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6. AUTHOR(S)
Derek R. Lovley

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Massachusetts
Department of Microbiology
106N Morrill IV North
U of MA, Amherst, MA 01003

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13. ABSTRACT *(Maximum 200 words)*

The potential for anaerobic degradation of polycyclic aromatic hydrocarbons (PAHs) in contaminated harbor sediments was investigated. PAHs were anaerobically oxidized to carbon dioxide in petroleum-contaminated sediments from San Diego Bay, Tampa Bay, and Boston Harbor. Studies with molybdate, a specific inhibitor of sulfate reduction, as well as investigations in which sulfate levels were varied, demonstrated that PAH oxidation was coupled to sulfate reduction. Pristine harbor sediments did not have a significant potential for anaerobic PAH oxidation, but could be adapted for PAH oxidation by exposure to PAhs or inoculation with PAH-oxidizing microrganisms. Exposure of pristine sediments to PAH resulted in the growth of PAH-oxidizing microorganisms. PAHs added to sediments as part of complex fuel mixtures were degraded. The alkanes in the complex fuel mixtures were also anaerobically degraded under sulfate reducing conditions. In estuarine sediments in which concentrations of sulfate were low, PAH and alkane degradation could be stimulated with the addition of sulfate. These studies have demonstrated for the first time that PAHs can be oxidized under the anaerobic conditions that predominate in most contaminated harbor sediments and suggest that anaerobic strategies for remediation of contaminated sediments and dredge spoils are possible.

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FINAL REPORT

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PRINCIPLE INVESTIGATOR: Derek R. Lovley

INSTITUTION: University of Massachusetts

GRANT TITLE: Anaerobic oxidation of hydrocarbon contaminants in marine and estuarine sediments

AWARD PERIOD: 15 November 1995-30 September 1998

OBJECTIVE: The purpose of this research is to investigate the potential for anaerobic degradation of polycyclic aromatic hydrocarbons (PAHs) under sulfate-reducing conditions in marine harbor sediments.

APPROACH: Rates of anaerobic PAH oxidation in harbor sediments are determined by measuring oxidation of [^{14}C]-PAHs to $^{14}CO_2$, or, when radiolabelled PAHs are unavailable, by measuring the loss of the PAHs over time. Enrichment and pure cultures of PAH-oxidizing microorganisms are established using adaptations of techniques in routine use in our lab. Sediments from sites with various degrees of PAH exposure and laboratory studies in which pristine sediments are amended with PAHs are investigated to determine the effect of prior PAH exposure on PAH degradation. PAH degrading populations are monitored with traditional most probable number techniques and molecular methods.

ACCOMPLISHMENTS:

Further studies on the potential for anaerobic PAH degradation in San Diego Bay sediments demonstrated that naphthalene and phenanthrene were consistently degraded in petroleum-contaminated sediments from the Naval Station site, but not in the more pristine sediments from the Shelter Island site. PAH oxidation was sulfate-dependent and also inhibited by molybdate, a specific inhibitor of sulfate reduction. These findings indicate that sulfate reducers are involved in PAH oxidation. Investigations into the range of PAHs that could be degraded revealed that methylnaphthalene, fluorene, and fluoranthene were also oxidized in the Naval Station sediments. Differences in the degradative capacity between the Naval Station and Shelter Island sites was related to the structure of the microbial community rather than unfavorable physical/chemical conditions at the Shelter Island site. This was demonstrated by the fact that if the Shelter Island sediments were inoculated with Naval Station sediments, then PAHs were readily degraded in the Shelter Island sediments. It was also found that the benzene- and PAH-degrading populations in these sediments were different. A paper summarizing these results was published in *Applied and Environmental Microbiology*.

Studies at a variety of harbor sites demonstrated that there is a direct link between prior hydrocarbon contamination of the sediments and the capacity for anaerobic PAH degradation. In sediments from Boston Harbor, Tampa Harbor, and Norfolk Harbor, [^{14}C]-PAHs added to sediments were readily oxidized to $^{14}CO_2$ in hydrocarbon-contaminated sediments. There was no PAH oxidation in nearby sediments which had not been contaminated. However, when uncontaminated sediments were anaerobically incubated in the laboratory with added PAH, then within months, these sediments were also capable of anaerobic PAH oxidation. Studies with a novel method for enumerating anaerobic PAH-degrading microorganisms demonstrated that the increase in capacity for PAH degradation upon exposure to PAHs was associated with an increase in PAH-degrading microorganisms. A paper summarizing these results has been accepted for publication in a special issue of *Chemical Geology*.

Studies on other environmental factors that might influence PAH degradation in harbor sediments demonstrated that the sulfate concentration was key in determining the extent of PAH degradation. In estuarine sediments in which sulfate levels fluctuate, it was found that addition of sulfate to the sediments greatly stimulated not only the degradation of PAHs, but also other hydrocarbons such as alkanes. Gypsum, an insoluble sulfate source, was very effective in stimulating hydrocarbon degradation. These results suggest that insoluble sulfate might be readily added to harbor sediments in order to enhance hydrocarbon degradation in instances in which sulfate concentrations are low due either to rapid consumption of sulfate in heavily contaminated sediments or lower sulfate levels in the overlying water because of freshwater inputs. A manuscript summarizing these results is in preparation.

In fuel-spill contamination of harbor sediments, PAHs are introduced into sediments as a component of complex fuel mixtures. Therefore, studies on the degradation of complex mixtures of hydrocarbon components, including PAHs and alkanes was investigated. It was found that, in addition to the PAHs, much of the alkane component of the fuel was degraded anaerobically. Although degradation of alkanes coupled to sulfate reduction has been well-documented in pure culture, this metabolism has not been intensively investigated in sediments. Studies with radiolabelled hexadecane confirmed that alkanes could be oxidized under sulfate-reducing conditions and that this metabolism was linked to sulfate reduction. These results were summarized in a paper in *Applied and Environmental Microbiology*.

Methodologies for evaluating the microbial populations in contaminated harbor sediments in order to determine the organisms associated with PAH degradation were further developed. PCR of 16S rRNA genes followed by denaturing gradient gel electrophoresis to separate the PCR products and sequencing was found to be an excellent method for evaluating populations in marine sediments. However, due to the great diversity of microorganisms, great care in the selection of the PCR primers is required. This suggests that other approaches,

such as evaluation of genes specific to sulfate-reducing microorganisms, may be a better strategy.

In order to better study the distribution of anaerobic PAH-oxidizing microorganisms in the sediments, attempts were made to enrich for and isolate these organisms in culture. An organism was isolated from Potomac River Estuary sediments which exhibited anaerobic, benzene-dependent growth. Although this organism has yet to be characterized in detail, it is the first example of an organism in culture that can grow on an unsubstituted aromatic hydrocarbon under anaerobic conditions. Using similar protocols, a sediment-free enrichment culture that exhibited naphthalene-dependent growth was obtained. It is expected that gene sequences from these organisms will aid in determining their distribution in sediments and help predict where anaerobic PAH degradation is likely to take place.

CONCLUSIONS: These studies demonstrate for the first time that PAHs can be oxidized to carbon dioxide under anaerobic conditions in contaminated marine sediments. The anaerobic PAH oxidation is carried out by as-yet-to-be-identified sulfate reducers. Although pristine sediments do not exhibit significant potential for anaerobic PAH oxidation, once sediments are exposed to PAHs, the growth of PAH-degrading sulfate reducers is stimulated and PAHs can be degraded.

SIGNIFICANCE:

PAHs are a major contaminant of concern in harbor sediments because they are toxic to bottom-feeding fish and sediment-dwelling organisms. Furthermore, PAH contamination in dredge spoils presents a disposal problem. Until our finding of PAH oxidation coupled to sulfate reduction, it was generally regarded that PAH degradation in marine sediments required molecular oxygen which is unavailable in most contaminated harbor sediments. If it is found that PAHs will naturally degrade over time in contaminated harbor sediments then the need for expensive remediation programs might be avoided. Anaerobic treatment of PAH-contaminated dredge spoils could prove to be a less expensive remediation option than aerobic methods. These studies with marine sediments have transfer value for other environments. For example, from our finding that aromatic hydrocarbons can be oxidized with the reduction of sulfate, we designed a novel bioremediation strategy that successfully remediated a petroleum-contaminated aquifer for Conoco, Inc. This strategy could be applied to Department of Defense sites.

PATENT INFORMATION: None.

AWARD INFORMATION: Elected a Fellow in the American Academy of Microbiology

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