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Toxicity Reduction Associated with Bioremediation of Gasoline-Contaminated Groundwaters

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In-situ biodegradation has received increasing attention as a method to remediate gasoline-contaminated soils and groundwaters. Typically, oxygen is added to enhance aerobic biodegradation. However, since oxygen is not very soluble in water and is difficult to distribute uniformly throughout an aquifer, nitrate has been investigated as an alternate electron acceptor. Nitrate has recently been used to stimulate BTX (benzene, toluene and xylene) biodegradation in the laboratory (Zeyer et al. 1986; Major et al. 1988; Gersberg et al. 1991) and in field-scale tests on gasoline contaminated aquifers (Berry-Spark and Barker 1986; Sheehan et al. 1988)

Since there are hundreds of organic compounds in gasoline, the possibility exists that there are toxic intermediate metabolites or byproducts formed by biodegradation which may still exert toxicity. Unfortunately there is no information on the degree of toxicity reduction associated with BTX biodegradation under denitrifying conditions. This study used the Ceriodaphnia acute toxicity test to determine the degree of toxicity reduction associated with remediation of gasoline-contaminated groundwaters under denitrifying conditions, and compared these results to those for the aerobic process.

MATERIALS AND METHODS

Biodegradation of BTX was enhanced under different treatment regimes in the laboratory and the decrease in both BTX levels and toxicity was measured as biodegradation proceeded. Regression lines were plotted using time as the independent variable and LC50 (lethal dose for 50% of the organisms) as the dependent variable.

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A comparison of slopes of the regression lines of the different treatments was made in order to test for significant differences in effectiveness between the treatment methods and between the treatment methods and the control. Tests to determine significant differences between slopes were done by comparing the critical value for the Student-t distribution with the test statistic for each pair of slopes contrasted.

Each experiment consisted of two different groundwater nutrient enrichment treatments and a control, in which no enrichment was made. Three different enrichment strategies were evaluated. For the "nitrate treatment" bottles were spiked with 50 mg L^{-1} nitrate-N (as $NaNO_3$) and 10 mg L^{-1} phosphate-P as (K_2HPO_4). For the "nitrate and peroxide treatment", bottles were spiked with the same amount of phosphate and nitrate and 150 mg L^{-1} of hydrogen peroxide. For the "ammonia and peroxide" treatment, water was spiked with the same amount of phosphate and hydrogen peroxide and 50 mg L^{-1} of ammonia-N (as NH_4Cl).

Gasoline-contaminated groundwater was sampled from below a leaking underground storage tank site in San Diego, California. Groundwater was first bailed from the well, (by purging at least 3 well-bore volumes) and then sampled using 1-gal glass bottles filled with no headspace. These were immediately taken to the laboratory, where they were refrigerated at 4°C until incubation or until the performance of the toxicity test (within 24 hr). Prior to incubation water was transferred to 125-mL bottles, filled to the top and sealed with teflon septum closures. Transfer of the groundwater into these bottles was carried out so as to minimize the introduction of oxygen, and indeed oxygen measurements using the Winkler method showed levels below 1 mg L^{-1} after transfer. At these low O_2 levels, denitrification will proceed if an alternate electron acceptor such as nitrate is available. According to stoichiometry, small amounts of oxygen (1 mg L^{-1}) present at the start of incubation will be used up rapidly after the consumption of less than 1 mg L^{-1} of BTX.

BTX disappearance was followed by peak attenuation on a gas chromatograph (Varian Model 3300, Varian Instruments, Sunnyvale, California) equipped with purge and trap (Model ATOC-1, Valco Instruments Co., Inc., Houston, Texas), a flame-ionization detector and a 2-m 5% SP-1200 packed column (Supelco Inc., Bellefonte, Pennsylvania). The operating conditions were: purge, 60 mL min^{-1} for 12 min; sample size, 5 mL; He, 40 mL min^{-1}; H_2, 100 mL min^{-1}; air, 250 mL min^{-1}; column temperature, 150°C; integrator, Varian 4290.

Ceriodaphnia toxicity testing was performed following the EPA Guidelines (USEPA 1985). Each toxicity test consisted of a minimum of five test concentrations and a control. Three replicate groups of 10 third generation neonate (<24 hr old) daphnids were exposed to each test concentration and control. The duration of the test was 24 hr. Immobilization was measured at 24 hr and the LC50's were calculated for each replicate test by the Moving-Average Angle Method.

Individual test containers (40-mL vials filled with no headspace and fitted with teflon septa) were partially submersed in 10-gal water baths for temperature control. Water baths and test containers were maintained at a constant temperature of 25^0C. The test organism used was *Ceriodaphnia dubia*. All daphnid cultures from which neonates where obtained were fed on a trout chow-Cherophyl-yeast (TCY) diet and 3.5 million cells of the green alga *Selenastrum capricornotum*. *Ceriodaphnia* neonates were not fed during the tests. Water quality characteristics were determined at the beginning of each test and ranged from 6.71-8.55 for pH, 140-290 mg L^{-1} for alkalinity, 200-475 mg L^{-1} as $CaCO_3$ for hardness. Dissolved oxygen levels were mantained above 40% saturation throughout the tests.

RESULTS AND DISCUSSION

Accurate prediction of the toxicity reduction to be expected by BTX bioremediation is difficult due to the presence of other organic compounds, some of which are volatile. Toxicity tests were performed on gasoline-contaminated groundwater before and after aeration of this water to estimate the contribution of the volatile organic fraction (a large proportion of which is BTX) to the overall toxicity. Toxicity tests were also performed with uncontaminated groundwater to assess the natural toxicity of the groundwater. Uncontaminated water showed some toxicity, resulting in an LC50 of 74.13% effluent (Table 1). When contaminated groundwater was aerated, thus volatilizing the purgeable hydrocarbons such as BTX, the toxicity tests showed LC50's which varied from 58.47±4.44% effluent in March 1990, to 70.33±4.04% in May 1990, and 76.66±7.63% in October 1990. The LC50's before aeration treatment in the dates above were 5.4±0.67% effluent in March, 8.57±1.05% in May and 12.74±0.76% in October of 1990. On the latter two dates tested, aeration of the water which removed the volatile hydrocarbons, reduced the toxicity of the gasoline-contaminated water to near the level of the uncontaminated groundwater, suggesting that the non-volatile organic compounds contribute only slightly to the overall toxicity (Table 1).

The toxicity tests performed with dilution water spiked with 70 mg L^{-1} BTX, in levels and proportion of each of the monoaromatic hydrocarbons, similar to that which existed in the actual aquifer, resulted in an LC50 of 16.9±1.6% effluent as compared with 12.7±0.8% for the gasoline-contaminated groundwater (Table 1). This supports our conclusion that the BTX fraction of the total volatile hydrocarbons accounts for most of the toxicity in these gasoline-contaminated groundwaters.

Table 1. Toxicity of groundwater contaminated with gasoline or enriched with BTX

	*LC50'S March 90	LC50'S May 90	LC50'S Oct. 90
Contaminated Groundwater			
Before Aeration	5.4±0.7	8.6±1.0	12.7±0.8
After Aeration	58.5±4.4	70.3±4.0	76.7±7.6
Uncontaminated Groundwater (sampled upgradient of site)	-	-	74.1±0
Uncontaminated Groundwater (enriched with 70 mg L^{-1} BTX)	-	-	16.9±1.6

* LC50's calculated as percent effluent.

Since BTX appears to be responsible for a major fraction of the toxicity, it is important to evaluate how much toxicity reduction can be achieved by BTX biodegradation. This was done by incubating gasoline-contaminated groundwaters with varying regimes of nutrient and electron acceptor enrichments. These regimes were (1) nitrate and phosphate enrichment, whereby nitrate acted as both a nutrient and electron acceptor and phosphate acted solely as a nutrient, (2) similar to (1) above, but with the addition of hydrogen peroxide which upon decomposition acts as an oxygen source, and (3) phosphate, and ammonia and peroxide enrichment, whereby ammonia was used instead of nitrate as a source of the nutrient nitrogen.

Toxicity tests were performed at different stages of the incubations, and the 24-hr LC50 was calculated. BTX levels were also measured (Table 2). BTX losses ranged from 82%-88% in the "nitrate" treatments and 87%-95% in the "nitrate and peroxide" treatments to 95% in the "ammonia and peroxide" treatment. BTX was lost even in the control due to background biodegradation and/or physical losses such as volatilization and adsorption to glass (Gersberg et al. 1991).

Table 2. BTX loss during toxicity reduction tests

	Initial BTX (mg L^{-1})	Final* BTX (mg L^{-1})			
Expt		Control	Nitrate	Nitrate + Perox.	Ammonia + Perox.
#1	58.5 (±0.4)	30.6**	6.9 (±5.25)	5.1 (±0.9)	
#2	72.7 (±12.9)	30.7 (±1.5)	8.2 (±6.8)	7.6 (±8.8)	
#3	80.6 (±9.7)	20.2 (±1.5)	9.5 (±14.4)	9.9 (±5.2)	
#4	66.8 (±3.3)	36.4**		4.5**	2.9 (±2.5)

* 12 wk incubation ** only one sample analyzed

The regression lines representing the toxicity reduction in the different treatments and the control are shown in Figs. 1-4. Statistical analyses of data presented in Figs. 1-4 show that reduction in toxicity in the "nitrate" and "nitrate and peroxide" amended groundwater occurs at a significantly higher rate than in the non-enriched gasoline-contaminated groundwater used as control ($P < 0.005$).

Statistical comparison of treatments did not show that the addition of nitrate and peroxide is more effective than the addition of nitrate alone ($P > 0.15$). In this respect these results show that denitrification is nearly as efficient in toxicity reduction as the aerobic process. This might be expected, since from thermodynamic calculations, the energy yield under denitrifying conditions is nearly 80% of the maximum aerobic yield (Payne 1981).

Statistical comparison between the "nitrate and peroxide" versus the "ammonia and peroxide" treatment showed that the latter regime was significantly more effective in reducing the toxicity ($P < 0.005$) (Fig. 4), this is most

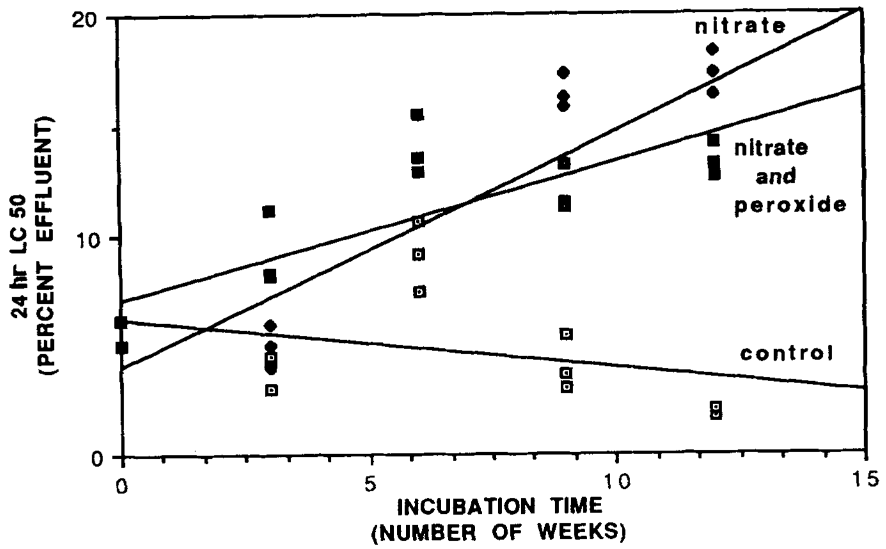

Figure 1. Linear regression analysis of the relationship between toxicity reduction and time of incubation for the "nitrate" and the "nitrate and peroxide" enrichments.

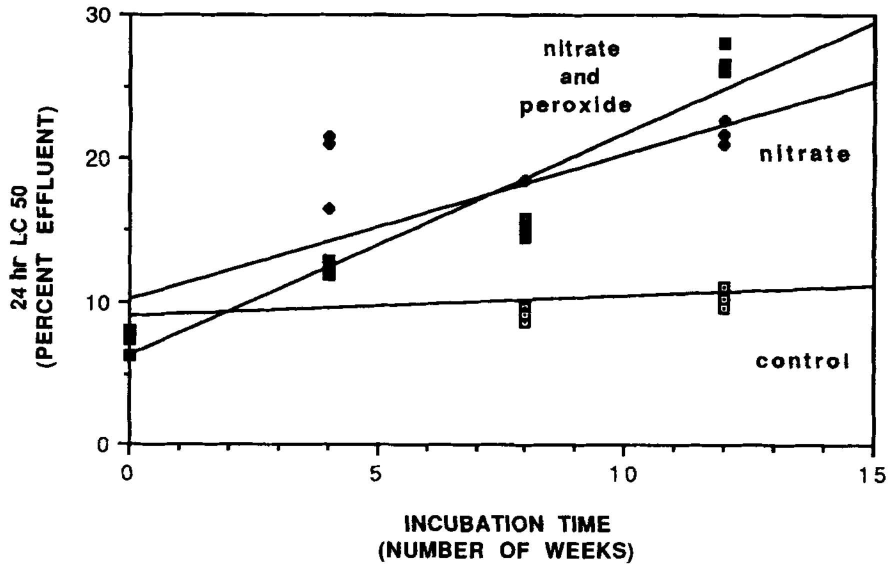

Figure 2. Linear regression analysis of the relationship between toxicity reduction and time of incubation for the "nitrate" and the "nitrate and peroxide" enrichments.

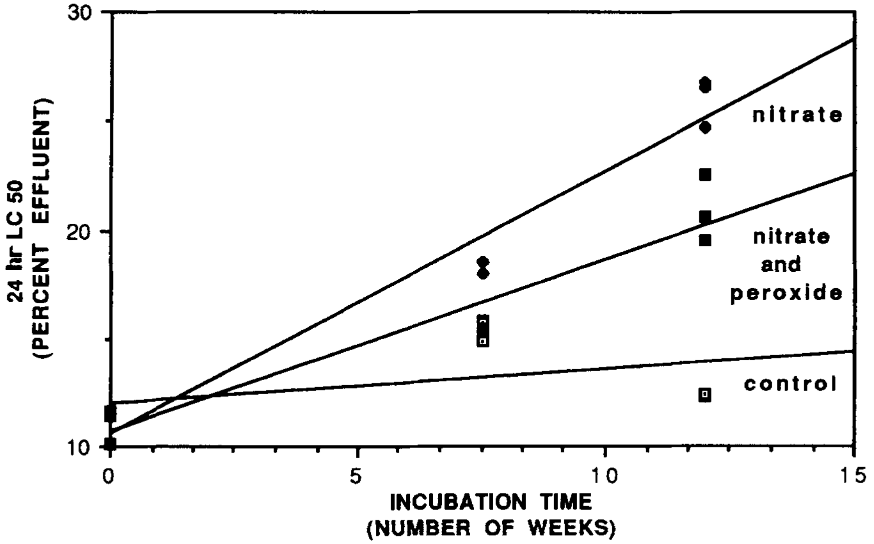

Figure 3. Linear regression analysis of the relationship between toxicity reduction and time of incubation for the "nitrate" and the "nitrate and peroxide" enrichments.

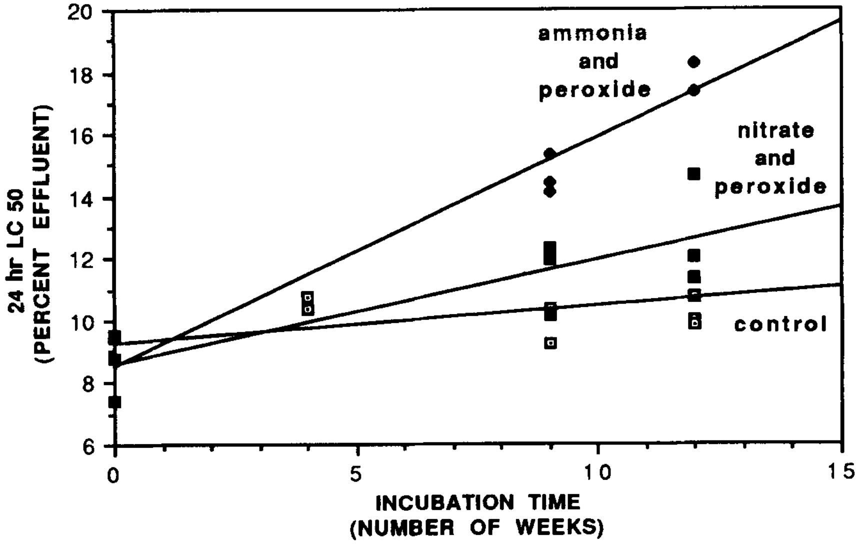

Figure 4. Linear regression analysis of the relationship between toxicity reduction and time of incubation for the "ammonia and peroxide" and the "nitrate and peroxide" enrichments.

probably due to the fact that in the form of ammonia, the nutrient nitrogen is already reduced, and therefore more easily assimilated by bacteria into biomass.

This study provides evidence that the Ceriodaphnia toxicity bioassay can be a useful tool in a toxicity reduction evaluation of gasoline-contaminated groundwaters. Additionally, such biomonitoring can be valuable in evaluating alternative bioremediation strategies, especially when the byproducts and intermediates of biodegradation have not been well identified.

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