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A Benzene–Toluene Mixture to Adapted Microbial Communities

Dr. J. Venkata Rajkumar

Assistant Profesor, Avanti institute of Pharmaceutical Sciences, Gunthapalli, Abdullapurmet, Rangareddy, Telangana, India.

***Corresponding Author**: J. Venkata Rajkumar, Assistant Profesor, Avanti institute of Pharmaceutical Sciences, Gunthapalli, Abdullapurmet, Rangareddy, Telangana, India.

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Abstract

The rate of degradation of a benzene–toluene mixture in aerobic microcosms prepared with samples of an aquifer that lies below a petrochemical plant (SIReN, UK). Five samples exposed to different concentrations of benzene (from 0.6 to 317 mg l−1) were used. Fast degradation (approx. 1–6 mg l−1 day−1) of both contaminants was observed in all groundwater samples and complete degradation was recorded by the seventh day except for one sample. We also identified the microbial community in each of the samples by culture-independent techniques.

Keywords: biological active compounds; subarctic origin; medicinal plant

Introduction

Many contaminated subsurface environments are impacted by a mixture of the gasoline oxygenates, benzene, toluene, ethylbenzene, xylenes (BTEX), alkanes and polyaromatic hydrocarbons as well as a range of other contaminants. BTEX compounds are of particular interest because they are highly soluble in water and highly toxic. Several technologies are currently applied to remediate contaminated areas by the oil industry. However, BTEX contamination is still an issue of paramount importance, the better understanding of contaminated site communities and their particular behaviors towards different compound mixtures is critical in order to select the best bioremediation strategy.

Materials and Methods

Groundwater was sampled on 15 April 2003; all samples (309d, W18i, 309s and 308i) were collected with a bladder type pump except DW3s that was collected with a peristaltic pump. Aerobic microcosms were prepared in triplicates by dispensing 20 ml of the groundwater from wells 309d, DW3s, W18i, 309s and 308i into 110-ml serum bottles. The groundwater samples had been previously stored in the laboratory at 12°C and no residual benzene or toluene were detected when measured by GC-FID. The microcosms were spiked with benzene and toluene to give each a concentration of 25 mg l−1 in order to have a medium concentration of the contaminants and observe which one is degraded preferentially when the starting concentration is the same. Microcosms were later crimp-sealed with PTFE-lined silicone septa. The amount of oxygen in this volume of headspace (90 cm3) had previously been shown to be sufficient to allow complete aerobic degradation of benzene at this concentration without depletion of oxygen.

Gas chromatography was used to measure the benzene and toluene concentrations in microcosms with a Unicam 610 Series GC fitted with a 4-mm internal diameter, glass packed column (10% apiezon on chromosorb W) and a flame ionization detector (FID). The temperatures were at the injector 250°C, column 155°C and detector 250°C, and detection was set at medium sensitivity. Hydrogen was used as carrier gas at a flow rate of 1.0 ml min−1. Autoclaved microcosms were used as controls throughout the experiment. Standards and controls all had the same liquid/headspace ratio, and were held at the same temperature as the test samples. Microcosms were measured at time zero and every 2 days until hydrocarbons had been degraded. Degradation rates were calculated from linear regression of benzene or toluene concentrations curves by using Origin 7.

DNA extraction

Microbial community DNA was extracted from the microcosms as previously described. Nucleic acids were extracted from the start of the degradation (2 days) and every 2 days until degradation was complete, one microcosm was sacrified for each DNA extraction. Cells were pelleted by centrifuging 20 ml of groundwater at 3,200 g for 10 min at 4°C, the pellet was resuspended in 0.5 ml potassium phosphate buffer 240 mM (pH 8.0) and transferred to a Lysis matrix B (Bio Gene). Next, 450 µl of Phenol:Chloroform:Isoamylalcohol (25:24:1, pH 8), mix was added and the matrix was then shaken in a bead beater at 2,000 rpm for 30 s. The mixture was centrifuged at 4°C for 60 s [RCF (×g)] and 450 µl from the aqueous top layer was collected. An equal volume of Chloroform:Isoamylalcohol (24:1) was added and mixed by inversion. A volume of 400 µl of the top aqueous layer was again collected and transferred to a new micro-centrifuge tube. Exactly 40 µl of 3 M sodium acetate pH 5.2 and 1 ml of ice-cold 100% ethanol were added to be incubated on ice for 30 min and later centrifuged at 15,000 g for 25 min at 4°C. The supernatant was discarded and the pellet washed with 70% ethanol in DEPC-treated water, and air dried for 30 min. The DNA was resuspended in 30 µl of DEPC water.

Results

When groundwater samples were supplemented with the toluene and benzene mixture (25 mg l−1 each) fast degradation of both contaminants was observed with a constant rate; complete degradation of both contaminants was observed by the seventh day for all samples but 309d. The DGGE profile of the bacterial communities during the degradation experiments shows that the community from each well was different, but for each well it remained stable during the period of incubation, suggesting that the degrading community is established early on. Cluster analysis of the resulted DGGE gel confirmed this observation. Moreover, the dendrogram shows that there is 100% similarity between days 2 to 6 in samples 309d, 308i and W18i, while the community changes slightly on day 6 in samples 309s and DW3s.

Discussion

The microcosms for this study were prepared with groundwater samples from the wells 309d, 308i, 309s, DW3s and W18i at the SIReN site. All locations were exposed to hydrocarbons in situ. Upon addition of the hydrocarbons benzene and toluene to microcosms, the lack of a lag phase and the fast degradation of both hydrocarbons can be explained by the fact that the groundwater had developed an aerobic bacterial community readily adapted to the hydrocarbons. Several factors, such as the availability of organisms as well as enzymes and the presence of other substrates, have been shown to influence

the relative rates of degradation of benzene and toluene, including the enhanced degradation of benzene in the presence of toluene.

Conclusion

The addition of a benzene–toluene mixture to adapted microbial communities results in a rapid and concomitant degradation of both contaminants (7 days) with rates of degradation of 1 to 6 mg l−1 day−1; these rates are independent of the level of contamination to which the samples were exposed.

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