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Microbial Degradation of Benzene and Toluene in Groundwater

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Certain organic pollutants reaching the groundwater are subject to biotransformations. Often many of these contaminants are detected in the subsurface environment within a range of 200-1000 $\mu g\ L^{-1}$ which may lead to potential health risks (Tabak et al., 1981). Currently, remedial measures promoting microbial degradation of pollutants are becoming very attractive because of their cost-effectiveness in removal of the contaminants (Delfino and Miles, 1985; Jamison et al., 1974).

In case histories, the native microbial population within aquifers has been stimulated by the addition of nutrients when deficient, or by the addition of microorganisms acclimated to the contaminants (Lee and Ward, 1984). Current technology for reclaiming groundwater polluted with petroleum hydrocarbons involves i) pumping the water into an aerated stripping tower, ii) removal by sorbents, or iii) biodegradation in situ or pumped into a bioreactor (Nyer, 1985). Among the bioreactors, fixed film and suspended growth reactors are the most popular systems.

Gasoline contamination of groundwaters is becoming an alarming and widespread problem. Petroleum hydrocarbons are capable of migrating from leaking underground storage tanks and pipelines through the soil matrix within the saturated zone. A major concern with petroleum contamination is the benzene, toluene and xylene (BTX) content reaching the groundwater because of their solubility and high toxicity (Ogawa et al., 1981). The state of California Department of Health Services now recommends that remedial action be taken when the concentration of benzene and toluene exceeds 0.7 and 100 $\mu g\ L^{-1}$, respectively. The purpose of this study was to assess biodegradation of benzene and toluene in groundwater upon amendment with nutrients and an enriched hydrocarbon oxidizing culture.

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MATERIALS AND METHODS

Gasoline-contaminated groundwater was collected from a monitoring well at a depth of 12-15 ft in Los Angeles, CA. Upon collection, the water sample was stored at 4°C in tightly capped containers with no headspace to prevent loss by volatilization. The groundwater sample had the following chemical and biological properties: pH, 7.93; total organic carbon (TOC), 23 $\mu g\ mL^{-1}$; NH_4-N, 0.22 $\mu g\ mL^{-1}$; NO_3-N, 0.21 $\mu g\ mL^{-1}$; total P, 0.37 $\mu g\ mL^{-1}$; petroleum hydrocarbon content, 6.2 mg L^{-1}; benzene, 477 $\mu g\ L^{-1}$; toluene, 561 $\mu g\ L^{-1}$; xylenes, 153 $\mu g\ L^{-1}$; gasoline degrading bacterial population, 710 colony-forming units (CFU) mL^{-1}; total heterotrophic population, 3430 CFU mL^{-1}. In the chemical analyses reported above, tests were performed as described in Standard Methods for the Examination of Water and Wastewater (1985). Total petroleum hydrocarbon content was determined by the EPA method 418.1 (US EPA, 1983).

Petroleum degrading bacteria in groundwater were determined by the spread plate technique. Various dilutions of water samples were added to standard media consisting of the following per liter: 1 g NH_4NO_3, 10 mg yeast extract, 15 g agar, 1.1 g K_2HPO_4, 0.8 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.5 g $CaCl_2 \cdot H_2O$, 2.0 mg $FeSO_4 \cdot 7H_2O$, 2.0 mg $MnCl_2 \cdot 4H_2O$, 1.0 mg $CuSO_4$, 1.0 mg H_3BO_3, and 1.0 mg $ZnSO_4 \cdot 7H_2O$. The pH was adjusted to 7.0 prior to autoclaving. Gasoline vapors served as the C source. A sterile cotton plug saturated with gasoline was placed in the lids of the inverted plates. After 5 days of incubation, the bacterial colonies were counted.

Soil bacteria capable of utilizing gasoline as a sole carbon source were also isolated from a site previously exposed to gasoline contamination. Organisms were enriched on the same mineral salt medium, as described above, with carbon being provided in the form of hydrocarbon vapors evolving from cotton plugs saturated with gasoline. This mixed culture was used as an inoculum to accelerate the rates of biodegradation of benzene and toluene.

Benzene, toluene and xylenes were measured by gas chromatography. The gas chromatograph (Shimadzu) was equipped with a flame-ionization detector (FID) and a 2-m 3% OV-1 on chromosorb WHP (80/100 mesh) stainless steel packed column. A typical elution sequence in terms of retention time (min) of BTX consisted of benzene, 1.26; toluene, 2.28; xylenes, 4.43 and 4.84 and aliphatics (C_{8+}) 8.95 to 26.17. The operating conditions consisted of the following: sample size, 1 μL; N_2, 13 mL min^{-1}; H_2, 50 mL min^{-1}; air, 500 mL min^{-1}; column temperature, 50-325°C, 15°C min^{-1}; detector temperature, 340°C; integrator, HP 3390A. Peak areas and retention times were compared to reference standards.

Benzene and toluene in gasoline contaminated groundwater were monitored to follow the rates of biodegradation in batch flasks with various amendments. After filtration (Whatman #2), 50-mL samples of contaminated groundwater were added to 250-mL side-arm Erlenmeyer glass flasks and stoppered with a Mininert Teflon septum. The treatments consisted of a i) sterile control (filter-sterilized, 0.22 μm), ii) unamended groundwater, iii) amended with 100 mg L^{-1} NH_4NO_3-N, and iv) amended with NH_4NO_3-N (100 mg L^{-1}) plus a 1-mL inoculum of an enrichment culture consisting of gasoclastic microflora. The flasks were shaken (120 rpm) at ambient temperature (ca. 23°C). Microbial growth was monitored through the sidearms, without opening the flasks, using a Bausch and Lomb Spectronic 20 spectrophotometer at 535 nm. The benzene and toluene content was determined by taking 1-μL samples through the septa and direct injection into the GC.

RESULTS AND DISCUSSION

In all non-sterilized treatments, the toluene concentration dropped dramatically within 30 h (Fig. 1). The amount of time required for toluene to decrease to acceptable limits (100 μg L^{-1}) by microbial degradation was 23, 17, and 14 h for the unamended sample, groundwater plus a N source, and groundwater inoculated with the enriched culture of hydrocarbon oxidizers plus a N source, respectively. It was evident that with turbulent mixing (which promotes O_2 transfer) on-going biodegradation can be enhanced. The rates of toluene removal for each treatment were as follows: sterile control, 5.2 μg L^{-1} h^{-1}; unamended, 18.8 μg L^{-1} h^{-1}; plus N, 29.1 μg L^{-1} h^{-1}; and plus N and inoculum, 45.9 μg L^{-1} h^{-1}, respectively.

The benzene content slowly degraded in the unamended sample, but dramatically dropped in concentration in the N-treated water and in the inoculated plus N-amended water (Fig. 2). During 48 h of incubation the benzene concentration in the unamended water dropped from 480 to 218 μg L^{-1}. Upon inoculation with the enriched mixed culture plus 100 mg N L^{-1}, the benzene level dropped to 35 μg L^{-1} in 20 h. However, after 20 h of incubation there was little change in the benzene concentration. Apparently 22-35 μg L^{-1} of benzene did not provide enough C to sustain an active microbial population for degradation in groundwater and never did approach a level considered to be non-hazardous (0.7 μg L^{-1}). The rates of benzene removal for each treatment were as follows: sterile control, 6.4 μg L^{-1} h^{-1}; unamended, 5.7 μg L^{-1} h^{-1}; plus N, 12.7 μg L^{-1} h^{-1}; and plus N and inoculum, 25.4 μg L^{-1} h^{-1}.

The bacterial population capable of utilizing gasoline as a C and energy source was relatively low (710 CFU mL^{-1}), but comparable to that reported for groundwater samples involving a gasoline pipeline leakage near Ambler, Pennsylvania in July, 1971 (Jamison et al., 1974). Maximum degradation rates of toluene were observed from the onset of the experiment to 30 h for the unamended sample

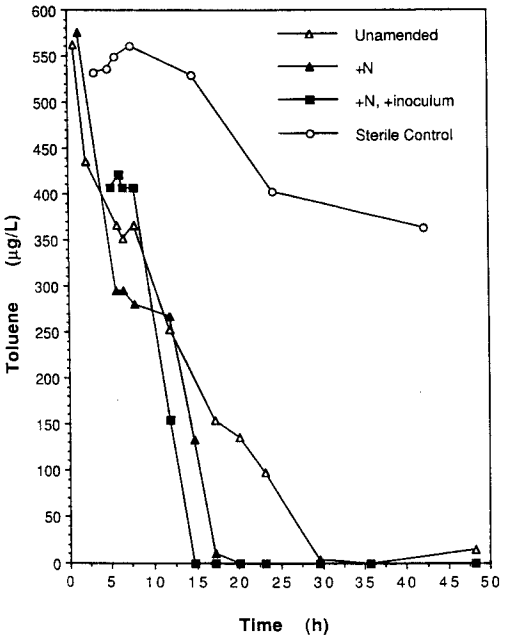

Figure 1. Removal of toluene from petroleum-contaminated groundwater upon incubation in a closed agitated system.

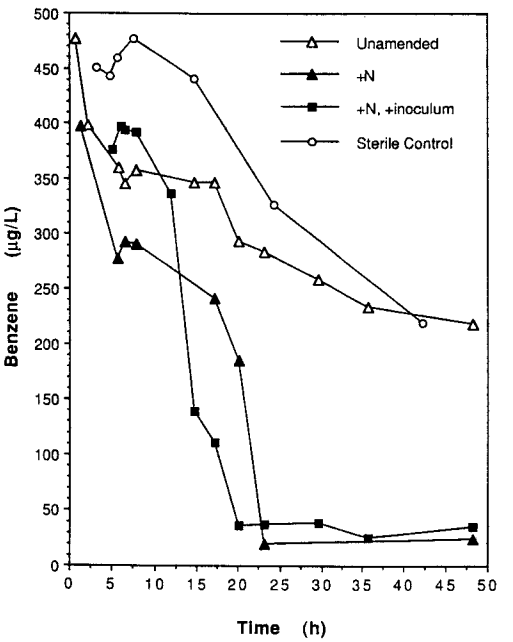

Figure 2. Removal of benzene from petroleum-contaminated groundwater upon incubation in a closed agitated system.

and up to 15 h with the seeded hydrocarbon oxidizers plus N. Maximum degradation rates of benzene occurred up to 23 h. The seeded bacteria responded earlier than the indigenous microflora as evident by less of a lag phase in the growth cycle (Fig. 3). Maximum biodegradation rates of toluene and benzene coincided with the bacterial exponential growth phase. The rate of bacterial growth declined as the concentration of the benzene and toluene decreased.

The level of BTX on site is often monitored as an index of the degree of petroleum contamination since these constituents are typical aromatics found in petroleum. Generally, in groundwater, the nitrogen content is too low to support appreciable microbial growth to utilize petroleum as a carbon and energy source, thus a N amendment is often needed to enhance biodegradation. Molecular oxygen is required for the microbial oxidation of hydrocarbons. This study reveals that both components (oxygen and nitrogen) were major limiting factors in the biodegradation of benzene and toluene. A natural mixed cocktail of adapted microorganisms obtained by selective enrichment was used as the inoculum to accelerate the biochemical breakdown of benzene and toluene in groundwater. The inoculum plus N treatments enhanced the biodegradation rate of benzene 4.5-fold and toluene, 2.4-fold. More than 95 and 100% of the benzene and toluene in groundwater, respectively, were removed through microbial action within 73.5 h.

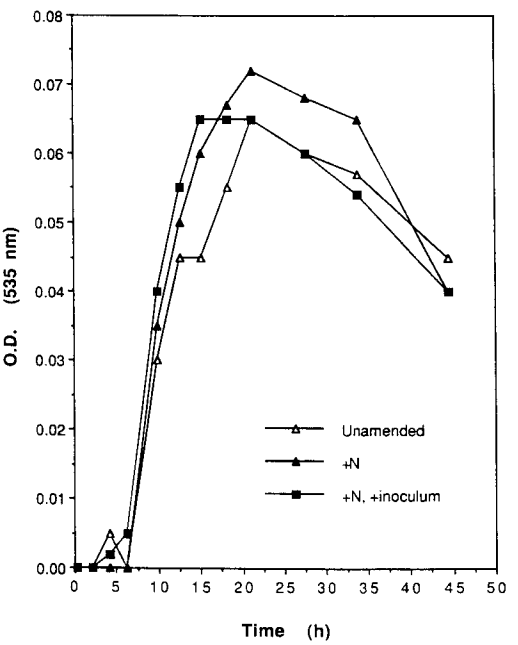

Figure 3. Optical density of contaminated groundwater upon incubation in a closed agitated system.

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