

BIODEGRADATION OF MTBE AND GASOLINE IN VARIOUS SOIL SAMPLES FROM POLLUTED SITES IN MEXICO

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ABSTRACT

The problems associated with contamination of groundwater and drinking water by MTBE have been increased the need for a better understanding of the process that control its environmental fate and the potential for natural attenuation. As a result, the biodegradability of MTBE has received considerable attention. In this work a compilation of the available information about the presence of MTBE in environment demonstrates that MTBE pollution exists but the problem has not been analyzed in Mexico. In order to evaluate the potential of biodegradation in soil samples from gasoline polluted sites in Mexico microcosm under both aerobic and anaerobic conditions were done. Up to date the results of microcosm experiments suggest that biodegradation of MTBE was present in the most of the samples only in aerobic conditions and in cometabolism with other gasoline compounds.

INTRODUCTION

Fuel oxygenates are added to gasoline to increase combustion efficiency and to reduce air pollution. Methyl tert-butyl ether (MTBE) is the most commonly oxygenate used because its high octane level, low cost, ease of blending with gasoline. However, due to its unique physical and chemical properties relative to other gasoline components, MTBE has shown to migrate in subsurface at speeds similar to groundwater with minimal retardation. It is well known that the current practice of amending gasoline with MTBE has caused a widespread contamination of groundwater (1). The use of oxygenated gasoline in Mexico, as a control strategy to improve air quality, started in 1991 and the current concern on MTBE contamination in the USA have provoked that Latin-American countries are aware of the MTBE ban, although the pollution problem has not been yet formally recognized in those countries.

Biodegradability tests are a good indication of the persistence and recalcitrance of the compounds in the environment. Some studies have been conducted on the biodegradability of MTBE under both aerobic and anaerobic conditions. The recalcitrance of this compound was clearly demonstrated when first studies were published and no degradation was observed, however recent studies have shown that MTBE is biodegradable preferentially under aerobic conditions, using MTBE as a sole carbon source (2, 3, 4) or in cometabolism with other compounds such as alkanes (5, 6, 7, 8). More recently few studies have reported the MTBE degradation by anaerobic conditions under Fe(III) (9) and nitrate reduction (10).

The aim of this work is to analyze the situation of use, pollution and the potential of biodegradation of MTBE in gasoline from polluted sites in Mexico. In order to achieve this objective, three main aspects are analyzed in this work: 1) The potential risk of groundwater pollution in Mexico based on data of actual production, demand of MTBE and number of gas stations and drinking water wells, 2) Some scarce studies about its presence in the environment as results of pipeline leaks and storage tank spills and evaporative emissions and 3) Potential of biodegradation of samples collected in polluted sites.

MTBE production and pollution in Mexico

Two kinds of gasoline are used in Mexico, PEMEX Magna and PEMEX Premium; they contain aromatics, 29.5%; olefins, 8.5%; paraffins, 54% and MTBE, 9%. The gasoline demand in Mexico is approximately of 528 thousand of barrels per day (11) and the MTBE production in Mexico is only about 4.4 thousand of barrels per day (12) for this reason an important volume is imported from USA. Globally Latin America consumes 4% of the MTBE world production (13). It is well known that gasoline and specifically MTBE reaches and pollutes groundwater and it is an important water source. The water supply for Mexico City is 65 m³/s and 70% is obtained from groundwater. Soto *et al.*, 2000 (14) consider that Mexico City is a representative example for the risk of pollution because of high population and gas station densities; in Mexico City there are 241 gas stations and 752 drinkable water wells, besides some indications exist of groundwater pollution due to the presence of organic compounds. There are some reports about MTBE presence in environment around gas stations. Concentrations in air about 11.5 mg/m³ has been monitored in service station in Chihuahua city (15) and MTBE concentrations of 4.4 ppb have been detected in crossroad by monitoring emission to on-road vehicles (16). Additionally distribution and storage stations are also a pollution source and MTBE has been detected (17, 18, 19) (see Table 1). Only one serious study has been conducted in order to try to evaluate the MTBE pollution in Mexico City groundwater. It was carried out by a governmental department. A total of 345 samples were taken in the underground and groundwater around gas stations and concentrations in the range of 4-87 mg/L (see Table 2) were detected in some samples, fortunately no MTBE was found in any of the 33 monitored drinkable water well (20). In Mexico there is no legislation about gasoline releases and MTBE limits in groundwater, however it could be seen that all reported concentrations are above the limit of 20 µg/L established by USEPA.

MTBE is facing out in USA, however the MTBE substitution seems unlikely to be adopted in the short term in Latin America countries because of there are only few studies on MTBE presence in the environment that support a stringent regulation and therefore no penalties exist for organisms involved in production, distribution and releases of the oxygenated fuel. In addition, economic aspect involved in changes of production and distribution units is an important point furthermore the price increase associated to the introduction of other compounds instead of MTBE in gasoline.

Therefore more studies are necessary to prove the contamination for this compound and to study alternatives for cleanup.

The following section will show the potential of bioremediation on gasoline polluted sites.

MATERIALS AND METHODS

Soil Sampling.

Soil Samples from gasoline polluted sites were obtained in the southeast region of the country. The spills were result of illegal fuel intake in pipelines. The samples were aseptically taken to depths of 1.50 m by a soil sampler with and conserved to low temperature during transport.

Location of sampling points and soil characterization.

The sampling points were located along the gasoline pipeline Minatitlán-Mexico. Samples of three bioremediated sites and three polluted sites were collected (Table 3). The polluted sites belong to the southeast region, the mayor soil activity was for agricultural purpose. The spill dates were between October 1998 and February 2000. The gasoline spill volume (data by PEMEX, Mexican Petroleum) was between 40,922 and 556,500 liters.

It was made a physicochemical and microbiological characterization of the samples. Organic matter, total nitrogen, pH and moisture content were analyzed according to standard methods (21, 22). The soil type was analyzed by the texture technique in a certified laboratory. Total microbial counts and gasoline degraders were done on bacteriological agar and noble agar in gasoline atmosphere respectively.

Microcosms.

The microcosms were carried in 125 mL serum bottles sealed with mininert valves, in both anaerobic and aerobic conditions. Aerobic tests were inoculated with microbial consortia grown in plates. The initial biomass was of 20 mg_{protein} /L, with 20 mL of mineral medium and 5 uL of gasoline (Magna SIN). The composition of the mineral medium was (g/L): K₂HPO₄,2; KH₂PO₄, 1; NH₄Cl,0.75; MgSO₄,0.5; CaCl₂, 0.018; element trace and pH of 7. The experiments were carried out at a constant temperature of 30 °C and 100 rpm. Sterile microcosms with gasoline or MTBE were used as blanks.

Anaerobic microcosms to assay MTBE degradation were inoculated with soil and the mineral medium was amendment with different electron acceptors. It was tested for nitrate and sulfate reduction, methanogenic conditions, Fe (III), Mn(IV) and humic acids.

Maximal degradation rate was calculated by Gompertz model as it was described by Acuña *et al.*, 1996 (23).

Analytic Techniques:

Gas chromatography: For gasoline quantification, samples in headspace were analyzed by a gas chromatograph GC -FID (Hewlett Packard 6890) with a column HP-1 of 30 m x 320mm x 250 um, the flow of the carrier gas, helium, was of 1.5 ml/min, hydrogen flow of 35 ml/min and air flow of 400 ml/min. The initial temperature was 40°C and the final temperature was 120 °C at a rate of 2 °C per minute. CO₂, O₂ analysis was done by a GC-TCD (Gow-Mac 580) at ambient temperature and carrier gas flow of 65 ml/min. The column was a CTR-I (Alltech).

Biomass. Coomassie brilliant blue assay was used to quantify protein, the biomass was hydrolyzed with NaOH 0.1 N after reaction it was read in a UV-Spectrometer at 620 nm (24).

RESULTS AND DISCUSSION

Soil characterization.

The most of the soil samples were of clay type with sand or slime fraction (Table 4), the pH close to neutral or slightly basic. The nitrogen content was in the range of 0.03 to 0.130 %, these values classify the soil between poor and slightly rich (25), the samples 243 and 132 km were the richest according to the nitrogen content. The organic matter was between trace and 1.77%, these values are in the range of very low to low according to the classification by Aguilera, 1989 (26). The moisture content was between 10-30% these values are considered acceptable to support microbial activity in soil (27). Although the soil was for agricultural activities the matter content and nitrogen was no as high as it was expected. The soil samples with the highest organic matter and nitrogen content were 132 km and 243 km.

Microbiological counts

All soil samples presented an acceptable count (see Table 5). The soil sample with the highest total microbial count was 243 Km and the highest gasoline degrader count was for the sample 214 Km. In general, the total microbial counts were between 2.38×10^6 and 6.92×10^8 cfu/g these values correspond to an adapted microbial population (28). Gasoline degraders counts were in the range of 1.12×10^6 and 3.24×10^7 cfu/g. It can be observed that a great fraction of the microbial population is gasoline degrader. It can be concluded from the high percentage between the total and gasoline degrader populations. The best percentage was approximately 80% for the sample 214Km followed by the samples 132 Km and 242 Km with percentage of 45%, the sample with the lowest ratio was 243 Km where less than 1% of the microbial population is gasoline degrader. However, in this case the gasoline degrader count was in the same magnitude order as the other samples. The counts were quite acceptable and this shows a microbial population highly specialized in the degradation of gasoline compounds.

Microcosm

The gasoline degradation rate was measured for all the samples. As it can be seen in Table 6, the samples 214, 396 and 132 were the most active and the degradation rates were between 0.93 and 1.34 mg/mg_{protein}/h for gasoline. MTBE degradation was also quantified by chromatographic analysis and the highest degradation rates correspond to the same samples. The MTBE degradation rate was in the range of 0.032-0.166 mg/mg/h. In general, the sample 214 Km showed to have the best activity in both microbial counts and kinetics. In Figure 1 it can be observed the evolution of the gasoline and MTBE and oxygen consumption and the CO₂ production and microbial growth for sample 214 Km. A complete degradation of MTBE was observed in approximately 100 hours. Similar degradation rate was reported by Hernández *et al.*, 2001 (29) in microcosms experiment inoculated with biofilter packing material treating gasoline vapors.

There was no changes in gasoline concentrations in the blanks and also for anaerobic microcosm under different acceptor electrons acceptors.

No degradation was observed in microcosm with MTBE as sole carbon source and in anaerobic conditions.

The results indicate cometabolism degradation of MTBE; some reports have demonstrated MTBE degradation by cometabolism with some gasoline compounds and alkanes such as: propane (5), butane (6) and pentane (8). The MTBE degradation rates reported are in the range of 0.92-44 mg_{MTBE}/g_{cell}/h (30). As it can be observed the degradation rate for MTBE in this work is higher.

Other compounds degradation

In order to study the degradation of specific compounds, the samples were grouped in two microbial consortia: 1) Contaminated soil and 2) bioremediated soil for further studies named as M1 (samples 132, 181, 242 Km) and M2 (214, 243, 396 Km) respectively.

For M1 and M2, the gasoline degradation was 0.821 and 1.103 mg/mg_{protein}/h respectively and the MTBE degradation rate was 0.117 and 0.080 mg/mg_{protein}/h. Other compounds were specifically measured: pentane, hexane, toluene and isooctane. In the case of the pentane and toluene degradation was slightly faster than the MTBE degradation but for hexane and isooctane degradation it was lower (Table 6). The Figure 2 shows the pentane, hexane, isooctane and toluene degradation consumption. Only few studies (31, 32) have reported the degradation of specific compound in the gasoline mixture.

FINAL REMARKS

The analysis about the current situation of the contamination with MTBE shows a not recognized potential risk for using MTBE as gasoline oxygenate in Latin American

countries due to the legislation lack. The MTBE pollution exists and it can affect the groundwater.

About potential of degradation of MTBE, it was observed that the degradation of this compound can be carried out in presence of other gasoline compounds by cometabolism. This kind of degradation seems widespread and some studies report MTBE cometabolism with alkanes. Possible reasons for the absence of direct metabolism of MTBE can be: the short period of exposure of the soil to the MTBE, the collected samples were shortly exposed and adapted to MTBE.

The microcosm experiments using MTBE as a sole carbon source and under anaerobic conditions were negative during the test period. Some reports exist about positive results on MTBE biodegradation and more recently in anaerobic conditions but in many cases the activity was present after long adaptation and enrichment time. In this work, further studies need to be done about cometabolism with specific compounds and to search for intermediates as TBA.

ACKNOWLEDGMENTS AND REFERENCES

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Figure Legends

Figure 1. Gasoline, MTBE and O₂ consumption and CO₂ , biomass production of 214 Km soil sample

Figure 2. Evolution of pentane, hexane, isooctane and toluene consumed on sample M1

Table Legends

Table 1. MTBE in soil samples from distribution and storage tanks

Table 2. Underground and groundwater analysis around gas station (20)

Table 3. Location of the sampling points from gasoline spill sites.

Table 4. Physicochemical Soil characterization

Table 5. Microbial Counts and degradation rates of soil samples

Table 6. Summary of degradation rates of samples. M1: mixture of polluted sites, M2: mixture bioremediated sites

Table 1. MTBE in soil samples from distribution and storage tanks

Number of sampling points	Number of samples with MTBE	MTBE concentration	Location	Reference
41	5	0.03 mg/Kg	Morelia	Iturbe <i>et al.</i> , 2001a (17)
46	4	0.17 mg/Kg	Zacatecas	Iturbe <i>et al.</i> , 2001b (18)
30	1	3.35 mg/Kg	Puebla	Flores <i>et al.</i> , 1999 (19)

Table 2. Underground and groundwater analysis around gas station (20)

Tested place		Number of service station	Number of service station with MTBE	Maximal MTBE concentration (mg/L)
Gas station underground		54	40	52.1
Phreatic water below gas station		47	39	87
Drainage around gas station (residual water)		48	34	3.96
Gas station in zone of aquifer recharge	Underground	7	5	5.58
	Phreatic water	7	5	7.22
Discharge of residual water in gas station	Drainage	58	46	9.20
	Underground	4	4	6.04

Table 3. Location of the sampling points from gasoline spill sites.

Location	Spill date	Spill volume (liters)	Actual status
132 Km	June 2000	-	Polluted
242 Km	February 1999	-	Polluted
181 Km	February 2000	-	Polluted
214 Km	October 1999	556,500	Bio restored
396 Km	October 1998	40,922	Bio restored
243 Km	April 1999	80,000	Bio restored

Table 4. Physicochemical soil characterization

Location	Soil type	pH	Organic matter (%)	Total nitrogen (%)	Moisture content (%)
132 Km	Clay-slime	7.43	1.77	0.120	28.09
242 Km	Sand-clay	7.40	0.20	0.030	13.52
181 Km	Clay-medium	7.40	1.00	0.090	20.12
214 Km	Clay-medium	6.90	1.60	0.070	15.06
396 Km	Clay-sand	7.70	Trace	0.03	20.32
243 Km	Clay-sand	8.00	1.50	0.130	10.40

Table 5. Microbial Counts and degradation rates of soil samples

Location	Total count CFU/g dry soil	Gasoline count CFU/g dry soil	Gasoline degradation rate mg/mg _{protein} /h	MTBE degradation rate mg/mg _{protein} /h
132 Km	4.20 E+07	1.84 E+07	0.93	0.107
242 Km	2.38 E+06	1.12 E+06	0.80	0.032
181 Km	3.3 E+07	2.69 E+06	0.74	0.059
214 Km	4.26 E+07	3.64 E+07	1.34	0.166
396 Km	3.17 E+08	1.14 E+07	1.18	0.107
243 Km	6.92 E+08	1.24 E+06	0.67	0.05

Table 6. Summary of degradation rates of samples. M1: mixture of polluted sites, M2: mixture bioremediated sites

	M1	M2
Gasoline degradation rate (mg/mg _{protein} /h)	0.821	1.103
MTBE degradation rate (mg/mg _{protein} /h)	0.117	0.080
CO ₂ production rate (mg/mg _{protein} /h)	1.040	1.197
O ₂ uptake (mg/mg _{protein} /h)	0.211	0.266
Pentane degradation rate (mg/mg _{protein} /h)	0.17	0.226
Hexane degradation rate (mg/mg _{protein} /h)	0.044	0.026
Isooctane degradation rate (mg/mg _{protein} /h)	0.039	0.033
Toluene degradation rate (mg/mg _{protein} /h)	0.111	0.167

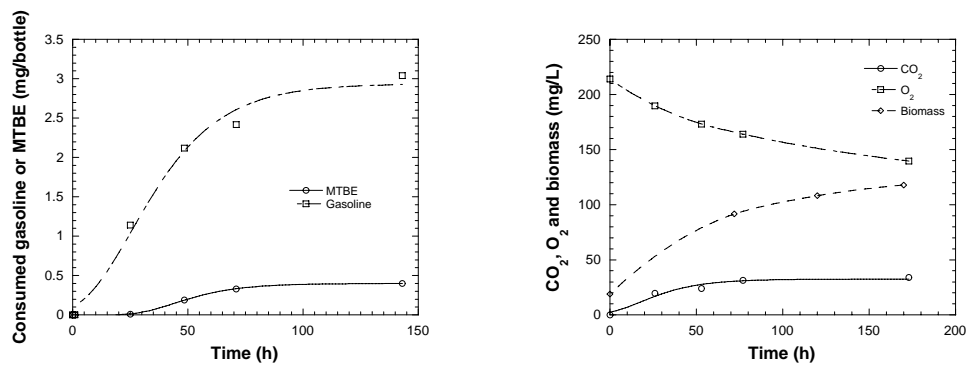


Figure 1. Gasoline, MTBE and O₂ consumption and CO₂, biomass production of 214 Km soil sample

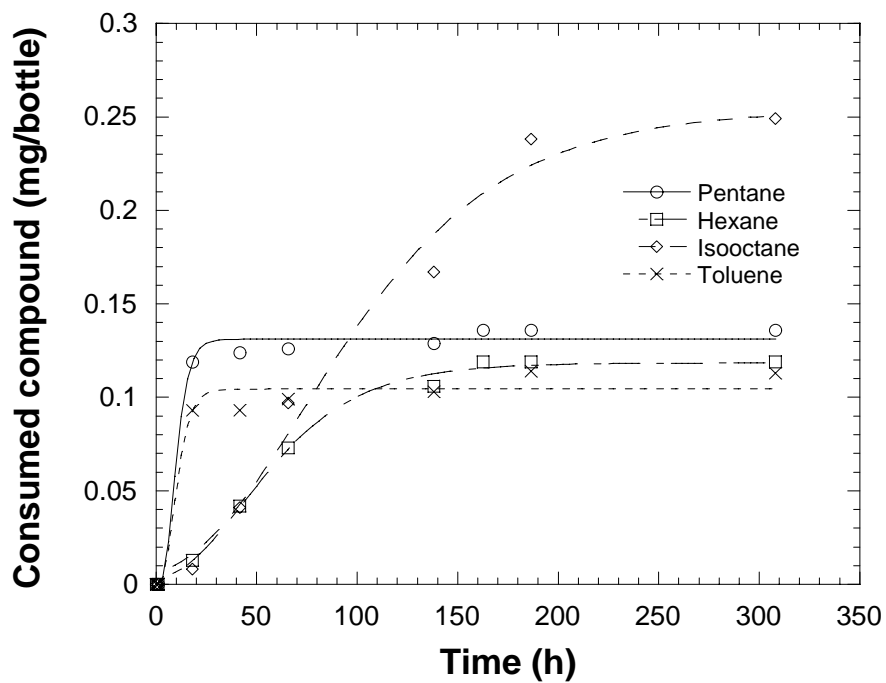


Figure 2. Evolution of pentane, hexane, isooctane and toluene consumed on sample M1