

Microcosm and Column Studies on the Biodegradation of Methyl Tert-Butyl Ether (MTBE) in Soil-Water Systems

Marcia Morales, and Marc A. Deshusses*

Department of Chemical and Environmental Engineering

University of California, Riverside CA 92521

Sergio Revah

Departamento de Ingeniería de Procesos e Hidráulica, UAM-Iztapalapa, Apdo.

Postal 55-534, 09340, México, D.F., MEXICO.

ABSTRACT

Microcosms and column experiments were carried out using soil and groundwater from a MTBE contaminated site or using distilled water amended with mineral salts. The biodegradation of MTBE was tested under aerobic and anaerobic conditions with an initial MTBE concentration of 25 mg/L. The water to soil ratio in the microcosm experiments was 1:2 by mass and the inoculum size was 0.3 g wet biomass/50 mL liquid. No MTBE degradation was observed in microcosms incubated with only the indigenous microbial population or under anaerobic conditions. MTBE degradation was only observed in the microcosms incubated under aerobic conditions and inoculated with our MTBE degrading consortium. In those microcosms, complete degradation of MTBE was observed in approximately 10 days. This represented a decrease of the MTBE biodegradation rate compared to previous experiments performed at 30 °C in a shaker and without soil. Also a decrease of the MTBE biodegradation rate was observed with time which was probably due to nitrite accumulation and low pH resulting from the nitrification of ammonia. Subsequently, a 0.7 L column was packed with the same soil, and synthetic groundwater with an MTBE concentration of 13 ppm was trickled through the column while maintaining aerobic conditions. The soil column was operated for more than 6 months, at variable loadings and MTBE removal efficiencies remained close to 100%. Experiments using radiolabeled MTBE were carried out. In the soil column, a very fast release of $^{14}\text{CO}_2$ was observed within the first 24 hours after the injection of ^{14}C -MTBE, and the $^{14}\text{CO}_2$ captured reached approximately 10% of the radioactivity injected within that time. Thereafter a very slow release was observed. The reasons for the very slow carbon dynamics remain to be understood. Nitrification of ammonia was also present in the column and a significant drop in pH was observed. Finally, a rapid response of the system was found when the MTBE supply was shut off and turned on again. The results appear to be very promising for future applications of MTBE bioremediation.

KEYWORDS:

MTBE, BTEX, bioremediation, microcosm, in-situ bioremediation, mineralization

INTRODUCTION

MTBE contamination of groundwater has become a major concern because its increasing appearance in urban and municipal water wells. MTBE was the second chemical most detected in shallow groundwater.¹ Among the potential point sources of MTBE environmental release are the production sites, storage areas, transportation, distribution and confinement sites of MTBE or oxygenated gasoline. Non-point sources may also contribute to the prevalence of MTBE in groundwater. An EPA study reported that nearly 3.5×10^6 lbs. of MTBE were released into the environment in 1995. Of this amount, nearly 3.4×10^6 lbs. of MTBE were released into the air due to air emissions as compared to about 15,000 lbs. released into the subsurface, about 4,000 lbs. on open land and nearly 80,000 lbs. released into surface waters. MTBE adversely impacts the taste and odor of water and it has been classified as a possible carcinogen.² To date, natural attenuation of MTBE has not been firmly demonstrated and several challenges exist before MTBE in-situ bioremediation can be deployed in the field. These include the inoculation with suitable microorganisms (bioaugmentation), and maintaining the activity of added microorganisms over time. The main objective of this work was to study the behavior of a MTBE degrading consortium in the laboratory, when added to soil and groundwater systems. For this, both microcosm and column experiments were carried out.

MATERIALS AND METHODS

Microorganisms. The consortium used for the experiments was initially enriched in a biotrickling filter³ and it was maintained in a 2 L Bioflo I reactor. The consortium was grown attached on the exterior surface of a silicon tubing while a small air stream was passed through the tubing to ensure adequate oxygen supply without stripping the MTBE. 1 mL of MTBE was added weekly and the mineral medium³ was periodically refreshed. Samples of wet biomass were taken from the reactors as needed and served for the inoculation of the microcosms or of the soil column.

Soil and groundwater. Groundwater and soil were from an actual contaminated site in Southern California. The soil was relatively sandy, its permeability was not determined. For all experiments with soil, the larger stones were removed. Groundwater samples were analyzed. The original MTBE concentration was about 2 ppm, the pH was 7.5, TDS was 800 mg/L but no nitrogen as ammonia or nitrate was detected in the native samples. Also, soil and groundwater samples were taken and extracted with sterile saline water. It was then attempted to grow indigenous organisms in mineral medium agarose plates with nitrate or ammonium as nitrogen source and kept in MTBE atmosphere. No microbial population that could grow on MTBE could be found, even after 6 months incubation. The absence of indigenous MTBE degraders was consistent with the results of microcosms presented in the results Section.

Microcosms. Microcosms experiments were performed in 250 or 500 mL sealed Mason jars. A 15 cm long piece of stainless steel tubing was placed through the lid, via a Shimadzu GC septum and was used for sampling and for MTBE additions. All the tests were carried out using actual contaminated soil and groundwater (ratio 1:2 weight) or using distilled water amendment with 50% strength mineral salts solution.³ Headspace (one half of the total volume), was kept to maintain aerobic conditions; thus the liquid volume was 50 and 150 mL for the 250 and 500 mL jars, respectively. Microcosms were inoculated with 0.3 g wet biomass of MTBE degrading

culture per 50 mL liquid. Some microcosms were carried out without inoculation to check for the presence of sufficient density of indigenous MTBE degrading microorganisms. MTBE degradation was tested both under aerobic and anaerobic conditions. For anaerobic conditions the Mason jars were incubated in a plastic disposable glove box flushed with a continuous nitrogen flow of 6 mL/min. To guarantee anaerobic conditions, all the materials were first placed in the jars, they were sparged with nitrogen in the glove box and inoculated with our consortium, sealed and MTBE was added at the end. Microcosms without soil were performed to test the possible adverse effect of the soil in the activity of the consortium, in all cases controls were set without inoculation or adding 2 g/L of HgCl_2 . The initial MTBE concentration in all the cases was approximately 25 mg/L. All the microcosms were done in duplicate and incubated at ambient temperature. The microcosms were gently shaken once per day and before sampling.

Column studies. A 0.7L glass column (4.5 cm I.D., 47 cm packing height) was packed with 998 g of soil and inoculated with a total of 1 g (wet basis) of biomass from both a microcosms and the Bioflo I reactor. The synthetic contaminated water consisted of 50% strength mineral medium³ and the inlet feed MTBE concentration was approximately 13 mg/L. The experimental setup is shown in Figure 1. The setup included one Masterflex pump fitted with two pump heads. The setup allowed to induce a positive air flow through the column (via a syringe needle at the top of the column) by using two different size tubing (1.6 mm and 3.1 mm I.D. Pharmed tubing) for the inlet and outlet streams. With this system, a constant liquid to air ratio (1:2.5) was maintained throughout the experiments. Stripping of MTBE was always less than 2% of the total feed. The MTBE loading was varied between 0.1-2.5 g of MTBE per cubic meter of soil bed per hour ($\text{g}/\text{m}^3 \cdot \text{h}$) by changing the liquid flow while maintaining a constant inlet MTBE concentration.

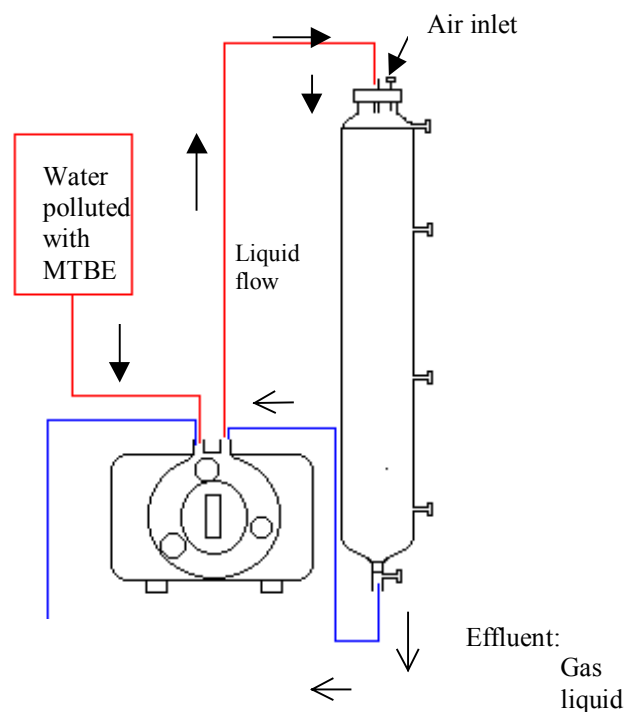


Figure 1. Experimental setup for MTBE biodegradation in a soil column.

¹⁴C-Experiments. An ethanol solution containing ¹⁴C-MTBE (NEN Life Science Products, Boston Mass.) with a specific activity of 5.0 mCi/mmol and a radiochemical purity of 99% was used to demonstrate mineralization in both the microcosm and in the soil column. To avoid altering the process culture metabolism, the ethanol was biodegraded prior to introduction into the soil column or into the microcosms. This was done by introducing 500 μ L of a microorganism suspension taken from a biotrickling filter used for toluene degradation. This microbial population was unable to degrade MTBE but degraded ethanol rapidly. For each microcosm experiments a specific activity of 1.97×10^{-6} mCi/mL was used and for the column experiments, pulses of a 13 ppm MTBE solution of mineral medium with a specific radioactivity of 7.1×10^{-6} mCi/mL were made. The total radioactivity introduced during the pulse was 2.4×10^{-4} mCi and the duration of the pulse was 90 minutes. The gaseous ¹⁴CO₂ generated from the MTBE biodegradation was captured in two successive 4M NaOH baths after the column. For the microcosm experiments, labeled MTBE was added initially and ¹⁴CO₂ was captured in 4mL 4M NaOH solution placed a small test tube placed in the jars. In microcosms, parallel experiments were conducted with cold MTBE and were analyzed by gas chromatography. For both microcosm and column experiments, aliquot samples of NaOH were taken and the volume was replenished. The NaOH samples were mixed with scintillation cocktail (ScintiVerse II) in a ratio 1:4 vol. and measured in a Triathler multilabel tester (Hidex Inc., Mustonkatu, Finland). Readings were taken after 2 minutes.

Analysis. Representative samples (soil-water) from the microcosms were centrifuged first and the supernatant was analyzed. For the column experiments, liquid samples were taken directly from the liquid influent and effluent. The liquid MTBE concentration and the gaseous CO₂ or MTBE were measured as before by gas chromatography and a FID detector on a HP 6890 GC and by a TCD detector on a HP 5890 GC, respectively.³ Nitrate was quantified by the salicylic-acid method,⁴ ammonia and nitrite were quantified by Vacu-vials kit (k-1523 and k-7003 Chemetrics, Calverton, VA) using the principle of nesslerization and the azo dye formation respectively. All liquid samples were diluted for nitrogen analysis to the adequate range.

RESULTS

Microcosms Experiments

MTBE biodegradation was only observed in those microcosms incubated under aerobic conditions and inoculated with our MTBE degrading consortium (Figure 2). No MTBE degradation occurred in the microcosms with indigenous soil microbial population or those incubated under anaerobic conditions.

Complete degradation of 20-25 ppm MTBE was observed in approximately 10 days. This represents a marked decrease in the MTBE biodegradation rate compared to previous experiments (not shown) without soil incubated at 30 °C where usual rates of up to 1 ppm/h were recorded. Here, in the microcosm without soil, MTBE was degraded in about 3 days. Hence, it is possible that mixing and higher temperature (250 rpm and 30°C) helped increasing the degradation rate.

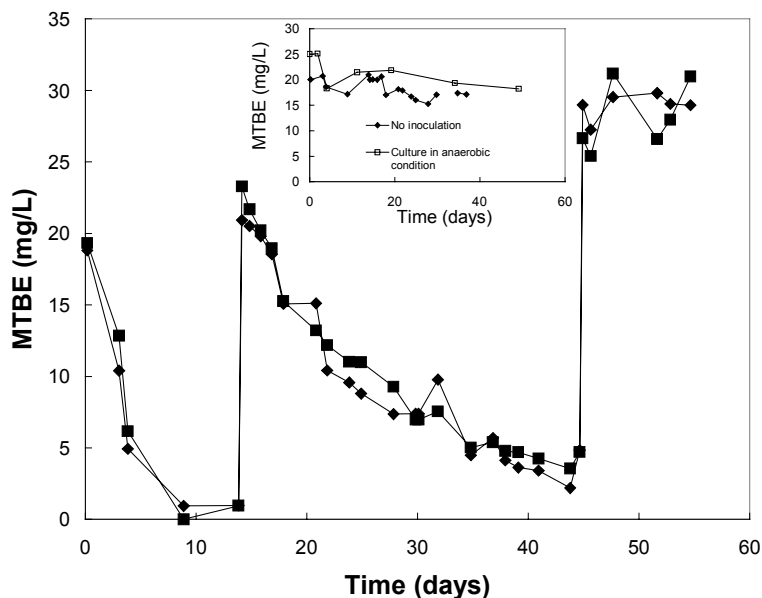


Figure 2. Degradation of MTBE in soil-groundwater microcosms incubated under various conditions. The initial MTBE concentration was 25 ppm. Main graph: duplicate of aerobic microcosms inoculated with our MTBE degrading consortium (two MTBE respikes were performed). The inset shows one uninoculated control and one anaerobic microcosm.

Still, the sole presence of soil reduced the biodegradation by a factor of about 3. The reasons are not yet fully understood but could be related to diffusion limitation of MTBE out of the pores of the soil. Also a clear decrease in the MTBE degradation rate was observed over the successive MTBE spikes (Figure 2). This could be linked to the nitrification of ammonia, which caused a drop in the pH and resulted in generation of toxic levels of nitrite (2 ppm). Other experiments⁵ revealed that less than 1 ppm N-NO₂⁻ inhibited MTBE degradation by the consortium. The presence of nitrifiers in the MTBE microbial population has been reported previously⁶ and the deactivation of MTBE degrading cultures was also reported⁷ in microcosms experiments. The accumulation of nitrite and the high sensitivity of MTBE degraders to nitrite could one of the reason for the difficulty generally experienced in enriching a successful MTBE degrading culture.

Once the inhibition by nitrite was identified, the mineral medium at the Bioflo I reactor for maintenance of the consortium was refreshed once a week instead of every 20 days. This resulted in a noticeable increase in the activity of the stock consortium. Selected microcosm experiments were redone and a significant reduction in the time necessary for complete MTBE biodegradation was observed as reported in Figure 3. In this Figure, one can see a decrease in the ammonium and the associated increase in the nitrate and nitrite ions as result of the nitrification process. The total balance of nitrogen was close to 100%. This is consistent with the fact that the MTBE consortium is very slow growing, hence that it did not incorporate a significant fraction of the nitrogen available in the medium. This further suggests that the nutrient requirements of the consortium must be very low. Detailed examination of Figure 3 reveals that after the nitrite reached a value close to 1ppm, the rate of MTBE degradation was seriously affected. Since the groundwater was slightly buffered, the pH decreased only from 6.9 to 6.5, thus the acidification of the system was not significant and not the cause of the consortium deactivation. Other experiments (not shown) revealed that the nitrate is not an inhibitor. In fact, nitrate is a better source of nitrogen for the MTBE degrading consortium. With nitrate, MTBE degradation was slightly higher than with ammonium and both pH control and nitrite inhibition problems were solved.

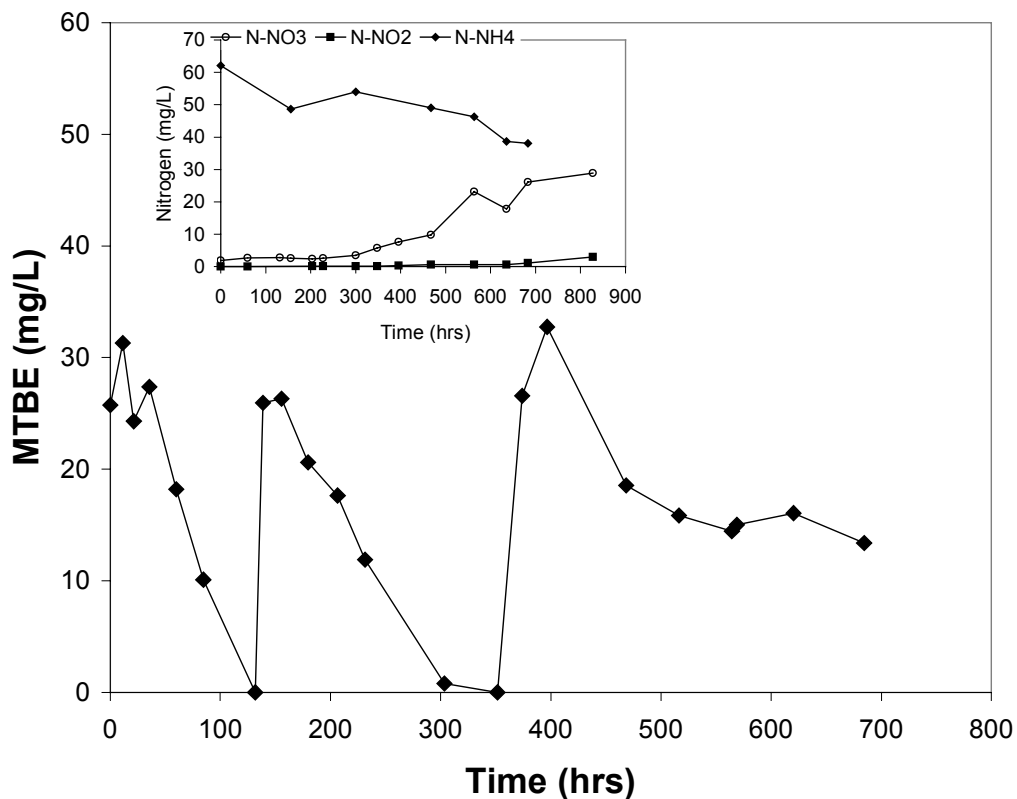


Figure 3. MTBE biodegradation in a soil-groundwater microcosm inoculated with our MTBE degrading consortium and nitrogen analysis as ammonium, nitrate and nitrite (inset). The MTBE initial concentration was 25 ppm.

Column Studies

MTBE Biodegradation Performance

The soil column was operated for approximately six months with MTBE loads (defined as flow \times inlet concentration / packed soil volume) ranging from 0.1 – 2.5 g/m³h. The load was varied by increasing the liquid flowrate, the empty bed liquid residence time (EBRT) ranged from 3.5 to 0.2 days. Different experimental setups were tested for the column before the configuration presented in Figure 1 was adopted. This is why the startup phase is not presented. However, a similar column was operated in parallel but it was not inoculated and served as a control. In that column, no difference was observed between the inlet and outlet MTBE concentration. Thus, the soil used in these studies had a very limited sorption capacity for MTBE and abiotic losses were minimal. A global balance over the period of operation of the column revealed that approximately 2156 mg of MTBE were eliminated in the column with removal efficiency close to 100%. Based on the amount of MTBE eliminated in the column 5391 mg of CO₂ should have been produced, however globally only 40% of that value was experimentally detected. Figure 4 shows a summary of the performance reported as the elimination capacity (i.e., flow \times (inlet -

outlet concentration) / packed bed volume) with respect to the load (flow \times inlet / packed volume) for the column. The elimination capacity (EC) represents the amount of MTBE degraded per hour per unit volume of soil. The MTBE removal efficiency was close to 100% over the entire range of MTBE loadings studied; the maximum performance was not determined.

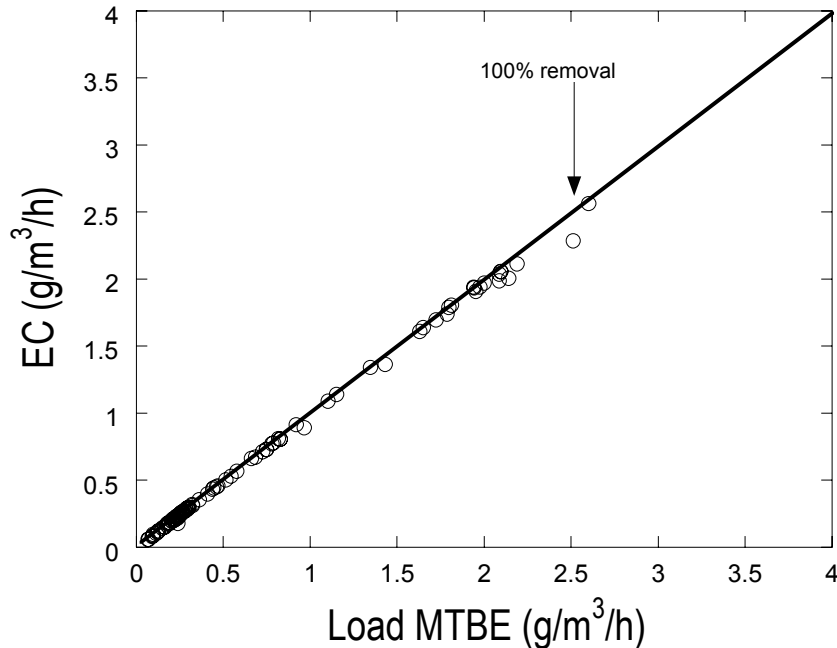


Figure 4. Elimination capacity versus MTBE load in soil column. The inlet concentration was approximately 13 ppm and the MTBE loading was varied from 0.1 to 3 g/m³h by changing the liquid flow.

The Effect of Nitrification on the Process

Nitrification was also observed in the column, however nitrite ion concentrations remained low (<0.18 ppm) and never reached toxic levels. Examination of Figure 5 reveals that the pH drop was more pronounced for low empty bed residence time. This means that nitrification was increased with the ammonia load. For the lowest flow corresponding to an EBRT=3.5 days, ammonia was stoichiometrically converted to nitrate (0.67 mg_{Nitrogen}/L.h). At higher nitrogen loads, the nitrification rate increased up to 6.13 mg_{Nitrogen}/L.h. Nitrification was observed even when the MTBE supply was stopped. This indicates that the nitrification process is not directly associated to the MTBE degradation. However, further research in the role of the nitrifiers in the MTBE degrading consortium is warranted.

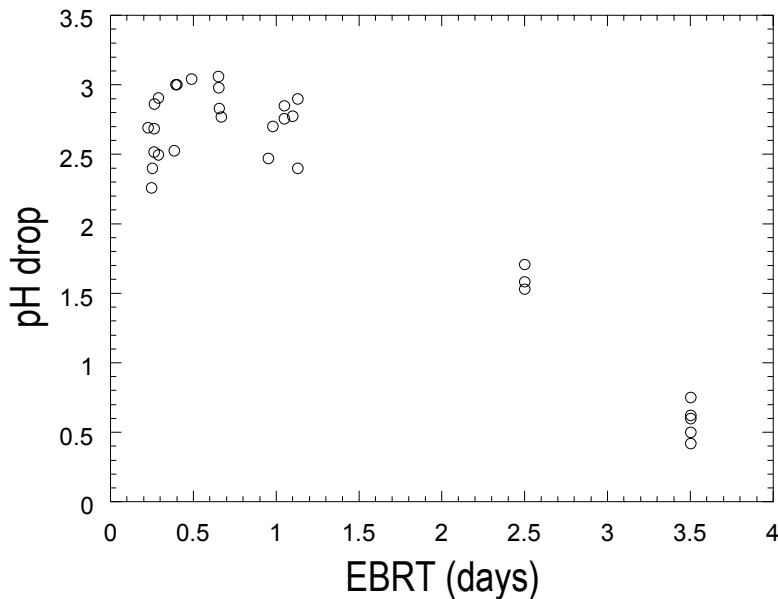


Figure 5. pH drop over the soil column as a measure of the nitrification activity. EBRT = empty bed retention time = soil bed volume / groundwater flow

MTBE Mineralization Studies

Mineralization experiments in soil microcosms (not shown) revealed that 70% of the ^{14}C -MTBE degraded was rapidly recovered as $^{14}\text{CO}_2$. This confirmed that MTBE disappearing was mineralized. Approximately 1% of the total radioactivity was recovered in the NaOH bath of the radiolabeled control without inoculation, which proved that the presence of MTBE in the bath was negligible (this quantity is the equivalent to the equilibrium MTBE distribution among the different phases). A different overall pattern was observed in the soil column (see Figure 6) where a very fast release of ^{14}C - CO_2 occurred during the first 24 hrs after the injection of ^{14}C -MTBE reaching approximately 10% of the radioactivity injected. This rapid release phase was then followed by a very slow but continuous release of labeled carbon dioxide over several weeks. The total radioactivity recovered was approximately 32% within 1500 hrs after the radiolabeled MTBE injection was done. Before the experiment, MTBE biodegradation measured by GC was close to 100% and no significant radioactivity was recovered in the liquid effluent. At this time, no good explanation exist for the missing radioactivity. It is possible that some radioactivity can be retained in the soil matrix (as carbonate or in another form) or incorporated into the biomass during growth of MTBE degraders or assimilation of CO_2 by nitrifiers. Since both nitrifiers and MTBE degraders are very slow growing organisms, any ^{14}C incorporated would be released slowly.

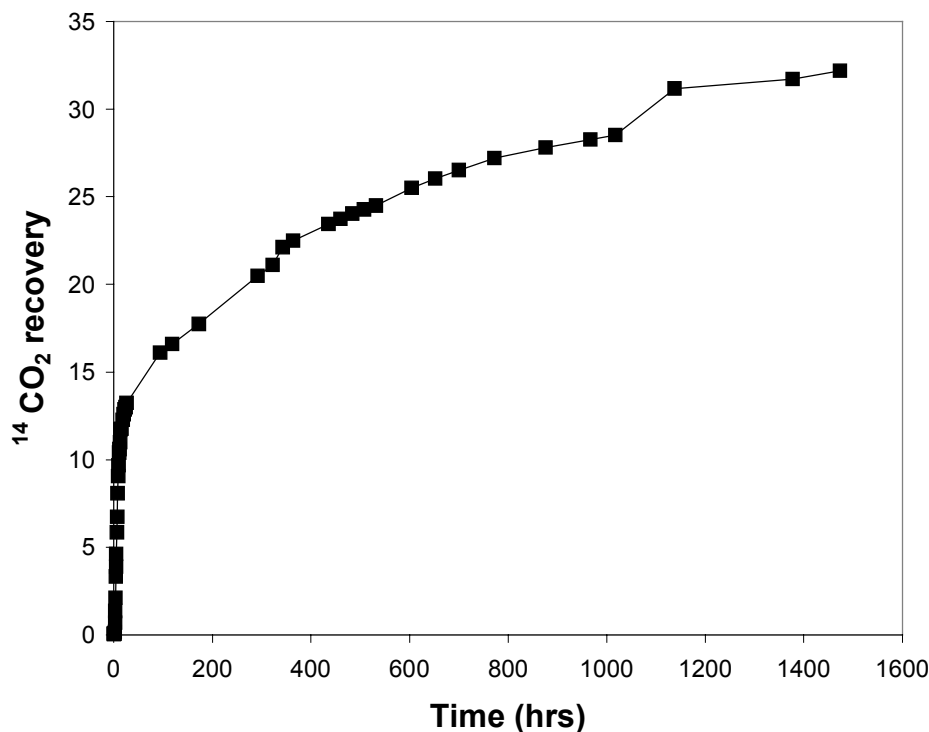


Figure 6. ¹⁴C-MTBE degradation experiment in the soil column. The percentage recovery of the injected ¹⁴C-MTBE as ¹⁴CO₂ is reported. A constant MTBE feed concentration of 25 ppm was maintained throughout the experiment; the total radioactivity added was 2.6×10^{-4} mCi over 90 minutes, liquid flow was 0.35 mL/min.

Dynamic Response of the Columns

The dynamic behavior of the soil column was studied to assess time constants associated with the treatment. Although the recovery of radioactivity as CO₂ was found to be very slow and only partial (see previous Section), a very fast response of the outlet CO₂ concentration was observed when the MTBE load was stopped and restarted. A typical response of the soil column is reported in Figure 7. Fifteen minutes after interrupting the MTBE feed, the outlet concentration of CO₂ started to decrease from the initial value of 1.3 gC/m³ and reached a constant level of CO₂ at 0.5 gC/m³ after 3 hours. The latter value is about 0.3 gC/m³ above the lab CO₂ concentration and is due to the endogenous respiration of the process culture. When the MTBE supply was resumed, an immediate response was observed and the CO₂ and MTBE concentration recovered the normal values also in approximately 3 hours. These results are important because they show a) a rapid response to changing conditions, and b) a clear relationship between the MTBE consumption and CO₂ production.

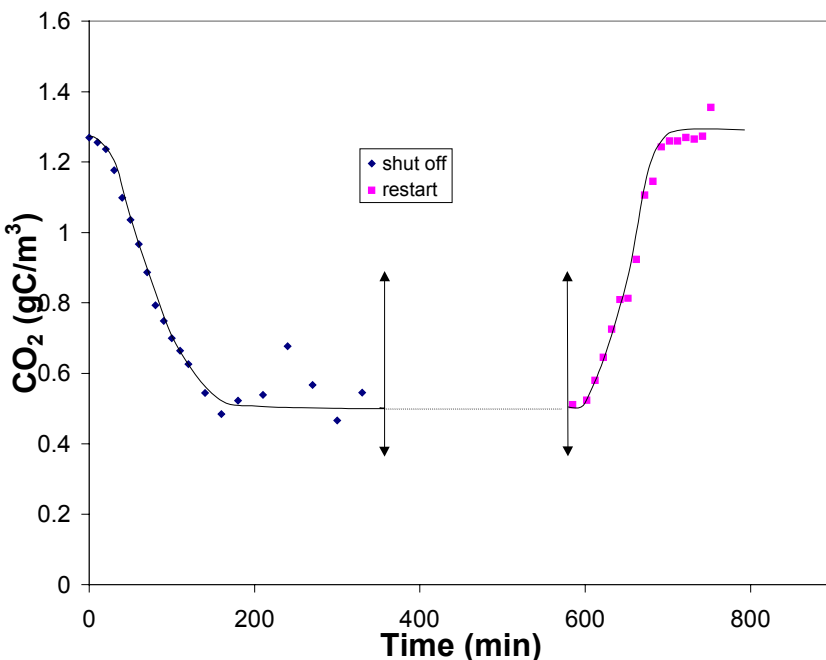


Figure 7. Dynamic response of the soil column after MTBE concentration supply (13 ppm) was stopped at time 0 and turned back on after 600 minutes. The liquid flow was kept constant at 1.9 mL/min. The vertical arrows indicate the time for a CO₂ pulse experiment (not shown).

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CONCLUSIONS

MTBE biodegradation and mineralization were demonstrated in soil-groundwater microcosms and in a column packed with soil through which synthetic groundwater was trickled. Effective MTBE biodegradation was maintained for over 6 months. Although the evolution of the microbial population was not determined, the fast biodegradation of MTBE in the different soil-groundwater systems suggests that our consortium adapted well to the new environment. Further studies are still necessary to understand some of the phenomena presented in this paper, such as nitrification and the low carbon recovery and their relation to MTBE biodegradation. Even so, the stability and the performance of MTBE biodegradation observed in these studies are very promising for the future implementation of either in-situ bioremediation or of engineered systems such as biobarriers for the treatment of groundwater polluted with MTBE.

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