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13. ABSTRACT (Maximum 200 words) Carnegie Mellon University and Stanford University investigated the anaerobic biodegradation of polycyclic aromatic hydrocarbons (PAHs) in contaminated sediments under bioslurry denitrifying conditions. Samples from sediment bioslurry tests and toxicity bioassays were used to assess PAH bioavailability as inferred from subparticle-scale measurement of PAH locations on sediment particles and association with sorbent organic matter. The research employed new microscale, analytic measurement techniques: microprobe, two-step laser desorption/laser ionization mass spectrometry; infrared microspectroscopy; wavelength dispersive X-ray analysis; and thermal desorption mass spectrometry. These techniques provide information on subparticle-scale PAH distributions in sediment. CMU researchers have reviewed relevant studies, cultivated <i>Pseudomonas putida</i> KBM1, a strain of microorganisms capable of degrading PAHs under denitrifying conditions, and conducted anaerobic and aerobic PAH degradation studies. R. G. Luthy has leveraged the experimental studies with a major Gas Research Institute initiative on environmentally acceptable endpoints. CMU and Stanford have examined PAH distribution on dredged Milwaukee harbor sediment obtained from the Jones Island confined disposal facility. Subparticle-scale analyses have shown that PAH sorption involves near-surface sorption processes, rather than particle-scale diffusion phenomena, and that coal-like particles contain many times more PAHs than silica particles. Results from anaerobic and aerobic biodegradation experiments conducted with two PAH contaminated sediments are presented in this final report.					
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Enclosure 1

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Honors/Awards:

Richard G. Luthy:

Elected member National Academy of Engineering, Feb, 1999

Richard N. Zare:

Distinguished Service Award, National Science Board, August, 1998

E. Bright Wilson Award in Spectroscopy, American Chemical Society, March, 1999

Scientific Progress and Accomplishments:

Results presented in this final report on screening bioslurry reactor tests of anaerobic treatment of sediments contaminated with Polycyclic Aromatic Hydrocarbons (PAHs) indicate possible degradation of PAHs under denitrifying conditions. During the two months of anaerobic degradation, total PAH removal was modest probably due to both the low availability of the PAHs and competition with other more easily degradable sources of carbon in the sediment. For both Harbor Point sediment (HPS-2) and Milwaukee Harbor sediment (MHS), PAH reduction was confined to 3- and 4-ring PAHs. There was virtually no change in concentration for the higher molecular weight PAHs in the sediment for the duration of the study. Comparing PAH reductions during two months of aerobic and anaerobic biotreatment of MHS, it was found that extent of PAH reduction was higher for aerobic treatment. Interestingly, removal of PAHs from sediment particle classes (by size and density) followed similar trends for aerobic and anaerobic treatment of MHS. The majority of the PAHs removed during biotreatment came from the clay/silt fraction. In an earlier study it was shown that PAHs associated with the clay/silt fraction in MHS were more available than PAHs associated with coal-derived fraction. Therefore, although total PAH reductions were small, the removal of PAHs from the more easily available sediment fraction (clay/silt) may result in a significant environmental benefit owing to a reduction in total PAH bioavailability.

Results in this study indicate that naphthalene could be degraded under anaerobic conditions. Naphthalene spiked into MHS was degraded below detection limit within 20 days with the accompanying reduction of nitrate. With repeated addition of naphthalene and nitrate, naphthalene degradation under nitrate reducing conditions was stable over one month. Nitrite, one of the intermediates of denitrification was detected during the incubation. Also the denitrification activity of the enrichment culture from MHS slurries was verified by monitoring the production of nitrogen gas in solid fluorescence denitrification medium. Microorganisms capable of degrading naphthalene via denitrification were isolated from this enrichment culture. In microcosms prepared with HPS-2 slurries, the added naphthalene was degraded under anaerobic conditions even though it did not seem to be coupled to nitrate reduction.

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Sincerely,

Enclosure 3

BIOSTABILIZATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) UNDER DENITRIFICATION CONDITIONS IN SEDIMENTS (DAAG SS-98-1-0395)

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Foreword

The U.S. Army Research Office Grant to Carnegie Mellon University (CMU) and Stanford University (Stanford) began in June 1998 and supported investigations on the anaerobic biodegradation of polycyclic aromatic hydrocarbons (PAHs) in contaminated sediments. The ARO study was central to connecting and leveraging experimental work on biological degradation of PAHs in contaminated sediments by linking experimental programs and test facilities among various institutions. Experimental work in the Department of Civil and Environmental Engineering at CMU focused on the biological processes and contaminant-sediment interactions associated with PAH degradation. Tests were performed with dredged sediment from a Milwaukee, WI confined disposal facility, and with sediment from Harbor Point, NY. Although this research was originally planned for a period of 2 years, funding from ARO was curtailed at the end of the first year. The PI however, was able to leverage the experimental studies in this research through the GRI research initiative on environmentally acceptable end-points. The research therefore continued for the second year without any funding from the ARO. This final report documents findings from the research conducted during the period of 1.5 years from June 1998 - Dec 1999. This report comprises two sections. The first section describes screening bioslurry studies to assess the remediation of sediments contaminated with polycyclic aromatic hydrocarbons (PAHs). This part of the report presents results of bioslurry studies to assess the biodegradation of PAH-contaminated sediments under denitrifying conditions. The second section describes microcosm studies to demonstrate the biodegradation under denitrifying conditions of naphthalene spiked into sediments, and experimental work to isolate microorganisms capable of degrading naphthalene via denitrification.

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1. Statement of the problem studied

PAHs enter the environment primarily through anthropogenic sources such as use in protective coatings, from coal by-products like coal tars, from coal processing operations like those at former manufactured gas plant sites, and from the use of fossil fuels. PAH contamination is also associated with wood treatment and preservation operations, as PAHs comprise 85% of creosote. PAHs are hydrophobic, exhibit low water solubility, and have a high affinity for sorption to solid materials. The low solubility and high sorptive properties have a strong affect on PAH remediation by reducing mobility and reactivity. Due to their toxicity, PAHs remain a human health threat even at low concentrations in the environment. For this reason, the EPA lists 16 PAHs as contaminants of concern on the priority pollutant list. PAHs comprise an important class of contaminants that the EPA has identified as bioaccumulative, persistent, and toxic.

Due to their low solubility and hydrophobicity, PAH contamination usually concentrates in sediments or soils. PAH removal through conventional pumping technologies is impractical, while excavation and thermal treatment is costly and often opposed by affected communities. For these reasons, there has been long-standing interest in assessing microbial degradation for removing PAH contaminants. However, considerable evidence from both field and laboratory studies shows that biodegradation may remove only a fraction of the total PAHs in sediment or soil sample. The remaining portion of the PAHs may be sequestered and unavailable, and thus pose much less risk than the untreated material. This concept is referred to as biostabilization. Research is needed to advance the concept of biostabilization and address conditions under which the biostabilization of PAHs may be favorably realized in sediments.

Bioremediation and PAH Sorption and Sequestration

Bioremediation through monitored natural processes or engineered systems is a potentially cost-effective treatment for PAH-contaminated sediments that exploits microorganisms to destroy or modify PAHs to less harmful products (1, 2, 3, 4). The most fundamental long-term issue confronting sediment bioremediation is the lack of understanding of contaminant-sediment interactions (5). Little is known about the mechanisms of PAH and other hydrophobic organic compound (HOC) sequestration and aging in sediments and the resulting effects on chemical and biological availability (6). A major factor in the success of sediment bioremediation is the

availability of contaminants to microorganisms, which may be affected by the particle type and the character of sorbent organic matter. Likewise, contaminants that are strongly sorbed may not be available for a toxic response (7, 8). Thus, knowledge of how and where PAHs are bound to sediment material is necessary to assess the efficacy of sediment bioremediation and to correlate this knowledge with reductions in availability, mobility, and toxicity.

Three fundamentally different mechanisms that may describe sorption of PAHs on sediments are: 1) surface sorption processes near the external regions of organic or inorganic particles; 2) diffusion processes leading to penetration within the interior regions of porous particles; and 3) diffusion within organic matter (9, 10, 11). Weber and Huang (12), Pignatello (9), Brusseau et al. (13) and Carroll et al. (14) emphasize intra-particle organic matter diffusion to explain slow desorption of HOCs from soils and sediments. Particle-scale pore diffusion and organic matter diffusion are similar mathematically and difficult to distinguish from model fitting of desorption kinetic data (6).

Current methods that employ macroscopic techniques for assessing sorption and sequestration of HOCs, like PAHs, on geosorbents do not provide information on where toxic contaminants lie and what their associations are, nor do such methods adequately discern the underlying fundamental mechanisms governing PAH sorption. These concerns indicate the need for improved understanding of contaminant-geosorbent interactions and sequestration processes and the effect of such processes on bioavailability and toxicity of PAHs in geosorbents (5, 6). Such knowledge has enormous impacts on policy decisions for setting soil and sediment quality criteria, defining cleanup goals, and establishing priorities among various environmental problems. The research being conducted under ARO sponsorship addresses these concerns by providing more fundamental information at the sub-particle scale regarding where and how PAHs are bound to sediments and how this affects treatment and toxicity.

PAH Biodegradation

Aerobic degradation of PAHs is fairly well documented (15, 16), especially in the case of microcosm studies. Yet, maintaining a sufficient oxygen concentration in sediments is very difficult and expensive. Thus, alternative processes involving electron acceptors, such as NO_3^- ,

SO_4^{2-} , ferric iron (Fe(III)), or methanogenesis, have potential advantages for in-situ treatment. When present, higher yield (higher energy) terminal electron acceptors (TEAs) are used first. Thus, the zones moving away from the source of contamination are methanogenic, SO_4^{2-} -reducing, Fe(III)-reducing, NO_3^- -reducing, and oxic depending on the availability of TEA and the associated microbial population (17). Usually, due to availability of TEA, sulfate reduction is characteristic of marine sediments, while nitrate reduction is more characteristic of soil and fresh water sediments.

Anaerobic microbial degradation of PAHs is not well understood, whether for soils or sediments. The beginning step in the microbial work at CMU involved a critical review of previous work on anaerobic microbial degradation of PAHs. Additionally, microcosm studies were initiated using microbes that were successful in degrading naphthalene, phenanthrene, and pyrene under NO_3^- -reducing conditions (18).

Review of Previous Studies

Anaerobic Degradation

Although aerobic degradation of PAHs is well documented, anaerobic degradation still lacks broad evidence. Nonetheless, sufficient evidence exists, particularly from controlled laboratory microcosm studies with using field samples that point toward the possible complete degradation of certain PAHs under anaerobic conditions. These successful studies provide a starting point from which to further study the important parameters and criteria for PAH degradation under anaerobic conditions. A previous, in-depth, literature review of experiments involving PAH degradation under anaerobic conditions for both field (14 studies) and microcosm (10 studies) studies showed little confidence in the existing literature (19). The authors collected information on a few low molecular weight PAHs including naphthalene, phenanthrene, acenaphthene, fluorene, and 1-methylnaphthalene. While some of the site studies indicate that removal occurred, other studies were contradictory and some sources that claimed degradation lacked sufficient evidence to prove anaerobic degradation.

In a field-type experiment, Durant et al. (20) conducted laboratory microcosm studies using PAH-impacted soil samples taken from a manufactured gas plant site near Baltimore, MD to

determine if degradation occurred and, if so, under what prevailing TEA conditions. Durant compared the results to prevailing subsurface conditions in the bores at the site from which the sample cores were taken. The microcosm studies showed the potential for both aerobic degradation of naphthalene and phenanthrene and anaerobic naphthalene degradation under NO_3^- -reducing conditions. The dominance, or presence, of either process in the field was inconclusive as pH, redox potential, microbial population, and chemical composition varied between samples.

Several microcosm studies have examined the anaerobic biodegradation of PAHs, mainly naphthalene and phenanthrene, using ferric iron, nitrate, and sulfate as the TEA. A few microcosm studies have utilized PAH-impacted sediment as a microbial source. Coates et al. experimented with ferric iron reduction in marine sediments from San Diego Bay, CA and found that SO_4^{2-} reduction out-competed Fe(III) reduction (21). Various researchers have substantiated the removal of naphthalene and/or phenanthrene under sulfate-reducing conditions (22, 23, 24, 25, 26). Rockne et al. reported specific removal rates for creosote-contaminated sediment from Eagle Harbor, WA in fluidized-bed reactors (22). Rates for sulfate reduction were 50-80% slower than those reported for nitrate reduction as may be expected by the lower energy yield of sulfate reduction. As with Rockne, Coates et al. (24) linked PAH degradation and sulfate reduction by the addition of molybdate, a sulfate reduction inhibitor, to tie PAH removal with sulfate as the TEA. Coates et al. reported a lag phase for the inoculum during some initial trials with degradation proceeding immediately for subsequent PAH additions. Coates reported degradation of methylnaphthalene, fluorene, and fluoranthrene under sulfate-reducing conditions. However, pyrene and benzo(a)pyrene were not degraded and this difference may indicate limitations for degrading the higher molecular weight PAHs anaerobically. Bedessem et al. (25) and Zhang and Young (26) provide further proof of biological degradation of PAHs in microcosms through radio-labeling. Bedessem et al., using sediment from two sulfate-rich, coal tar-contaminated aquifers enriched over a 3-year period measured conversion of sulfate to sulfide with a radioactive tracer, $^{35}\text{SO}_4^{2-}$, while monitoring naphthalene utilizing HPLC. Zhang monitored radio-labeled PAH to obtain the percent utilization of naphthalene and phenanthrene through conversion to $^{14}\text{CO}_2(\text{g})$ in SO_4^{2-} -reducing microcosms enriched with sediment collected from the Arthur Kill in NY/NJ harbor.

Studies under NO_3^- -reducing conditions include those by Rockne et al. (23) and Mihelcic and Luthy (27). Rockne, in microcosms with Eagle Harbor, WA sediment, reported a nitrate-reducing removal rate constant for phenanthrene, $k=1.12 \mu\text{g PAH/mg VSS-day}$. Naphthalene was also degraded, but the rate constant was not reported. Mihelcic reported degradation in soil slurries under NO_3^- -reducing conditions for naphthol, naphthalene, and acenaphthene from initial concentrations of 8, 7, and 0.4 mg/L to non-detect levels within 16, 45, and 40 days. The lag period for naphthalene and acenaphthene was two weeks. Mihelcic also reported the effects of microbial acclimation to PAH exposure and mineralization of a portion of soil organic carbon on the nitrate requirement. Incorporating estimates for the effect of soil organic carbon, approximately 50 to 90% of naphthalene was mineralized completely to CO_2 .

A more recent study by McNally et al. served as the basis for the current biological work at CMU (18). The findings of that study support the degradation of PAHs under denitrifying conditions in soil-water, laboratory microcosms. Anthracene, phenanthrene and pyrene were degraded under strict anaerobic, denitrifying conditions using three pseudomonad strains: *stutzeri*, *fluorescens*, and *putida*. Two of the strains, *P. stutzeri* SAG-R and *P. fluorescens* W-2, were isolated from contaminated sites. All three strains were chosen for their ability to degrade PAHs aerobically and to grow under denitrifying conditions. Each strain was tested for the ability to grow under anaerobic, denitrifying conditions by monitoring nitrate utilization through N_2O production. The primary difference from previous experiments was the use of high cell densities ($\sim 2.5 \times 10^8$ cells/mL pure culture) of pure, well-adapted, microbial strains. Another difference from previous microcosm studies was the observation of anaerobic degradation of pyrene. The high cell density and isolation from contaminated sites reduced adaptation time for the microbes and optimized conditions for potential PAH degradation. Possibly due to such optimization, McNally et al. achieved decreases of PAHs with more than three rings and a degradation rate of PAHs under anaerobic, denitrifying conditions faster than aerobic degradation. This was particularly true for the *Pseudomonas stutzeri* SAG-R strain that degraded all three PAHs faster under anaerobic conditions.

Another recent study by Johnson and Ghosh (28) is also directly related to the biological research at CMU funded through the ARO. As with CMU, the focus of that study was the biological degradation of PAHs in the Milwaukee harbor sediments contained in the Jones Island CDF. Johnson and Ghosh tested natural degradation of 15 PAHs under anaerobic conditions with and without the addition of various electron acceptors. The PAHs ranged from naphthalene through benzo(ghi)perylene. The natural levels of NO_3^- and SO_4^{2-} were 12.5 mg/L and 175 mg/L respectively while the total PAH mass was 124 mg/kg. The findings support degradation of all 15 PAHs by the native microbial flora in an anaerobic bioslurry process without addition of TEA. The removal ranged from 79% for acenaphthene to 8% for indeno(123cd)pyrene. Neither NO_3^- addition nor conversion to methanogenic conditions enhanced degradation compared with unamended, anaerobic conditions. However, SO_4^{2-} addition improved removal compared with the unamended reactors. This suggests that either sufficient nitrate exists in the natural sediment for biological remediation or that the native microbial flora utilizes SO_4^{2-} for degradation of the PAHs.

More fundamental research is needed to further explore anaerobic biodegradation pathways and to understand physiochemical processes affecting bioremediation and biostabilization of PAH contamination in CDFs. For the ARO-funded study at CMU, the field samples are obtained from dredged Milwaukee, WI harbor sediment contained in the Jones Island confined disposal facility (CDF) (28) and contaminated river sediment from Harbor Point, NY, a historic location of manufactured gas plant operations.

Potential Degradation Pathways

While substantial evidence exists to support oxidation of PAHs under NO_3^- - and SO_4^{2-} -reducing conditions, potential pathways are just beginning to be explored. Leigh states (29), "The biochemical pathway for the aerobic degradation of PAHs with fewer than 5 rings is well understood. The initial attack for unsubstituted PAH involves the hydroxylation of the ring using O_2 followed by ring cleavage and complete oxidation to carbon dioxide and cell material." However, the stability of the unsubstituted aromatic rings makes activation of the ring structure difficult for microorganisms, especially in the absence of oxygen. Only Bedessem et al. (25) and Zhang and Young (26) provide insight into the anaerobic degradation pathways. These sources

provide direct evidence of potential PAH degradation pathways under anaerobic conditions by measuring metabolites and sequential testing of similar compounds. Bedessem and Zhang proposed two initial steps using these techniques. The first involves a process similar to that of aerobic degradation, hydroxylation. The second method involves carboxylation of the ring structure. Both pathways serve to destabilize the aromatic structure and enable further attack.

Bedessem et al. (25) used GC/MS to screen for potential metabolites of naphthalene degradation under SO_4^{2-} -reducing conditions. Naphthol was tentatively identified as an intermediate through comparison of the GC/MS with standards. Naphthol was also detected only in microcosms that exhibited naphthalene transformation. Such conversion suggests that hydroxylation is a first step in the degradation of naphthalene. Zhang and Young (26) cite evidence supporting an alternative pathway involving carboxylation of naphthalene. The detection of 2-naphthoate (2-NA) through GC/MS hinted at carboxylation as an initial step. Subsequent sequential testing of possible metabolites also supported this pathway. 2-NA was almost completely degraded in naphthalene-degrading cultures, while 1-naphthol, 2-naphthol, and 1-naphthoate remained unconverted. Addition of $^{13}\text{CO}_2(\text{g})$ produced the final evidence in favor of carboxylation. GC/MS detected 2-naphthoate with radio-labeled ^{13}C attached at the 2 position. Radio-labeled carbon dioxide is included in the 2-naphthoate intermediate. Further testing concluded that carboxylation was specific to the 2-position as that carbon is the most electronegative (-0.0579). The electronegativity reflects the potential for nucleophilic attack on the benzene structure. As with Bedessem et al., Zhang and Young did not specify nor suggest subsequent degradation reactions.

Anaerobic Degradation Pathways for Monocyclic Compounds

The anaerobic oxidation of benzene provides clues to the biodegradation pathway for polycyclic compounds. Leigh et al. (29) used the benzene pathway to surmise potential PAH initiation steps. Evidence exists for anaerobic attack on benzene through both hydroxylation and carboxylation of the ring. Experiments cited earlier (25, 26) provided evidence for such attacks on naphthalene. This evidence, as well as comparison between the aerobic pathways for the compounds, shows the applicability and possible connections between the mono- and polycyclic aromatic pathways.

Under anaerobic conditions, microbes tend to convert substrates into hydroxylated or carboxylated derivatives with conversion of parent compounds through a few common central intermediates. After substitution, cleavage of the ring and complete mineralization of the compound occur. For benzene, suggested intermediates prior to ring saturation have been benzoate (benzoyl-CoA) and phenol. Hollinger and Zehnder (30) propose a possible pathway utilizing the central intermediate, benzoyl-CoA. Hollinger and Zehnder also propose that phenol is converted through such a pathway. Likewise, Young and Kuhn (31) support the carboxylation of phenol. Therefore, phenol and benzoate may exist as common metabolites in a pathway that suggests benzene degradation under anaerobic conditions.

The experimental evidence from PAH degradation studies shows that microbial degradation is possible under anaerobic conditions. Although specific pathways are only partially explored, microcosm studies document the removal of low molecular weight PAHs under NO_3^- - and SO_4^{2-} -reducing conditions. Further investigation of the pathways through testing for intermediates and comparison with benzene degradation will enhance understanding of the conditions required for optimizing the degradative process. A primary factor of concern for optimization of experimental microcosm studies is the bioavailability of the PAHs, as well as the presence of both ample reducing agents and a sufficient microbial population. Many studies utilize soil/sediment samples from areas that are already contaminated with PAHs in which a native microbial population may be already adapted to obtaining energy through PAH removal. When supplied with ample TEA, degradation may occur.

2. Summary of results

This report comprises two sections and is presented in detail in Appendices I & II. The first section describes screening bioslurry studies to assess the remediation of sediments contaminated with polycyclic aromatic hydrocarbons (PAHs). This part of the report presents results of bioslurry studies to assess the biodegradation of PAH-contaminated sediments under denitrifying conditions. The second section describes microcosm studies to demonstrate the biodegradation under denitrifying conditions of naphthalene spiked into sediments, and

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Results presented in this final report on screening bioslurry reactor tests of anaerobic treatment of sediments contaminated with Polycyclic Aromatic Hydrocarbons (PAHs) indicate possible degradation of PAHs under denitrifying conditions. During the two months of anaerobic degradation, total PAH removal was modest probably due to both the low availability of the PAHs and competition with other more easily degradable sources of carbon in the sediment. For both Harbor Point sediment (HPS-2) and Milwaukee Harbor sediment (MHS), PAH reduction was confined to 3- and 4-ring PAHs. There was virtually no change in concentration for the higher molecular weight PAHs in the sediment for the duration of the study. Comparing PAH reductions during two months of aerobic and anaerobic biotreatment of MHS, it was found that extent of PAH reduction was higher for aerobic treatment. Interestingly, removal of PAHs from sediment particle classes (by size and density) followed similar trends for aerobic and anaerobic treatment of MHS. The majority of the PAHs removed during biotreatment came from the clay/silt fraction. In an earlier study it was shown that PAHs associated with the clay/silt fraction in MHS were more available than PAHs associated with coal-derived fraction. Therefore, although total PAH reductions were small, the removal of PAHs from the more easily available sediment fraction (clay/silt) may result in a significant environmental benefit owing to a reduction in total PAH bioavailability.

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The anaerobic biotreatment of contaminated sediment shows promise, but the results are not dramatic for source materials.

3. List of publications and presentations

Microscale Location, Characterization, and Association of Polycyclic Aromatic Hydrocarbons on Harbor Sediment Particles. Upal Ghosh, Richard G. Luthy, J. Seb Gillette and Richard N. Zare. *Environmental Science and Technology*, 34, 1729-1736, 2000.

Direct Observation of Polycyclic Aromatic Hydrocarbons on Geosorbents at the Sub-Particle Scale. Gillette, J.S., R.G. Luthy, S.J. Clemett, and R.N. Zare. *Environmental Science and Technology*, 33:8, pp. 1185-1192, 1999.

Microscale Investigation of Bioavailability and Biostabilization of PAHs in Sediments. Richard G. Luthy, Upal Ghosh, Jeffrey W. Talley, J. Seb Gillette and Richard N. Zare. Milwaukee Port Authority/US Army Corps meeting on beneficial uses for dredged sediments. Milwaukee Port Authority, Milwaukee, WI, September 23, 1999.

Analytical Tools to Assess Sequestration and Bioavailability of Organic Compounds. R. G. Luthy, U. Ghosh. AEESP Research Frontiers Conference. Aug 1-3, 1999, Pennsylvania State University, PA.

Microscale Characterization of PAH Sequestration on Sediments. U. Ghosh, R.G. Luthy, J.S. Gillette, R.N. Zare, J.W. Talley. Invited presentation and Proceedings paper. In Situ and On-Site Bioremediation, The Fifth International Symposium. April 19-22, 1999. San Diego, CA.

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Appendix I.

**SCREENING BIOSLURRY TREATMENT ASSESSMENTS OF REMEDIATION OF
PAH CONTAMINATED SEDIMENTS UNDER DENITRIFYING CONDITIONS**

Appendix II

**MICROCOSM AND MICROBIAL CULTURE ASSESSMENT OF NAPHTHALENE
DEGRADATION UNDER DENITRIFYING CONDITIONS BY MICROORGANISMS
FROM PAH CONTAMINATED SEDIMENTS.**

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