Anaerobic Benzene Degradation in Petroleum-Contaminated Aquifer Sediments after Inoculation with a Benzene-Oxidizing Enrichment

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Sediments from the sulfate-reduction zone of a petroleum-contaminated aquifer, in which benzene persisted, were inoculated with a benzene-oxidizing, sulfate-reducing enrichment from aquatic sediments. Benzene was degraded, with apparent growth of the benzene-degrading population over time. These results suggest that the lack of benzene degradation in the sulfate-reduction zones of some aquifers may result from the failure of the appropriate benzene-degrading sulfate reducers to colonize the aquifers rather than from environmental conditions that are adverse for anaerobic benzene degradation.

Persistence of benzene under sulfate-reducing conditions in petroleum-contaminated aquifers. There are extensive anoxic zones in many petroleum-contaminated aquifers (1, 7). Although anaerobic microbial processes can remove alkylated monoaromatic hydrocarbons from petroleum-contaminated aquifers, highly toxic benzene often persists under in situ anaerobic conditions (7). For example, benzene appears to be degraded slowly, if at all, under sulfate-reducing conditions in petroleum-contaminated aquifers (2, 14, 16). This is despite the fact that the potential for benzene oxidation coupled to sulfate reduction to carbon dioxide, with sulfate serving as the sole electron acceptor, according to the following reaction: 4C₆H₆ + 15HSO₄⁻ + 15H⁺ + 4H₂O → 24HCO₃⁻ + 15HS⁻ + 9H⁺. Similar percentages of benzene-dependent sulfate reduction have been observed in studies with benzene-adapted marine and estuarine sediments (9, 17).

The results with the freshwater aquatic sediments demonstrate that microorganisms that can oxidize benzene with the reduction of sulfate can flourish under freshwater conditions. Thus, a lack of appropriate salinity cannot account for the persistence of benzene in freshwater petroleum-contaminated aquifers. Another possibility for the persistence of benzene in the aquifer sediments was that even though environmental conditions might be suitable for benzene oxidation coupled to sulfate reduction, the sediments lack the appropriate benzene-degrading sulfate-reducing microorganisms. In order to evaluate this, aquifer sediments were inoculated (10% [wt/wt]) with the benzene-adapted freshwater aquatic sediments (Fig. 1A). Following inoculation, benzene degradation proceeded without a lag. With the depletion of the benzene initially present in
the sediments, more benzene was added, resulting in continued degradation (Fig. 1A). After the inoculated aquifer sediments of Fig. 1A had been refed benzene five times, this sediment was used to provide a 10% inoculum for another bottle of unadapted aquifer sediments, which then also rapidly metabolized benzene (Fig. 1B). This procedure was repeated two more times, with continued rapid benzene degradation in all inoculated sediments (Fig. 1C and D). This was the case even in the final transfer (Fig. 1D) in which the amount of aquatic sediment from the initial benzene-degrading inoculation constituted less than 1 part per 10,000 of the sediment mass.

The finding that benzene continued to be rapidly degraded after the aquatic sediment had been effectively diluted out indicates that the addition of the aquatic sediments did not stimulate benzene degradation by changing the physical-chemical characteristics of the aquifer sediments. The fact that the capacity for benzene degradation was maintained with successive transfers of adapted sediments into unadapted aquifer sediments suggests that the factor responsible for benzene degradation was capable of replication, i.e., that it was a benzene-degrading microorganism that originated from the freshwater aquatic sediments.

Previous studies have demonstrated that benzene also persists in anaerobic sediments from the Fe(III) reduction zone of this aquifer unless the availability of Fe(III) is artificially enhanced with the addition of Fe(III) chelators or humic substances (12, 13). Sulfate reduction is generally inhibited in the presence of Fe(III) because Fe(III) reducers outcompete sulfate reducers for electron donors (11). However, it seemed possible that benzene degradation in the Fe(III)-reducing aquifer sediments could also be stimulated with the benzene-oxidizing, sulfate-reducing inoculum since previous studies (12, 13) had indicated that there should be no Fe(III) reducers that would be able to compete with the sulfate reducers for benzene.

In order to evaluate this, aquifer sediments in which Fe(III) reduction was the terminal electron accepting process (TEAP) were amended with 20 mM ferrous sulfate and then inoculated with benzene-adapted aquatic sediments as described above. Inoculation of the Fe(III)-reducing sediments stimulated benzene degradation just as it had in the sediments in which sulfate reduction was the TEAP. Once the inoculated Fe(III)-reducing sediments were adapted for rapid benzene degradation, they could be used as inocula to stimulate benzene degradation in unadapted aquifer sediments. The involvement of
sulfate reduction in this benzene degradation was evaluated with molybdate after the third such 10% transfer, when the volume of the original aquatic sediment inoculum was no more than 1 part per 1,000 of the sediment mass. Molybdate inhibited both the loss of benzene over time and the production of $^{14}$CO$_2$ from $[^{14}$C]benzene (Fig. 3).

These results demonstrate that the inoculated benzene-degrading sulfate reducers were effective in stimulating benzene oxidation coupled to sulfate reduction in Fe(III)-containing sediments and Joan Woodward for technical assistance.

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**REFERENCES**


