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Effect of toluene as gaseous cosubstrate in bioremediation of hydrocarbon-polluted soil

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Abstract

The stimulation of the microbial population by a more bioavailable supplementary carbon source and by a surfactant pretreatment was studied in petroleum hydrocarbon-polluted soils bioremediation. Two types of soils were used, Soil A which had been recently polluted and the aged Soil B. They contained 52.4 and 50.4 g of total petroleum hydrocarbons per kg of dry soil, respectively. The effect of passing a continuous small stream of air containing a low concentration of gaseous toluene through packed 0.5 l (Ø = 5.5 cm) columns was studied. For Soil A, after 62 days the THPs degradation was 28% higher in the toluene treated columns than in controls. In aged Soil B the effect of toluene was not significant, probably due to bioavailability limitations. With Soil B, the combined effect of toluene as cosubstrate and a surfactant pretreatment was studied and the hydrocarbons degradation was 29% higher in the toluene-amended columns than in the controls. Toluene removal was higher than 99% in all cases. Surfactant addition increased hydrocarbon degradation when toluene was also added suggesting that the biological reaction was the limiting process. The study shows the possibilities of using gaseous substrates, such as toluene, for the in situ or ex situ treatment of petroleum hydrocarbon-polluted soil in processes limited by the biological reaction. The main advantage of the treatment is that the compound can be easily and directly delivered to the polluted soil through the venting system.

Keywords: Biodegradation; Cosubstrate; Petroleum hydrocarbons; Soil; Surfactant; Toluene

1. Introduction

Petroleum hydrocarbon pollution is one of the main environmental problems, not only by the important amounts released but also because of their toxicity. According to the Mexican oil company PEMEX [1] in Mexico more than 8000 tons of hydrocarbons were released to the soil due to leaks and spills, in 2001. Furthermore, the Mexican environmental authority, estimates that 64% of the total continental Mexican surface is affected by some type of contamination [2].

In nature, petroleum hydrocarbons are moderately degraded and the biological reactions by microorganisms are one of the main mechanisms. Hydrocarbon biodegradation is enhanced by some microbial properties such as the production of natural surfactants and the hydrophobic composition of its cellular wall [3]. The microbial activity for crude-oil degradation has been documented [4,5].

Biodegradation in soil requires the bioavailability of the pollutants and the metabolic activation of the suitable microorganisms [6]. The low bioavailability of petroleum hydrocarbons has been mainly attributed to their limited solubility and their adsorption to the organic matter of the soil matrix [7] although, for some hydrocarbons intrinsic reaction limitation has also been reported [8]. Surfactants may enhance hydrocarbon bioavailability by increasing the apparent solubility in the aqueous system or the contact surface by means of the formation of stable emulsions [9]. Enhanced hydrocarbon biodegradation has been reported with added surfactants and biosurfactants but some studies report inhibition due to toxicity [9–11].

It has been reported that microorganisms are able to simultaneously consume different substrates when these are at very low concentrations [12]. For environmental applications, it has been

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reported that the addition of a second organic substrate can substantially alter the degradation kinetics of organic compounds in soil [13–15]. In a previous work on phenanthrene biodegradation in a model soil, it was demonstrated that the addition of toluene as gaseous cosubstrate enhanced the degradation rate and favored the mineralization of intermediates such as phthalic acid [16]. In a parallel work with petroleum hydrocarbon-polluted soils, various compounds including toluene, ethanol, glucose, pentane and hexadecane, were assayed in batch slurries to evaluate their effect on the degradation of total petroleum hydrocarbons (TPHs) [17]. Addition of low concentrations of toluene and ethanol increased the TPHs degradation by about 50%. Toluene has also been reported as a cosubstrate to support the degradation of some chlorinated compounds [13,18,19].

The objective of this work was to evaluate the effect of the addition of small amounts of gaseous toluene on the biodegradation of petroleum hydrocarbons in highly polluted soils. To assess the effect of bioavailability, an experiment with surfactant-pretreated soil was performed.

2. Methods and materials

2.1. Soil samples

Two different soils were used and their main characteristics are described in Table 1. Soils were collected from polluted sites in oil extraction and processing facilities in the states of Tamaulipas (Soil A) and Veracruz (Soil B) in Mexico. Soil A was collected from a recently polluted site while Soil B, an aged soil, was from a zone that has been affected by petroleum hydrocarbons for many years. The soils were dried at 30 °C and sieved. For some experiments, Soil B received a surfactant pretreatment by adding 0.3 l of a Tween 80 solution (2.5 g l^{-1}) to 0.7 kg of soil to give a final content of 1.07 g $kg^{-1}_{dry\ soil}$. This Soil was called Soil B-S.

Vermiculite (AYASA S.A. de C.V., México) with a particle size between 1.91 and 3.36 mm was used as an inert bulking agent to improve porosity and water retention. Its maximum water retention capacity was 65% (w/w).

2.2. Column experiments

They were performed in the experimental device previously described [16] and consisted in a ventilated temperature controlled chamber at 30 ± 2 °C. An air stream was provided by a compressor and controlled with two mass flow meters (Mathesson, USA, mod. 8141; Aalborg, USA, mod. GFC17). The air was bubbled in a diluted NaOH solution to remove inlet CO_2. A small air stream was sparged into a toluene evaporator and the main stream was humidified to avoid soil drying in the packed columns. One manifold was used to distribute the humid air into the control (toluene-free) columns. The humid airflow passed into another manifold where it was mixed with the toluene stream before entering the test (toluene-amended) columns. In all experiments the empty bed residence time (EBRT = volume of the reactor/gas flow) was 50 min. For the toluene-amended columns the inlet toluene concentrations and loads were 0.87 ± 0.2 g m^{-3} and 1.9 ± 0.2 g $m^{-3}_{reactor}$ h^{-1} for Soil A and 0.84 ± 0.3 g m^{-3} and 0.96 ± 0.3 g $m^{-3}_{reactor}$ h^{-1} for Soil B.

The 0.5 l (Ø = 5.5 cm) glass reactors were packed with the soils mixed with vermiculite in an 80:20 ratio (wet weight). Each column tested contained 100 and 160 g of dry Soil A and Soil B, respectively, to have a bed height of about 13 cm. The columns had hermetic Teflon caps and ports for inlet and outlet gas sampling. The initial water content of the mixtures (w/w) were close to the maximum as shown in Table 1 and were attained by adding the required amount of mineral medium to the dry soil. Duplicate columns were used for all experiments. The mineral medium contained (in g l^{-1}) 0.6 KH_2PO_4; 2.4 K_2HPO_4; 1.5 $MgSO_4{\cdot}7H_2O$; 0.15 $CaSO_4{\cdot}2H_2O$; 0.03 $FeSO_4{\cdot}7H_2O$; 3.0 $(NH_4)_2SO_4$. The ratio C:N in the columns were 260:1 for Soil A and 340:1 for Soil B, these values are in the ranges used in some hydrocarbon-polluted soil bioremediation treatments [20]. The experiments in packed columns were performed for 98 days with Soil A and for 150 days with Soil B and Soil B-S. At days 20, 62 and 98 for Soil A and at 30, 60, 103 and 150 for Soil B and Soil B-S, the columns were unpacked, the soil was mixed and 5 g soil samples were drawn for analyses before repacking the columns.

2.3. Microcosms experiments

Ten grams of soil were sampled at the end of each treatment from the columns and used in 125-ml Erlenmeyer flasks stoppered with Mininert valves (VICI Precision Sampling Inc., USA) for the microcosms experiments. Duplicates

Table 1
Soil composition and properties

	Soil A	Soil B
TPH concentration (g_{TPHs} $kg^{-1}_{dry\ soil}$)	52.4 ± 1.1 [43.5 ± 1.2][a]	50.4 ± 0.9 [40.2 ± 0.8][a]
Composition (%)		
Sand	87.1	70.6
Silt	1.8	20.0
Clay	4.0	3.6
Organic matter	6.9	5.8
Particle size (mm)	<2	<2
Water retention capacity (%)	25 [33][a]	20 [30][a]
pH	7.6	6.6
Soluble organic carbon (g C $kg^{-1}_{dry\ soil}$)	0.944 ± 0.03	0.910 ± 0.03

[a] Including added vermiculite.

were prepared for all experiments. The CO_2 production was evaluated as an indirect measure of microbial activity, this method is widely used for biomass activity estimation in soils [21,22].

2.4. Analyses

Toluene concentration was measured in duplicate daily with 500 μl gaseous samples for inlet and outlet streams of the packed columns by GC-FID (Hewlett-Packard 5890, USA) equipped with a 5 m Megabore HP-1 column. The oven, injector and detector temperatures were 120, 180 and 210 °C, respectively. Nitrogen was used as carrier at a rate of 1.5 ml min^{-1}.

TPH extraction from soil was performed according to the EPA-3540C method (EPA SW-846, 1996) and quantified gravimetrically. ANOVA statistical analyses of hydrocarbon consumption were performed using four data for each column (two experiments and two measures) to find significant differences between toluene treatment and controls and between types of soil using the Scheffe's Test at a significant level of 0.05.

The soluble organic carbon was measured according to the 9060 EPA method (EPA SW-846, 1996) using a total organic carbon analyzer instrument (Shimadzu TOC-5000A, Japan). Soluble organic carbon was extracted from the soil in distilled water using a relation of 1:10. It was continuously stirred for 5 min and filtered before analysis.

Carbon dioxide production was measured in duplicate with 500 μl in outlet gas samples by GC-TCD (Gow Mac series 550, USA) with a concentric column CTR-1 (Alltech, USA) with helium as carrier gas at a flow rate of 65 ml min^{-1}. The injector, detector and column temperatures were 50, 100 and 40 °C, respectively.

3. Results and discussion

3.1. Hydrocarbon degradation

The toluene effect on TPHs biodegradation treatment for both soil samples is shown in Fig. 1. In the test columns, toluene degradation close to 100% was observed after the first day and complete removal was sustained over all the experiment. This corresponds to a toluene uptake of 5.23 ± 0.16 mg $kg^{-1}_{dry\ soil}$ h^{-1}, (1.9 ± 0.2 g $m^{-3}_{reactor}$ h^{-1}), for columns packed with Soil A and 2.21 ± 0.18 mg $kg^{-1}_{dry\ soil}$ h^{-1}, (0.96 ± 0.3 g $m^{-3}_{reactor}$ h^{-1}) for Soil B. For Soil A, as seen in Fig. 1a, after 20 days, there was not a significant difference in hydrocarbon degradation between the toluene-fed and control columns ($P > 0.05$). However, after 60 days, the hydrocarbon degradation was significantly ($P < 0.05$) improved and was 28% higher in the toluene columns compared the controls. The final TPHs degradation was 37 and 35% for the toluene treated and the control columns, respectively.

For the aged Soil B after 30 days TPHs degradation was around 17% and no significant effect were observed, at a significant level of 0.05, when toluene was added (Fig. 1b). After 100 days, degradation attained around 25% and remained almost constant, final hydrocarbon degradation was 24 and 26% for controls and toluene-amended treatment, respectively.

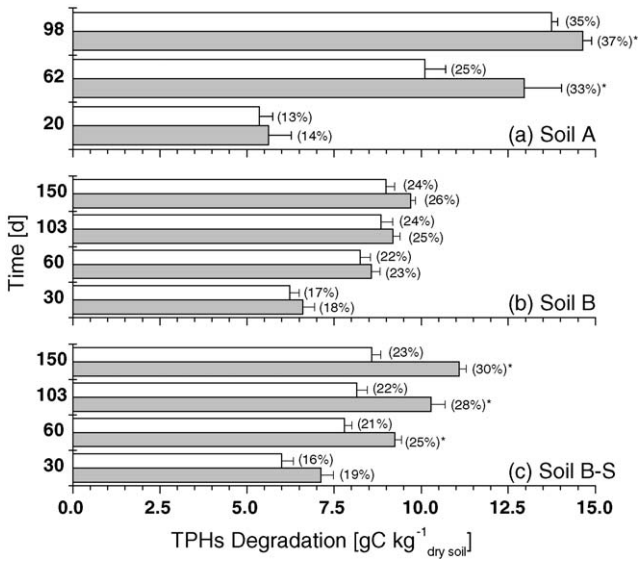

Fig. 1. TPHs biodegradation in packed columns for: (a) Soil A, (b) Soil B and (c) Soil B-S. (■), Toluene-amended and (□), Control. The percentages of TPHs elimination are shown in brackets. (*) Means significantly greater than open bars ($P < 0.05$). Considering a composition of TPHs of 90% carbon, initial concentration for Soil A was 39.15 ± 1.1 g C $kg^{-1}_{dry\ soil}$ and total toluene added per kg of dry soil was 12.42 ± 0.1 g in 98 days. Initial TPHs in Soil B was 36.18 ± 0.88 g C $kg^{-1}_{dry\ soil}$ in Soil B and total toluene added per kg of dry soil was 6.8 ± 0.5 g in 150 days. Error bars are one standard deviation.

The comparison between Soil A and Soil B shows that the TPHs elimination in 150 days in Soil B was considerably lower than in Soil A after 98 days. This result can be a consequence of the aging process in Soil B. It has been frequently reported that the biodegradation rate of organic compounds decreases with aging time until almost negligible consumption is detected even if microbial activity is present. This phenomenon has been attributed to a decreased bioavailability of the target pollutants and that a fraction of the easily metabolized compounds have already been transformed [23,24].

In both soils, TPHs degradation was higher in the gaseous toluene-amended columns than in the controls at a significant level of 0.05. The results were consistent to those previously reported [16], where the presence of toluene favored the biodegradation of phenanthrene and intermediates and mineralization increased from 39 to 86%. Several hypotheses have been suggested to explain the effect of toluene including increased biomass production and the activation of microorganisms or enzymes that improve the consumption rate of both the pollutants and their intermediates. Furthermore it is also possible that cometabolic reactions could be fostered by toluene, which would favor the petroleum hydrocarbon degradation.

The results suggest that sequestering of organic compounds could be limiting biodegradation in the aged Soil B and thus a surfactant pretreatment was evaluated to improve bioavailability. The initial amount of soluble organic carbon was 1.151 ± 0.003 g C $kg^{-1}_{dry\ soil}$ in the Soil B-S, which was 26% higher that that obtained with Soil B (Table 1). As seen in Fig. 1c, in the controls (without toluene) the final TPHs degradation was not significantly different ($P > 0.05$) for Soil B and Soil B-S at around 24%. The final hydrocarbon degradation, after 150

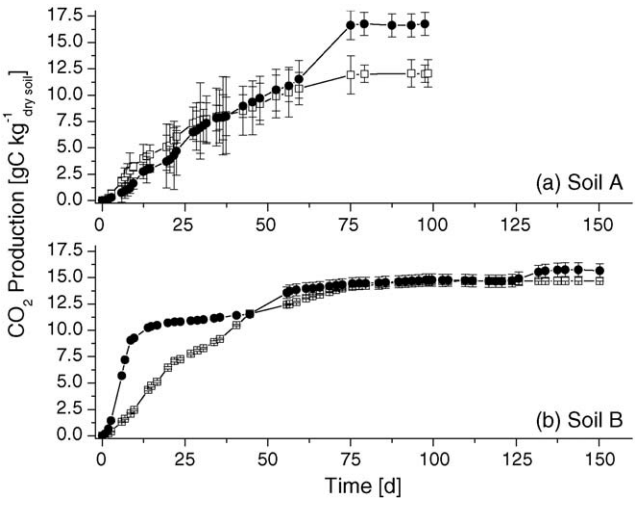

Fig. 2. CO_2 production in packed columns considering complete toluene mineralization. (a) Soil A and (b) Soil B. (□) Controls and (●) Toluene-amended. Error bars are one standard deviation.

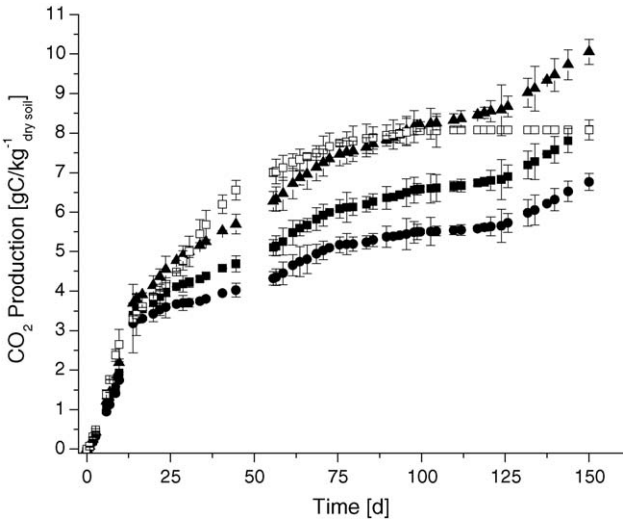

Fig. 3. CO_2 production in packed columns for Soil B-S. (□) Control. For toluene-amended considering (●) 100% toluene mineralization, (■) 80% toluene mineralization and (▲) 50% toluene mineralization. Error bars are one standard deviation.

days, in toluene-amended columns increased to around 30% with the surfactant pretreatment. These results indicate that surfactant addition facilitated the mass transfer but the process was then limited by biological reaction. The treatment with toluene as cosubstrate improved the transformation of the bioavailable compounds. Addition of surfactants, including some synthesized by microorganisms, has been studied as a method to increase biodegradation of hydrophobic compounds by increasing their apparent solubility [9]. On the other hand, surfactants may induce toxicity problems [10,11]. In this case, since the addition of surfactant improved only biodegradation in the toluene-amended treatment, it can be assumed that the limiting process was the biological reaction and not the mass transport. This was confirmed by the indirect measure of the microbial activity as will be discussed below.

3.2. Mineralization of hydrocarbons in columns experiments

Fig. 2 shows the CO_2 production for Soil A and Soil B. To calculate TPHs mineralization in the columns amended with toluene, its complete mineralization (seven CO_2 moles per toluene mol) was considered and this CO_2 was then subtracted from the total produced. In Soil A in 98 days, 12.42 ± 0.10 g of toluene per $kg_{dry\ soil}$ were added to each column while for Soil B, 6.8 ± 0.65 g of toluene per $kg_{dry\ soil}$ were added in 150 days. The CO_2 produced was higher in the amended columns than in the controls. However, the toluene effect on the CO_2 production was different for each soil. Soil A showed increased production at the end of the experiment while for Soil B this effect was observed at the onset of the treatment. In the recently polluted Soil A, the effect of toluene was observed when the easily available TPH had probably been depleted and presumably a certain amount of intermediates, some which may be recalcitrant, had been formed. For Soil B, a positive result of toluene on mineralization was observed at the early steps of the process due to the effect on the compounds already present that resulted from the prolonged aging process. However, at the end of the experiment the CO_2 production was equal for toluene-amended and control columns as the process became limited by either mass transport or the recalcitrance of the residual TPHs.

Comparing the controls from Soil B (Fig. 2b) with Soil B-S (Fig. 3), TPH degradation was similar $P > 0.05$, as shown in Fig. 1, but the surfactant pretreatment inhibited CO_2 production. This inhibition has been reported on the hexadecane and phenanthrene mineralization using a nonionic surfactant in soil and this effect was attributed to the alteration of the microbial population [25]. This effect seems to be accrued by the presence of toluene, despite the fact that TPH degradation was favored by the presence of the cosubstrate as seen in Fig. 1. The inhibitory effect on CO_2 production when toluene was added was reduced after day 125 and a CO_2 production rate of around $0.04\ g\ C\ kg_{dry\ soil}^{-1}\ day^{-1}$ was found while this rate remained close to zero in the control (Fig. 3). This behavior was consistent with the hypothesis that toluene favored the improved utilization of the formed intermediates as in Soil A. In our previous work on phenanthrene biodegradation, a mass balance of labeled-carbon (^{14}C) demonstrated the positive effect of toluene in the biodegradation and mineralization of soluble intermediates [16].

In the previous discussion, the complete mineralization of toluene was assumed, as the used methodology precluded the possibility to evaluate its real value. This consideration underestimates the CO_2 produced from the TPHs. In Fig. 3, two other scenarios are also presented assuming that only 80 or 50% of the toluene was mineralized. It can be seen that in 150 days equivalent mineralization is predicted if only 80% of the toluene carbon was mineralized and that 20% more CO_2 would be produced when mineralization attains 50%.

The TPHs mineralization was calculated from the evolved CO_2 considering a theoretical TPHs composition of 90% of carbon and 100% of toluene mineralization. Endogenous

respiration and mineralization of organic soil components was not considered. For Soil A at final time, in the toluene-amended columns about 43% of the initial TPH was mineralized and 31% for the control columns. In Soil B, final TPHs mineralization was 43 and 40% while for Soil B-S was 19 and 22% in the amended and control reactors, respectively. As the TPH degradation was similar for the controls and amended columns at final time for Soil A and Soil B (Fig. 1), the results confirm that there was an increased transformation of the intermediates produced during the TPH degradation when toluene was simultaneously degraded. Furthermore the effect may be more important if partial toluene mineralization is considered.

3.3. CO_2 production in microcosms experiments (biomass activity)

CO_2 production has been reported as a one of the simplest methods for assessing the overall activity of the soil community [21]. The results from the microcosm experiments performed with soil samples after the toluene treatment and the controls are shown in Fig. 4. The rates obtained for those soil samples treated with toluene were higher in all cases (200% for Soil A and around 50% for Soil B and Soil B-S). As the microcosms experiments were performed without toluene addition and when the TPH degradation rate was low, it is expected that the CO_2 production reflects the microbial endogenous respiration and this amount can be directly related to the active biomass present in the soil samples.

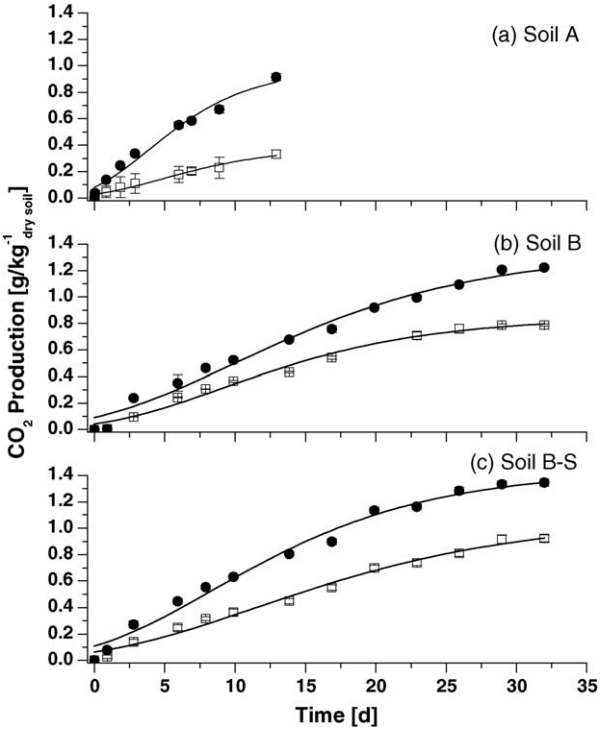

Fig. 4. CO_2 production in microcosms with samples from the column experiments after treatment, for (a) Soil A, (b) Soil B and (c) Soil B-S. (●) toluene-amended treatment and (□) control. Error bars are one standard deviation. No toluene was added.

Consequently, higher accumulated CO_2 implies increased microbial biomass. These results also confirm that toluene was partially used for biomass production and as a result, toluene mineralization was incomplete.

4. Conclusions

The addition of gaseous compounds allows homogeneous delivery of cosubstrates in low concentrations to promote enhanced biodegradation and biomineralization of hydrocarbons while avoiding competitive effects. The final results depend on soil composition, aging and the microbial population present. Previous research with a model soil containing phenanthrene showed that the gaseous cosubstrate treatment had a stronger impact on the mineralization of the intermediate phthalic acid which accumulated from the degradation of the PAH. The results in the treatment of two different hydrocarbon-polluted soils showed that toluene addition increased the microbial activity as shown in the microcosm experiments. The effect on the TPH degradation was more patent in Soil A and Soil B-S where bioavailability was higher confirming previous results that suggested that the addition of the cosubstrate may be more effective when degradation was reaction controlled. The reduced effect with Soil B can probably be attributed to the low bioavailability, due to the aging time, or that the remaining TPH were more recalcitrant, nevertheless in this soil toluene addition allowed stronger initial mineralization. The increased activity in the toluene-amended reactors suggests that a fraction of the carbon was incorporated as biomass and consequently TPHs mineralization was also favored in Soil B-S when toluene was added.

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