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Monitoring of the biodegradation of toluene-contaminated sand in columns by SIP measurements, CO_2 content and its $^{13}C/^{12}C$ isotopic signature.

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Hydrocarbon contaminated soils represent an environmental issue as it impacts on ecosystems and aquifers. Bioremediation uses the ability of bacteria naturally present in the ground to degrade hydrocarbons. It represents an effective solution to fight the pollution but *in situ* monitoring before and during soil treatment is difficult and challenging. Indeed, where significant subsurface heterogeneity exists, conventional intrusive groundwater sampling can be insufficient to obtain a robust monitoring as the information they provide is restricted to vertical profiles at discrete locations, with no information between sampling points.

In order to obtain wider information, complementary methods can be used like geo-electrical techniques. Induced polarization (IP) seems to be the more promising to study the effects of biodegradation processes. Indeed, laboratory and field experiments have shown an enhancement of real and imaginary parts of electrical conductivity while bacterial treatment is progressing (Abdel Aal et al., 2006 ; Atekwana et Atekwana, 2010).

Moreover, microbial activity induced CO_2 production and isotopic deviation of carbon (Aggarwal and Hinchee, 1991). The ratio $\delta^{13}C(CO_2)$ will come closer to $\delta^{13}C$(hydrocarbon).

From these findings, the French project *BIOPHY,* supported by the French National Research Agency (ANR), proposes to use electrical methods and gas analyses to develop a non-destructive method for monitoring *in situ* biodegradation of hydrocarbons in order to optimize soil treatment. Laboratory experiments in columns are carried out to demonstrate its feasibility.

Our objectives were to monitor aerobic microbial activity in toluene-contaminated sand columns using complex electrical resistivity measurements (SIP, Spectral Induced polarization and GEIS, Galvanostatic Electrochemical Impedance Spectroscopy) and measuring concentration and $\delta^{13}C$ isotopic ratio of produced CO_2.

MATERIAL AND METHODS

Toluene aerobic biodegradation by *Rhodococcus wratislaviensis* (*R. w.*) is studied. Columns (height 50cm ; diameter 15cm) are constructed in Kynar® and filled with sand from Fontainebleau. They are fed up with a fluid containing toluene (carbon source), bacterial growth medium and H_2O_2 in low concentration to supply O_2 and thus stimulate aerobic metabolic bioprocesses.

Complex electrical conductivity measurements are performed regularly to monitor the bio-activity and progress of toluene degradation. Two methods are used: SIP and GEIS. An electrical current with known amplitude and frequency is applied at two ring electrodes, positioned at both ends of the columns. This induces an electrical current in the porous media and the associated voltage is measured between two $Cu/CuSO_4$ potential electrodes. The change of amplitude and phase of the received waveform allows measuring complex conductivity σ*, expressed as a sum of the real (σ') and imaginary (σ'') parts: $\sigma^*(\omega) = \sigma'(\omega) + i\sigma''(\omega)$ with $i = \sqrt{-1}$ et $\omega = 2\pi f$.

CO_2 concentration and $\delta^{13}C(CO_2)$ is measured by infrared laser spectroscopy. Chemical composition of fluid is also monitored: toluene quantification, $\delta^{13}C$(toluene) and $\delta^{13}C$(DIC) (Dissolved Inorganic Carbon) and alkalinity.

RESULTS AND DISCUSSION

We show the results for two columns on Fig. 1: a biotic and an abiotic control.

For the biotic column, we notice an enhancement of σ'' during decrease of toluene concentration. Moreover, there is an increase of CO_2 concentration in the air just after increase of

HCO_3^- concentration in the pore fluid ; and $\delta^{13}C(CO_2)$ approaches $\delta^{13}C$(toluene). On the contrary, for the abiotic column, there is no significant variation of the different parameters. The $\delta^{13}C(CO_2)$ was not able to be measured because of the lack of CO_2 in the air of the column.

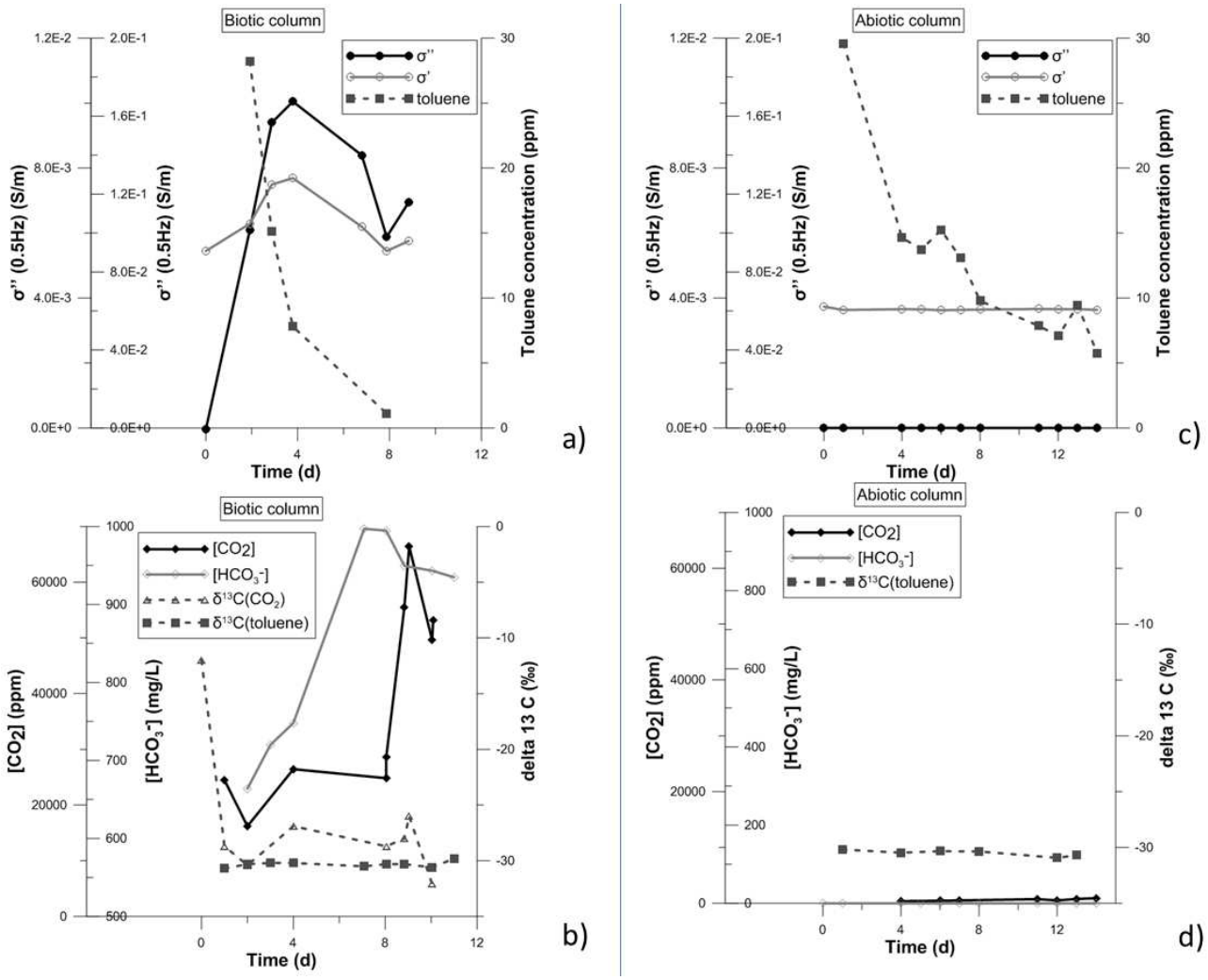

Fig. 1: Results for biotic (left) and abiotic (right) columns. a) and c) Variation over time of real and imaginary parts of complex conductivity at 0.5Hz and toluene concentration ; b) and d) CO_2 and HCO_3^-concentrations ; $\delta^{13}C(CO_2)$ and $\delta^{13}C$(toluene).

Regarding these results, we can say that microbial activity is characterized by CO_2 production and $\delta^{13}C(CO_2)$ isotopic deviation, and also by an evolution of complex electrical resistivity in correlation with chemical analyses. Aerobic biodegradation of toluene can be monitored by SIP and gas measurements at laboratory scale and it will be tested soon on the field scale.

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