AIM

Association for Information and Image Management

1100 Wayne Avenue, Suite 1100
Silver Spring, Maryland 20910

301/587-8202

Centimeter

Inches

MANUFACTURED TO AIM STANDARDS
BY APPLIED IMAGE, INC.

1 of 1

DOE|PC|96303-T11

Final Technical Report
DOE-FC22-90PC90303
+ 2nd yr report
April-June 1993
JUL 1

Biodegradation of Naphthalene from Coal Tar

Research Progress Report

February 7, 1994

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

Subhasis Ghoshal, Anuradha Ramaswami
and Richard G. Luthy

Department of Civil Engineering
Carnegie Mellon University
Pittsburgh, PA 15213

sb
DISSEMINATION OF THIS DOCUMENT IS UNLIMITED

Abstract

Biodegradation experiments were conducted to evaluate the mineralization of naphthalene released from coal tar entrapped in microporous silica media. Tests were performed with two coal tars recovered from former manufactured gas plant sites. Results from these tests showed that the degradation end point for naphthalene was significantly lower than the total amount of naphthalene present in coal tar. The role of physico-chemical and biological processes on the rate of biotransformation of naphthalene was evaluated. Mass transfer rates for dissolution of naphthalene from entrapped coal tar were measured in batch, flow-through systems. The rate of naphthalene mass transfer from the coal tar was found to be significantly greater than the rate of naphthalene biomineralization in batch slurry reactors. This implied that the rate limiting factor for the biodegradation process was related to biokinetic phenomena rather than mass transfer processes. Further tests indicated that conditions inhibitory to bacteria limited the biodegradation of naphthalene, and in some cases the inhibition was reversible upon dilution of the reactor contents.

1 Introduction

Coal tar is a mixture of various polycyclic aromatic hydrocarbons (PAHs) and is a contaminant found in the subsurface at some former manufactured gas plant (MGP) sites. In the subsurface, coal tar may remain imbibed in the macropores of soil/aquifer matrix or may exist as entrapped pools of dense non-aqueous phase liquid (DNAPL). Also, the presence of some surface active and polar compounds in the coal tar may cause it to be the preferentially wetting fluid and facilitate entry of coal tar into the micropores of the soil/aquifer matrix. Although relatively immobile in the subsurface, coal tars may serve as a continuous source of groundwater contamination due to dissolution of PAH compounds and other solutes. Bioremediation may be a feasible alternative for remediation of the coal tar-contaminated subsurface (Sherman et al., 1988). Factors controlling the biodegradation of naphthalene, a major constituent released from coal tar into the aqueous phase, has been examined in this study to explore the physico-chemical factors controlling the biotransformation process of the PAHs released from coal tar.

The consensus from various investigations with PAH compounds and soils is that 2- through 5-ring PAH compounds can be biodegraded in liquid cultures given appropriate microbial populations and environmental conditions. Higher-ring PAH compounds are probably not capable of sustaining microbial growth as these compounds are inadequate carbon sources due to their limited solubility. PAH contamination may exist in several forms in soils and aquifer matrix: (i) PAH sorbed onto nonporous matter (e.g, sand) (ii) PAH sorbed onto porous aggregates, and (iii) PAH residing in oils or tars as NAPLs. Studies on biodegradation of PAHs from contaminated MGP site soils (Smith and Weightman, 1988; Sherman et al., 1988) report unpredictable and incomplete degradation of the PAHs associated with soil, although PAHs present in the aqueous solutions may degrade readily. Such studies have hypothesized that mass transfer limitations due to NAPL being trapped inside soil aggregates or due to the PAHs being slowly desorbed from soil particles may cause the PAHs to be unavailable to microorganisms in the aqueous phase.

Nakles et al. (1991) reported results from laboratory biodegradation studies with contaminated MGP site soils in pan (unmixed) and slurry (mixed) systems. In soils containing 25% as fines less than 75 micron, the slurry system showed a larger extent and a faster rate of degradation of total PAHs

than unmixed systems. This was explained by the slurry reactor helping to promote disaggregation of larger aggregates, while providing no limitations to nutrient or oxygen mass transfer. In another soil containing entrapped NAPL, pan and slurry reactors approached the same overall extent of PAH removal, although the rate of degradation in the slurry reactor was greater than in the pan. It was concluded that mixing enhanced dissolution of PAHs from the NAPL and that the residual PAH was probably trapped inside the NAPL and would be solubilized very slowly.

Additional studies on PAH degradation from MGP site soils have described the aqueous-soluble fraction of PAHs as being 'readily available' and degradable with the residual PAHs as being a 'resistant fraction' (Morgan et al., 1992). The concentration of the aqueous-phase PAHs were found to decrease rapidly in slurry tests in contrast to the sorbed fraction from pan tests. Tests were performed to verify if the lack of degradation of the residual fraction was due to mass transfer limitations or toxicity by spiking some contaminated soil with phenanthrene (Morgan et al., 1992). Added phenanthrene was considered to be available to microorganisms. The tests showed that the freshly-added phenanthrene degraded readily and it was concluded from this test that the resistant PAH fraction in the contaminated soil was incapable of being degraded by the microbial population. This investigation pointed to mass transfer limitations as preventing degradation of PAHs from contaminated MGP site soils.

Erickson et al. (1993) observed no significant loss of PAHs from MGP site contaminated soil (including tar contaminated soil). Temperature, pH and supplements with additional substrates or inoculum had no significant effect on the results. The failure to observe losses of PAHs was postulated to be either due to the PAHs being not available to the microorganism in the aqueous phase or due to toxicity to the microbial population. Tests were carried out with different amounts of PAH-contaminated MGP site soils being mixed with a clean soil having a microbial population capable of degrading PAHs. Some of these soil mixtures were spiked with naphthalene and phenanthrene. Naphthalene and phenanthrene equal to the amount added were eliminated, while the concentrations of PAH equal to those originally present in the contaminated soil were not. This observation, and the fact that PAHs from the contaminated soil were not extractable by water, led to the conclusion that the contaminated soils were not toxic to the microorganisms but that the PAHs were bound to the soil in a way that they were unavailable to the microorganisms.

The above studies have indicated qualitatively that mass transfer limitations may prevent significant biodegradation of PAHs from MGP-site contaminated soils. However, biodegradation of complex chemical mixtures such as coal tar may be further complicated by substrate interactions causing unpredictable biodegradation patterns. Alvarez and Vogel (1991) have reported both inhibited and enhanced biodegradation of individual BTX compounds (benzene, toluene and xylene) in the presence of other BTX compounds. For example, degradation of benzene was enhanced by the presence of toluene but was inhibited in the presence of p-xylene.

Independent measurements of rates of dissolution and biodegradation are needed for a quantitative evaluation of the rate-limiting process governing biodegradation of PAH solutes from coal tar. As a working hypothesis, consistent with the findings on the biodegradation of sorbed hydrophobic organic compounds in soil-water systems (Ogram et al., 1985; Mihelcic and Luthy, 1991; Rijnaarts et al., 1990), it may be inferred that it is the aqueous phase organic substrate that is readily available to microorganisms. Thus in addition to knowledge of biotransformation rates, an understanding of the physical processes of equilibrium partitioning of solutes, and mass transfer of solutes between NAPL and aqueous phase is necessary.

This study focuses on identifying the nature of the rate limiting processes governing biodegradation of naphthalene from coal tar entrapped in a microporous matrix. Physicochemical and biokinetic rates are assessed by measuring rates of dissolution and biomineralization in mixed or slurry systems. A schematic of the system studied is shown in Figure 1. In this system, naphthalene and other solutes dissolve into the bulk aqueous phase from the entrapped coal tar-NAPL in the micropores. Only the bulk-phase naphthalene is degraded by bacteria, which are size excluded from the micropores.

2 Experimental techniques

2.1 Coal tar analysis

Analysis of the volatile organic compounds, polycyclic aromatic hydrocarbons, and different fractions in the coal tar was provided by EA Laboratories, Sparks, MD. Four coal tar samples were analyzed for selected volatile and semi-volatile compounds. The four samples were collected from monitoring well C4-WO6-D at the BG&E Spring Gardens, Maryland site. Sample 1 was collected on October 13, 1992 and coal tar from this sample was used in this research. This sample was analyzed by EA at the time of collection was also analyzed a second time by Atec Laboratories, Columbia, MD on June 15. Samples 2A, 2B and 2C were collected on June 2, 1993 from the same well to assess compositional variations in the coal tar samples from the same well. All four samples were analyzed for EPA Priority Pollutant volatiles and PAHs by EPA Methods 8240 and 8270, respectively. Sample 2A was analyzed by Atec Laboratories, Sample 2B was analyzed by EA Laboratories and Sample 2C was analyzed by Lancaster Laboratories, Lancaster, PA. Sample 1 was also analyzed for the asphaltene, polar compounds, saturates and aromatics by the Clay Gel Adsorption Method ASTM D2007 at Phoenix Chemical Laboratory, Chicago, IL.

A second sample of coal tar used in this study was collected from a manufactured gas plant site in Stroudsburg, Pennsylvania. Volatiles and PAHs in the tar were analyzed by GC/MS and the different fractions were analyzed by Method ASTM D2007 (Peters and Luthy, 1993).

2.2 Preparation of coal tar imbibed into microporous medium

Microporous silica beads (mean dia $\simeq$ 250 μ m, average pore dia = 140 Å) were used as a model porous medium. The silica beads were conditioned to be slightly hydrophobic by heating them to 800 C for about 2 hours in a muffle furnace to convert surface silica hydrophilic groups to silica oxide. The beads were allowed to cool to room temperature and were stored in air tight containers for future use. Radiolabeled coal tar was prepared by adding 1 μ Ci radiolabeled naphthalene to one

mL of coal tar. The radiolabeled naphthalene and coal tar was then mixed with 5 g of microporous silica beads in teflon-capped 50 mL centrifuge tubes. The coal tar-imbibed silica beads were mixed for approximately 24 hours on end-over-end rotators. After mixing the tubes were allowed to stand for 7 days to provide sufficient time for the coal tar to imbibe into the micropores. The fate of the ^{14}C naphthalene in the coal tar was assumed to be representative of the naphthalene present in the coal tar.

Some tests were carried out with coal tar that had been amended with addition of naphthalene. Sixty milligrams of naphthalene per mL of coal tar was added to a known volume of coal tar in small vials with teflon lined caps. The vials were then sonicated for a few minutes every hour for about two days until crystalline naphthalene was no longer visible on the walls of the vials. Radiolabelled naphthalene was then added, and the amended mixture was then applied to silica beads as described above.

2.3 Radiolabeled measurement techniques

The activity of ^{14}C compounds was measured using a Beckman LS 5000TD liquid scintillation counter (LSC). The instrument counts light emission as a measure of radioactivity from liquid samples. Pulse heights ranging from 0 to 670 (range of beta energies for ^{14}C) were recorded by the channel window. Corrections for quenching were automatically corrected by the H# method of quench monitoring. The H# of the samples was measured three times and the average value was used for determining counting efficiency. An internal quenching curve had been generated using different ^{14}C standard solutions and varying amounts of quenching agents. The activity of ^{14}C in the liquid samples was counted as disintegrations per minute (dpm) such that the 2σ error in dpm was less than 1%. Triplicates of every sample were counted and the average dpm was recorded after correcting for background activity (approximately 40 dpm).

Mixtures of radiolabeled naphthalene and coal tar were diluted by a factor of 100 with n-butylamine (a solvent that dissolves coal tar readily) to reduce color and opacity of the sample. Samples of volume less than 200 μL were added to 20 mL scintillation vial containing 15 mL of UI-

tima Gold scintillation liquid (Packard Instrument Co.). To reduce errors due to chemiluminescence and static, samples were stored overnight or longer before counting until the random coincidence monitoring (RCM) errors were less than 1%. Aqueous solutions containing ^{14}C compounds were counted by adding up to 1 mL sample volumes to 10 mL of Optifluor scintillation liquid (Packard Instrument Co.).

2.4 Equilibrium aqueous concentration of solutes from coal tar

The partitioning of aromatic compounds from coal tar was measured by both radiolabeled and chromatographic techniques. The equilibrium concentration of naphthalene partitioned from the coal tar into the aqueous phase was determined using radiolabeled techniques. For this, 50 mL teflon-capped centrifuge tubes containing 5 g silica beads were imbibed with 1 mL radiolabeled coal tar, and filled to zero headspace with deionized, autoclaved water. The tubes were then rotated for three days in end-over-end rotators. After this thorough mixing, the tubes were centrifuged, and the activity of 1 mL of the aqueous supernatant was measured after filtering through $0.2\ \mu\text{m}$ filters. PTFE filters were used to minimize sorption onto filters, and the filters were preconditioned by expressing at least 2 mL of the supernatant through the filter to waste. The aqueous concentration of naphthalene, C_s , was calculated using the Equation 1.

$$C_s = \frac{1000(dpm/mL)_{PAH}^w m_{PAH}^T}{dpm_{PAH}^T} \quad (1)$$

where C_s is in mg/L, m_{PAH}^T is the mass of naphthalene (mg) in 1 mL of the coal tar, and dpm_{PAH}^T is the total dpm of ^{14}C naphthalene added to 1 mL of coal tar.

Chromatographic techniques were used by EA to measure the equilibrium aqueous concentration of benzene, toluene, xylene, ethylbenzene, naphthalene, and 1- and 2- methylnaphthalene. In that work, 4 mL of coal tar were carefully injected to the bottom of a 40 mL tube partly filled with 24 mL of sterile, deionized water. The remaining volume was then filled with water to zero headspace and capped with teflon-lined septa. Care was taken to prevent free phase coal tar from forming globules, emulsions, or floating films in the aqueous phase. Six identical tubes were set up with HCl

added to inhibit bacterial activity. The tubes were wrapped with aluminum foil to prevent exposure to light and stored undisturbed. On days 7, 14 and 30 after the start of the experiment, two tubes were sacrificed and aqueous samples were withdrawn with a syringe by forcing the needle through the septa. The samples were analyzed for benzene, toluene, ethylbenzene and xylenes (BTEX) in addition to naphthalene and 1- and 2- methylnaphthalene. Analysis was done by GC using the purge-and-trap method for compound extraction (EPA Method 602). Samples had to be diluted 100 times for measuring naphthalene and 2-methylnaphthalene.

The equilibrium aqueous phase concentrations measured by both radiolabeled techniques and chromatography were compared with those predicted from Raoult's law,

$$C_{so} = x_{PAH} * C_{pure liquid}^{PAH} \quad (2)$$

where the equilibrium concentration of the compound in water, C_{so} , is equal to the mole fraction of the compound in the coal tar, x_{PAH} , times the aqueous solubility of pure liquid, or sub-cooled liquid in the case of naphthalene, $C_{pure liquid}^{PAH}$.

2.5 Naphthalene mineralization from coal tar

The mineralization of naphthalene from coal tars was measured under aerobic conditions, using radiolabeled techniques. The experiments were carried out in 250 mL biometer flasks fitted with a side tube. The side tube and the flask were sealed with neoprene stoppers covered with aluminum foil. The aluminum foil prevented any sorption of organic compounds onto the neoprene stoppers. Biometer flasks were autoclaved prior to use in the experiments. Nutrient media (17 mg KH_2PO_4 , 435 mg K_2HPO_4 , 85 mg NH_4Cl , 33.4 mg $Na_2HPO_4 \cdot 7H_2O$, 22.5 mg $MgSO_4 \cdot 7H_2O$, 27.5 mg $CaCl_2$, 0.25 mg $FeCl_3 \cdot 6H_2O$ in 1 liter deionized water) was autoclaved, and 50 mL of this solution was added to each biometer. Five grams of silica beads imbibed with 1 mL of coal tar prepared as described above was added to each of the biometers. The pH of slurry was then adjusted to approximately 7.2 using a few drops of 2M NaOH. The pH of the slurry was measured again after equilibration for 2 days and readjusted if necessary. Biometers were then inoculated with 2 mL of an actively growing bacterial culture of naphthalene degraders (RET PA101) containing approximately 5×10^7 cfu/mL

(as determined by multiple naphthalene plate counts). The microorganisms used for inoculation were grown in a supersaturated solution of naphthalene as sole carbon source in the nutrient media. Standard sterile techniques were followed for plate counts and inoculation. Five milliliters of 2 M NaOH was added to the side arm of the flask to trap any CO_2 evolved during mineralization. Biometers were continuously shaken in a gyratory shaker at approximately 60 rpm to ensure that the aqueous phase was well mixed. At least duplicates were set up for biometers representing a particular set of experimental conditions to assess variability in results. Sterile controls were employed to assess any abiotic mineralization. The control biometers were prepared exactly as for the mineralization tests but were not inoculated and had 200 mg/L mercuric chloride to prevent microbial activity.

Radiolabeled CO_2 from biomineralization of naphthalene was measured by periodically sampling the NaOH in the biometer side arm and counting the activity of the caustic solution. Triplicate samples of 0.5 mL NaOH were pipetted from the side arm and the activity of each was measured as described above. At each sampling period the remaining NaOH in the side arm was replaced with 5 mL of fresh NaOH, and the headspace of the flask was purged briefly with O_2 and the stopper replaced immediately.

The aqueous concentrations of naphthalene, BTEX and methylnaphthalenes in the supernatant of some biometers were analyzed by gas chromatography on the 14th day and the 30th day after inoculation. This was done to assess the availability of these compounds to the microorganisms. Supernatant samples from the biometers containing BG&E coal tar imbibed in silica beads were filtered through 0.2 μm PTFE filters into 40 mL vials with teflon lined caps. Filters were preconditioned by expressing 2 mL of supernatant to waste, and sample volumes between 2 and 6 mL were collected into the vials. The vials were tightly capped after the headspace was filled with deionized water and $HgCl_2$ to maintain a concentration of 200 mg/L of $HgCl_2$. This ensured that there was no additional biological activity in the vials during storage. Analysis of these samples were provided by EA.

2.6 Mass Transfer Experiments

Mass transfer tests were carried out in batch and flow-through systems. In batch tests, 5 g of silica beads imbibed with 1 mL of radiolabeled coal tar were contacted with 50 mL of water and the aqueous phase naphthalene concentration was measured at different times until equilibrium was attained. The measured equilibrium aqueous phase naphthalene concentration was compared with that predicted from Raoult's law (Equation 2).

In order to assess the effect of 'aging' (Luthy et al., 1993) on equilibrium partitioning and mass transfer rates, the dissolution of naphthalene from coal tar was studied in continuously stirred flow-through reactors. In the flow-through tests, the beads containing radiolabeled coal tar were stirred with a fixed volume of water, V , in an air-tight reactor vessel, and water was flushed through the system at different volumetric flow rates, q . The outflow of water creates a driving force for further dissolution, and by varying the flow rate, experiments may be conducted at different residence times, θ . The aqueous phase concentration of naphthalene in the reactor is monitored periodically by measuring the ^{14}C activity of the effluent. The change in aqueous phase naphthalene concentration, $C(t)$, with pumping time, t , may be described by writing a naphthalene mass balance equation for the water in the reactor vessel:

$$\frac{\partial C(t)}{\partial t} = k_{la}(t) [C_{eq}(t) - C(t)] - \frac{q C(t)}{V} \quad (3)$$

The second term on the right hand side of Equation 2 represents the rate of outflow of naphthalene from the stirred reactor due to flushing at a volumetric rate of q , while the first term represents the rate of input of naphthalene to the aqueous phase due to dissolution from coal tar. The dissolution rate is described using a lumped mass transfer rate coefficient, $k_{la}(t)$, and a linear driving force term that represents the departure of the measured aqueous concentration, $C(t)$, from equilibrium, $C_{eq}(t)$. Since most of the coal tar constituents are relatively insoluble in water, the naphthalene mole fraction in coal tar, and hence the equilibrium aqueous phase naphthalene concentration (as predicted from Raoult's law), should decrease continuously with pumping time as more naphthalene is flushed out of the reactor. Using Raoult's law, the change in equilibrium aqueous phase naphthalene concentration, $C_{eq}(t)$, is given by:

$$C_{eq}(t) = F_{PAH}(t) * C_{s0} \quad (4)$$

where C_{so} is the initial solubility of naphthalene in water, and $F_{PAH(t)}$ is the fraction of naphthalene remaining in coal tar at time, t . Thus, knowing the initial aqueous solubility of naphthalene, C_{so} , from batch solubilization tests, and by keeping track of the fraction of naphthalene remaining in coal tar at any time, $F_{PAH(t)}$, the time-varying equilibrium aqueous phase naphthalene concentration, $C_{eq(t)}$, may be estimated from Equation 4. Equation 3 is then used to evaluate the lumped mass transfer rate coefficient, $k_{la(t)}$ as it varies with time (aging). 'Fresh' systems were tested soon after contacting the coal tar-imbibed beads while in 'aged' systems the coal tar was in contact with water over periods of 1 week to 1 month before commencing the pumping test. Flow through tests were also conducted with beads obtained from the biometers at the conclusion of the mineralization experiments to evaluate the mass transfer rate coefficients.

2.7 Mineralization experiments using surfactants

The effect of two nonionic surfactants on mineralization of naphthalene from the coal tar imbibed in the microporous beads was evaluated in similar biometer tests. Two nonionic surfactants, Triton X-100 (a alkylphenol ethoxylate) and a 1:1 (v:v) mixture of Brij 30/35 (an alkylethoxylate) were used at doses that resulted in sub-CMC concentrations of the surfactants in the aqueous phase. Surfactant solutions (0.2% to 1%, v/v) were prepared by dissolving the required volume of surfactant into autoclaved nutrient media. The solutions were sonicated to ensure that the surfactants were completely dissolved, and 50mL of surfactant solution with nutrients was added to each biometer. Surface tension measurements of the supernatant fraction in the biometers were made for different surfactant doses to verify that the surfactant doses used were below the CMC.

2.8 Mass balance for ^{14}C

At the end of both mass transfer and biodegradation experiments, the residual radioactivity of the silica beads and of the supernatant in selected biometers was measured to check for mass balance of the radiolabeled naphthalene. The supernatant from each biometer was pipetted out, and the activity in 1 ml samples were counted in the LSC. Weighed samples of the remaining coal tar-

imbibed silica-bead slurry were combusted in a R.J. Harvey biological material oxidizer (R.J. Harvey Instrument Corp., Hillsdale, NJ). The $^{14}\text{CO}_2$ from combustion was trapped into vials containing a special cocktail (R.J. Harvey ^{14}C cocktail) and its activity was counted in the LSC counter. The total weight of the slurry being known, the total activity present in the slurry was calculated from the activity of the combusted samples. Recovery of ^{14}C from the systems ranged from 85% to 105% with an average of 93%.

3 Results

3.1 Coal Tar Composition

The composition and physical properties of coal tars have been reported to vary widely, depending on the gasification processes, e.g., water gas, carburetted water gas, or coal gas, and the type of coal and/or oil employed (Rhodes, E.O., 1979 and Harkins, S.M., 1988). Results from analysis of BG&E coal tar samples are shown in Table 1 and 2. Table 1 shows the composition of four the coal tar samples collected at monitoring well C4-W06-D. One analysis is for the sample of tar being used at CMU which was collected on October 13, 1992 and analyzed in June, 1993. The other three analyses are for tar samples from the same well collected in June, 1993. Comparison of the coal tar samples 2A, 2B and 2C collected on June 2, 1993 indicates compositional variation. The coal tar supplied to CMU shows a somewhat smaller BTEX fraction in comparison to the other samples, while the concentration of the PAHs are similar. The closest comparison between the tar supplied to CMU and the other samples from the same monitoring well, is with sample 2A as shown below.

Concentration (mg/Kg coal tar) of BTEX and PAH compounds in 2 samples of the BG&E coal tar		
Compound	October 13, 1992 (Sample 1)	June 2, 1993 (Sample 2A)
Benzene	1,000	2,700
Toluene	3,400	7,800
Ethylbenzene	440	840
Styrene	2,500	4,500
Total Xylenes	3,700	6,800
Naphthalene	100,000	100,000
2-Methyl Naphthalene	53,000	52,000
Acenaphthylene	13,000	13,000
Phenanthrene	20,000	24,000

The abundance of PAH compounds is characteristic of coal tars. Naphthalenes were the most predominant PAH compounds with naphthalene being the most abundant compound (10% of the

coal tar, w/w). The naphthalene concentration of 100,000 mg/Kg was used in all computations in this study as this concentration was reported in three of the four samples analyzed in June 1993, including the sample supplied to CMU. The classification of hydrocarbon types in Table 2 for the BG&E coal tar shows that aromatic compounds account for about 98% of the coal tar. Asphaltenes, a complex macromolecular fraction, believed to be important for interfacial film formation, accounts for about 1% of the coal tar mass.

The composition of the Stroudsburg tar is presented in Tables 2 and 3. The classification of the coal tar by characteristic groups, shown in Table 2 indicates that the fraction of aromatics in this tar was significantly less compared to the BG&E coal tar, accounting for only 41% of the total weight of the Stroudsburg coal tar. Asphaltenes, on the other hand, were a significant fraction. As with the BG&E coal tar, naphthalenes were the most abundant fraction of PAHs for the Stroudsburg coal tar, and naphthalene was the predominant PAH compound (2.16% w/w). The average molecular weight of the BG&E coal tar collected on October 13, 1992 was 226 g/mole while that for the Stroudsburg tar was 210 g/mole, based on vapor pressure osmometry. The knowledge of chemical composition of the tars was necessary for estimating aqueous concentrations of different solutes to determine the appropriate solute to be studied.

3.2 Equilibrium aqueous concentrations of PAH solutes

Measured and predicted equilibrium aqueous concentrations for volatile and semi-volatile solutes from the BG&E coal tar are shown in Table 4. The predicted values have been calculated using Raoult's Law (Equation 2) with the average molecular weight of the tar and the computed mole fraction of the solutes from the chemical analyses of the coal tar. Duplicate aqueous samples measured after equilibration for 7, 14 and 30 days matched the predicted equilibrium values reasonably well although deviations from predicted values were observed for some of the samples, especially 2-methyl naphthalene. Since the deviations were random and did not show any meaningful trend, it may be assumed that the variations are due to sampling inconsistencies or variability from purge and trap GC analysis. Equilibrium for all the solutes was attained within 7 days. The predicted equilibrium concentration of 18.5 mg/L naphthalene matched very well with the measured values. The measured

equilibrium aqueous concentrations of the solutes provided a verification of the concentration of the respective solutes in the BG&E coal tar. The equilibrium concentration of 2-methylnaphthalene is closer to the predicted value if the coal tar composition is taken as 36,000 mg/Kg, as measured in October 13, 1992.

Equilibrium aqueous concentration of naphthalene was measured in experiments with radiolabeled naphthalene using coal tar imbibed in microporous media. The coal tar-imbibed beads were thoroughly mixed during the equilibration period of 3 days. Prior tests had shown that the equilibration time was in fact as short as 2 hours. Data in Table show that the measured equilibrium aqueous concentrations of naphthalene matched very well with Raoult's Law predicted values for both the BG&E coal tar and the Stroudsburg coal tar. This implied that the naphthalene was in equilibrium between coal tar entrapped as a NAPL in the microporous media and the bulk aqueous phase. Thus the use of coal tar imbibed in microporous silica beads emulated the idealized system of coal tar NAPL in equilibrium with water as described in Figure 1. For the case where additional naphthalene was dissolved in the coal tars, the Raoult's law predicted value matched very well with measured concentrations for the BG&E tar but a variation in result occurred for the Stroudsburg tar. Such variation is probably due to errors in measurement of the total dpm actually present in the coal tar.

3.3 Naphthalene Mineralization : Initial studies

Experiments to assess microbial mineralization of naphthalene were designed to obtain information on mineralization rates and end points. Typical results from mineralization experiments with Stroudsburg and BG&E tars are shown in Figure 2. The mineralization pattern for both tars is characterized by an initial period of rapid mineralization over a few days, followed by gradual decrease in mineralization. An estimate of the first order biomineralization rate constant may be derived from the slope of the mineralization curve over the interval of day 2 to day 5, and knowledge of the initial naphthalene concentration, C_{so} . The equation :

$$V * \frac{dC}{dt} = - V * k_{bio} * C_{so} \quad (5)$$

is a first order expression which the pseudo-first-order mineralization rate constant is derived. The value of C_{so} is assumed to be equal to the Raoult's Law predicted aqueous solubility for the tar. The left hand side of the equation is the rate of change of the naphthalene mass in the aqueous phase and can be derived from the slope of the mineralization curve by multiplying the latter with m_{naph}^T , where m_{naph}^T is the total mass of naphthalene. In the above equation, V is the volume of water in the biometer. Thus the biomineralization rate constant k_{bio} is estimated by:

$$\frac{\text{slope of percent mineralization curve}}{100} = \frac{V * k_{bio} * C_{so}}{m_{naph}^T} \quad (6)$$

The k_{bio} for Stroudsburg tar was approximately 15/day and for the BG&E tar was about 3/day. The first order mineralization rate constants k_{bio} , are comparable, but slightly less for the BG&E tar compared to the Stroudsburg tar. The total naphthalene in the BG&E tar degraded to only about 15%, whereas for the Stroudsburg tar mineralization ceased after approximately 60% of the naphthalene was depleted.

The possibility that partial degradation of naphthalene, rather than complete mineralization was occurring for a significant mass of naphthalene was evaluated by measuring the activity of ^{14}C in the supernatant. Apart from the dissolved solutes and soluble metabolic byproducts of naphthalene, the supernatant contained some colloidal particles with naphthalene and other solutes sorbed on it. If substantial metabolic intermediates were formed in aqueous solution, these would result in a very high dpm count for the aqueous supernatant. The total dpm in the supernatant for biometers containing Stroudsburg tar was about 10% and for the biometers containing the BG&E tar was about 5%. These fractions are greater than the 0.3% and 0.8% that was expected from the equilibrium aqueous concentration of naphthalene from the Stroudsburg tar and the BG&E tar respectively. However, the values were not high enough to account for the fraction of naphthalene not mineralized and part of this high dpm was as a result of naphthalene sorbed onto colloidal matter. In fact, the significant fraction of residual radioactivity was found in the coal tar imbibed-silica bead slurry.

Bacterial counts of 10^6 to 10^7 cfu/mL were obtained from supernatant samples obtained at the end of the test. This indicated the presence of viable organisms even after naphthalene mineralization ceased. The inoculum size of 10^8 cfu would result in bacterial counts of about 2×10^6 cfu/mL after

dilution in the nutrient media in the biometers at the start of the test. The comparison of bacterial counts at the beginning and end of the experiment indicated no substantive bacterial growth. The presence of a viable bacterial population at the point where mineralization activity in the biometer had ceased, indicated that it was not the lack of potentially active bacteria that was responsible for the attenuation in mineralization activity. This was further verified by reinoculating biometers at the time naphthalene mineralization had ceased, and addition of inoculum did not recommence mineralization.

The important observation from the mineralization data is that for both the tars mineralization end points were notably less than the total naphthalene present. In light of the assumption that the microorganisms only degrade compounds dissolved in the aqueous phase, and that the microorganisms were size excluded from the pores of the silica beads, issues relating to the bioavailability of naphthalene were examined to interpret the above results.

Concentrations of BTEX, naphthalene and methylnaphthalenes in the aqueous bulk phase were measured at the 14th day and 30th day of the biodegradation test. Filtered supernatant of biometers containing BG&E coal tar imbibed in silica beads, were analyzed by GC. All solutes except naphthalene were either at very low concentrations or below detection limits (Table 6). The naphthalene concentrations in aqueous solutions from the BG&E samples confirmed that ample naphthalene was available to the microorganisms in the biometers at the point where mineralization had ceased, and thus bioavailability of naphthalene was not limiting degradation. The absence of the benzene and 1-methylnaphthalene indicates that these solutes were not preventing mineralization of naphthalene by being preferred substrates or by being inhibitory to the microorganisms by toxic effects. The concentration of naphthalene in the aqueous phase ranged between 9 and 16 mg/L. This concentration is in the same range as a predicted concentration of 15.7 mg/L, calculated using Raoult's Law for the 85% naphthalene remaining in the biometer. The fraction of naphthalene remaining in the biometer was estimated from the difference between the activity of ^{14}C naphthalene initially added to the biometer and the cumulative activity of the $^{14}\text{CO}_2$ sampled. The discrepancy in the aqueous concentration is probably due to uncertainty in the estimate due to naphthalene incorporated into biomass or converted into metabolic intermediates. The Raoult's Law predicted concentration for the Stroudsburg coal tar following 60% mineralization is approximately 1.5 mg/L.

3.4 Mass Transfer Experiments

Mass transfer tests were conducted to measure the rate of release of naphthalene from coal tar. Experiments were conducted with coal tar imbibed in microporous silica beads which had been 'aged' for a week and with unaged or 'fresh' imbibed coal tar. The appearance of interfacial films (Luthy et al., 1993) at the coal tar water interface when coal tar is aged over a few days was the motivation for assessing mass transfer rates from coal tar imbibed in silica beads that had been aged in water. The mass transfer rate coefficient was observed to decrease with time, and this is believed to be a result of the interfacial 'aging' phenomenon. Results from the flow-through mass transfer experiments are shown in Figures 3 and 4. Figure 3 demonstrates the different response of 'fresh' and 'aged' systems to flushing by water at a residence time of 1 min. The change in the measured effluent concentration, as well as the corresponding decrease in predicted equilibrium concentration, is plotted as a function of pumping time. The change in the equilibrium aqueous phase naphthalene concentration with pumping time was estimated from Equation 4, and confirmed by letting the contents of the reactor re-equilibrate after pumping was terminated at the end of the experiment. 'Aged' systems (Figure 4) showed a greater departure from equilibrium compared to 'fresh' systems (Figure 3). Similar experiments were conducted with systems in which the beads imbibed with coal tar had aged in water over periods of time ranging up to 1 month, and with coal tar imbibed silica bead slurry samples obtained from the biometers (Figure 4). Lumped mass transfer rate-coefficients were then estimated for the different systems, using Equation 3. The lumped mass transfer rate coefficient, $k_{la(t)}$ determined from the flow-through experiments ranges from 3000/day for 'fresh' systems at the start of pumping, to about 500/day for 'aged' systems at the end of the pumping test. The lumped mass transfer rate-coefficient was approximately 700/day for BG&E tar-silica bead slurry samples, removed from biometers after the mineralization test. Thus, while the aging phenomenon results in a significant reduction in mass transfer rates, the rate of mass transfer remains much higher than estimated biomineralization rates of 3/day to 15/day as measured from mineralization data.

3.5 Naphthalene Mineralization: Effects of Surfactants

Surfactants are molecules characterized by structural moieties of different polarity. A relatively polar 'head' and an apolar 'tail' causes such molecules to partition into NAPL-water interfaces with their polar heads in the aqueous phase and their apolar tails in the NAPL phase. Their presence at the NAPL water interface alters interfacial properties such as interfacial tension. The effect of surfactants on naphthalene mineralization was studied to verify if altering interfacial characteristics significantly altered mineralization patterns.

Numerous studies have been reported in the literature on the effect of surfactants on biodegradation of sorbed compounds (Laha and Luthy, 1991; Aronstein et al., 1991; Guerin and Jones, 1988) and on biodegradation of compounds from NAPLs (Efroymson and Alexander, 1991; Foght et al., 1989). No definite conclusions can be made, as the effect of surfactants on biodegradation differed from one study to the other. In the experiments conducted in this study, only sub-CMC concentrations of surfactants were used, as high surfactant concentrations could lead to release of coal tar from the micropores of the matrix. Also, micellar concentrations have been reported to inhibit to PAH degrading organisms, perhaps through use of surfactants as competitive substrates (Laha and Luthy, 1992).

The naphthalene mineralization pattern was not consistent in the presence of surfactants with different samples of Stroudsburg tar. In some cases, mineralization was enhanced when surfactants were present. However, this pattern was not reproducible through replicate identical experiments (Figure 5 and 6). Different doses of surfactants at sub-CMC concentrations did not result in any consistent trends in naphthalene mineralization. For the BG&E coal tar, there was no beneficial effect of surfactants on naphthalene mineralization (Figure 7). The reasons for enhancement in naphthalene mineralization, as observed for some cases for the Stroudsburg coal tar is unclear and is not bioavailability related, as equilibrium solubility of naphthalene from the coal tar does not change with addition of sub-CMC concentration of surfactants.

In conclusion, the nonionic surfactants did not affect the mineralization patterns consistently, except that nonionic surfactants did not suppress mineralization significantly in any case.

3.6 Naphthalene mineralization : Further studies

Aqueous naphthalene concentrations and rates of mass transfer of naphthalene from imbibed coal tar to the aqueous phase and were assessed for samples taken from biometers at the end of active mineralization period. The results from these experiments as discussed in the previous sections give a definite indication that mineralization end points were not caused by mass transfer limitations or non-availability of naphthalene as substrate to microorganisms.

The predicted mineralization curve was generated from the results of a simple dissolution-degradation model. As shown in Figure 1, the release of naphthalene from the coal tar present in the micropores occurs through a sequence of several mass transfer steps such as dissolution at the intrapore tar water interface, pore sorption-diffusion, and particle surface to bulk water transport. All these microscale processes are lumped together and the mass transfer rate coefficient $k_{la(t)}$, defined in Equation 3, is used to describe the rate of release of naphthalene from coal tar to the bulk aqueous phase. The rate of bacterial degradation of the aqueous phase naphthalene in the buffer solution is assumed to be first order with respect to naphthalene concentration with k_{bio} as the pseudo-first-order rate constant. The rate constants $k_{la(t)}$ and k_{bio} were measured from mass transfer experiments and mineralization tests, respectively. The change in aqueous phase concentrations in the biometer is thus given by:

$$\frac{\partial C(t)}{\partial t} = k_{la(t)} [C_{eq(t)} - C(t)] - k_{bio} C(t) \quad (7)$$

The above equation couples the processes of mass transfer and biodegradation that take place in the biometer. The overall rate of biotransformation is controlled by the slower of the two processes. In this case the biodegradation process is slower of the two processes as evident from the values of $k_{la(t)}$ and k_{bio} obtained from experiments. When $k_{la(t)} \gg k_{bio}$, the aqueous phase concentration of naphthalene in the reactor, $C(t)$, is very close to the equilibrium concentration $C_{eq(t)}$. The first term on the right hand side of the Equation 7 can be neglected, and the expression reduces to:

$$\frac{\partial C(t)}{\partial t} = - k_{bio} C(t) \quad (8)$$

Figure 8 compares experimental data for mineralization experiments with the Stroudsburg tar to that

predicted with a first order mineralization rate constant k_{bio} equal to 5/day. The predicted mineralization curve is obtained by converting the predicted values of values of $C(t)$ to mass of naphthalene remaining in the coal tar, using Raoult's Law. The experimental data show limited biodegradation after a period of approximately 15 days. In comparison, the predicted mineralization curve showed continued mineralization with time as it did not account for any limiting biodegradation factors. Such difference in mineralization patterns and the fact that bioavailability was not limiting naphthalene mineralization motivated further investigation of factors that may limit the biodegradation process.

Excess naphthalene was dissolved into the Stroudsburg and BG&E coal tars to assess if altering the total naphthalene mass in the coal tar had any effect on mineralization rates or end points. Dissolving excess naphthalene into the coal tar resulted in a higher equilibrium aqueous concentrations of naphthalene, proportional to the increase in the mole fraction of naphthalene in the coal tar. The mass of naphthalene in both the tars was increased by 58 mg per mL of tar. Mineralization tests with these tars amended with excess naphthalene, showed a decrease in the fraction of naphthalene mineralized. The mass of naphthalene that was mineralized from these amended tars is compared in Figure 9 and 10. For the Stroudsburg tar, increasing the mass of naphthalene in 1mL samples from 22 mg to 80 mg resulted in only a small (15 mg) increase in the amount of naphthalene mineralized. For the case of the BG&E tar, increasing the mass of naphthalene from 100 mg to 158 mg in 1mL samples resulted in only a 4 mg increase in the amount of naphthalene mineralized. It is apparent that increasing the mass of naphthalene in these tars does not proportionally affect the total amount mineralized. The higher concentration of naphthalene in the aqueous phase did not cause a proportional increase in mineralization rate or end point. In biometers where 2 mL of Stroudsburg coal tar was imbibed into the silica beads, the mass of naphthalene degraded was approximately 12 mg. This was the same mass of naphthalene that was degraded for the case where only 1 mL of tar was present in the silica beads.

This data suggests that naphthalene mineralization ceases after a certain period of time, before which the the mass of naphthalene degraded ranges from 7 to 26 mg. With the knowledge that there were no mass transfer limitations in the system studied, it was postulated, that some form of toxic inhibition was responsible for a relatively constant mass of naphthalene being degraded. This was studied by conducting dilution experiments and by addition of salicylic acid.

Biometer contents were diluted with an additional 50 mL of nutrient media after the time at which $^{14}\text{CO}_2$ generation had become negligible i.e. 30 days. In these cases dilution resulted in a recommencement of mineralization implying that the toxic condition was reversible upon dilution. Naphthalene mineralization was sustained for a few days before ceasing again. Results from this experiment are shown in Figure 11, for both the Stroudsburg and the BG&E tar.

On dilution, the naphthalene concentration in the aqueous phase of the test system would rapidly reequilibrate to essentially the same concentration as before dilution. This is because the equilibrium aqueous concentration was determined by the mole fraction of naphthalene present in the coal tar (Raoult's Law) and not explicitly by the small volume of water. Only compounds which are not partitioned into the NAPL were effectively diluted. It is probable that some inhibitory products formed from metabolites (not necessarily from naphthalene degradation) were diluted on addition of the aqueous media and enabled further mineralization. Although this phenomena was repeatable between experiments, mineralization activity in some duplicate biometers did not respond to dilution.

Toxicity-limited mineralization of naphthalene was verified by supplementing some of the biometers with salicylic acid after naphthalene mineralization had ceased. Salicylic acid is a metabolic intermediate of PAHs and it is readily metabolized by PAH degrading organisms (Cerniglia, 1984). Separate experiments were conducted to ensure that PAH degraders used in this study mineralized salicylic acid rapidly and extensively. Salicylic acid was added to sets of duplicate biometers for systems containing Stroudsburg and BG&E coal tars. In one of the duplicate biometers a stock solution of salicylic acid in nutrient media was added such that the concentration of the salicylic acid was 200 mg/L. In the other biometer a similar solution was prepared but containing 2 μCi of ^{14}C salicylic acid in addition to the unlabeled salicylic acid was added. The fraction of salicylic acid mineralized was obtained by measuring the activity of the $^{14}\text{CO}_2$ in the NaOH from the biometer containing ^{14}C salicylic acid and subtracting from it any activity in the biometer containing unlabeled salicylic acid. Figure 12 shows the mineralization of salicylic acid. Daily samples taken subsequent to addition of salicylic acid showed slow mineralization of salicylic acid in the biometers containing Stroudsburg tar and insignificant mineralization from biometers containing BG&E coal tar. However, upon dilution with an additional 50 mL nutrient media the mineralization of salicylic acid occurred very rapidly, although, mineralization in some sets of biometers containing BG&E coal tar did not respond to the dilution.

A possibility exists that nutrient limitations may have caused mineralization activity to cease. Although not likely, this will be evaluated in subsequent tests.

The lack of mineralization of salicylic acid prior to dilution indicated that the microorganisms were inactivated, and upon dilution became active in most cases. This suggests a toxic condition or a nutrient limited environment for the microorganisms. The reasons for inhibition and the compounds responsible for it have not been identified. It is difficult to speculate on any specific factor that may be responsible for the inhibitory conditions because of the complex chemical nature of the aqueous phase. The multitude of compounds released from coal tar into the aqueous phase make identification of toxic compounds or reaction products difficult. However, that biological phenomena was limiting mineralization of naphthalene was empirically determined through these experiments.

4 Summary

This work describes an investigation of some of the rate limiting factors in the biodegradation of naphthalene from coal tar-NAPL. Two coal tars were used in these studies, and were obtained from two former manufactured gas plant sites. The aromatic constituents of these tars were analyzed and naphthalenes were found to be the most abundant aromatics.

Biodegradation tests showed that mineralization of naphthalene ceased after a fraction of naphthalene had been mineralized. Approximately 60% of the naphthalene in the Stroudsburg coal tar and up to 15% of the naphthalene present in the BG&E coal tar was mineralized over 10 to 15 days, after which mineralization became negligible. The first order rate constant, derived from the mineralization data, was 3 /day and for the BG&E was 15 /day. The physico-chemical phenomena of equilibrium partitioning and mass transfer of naphthalene from coal tar imbibed in microporous silica have been investigated to address their role in the biodegradation of naphthalene. Measured equilibrium aqueous concentrations of naphthalene matched Raoult's Law predicted values. Naphthalene mass transfer rates were measured for both BG&E and Stroudsburg coal tars. Minimum values of mass transfer rate coefficient in these tests were about 500/day. Thus mass transfer was not rate limiting, as the mass transfer rate coefficient were much greater than the biomineralization rates coefficients of 3/day to 15/day. Further investigations revealed that the mass of naphthalene degraded remained fairly constant irrespective of the total naphthalene content of the tar.

The biodegradation test systems were evaluated as to whether toxic and inhibitory conditions may have limited naphthalene mineralization. Reinoculating with 10^8 cfu naphthalene degrading microorganisms did not restart mineralization. Dilution experiments where nutrient media was added to the biometers at the time when mineralization had ceased, revealed that in many cases dilution resulted in reviving mineralization. Salicylic acid, an easily-degradable compound that is in an intermediate in the degradation pathway of naphthalene, was scarcely degraded when added to the biometers after the appearance of negligible naphthalene mineralization. In some biometers, dilution with nutrient media was followed by rapid mineralization of salicylic acid, suggesting that dilution eliminated the inhibitory conditions. All these observations indicate the presence of conditions inhibitory to the microorganisms.

These results have revealed that the degradation of naphthalene from coal tar is not limited by 'bioavailability' and mass transfer constraints for the small size of the microporous media used. Despite the complexity of the composition of coal tars and the compositional variations of coal tars from different sources, the observations of limited mineralization of naphthalene due to inhibitory conditions has been consistent between the two coal tars studied.

Aronstein, B.N.; Calvillo, Y.M.; Alexander, M. 1991. Environ. Sci. Technol. 25:1728-1731.

Alvarez, P.J.J.; Vogel, T.M. 1991. Appl. Environ. Microbiol. 57:2981-2985.

Cerniglia, C.E. 1984. Adv. Appl. Microbiol. 30:31-71.

Efroymson, R.A.; Alexander, M. 1991. Appl. Environ. Microbiol., 57:1441-1447.

Erickson, D.C.; Loehr, R.C.; Neuhauser, E.F. 1993. Water Research. 27:911-919.

Foght, J.G.; Gutnick, D.L. Westlake, D.S.W. 1989. Appl. Environ. Microbiol. 55:36-42.

Guerin, W.F.; Jones, G.E. 1988. Appl. Environ. Microbiol. 54:937-944.

Harkins, S.M.; Truesdale, R.S.; Hill, R.; Hoffman, P.; Winters, S. 1988. *U.S. Production of Manufactured Gases: Assessment of Past Disposal Practices*; US Environmental Protection Agency, Cincinnati, OH, EPA/600/S2-88/012.

Laha, S.; Luthy, R.G. 1991. Environ. Sci. Technol. 25:1920-1930.

Laha, S.; Luthy, R.G. 1992. Biotechnol. Bioeng. 40:1367-1380.

Luthy, R.G.; Ramaswami, A.; Ghoshal, S.; Merkel, W. 1993. Environ. Sci. Technol., 27:2914-2918.

Mihelcic, J.R.; Luthy, R.G. 1991. Environ. Sci. Technol., 25:169-177.

Morgan, D.J.; Battaglia, A.; Hall, B.J.; Vernieri, L.A.; Cushney, M.A. 1992. *The GRI Accelerated Biotreatability Protocol for Assessing Conventional Biological Treatment of Soils : Development and Evaluation using Soils from Manufactured Gas Plant Sites*; Gas Research Institute: Chicago, IL, GRI-92/0499.

Nakles, D.V.; Linz, D.G.; Murarka, I.P. 1991. *"Bioremediation of MGP Soils: Limitations and Potentials"*, Paper presented at EPRI Technology Transfer Seminar on Management of Manufactured Gas Plant Sites, Atlanta, GA, April 2-3.

Ogram, A.V.; Jessup, R.E.; Ou, L.T.; Rao, P.S.C. 1985. Appl. Environ Microbiol., 49:582-587.

Rhodes, E.O. 1979. In *Bituminous Materials: Asphalts, Tars, and Pitches*; Kreiger Publishing Co.: Huntington, NY. Vol III. Chapters 1 & 2.

Rijnaarts, H.H.M.; Bachmann, A.; Jumelet J.C.; Zehnder, A.J.B. 1991. Environ. Sci. Technol., 24:1349-1354.

Peters, C.; Luthy, R.G. 1993. Environ. Sci. Technol., 27:2831-2843.

Sherman, D.F.; Loehr, R.C.; Neuhauser, E.F. 1988. *Proceedings of the International Conference on Physicochemical and Biological Detoxification of Hazardous Waste*, Atlantic City, N.J., May 3-5; Technomic Pub.: Lancaster, PA; pp.1035-1045.

Smith, J.R.; Weightman, R.L. 1988. *Co-Treatment of Manufactured Gas Plant Site Groundwaters with Municipal Wastewaters*. Final Topical Report to the Gas Research Institute, Chicago, IL, Contract No. 5086-254-1350, August.

**RESULTS OF ANALYSIS OF COAL TAR SAMPLES
COLLECTED FROM MONITORING WELL C4-W06-D**

	Sample 1 (CMU)	Sample 2-A	Sample 2-B	Sample 2-C
Date Collected	27 May 1993	2 June 1993	2 June 1993	2 June 1993
Date Analyzed	15 June 1993	15 June 1993	16 June 1993	29 June 1993
<u>VOLATILES mg/kg</u>				
Benzene	1.000	2.700	7.100	2.400
Toluene	3.400	7.800	20.000	6.400
Ethylbenzene	440	840	2.300	760
Styrene	2.500	4.500	NA	NA
Total Xylenes	3.700	6.800	19.000	5.300
<u>PAHs mg/kg</u>				
Naphthalene	100.000	100.000	150.000	100.000
Acenaphthylene	13.000	13.000	4.500	84.000
Acenaphthene	1.800	1.800	17.000	<30.000
Fluorene	1.600	1.600	22.000	<20.000
Phenanthrene	20.000	24.000	28.000	35.000
Anthracene	5.500	5.000	11.000	4.600
Fluoranthene	3.200	3.200	5.600	<10.000
Pyrene	10.000	8.800	11.000	<7.000
Benzo(a)anthracene	3.600	3.500	11.000	2.500
Chrysene	4.000	3.600	5.200	1.700
Benzo(b)fluoranthene	3.600	3.300	2.200	1.100
Benzo(k)fluoranthene	1.600	1.300	740	560
Benzo(a)pyrene	3.600	3.200	3.400	2.300
Dibenzo (a,h)anthracene	400	670	370	<300
Benzo (g,h,i)perylene	1.300	820	1.500	<2.000
Indeno (1,2,3-cd)pyrene	1.300	770	1.500	1.700
2-Methyl Naphthalene	53.000	52.000	NA	NA

Notes:

NA : Not Analyzed

* : Hydrocarbon types analyzed by the ASTM 2007

All other priority pollutant volatiles were below method detection limit.

Table 1. Results of analysis of coal tar samples collected from monitoring well C4-W06-D.

Classification	Wt%, BG&E Tar	Wt%, Stroudsburg Tar
Asphaltenes — the n-pentane insoluble faction	1.1%	34%
Polar compounds — material retained on adsorbent clay after percoaltion of the samples in a n-pentane eluent	0.4%	17%
Aromatics — material that passes through a column of adsorbent clay in a n-pentane eluent, but adsorbs on silica gel	98.4%	41%
Saturates — material in a n-pentane eluent that is not adsorbed on either the clay or the silica gel	0.1%	8%

Table 2. Classification of Stroudsburg and BG&E coal tar into characteristic groups using ASTM method D2007.

	MOLECULAR WEIGHT	WEIGHT PERCENT	ANALYTIC REFERENCE ^a
benzene	78	0.050	K
toluene	92	0.094	K
m-p-xylene ^b	106	0.32	K
o-xylene	106	0.41	K
naphthalene	128	2.16	P
2-methylnaphthalene	142	3.75	EP
1-methylnaphthalene	142	3.80	P
acenaphthylene	152	0.68	EP
biphenyl	154	0.50	P
1,2-dihydroacenaphthylene	154	1.2	T
acenaphthene	154	1.52	P
2-ethylnaphthalene	156	1.84	P
1-ethylnaphthalene	156	0.45	P
2,6-dimethylnaphthalene	156	1.99	P
9H-fluorene	166	1.4	T
1H-phenalene	166	0.4	T
trialkylated naphthalenes	170	4.3	T
phenanthrene	178	2.12	P
anthracene	178	0.59	P
methyl-9H-fluorenes	180	1.7	T
4-methyl-1,1'-biphenyl	184	0.2	T
dibenzothiophene	184	0.22	P
3-methylphenanthrene	192	0.55	EP
2-methylphenanthrene	192	0.43	EP
2-methylantracene	192	0.31	EP
4 H-cyclopenta[def]phenanthrene and 9-methylphenanthrene ^b	192	0.57	EP
1-methylphenanthrene	192	0.33	P
methyl dibenzothiophene	198	0.3	T
fluoranthene	202	0.30	EP
aceanthrylene	202	0.29	EP
pyrene	202	0.50	EP
1-phenylnaphthalene	204	0.29	EP
2-phenylnaphthalene	204	0.22	EP
9-ethylphenanthrene	206	0.17	EP
2-ethylphenanthrene	206	0.20	EP
dimethylphenanthrene	206	2.3	T
methylpyrene	216	3.9	T
benzo[a]anthracene	228	0.31	P
acetyrene	228	0.4	T
chrysene and triphenylene ^b	228	0.27	P
methylchrysenes	242	4.4	T
benzopyrenes	252	1.8	T
picene	278	0.13	P
total		47.66	

^a K = Killam Associates, P = PETC, EP = Estimated from PETC data, T = Texaco
^b Elute together.

Table 3. Stroudsburg coal tar composition from GC/MS analyses
(From Peters and Luthy, 1992).

Compound	Mole fraction	Pure compound Liquid Solubility mg/L	Predicted Conc. mg/L	Measured Conc. Day 7 mg/L	Measured Conc. Day 14 mg/L	Measured Conc. Day 30 mg/L
Benzene	0.021	1,780	36.94	35.5	39.5	34.5
Toluene	0.049	515	25.53	29.5	33	28
Ethyl-benzene	0.005	152	752	1.05	1.2	1.15
Meta & Para Xylenes	0.214	199	4.26	5.3	6.0	5.75
Ortho Xylene	0.039	175	6.78	6.1 ^a 4.8	6.2	5.8
Naphthalene	0.178 ^b	104 ^c	18.5	18.0	20.5	17
1-Methyl-naphthalene	NA	28.5	NA	0.87 ^a 0.22	<500	<500
2-Methyl-naphthalene	0.084	29.7 ^c	2.48	1.8	2.5	1.45

a: Measured values showing more than 25% variation have not been averaged.

b: Naphtalene mass in coal tar taken as 100,000 mg/Kg which is different from value from obtained by analysis of Sample 2B.

c: Liquid solubilities are obtained by multiplying the pure solid solubilities by the appropriate fugacity ratios. Fugacity ratio for naphthalene is 3.28 and for 2-Methylnaphthalene is 1.17

Table 4. Comparison of measured solute concentrations and Raoult's Law predicted concentrations from BG&E coal tar. Each measured concentration is an average of measurements from identical two test systems unless otherwise indicated. Mole fractions have been calculated based on analysis of Sample 2A.

Coal Tar	Naphthalene mass per mL coal tar <i>mg/mL</i>	Raoult's Law Predicted Conc. <i>mg/L</i>	Naphthalene Conc. from Equation 1 <i>mg/L</i>
BG&E Coal Tar	100	18.52	18.78
Stroudsburg Coal Tar	22	3.75	4.87
BG&E Coal Tar amended with 58 mg naphthalene	157.9	27.64	28.45
Stroudsburg Coal Tar amended with 58 mg naphthalene	80	12.9	17.7

Table 5. Comparison of Raoult's Law predicted naphthalene concentrations and equilibrium aqueous naphthalene concentrations measured by radiolabeled techniques.

Aqueous Solute Concentrations, $\mu\text{g/L}$						
Compound	Biometer 1 Sample 1 Day 14	Biometer 1 Sample 2 Day 14	Biometer 2 Sample 1 Day 14	Biometer 2 Sample 2 Day 14	Biometer 3 Sample 1 Day 30	Biometer 3 Sample 2 Day 30
Benzene	<100	<100	<40	<40	<700	<700
Toluene	728	702	449	182	<700	<700
Ethylbenzene	100	111	83	71	<700	<700
Meta & Para Xylenes	621	744	589	489	<700	<700
Ortho Xylene	492	539	428	407	<700	<700
Naphthalene	12840	13696	10379	15693	12840	9273
1-Methyl -naphthalene	<270	<270	139	346	<3570	<35700
2-Methyl -naphthalene	1284	1284	1070	2242	<3570	<35700

Note: Detection limit varied on different analysis dates

Table 6. Solute concentrations from supernatant samples from biometers containing BG&E coal tar. Samples were taken at day 14 and day 30 of a mineralization test.

Figure 1. Schematic diagram showing dissolution, mass transfer and biodegradation of PAH compounds from coal tar imbibed in microporous media.

Figure 2. Mineralization patterns of naphthalene from the Stroudsburg and BG&E coal tar imbibed in microporous silica beads. The estimated first order biomineralization rate constant k_{bio} for the Stroudsburg tar is 15/day and for the BG&E tar is 3/day.

Figure 3. System operated at a residence time of 30 minutes with 'fresh' Stroudsburg coal tar appears to have attained equilibrium. Equilibrium concentrations could be predicted fairly accurately from Raoult's law.

Figure 4. Aqueous phase naphthalene concentration in flow-through stirred tank reactors containing BG&E coal tar imbibed in microporous silica beads. The system was operated at a residence time of 1 minute. 'Aged' system shows a greater departure coal tar shows departure from equilibrium compared to the fresh systems.

Figure 5. Effect of surfactants on naphthalene mineralization from the Stroudsburg coal tar: Some tests showed enhancement in mineralization of naphthalene in the presence of sub-CMC concentrations of nonionic surfactants.

Figure 6. Effect of surfactants on naphthalene mineralization from the Stroudsburg coal tar: These results do not show an enhancement in mineralization.

Figure 7. Effect of surfactants on naphthalene mineralization from the BG&E coal tar: typical results. These tests consistently showed no significant effect on mineralization of naphthalene in the presence of sub-CMC concentrations of nonionic surfactants.

Figure 8. Comparison of simplified model mineralization predictions with mineralization test data.

Figure 9. Effect of varying naphthalene mass in Stroudsburg coal tar on naphthalene mineralization. Different naphthalene contents of the tar results in approximately the same naphthalene mass being mineralized. Values in parenthesis indicate initial naphthalene mass is the coal tar. The coal tar was imbibed in the silica beads.

Figure 10. Effect of varying naphthalene mass in Stroudsburg coal tar on naphthalene mineralization. Different naphthalene contents of the tar results in approximately the same naphthalene mass being mineralized. Values in parenthesis indicate initial naphthalene masses in the coal. The coal tar was imbibed in the silica beads.

Figure 11. A recurrence in naphthalene mineralization following dilution with nutrient media.

Figure 12. Enhancement in salicylic acid mineralization on dilution with nutrient media following a 44 day test period.

**DATE
FILMED**

9/22/94

END