/L). All the available electrons acceptors are depleted in the contaminated regions. Consequently, in this case study, intrinsic biodegradation seems primarily to be limited by electron acceptors availability.

Biodegradation under aerobic, denitrifying and mixed microaerophilic/denitrifying conditions have been evaluated in laboratory microcosms. The intrinsic biodegradation capacity of the indigenous microflora was examined using plume water as inoculum and the water soluble hydrocarbon fraction from local gasoline as sole carbon and energy source. Analytical method combining SPME/GC and use of Hydro*CARB*® software allowed detailed information at low concentrations on about 200 individual hydrocarbons. These laboratory results showed interesting biodegradation capacities under aerobic conditions but no degradation in strictly denitrifying conditions. Furthermore a benefit appeared in providing both nitrate and oxygen to enhance the biodegradation of a mixture of monoaromatics.

INTRODUCTION

Intrinsic biodegradation represents the key process in natural attenuation. It is increasingly considered as an alternative to more active measures for the remediation of contaminated sites. In the case of petroleum distillation products like gasoline, the selective dissolution process occurring at the groundwater/product interface leads to a groundwater pollution mainly characterized by monoaromatic compounds including BTEX and others substituted monoaromatics. The ability to degrade aromatic compounds under anaerobic conditions is limited to a few strains, and the degradation is typically much slower than under aerobic conditions. This anaerobic biodegradation has been established for aliphatic compounds (Van Hamme *et al.*, 2003; Widdel et Rabus, 2001) but due to very low kinetics is not relevant for subsurface processes. In this site, the pollutant profile alteration is expected to be strongly influenced by aerobic metabolism. Contaminated aquifers are often characterized by their low dissolved oxygen level (< to 1-2 mg) in contrast to non-contaminated regions.

MATERIALS AND METHODS

Site Investigations. Water samples were collected from a net of 30 wells settled mostly in lines perpendicular to the flow direction of the aquifer (Figure 1). For microbiological studies, sterile glass bottles with watertight Teflon®-coated caps were filled up with water avoiding any gas phase and stored immediately at 4°C in the dark. In the laboratory, a fraction of each water sample was dispensed in an anaerobic chamber into vials that were sealed with butyl rubber stoppers. Gas phase in the vials was exchanged with N_2, outside the chamber, using a gazing manifold. These vials were used for anaerobic microbiology. The bottles and vials were stored at 4°C until use.

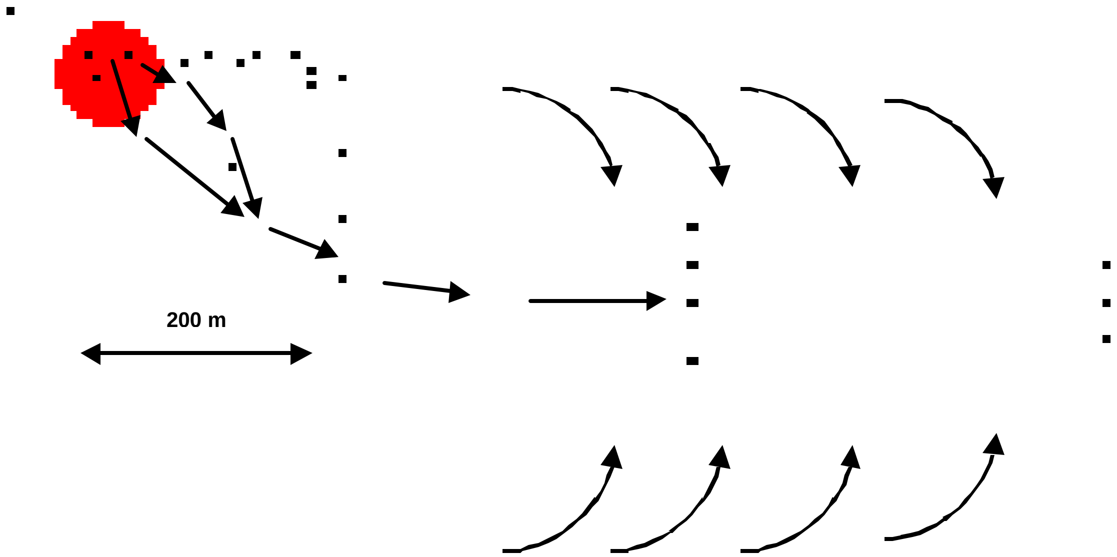

FIGURE 1. Monitoring wells and groundwater flow directions

Biodegradation in Aerobic Conditions. Experiments were performed in Erlenmeyer flasks with a working liquid volume of 75 mL, and a gas phase (air) of 45 mL, providing enough O_2 for total carbon oxidation. Hydrocarbons are supplied with 25 mL of a gasoline/water phase contact (Figure 3). Water samples (25 mL) collected from observation wells were used as inoculum. 25 mL of water, or mineral salt solution ([*3]) completed the working volume. The flasks were closed with Teflon® butyl stoppers. Aerobic control received 250 µL of a H_2SO_4 4N solution. Essay and control (both triplicate) prepared for each point of the kinetic were gently shaken at 20°C.

Biodegradation in Denitrifying Conditions. Experiments were performed in test tube (Bellco), with a working liquid volume of 10 mL, and a gas phase (N_2) of 15 mL. 3,3 mL of MDB mineral medium ([*3]) (Rabus and Widdel, 1995) were added. Hydrocarbons are supplied with 3,3 mL of a gasoline/water phase contact. Water samples (3,3 mL) collected from observation wells were used as inoculum. Addition of KNO_3 was added in water phase used for the contact with gasoline, in order to obtain in test tube a concentration of 120 µM of NO_3^-. All these solutions were prepared under anoxic conditions. Essay and control (both five replicates) were carried out at 25°C for a month.

Analytical Methods. Soluble hydrocarbons were analyzed on essay and control flasks. The analytical method combined SPME (solid phase micro extraction) technique with GC-FID. The SPME headspace procedure involved a fiber coated with a 100-µm thickness film of polydimethylsiloxane phase according to the methodology described by Pawliszyn (1997). The use of Hydro*CARB*® software based on a retention index principle allowed detailed information at low concentrations on about 200 individual hydrocarbons. External calibration was done using a commercial mixture of identified compounds (502/524 Volatil Organic Calibration Mix, Supelco).

RESULTS AND DISCUSSION

A fuel storage site has been contaminated in 1962 with about 10 to 20 m^3 of mogaz: a particular gasoline. Due to fractured bedrock, the oil phase was nearly not subjected to weathering. This ensured for over 40 years a limited but constant release of hydrocarbons in groundwater. Major upwelling events of the groundwater table (more than 14 m) were due to a hilly topography.

About 5 tons of the mobile gasoline phase were removed by pumping over a period of 5 years. Plume expansion (approximately 1 300 m length) and dissolved hydrocarbons have been monitored through a net of 30 wells settled mostly in lines perpendicular to the flow direction (Figure 1).

The oil phase recovered from this site contained 26% n-paraffins, 35% isoparaffins, 17,4% naphthenes, 11,6% aromatics, 9,3% olefins, 0,5% C15+ hydrocarbons and less than 0,5% of unidentified compounds. This composition, in particular the presence of olefins, the low content of aromatics and the high content of normal paraffin, clearly indicate that this contaminant is an old gasoline: the mogaz from 1962 reported as having been released in February of this year. The presence of light hydrocarbons (olefins with less than 5 carbon atoms) thus indicates that the oil phase was only subjected to a limited volatilization and solubilization. The most important hydrocarbons present in this oil phase regarding its impact on the groundwater are the most water soluble monoaromatic hydrocarbons illustrated in Figure 2.

A gasoline/water phase contact using free oil phase recovered from the site was done. The hydrocarbons transferred to the aqueous phase in this laboratory experiment showed a very similar profile to the hydrocarbons present in the plume of the site. The concentration obtained in the sample originating from the site in the heart of the hotspot are about a third than those measured in the laboratory experiment. These data are presented in Figure 3. The hydrocarbons transferred in the aqueous phase are mainly monoaromatic compounds (73% of total hydrocarbons). Twenty major compounds represent 91% of the aromatic fraction.

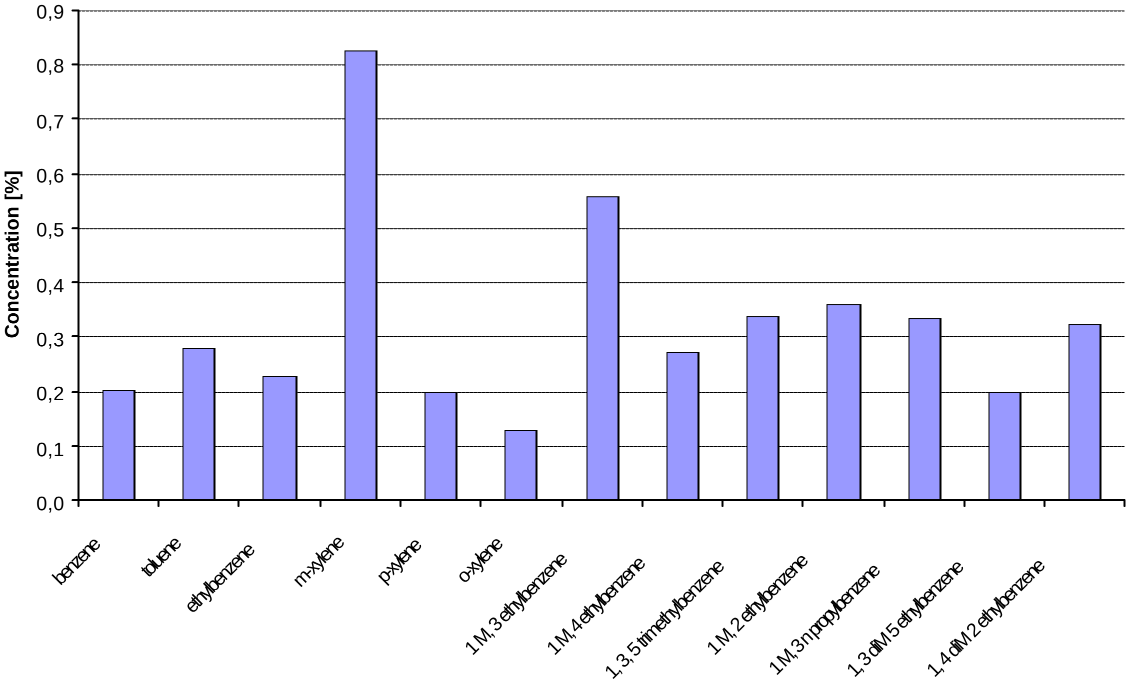

FIGURE 2. Composition of the major monoaromatic compounds present in the oil phase contaminating the site.

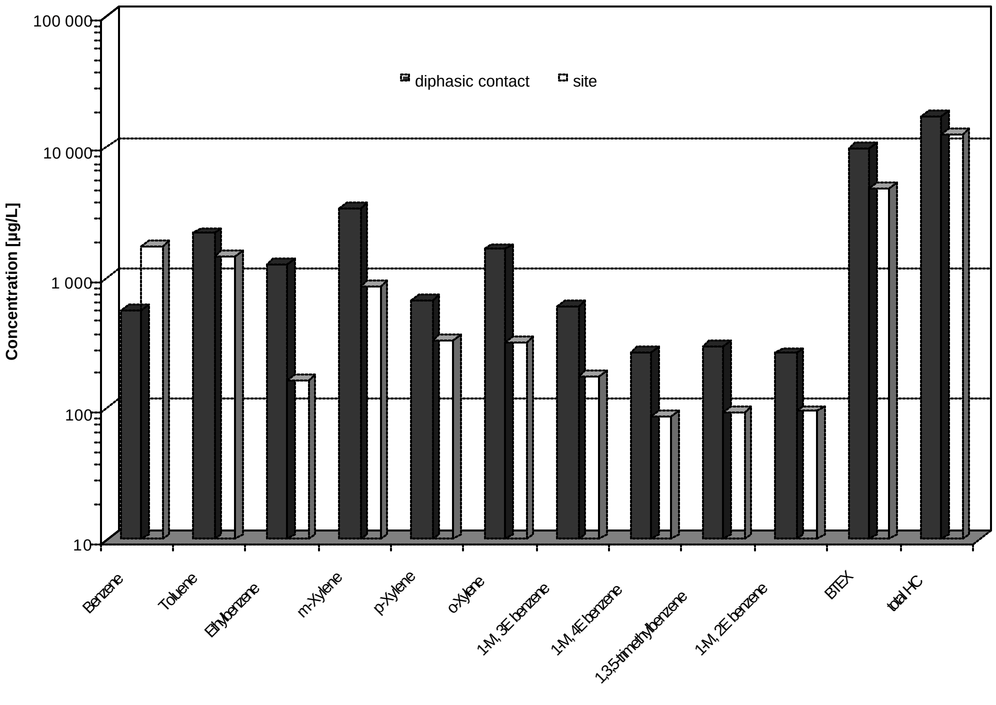

FIGURE 3. Concentration of dissolved hydrocarbons in the aquifer sampled right under the contamination source and in the gasoline/water contact using free oil recovered on the site.

The plume extension has been monitored through a net of 30 wells settled mostly in lines perpendicular to the flow direction. Figure 4 shows the plume of dissolved hydrocarbons in the aquifer downstream of the contaminated hot spot. This plume appears to be very long: about 1 300 m.

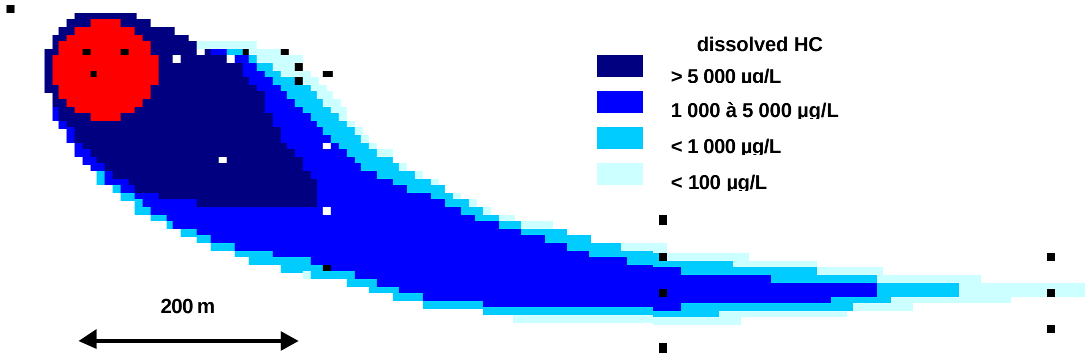

FIGURE 4. Plume of dissolved hydrocarbons in the aquifer downstream of the contamination source.

The results of the detailed hydrocarbon analyses from water samples are presented in Figure 5. The samples have been taken from wells located in the center of the hydrocarbon plume at increasing distances from the contaminated soil (0, 50, 150, 700, and 1 300 m). These results show that the concentration of most of the main hydrocarbons

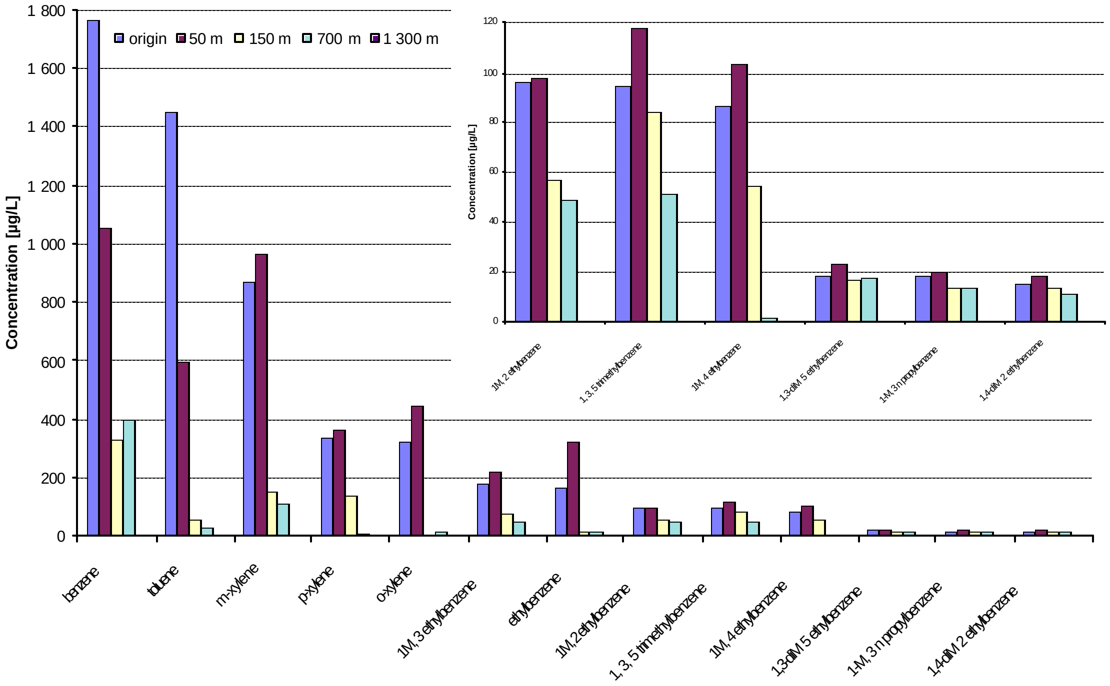

FIGURE 5. Concentration of dissolved hydrocarbons in the plume at increasing distances from the contamination source.

decreases with increasing distance from the contamination source. The biodegradation of the hydrocarbons dissolved in the plume can be evaluated through the evolution of ratios of particular compounds. For compounds presenting comparable octanol-water partition coefficients, significant differences in disappearance velocities indicate that intrinsic biodegradation occurs. In the present case 8 out of the 11 preponderant compounds are very rapidly biodegraded. On the other hand, four compounds: 1,3,5-trimethyl-benzene, 1-methyl, 2-ethyl-benzene, 1,2,3-trimethyl-benzene and *n*-propylbenzene, are still persistent (less than 50% decrease) at 700 m.

The oxygen concentration in the plume is very low, and only nitrate is present as alternative electron acceptor (maximal concentration of 15 mg/L). All the available electrons acceptors are depleted in the plume until the well located at 700 m downstream of the contamination source. The electron acceptors potentially used by the autochthonous microflora are mainly originating from lateral water fluxes arriving in the thalweg where the plume is located. These water fluxes are contributing to dilution effects and thus providing electron acceptors necessary to the biodegradation of the hydrocarbons present in the plume.

Decreasing pollutant concentrations (from 30 mg/L to µg/L range) with increasing plume length were mostly attributed to biodegradation since only limited dilution and dispersion occurred.

The potential of biodegradation of the indigenous microorganisms was investigated in the laboratory under aerobic and denitrifying conditions. The aqueous phase obtained from the diphasic contact (Figure 3) was used as hydrocarbon source. This approach allowed on one hand to study the biodegradability of individual components that are not always available as commercial products, and on the other hand to have exactly the same

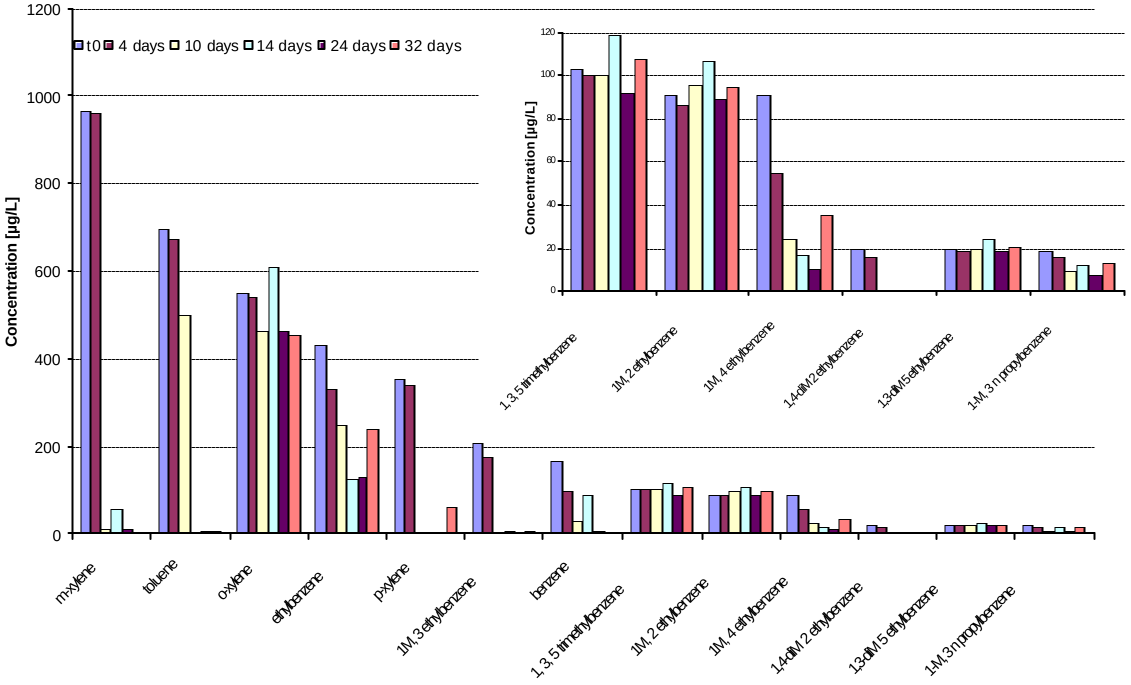

FIGURE 6. Evolution of hydrocarbon concentration in aerobic biodegradation experiments.

relative proportion of individual compounds than in the polluted aquifer. This approach gives more realistic kinetic information about the biological removal of the pollution and is furthermore suitable to evaluate the substrate interactions observed by Chang *et al.*, 1993 and Oh *et al.*, 1994 and susceptible to lead to inhibition e.g. co-metabolism. These authors have shown that hydrocarbon mixtures may affect the rates of disappearance as well as the overall behavior of theses compounds during biological degradation. Therefore it is preferable to study the degradation of the whole water-soluble fraction of the gasoline, rather than individual hydrocarbons.

The biodegradation tests were carried out under aerobic conditions at 20°C during 32 days. Two sets of experiments were done, one with complementation with mineral salts medium, the other without to simulate plume conditions. Polluted water collected on observation wells at 700 m from the contamination source was used as inoculum. Water samples were used directly (without enrichment steps) to avoid selection pressure and evolution of the diversity of the microbial consortium. Respective concentrations of hydrocarbons tested were the third of the saturated concentrations obtained with the contact gazoline/water and are of the same order of magnitude than those found in the plume, in particular downstream of the gasoline source (decreasing values from 30 mgL^{-1} to µg.L^{-1} range). The hydrocarbon concentrations obtained in the biodegradation tests are average values of at least three replicates. Abiotic loss of hydrocarbons, and specially of volatile aromatics was evaluated on controls for each point of the kinetic. Due to low initial concentration of the individual components (between 20 to 1 000 µg.L^{-1}) the highest attention has been given to validate the biodegradation especially for weak degradation levels. In both set of experiments (with or without addition of salts) the capacity of the microbial consortium was similar (data not shown). Differences are observed only in term of kinetic. The BTEX (benzene, toluene, xylene and ethyl benzene), except o-xylene, and three other substituted benzene compounds, 1-methyl, 3-ethylbenzene, 1-methyl, 4-ethylbenzene and 1,4-dimethyl, 2-ethylbenzene, are biodegraded. This last compound with an initial concentration of 20 µg.L^{-1} illustrates the sensitivity of the methodological approach including the analytical method combining SPME/GC and Hydro*CARB*® software. Compounds as like 1,-3,-5 trimethylbenzene and 1-methyl, 2-ethylbenzene, present in relatively high proportion in the aromatic fraction (90-100 µg.L^{-1}) are not biodegraded in this test. The degradation of gasoline by a microflora originating from a urban waste water treatment plant was investigated by Solano-Serena et *al.* (1999). They found that all the aromatic compounds usually considered as recalcitrant were entirely biodegraded but with a high inoculum level (100 mg dry matter per essay). Interestingly, the two components not completely biodegraded in these optimized conditions were also 1-methyl, 2-ethyl benzene and 1,-3,-5 trimethylbenzene.

The biodegradation was also tested under strictly anaerobic denitrifying conditions. No biodegradation was observed with the native microorganisms from the polluted plume. As control, a *Thauera aromatica* strain isolated from production water collected from oil wells was used (Grabowski *et al.*: to be submitted). This strain degraded only the toluene among the aromatic mixture tested.

CONCLUSION

The plume extension has been monitored in a gasoline contaminated site. The hydrocarbon composition and the electron acceptors indicate that biodegradation and dilution mechanisms are both contributing to natural attenuation along the first 700 meters of the plume. Furthermore, the biodegradation process seems to be limited by the availability of the electron acceptors. Beyond this first zone, lateral water fluxes arriving in the thalweg were increasingly contributing to dilution effects and thus providing electron acceptors, both leading to an extensive hydrocarbon disappearance in the plume.

Biodegradation experiments involving the indigenous microflora were performed in laboratory. The determined biodegradation capacity is in good agreement with the disappearance of hydrocarbons in the first zone of the plume. Some major compounds like 1,-3,-5 trimethylbenzene, 1-methyl, 2-ethyl benzene, 1,-2,-3 trimethylbenzene and *n*-propylbenzene appeared to be more recalcitrant to biodegradation under conditions limited by electron acceptors.

The important upwelling events of the ground water table occurring in this fractured chalk site sporadically favors an important transfer of hydrocarbons from the source to the aquifer. Nevertheless the dissolved hydrocarbons monitored in the aquifer over several years indicate that the plume is not expanding despite the hydrogeological characteristics of this site. As a consequence, the natural attenuation illustrated in this work clearly contribute to limit the extension of the plume.

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