STATUS REPORT
AND REVISED LAGOON CLOSURE PLAN
FOR
BUCKLEY & MANN, INC.
NORFOLK, MASSACHUSETTS

OCTOBER 1990

CAMP DRESSER & McKEE INC.
Cambridge, Massachusetts

INTRODUCTION

This report has been prepared to update the status of the closure of the former wastewater treatment lagoons at Buckley & Mann, Inc. (B&M) and to revise the previous closure plan to reflect the current conditions at the site.

The Norfolk Board of Health's last action on the project was to issue a Site Assignment pursuant to Massachusetts General Laws Chapter 111 Section 150A in July 1988 for a pilot composting experiment. Since then, new information on the actual conditions of the lagoons has shown that composting is no longer feasible. This report describes the new information, the results of bench scale experiments on biodegradation of the organic material in the lagoon subsoils, and presents a revised plan. B&M seeks Board approval of the revised closure plan presented below, including both the biodegradation process and the final disposal recommendation.

For the convenience of the reader, the history of the site and previous closure-related actions are summarized below. More details are available in a series of reports prepared by Camp Dresser & McKee Inc. (CDM) and listed in the Appendix.

RECENT HISTORY OF THE SITE

B&M has manufactured textile products for over 100 years at its facility in Norfolk, Massachusetts, northwest of the junction of Park and Lawrence Streets. Current operations at the plant do not generate process wastewaters. Formerly, two operations generated wastewaters which were treated in earthen lagoons. **Figures 1 and 2** show the location of the lagoons on the property.

Until 1986, the company operated a small dyehouse which discharged approximately 40,000 gallons per week to Lagoons #1 and #2 for settling and facultative biological treatment. The lagoons operated

in series and discharged by percolation to the ground adjacent to the Tail Race, a manmade brook paralleling to the Mill River.

Lagoon #1 was built prior to 1950. The bottom had been scraped once prior to 1975 to remove a sludge layer and improve percolation. Approximately 100 cubic yards of soil/sludge from this operation were stockpiled just south of the lagoon (Pile A, Figure 2). By 1980, this material had the appearance of clean sand with small fragments of decayed rags. Any sludge formerly in this material was thoroughly decomposed to inorganic constituents. In late 1986, the trench leading from the dyehouse to Lagoon #1 was scraped to remove leaves, textile fiber and some of the underlying soils. Approximately 200 cubic yards of this material were stockpiled just south of Lagoon #1 (Pile B, Figure 2). The trench was filled with clean sand and regraded to eliminate it as a source of surface runoff to Lagoon #1.

Lagoons #2 and #3 were constructed in 1978. Lagoon #2 received the effluent from Lagoon #1. Lagoon #3 never received wastewater, but served as a diversion ditch for a groundwater spring that was fed from the surrounding hillsides.

B&M operated a carbonizer process to reclaim wool from old garments. The carbonizer process was active through the 1940s and diminished thereafter. The carbonizer building was demolished in 1965. The process consisted of passing the stock through hot acid to char the cotton threads on the seams, zippers, buttons, etc., and facilitate their separation from the wool. The wool was then neutralized and rinsed, and the solid residue consisting of fiber and fasteners was discarded on site. The wastewater was discharged to the Carbonizer Lagoon for settling and facultative biological treatment.

GEOLOGY AND HYDROLOGY OF THE SITE

The Mill River and the Tail Race drain the surface runoff from the surrounding low hills, which consist of rhylite and shale bedrock overlain by glacial till and stratified drift. Precipitation on the

hillsides which does not run off or evapotranspirate percolates into and through the unconsolidated soils and into joints/fractures in the bedrock to recharge groundwater. The groundwater then flows to the Mill River, which is the discharge point for the region. The lagoons are in the bottom of the valley, near the River, where the groundwater table intercepts the surface and discharges, rather than recharges.

MASSACHUSETTS DEPARTMENT OF ENVIRONMENTAL PROTECTION (DEP) INVOLVEMENT

Pursuant to an agreement between B&M and the DEP's Division of Water Pollution Control (DWPC), CDM conducted a site investigation in 1986. This investigation included installation of seven new monitoring wells, followed by extensive groundwater, surface water, soils and lagoon sludge sampling. The results were submitted to the DWPC and the Board in a 1986 report by CDM. The DWPC agreed with CDM's conclusions that the lagoons had no significant adverse impact on groundwater or surface water.

As part of the agreement, B&M was required to develop a closure plan for the lagoons, including remediation of sludges from the Lagoons #1 and #2. The 1986 analyses showed that the sludges contained traces of dye carriers. The sludges, and stockpiled soils from the scraping of Lagoon #1 prior to 1975, also contained some metals (primarily trivalent chromium) incidental to the former dye operations.

Tests of the bottom soils in the Carbonizer Lagoon did not show any contamination with dye carrier compounds, and no remediation or closure actions were required by the DWPC.

NORFOLK BOARD OF HEALTH INVOLVEMENT

In 1987, CDM estimated that there were up to 250 cubic yards of dilute sludge in the lagoons, based on measurements taken when the lagoons were still in service. CDM recommended that B&M excavate the sludge to elevate it above the groundwater table and then compost it to stabilized the organic material which include approximately 150 mg/kg

dry weight of dye carriers. CDM prepared a work plan for composting and in 1987, B&M requested a Site Assignment from the Norfolk Board of Health. In July 1988, the Board issued an Assignment for the proposed composting, subject to the requirement that a pilot experiment be completed first with a small portion of the sludge. A copy of the Assignment and B&M's comments on the Assignment's conditions is included in the Appendix. Composting never took place, as explained below.

REVISION OF SLUDGE QUANTITY ESTIMATES AND EXCAVATIONS

CDM reinspected the lagoons in October 1988 after a period of dry weather, when the actual conditions of the bottom materials could be easily determined. Lagoon #3, the groundwater diversion lagoon, was dry.

The bottom of Lagoon #1 was covered by less than 1" of a sludge/leaves mixture, in which there was a blue pigment-like layer less than 1/8" thick. B&M scraped up the sludge/leaves mixture and a thin layer of the underlying sand with hand tools for a total of approximately 20 cubic yards. The combined predominately sandy material was stored in drums and on a plastic sheet adjacent to the Lagoon #1. The Lagoon #1 bottom sand bed exposed after the scraping had a dark color from anaerobic decay of leaves and other organic material. The black color faded and the sand returned to its natural light tan color within about a day after the sand was piled above the water table, in conditions which allowed the water to drain and exposed the organic material to aerobic conditions. A sample of the material analyzed by CDM contained 85% solids (dry weight at 103°C) and 0.5% organic material (measured as "volatile solids" by loss after heating to 550°C), far too low for composting, which typically requires 20 to 40% volatile solids prior to mixing with a bulking agent like wood chips.

Approximately 20% of Lagoon #2 was dry and the remainder was covered with water at a depth of between 3" and 18". Weeds were encroaching in the shallow areas. There were no sludge deposits visible.

Based on the October 1988 field conditions, CDM concluded that the former 250 cubic yard estimate of sludge was no longer accurate. That estimate had been made when the lagoons were full of water, which made sampling and bottom layer measurements difficult. Also there had been time for any sludge to biologically degrade over the 2.5 years since the wastewater discharge to the lagoons was terminated. Nevertheless, there were still traces of dye carrier in the soils and these required further degradation as part of the overall closure of the lagoons.

PILOT EXPERIMENT

In December 1988, CDM recommended that B&M expose the soils to moist, aerobic conditions to biologically oxidize the dye carrier compounds. The recommendation was based on literature reports describing treatment of similar compounds and the consideration that composting was no longer applicable. Excerpts of these literature reports are included in the Appendix. The majority of the dye carrier compounds in the untreated soils had very low vapor pressures at room temperature, low water solubilities and high adsorption affinity for the soil. Consequently, CDM anticipated that the removal of these compounds under the test conditions would be caused by biological oxidation rather than evaporation or extraction into the leachate.

At CDM's direction, B&M conducted two bench scale experiments from May 1989 to May 1990 to test the recommended process. The test soil was taken from Lagoon #1 subsoils after the lagoon had been scraped in 1988. One of two experimental soil systems was supplemented with a small dose of nitrogen and phosphorous fertilizer.

The experimental apparatus for each test was a 3 gallon polyethylene tub with a layer of crushed stone to act as a "leachate" reservoir in the bottom. About 10" of test soil was placed over the stone, as shown in **Figure 3**. A small volume of water was applied to the soil initially and was supplemented as needed to make up for evaporative losses. Leachate was drained through a spigot at the bottom of the tub and recycled onto the soil periodically to keep the soil moist.

Treatment progress was monitored by analysis of test soil samples for:

1. Total petroleum hydrocarbons (TPH), measured by infrared absorbance of solvent extracts of the soil (EPA Method 9071/418.1).

   The total petroleum hydrocarbon (mg/kg dry weight) results are summarized below. The total solids (dry weight at 103°C) increased from 85 to 97% over the course of the experiment. Blank spaces indicate that no test was performed.

Date	Lagoon field Sample	Non-Fertilized Bench Pilot	Fertilized Bench Pilot
11/88	210		
5/89(Start)	90		
8/89	32		
3/90	73	130	
5/90		<50	

   A sample of the recirculated "leachate" analyzed during the third month of the experiment contained less than the 5 mg/l detection limit for TPH, indicating that the TPH compounds were not being extracted into the water.

2. Specific compounds, including dye carriers, measured by gas chromatography/mass spectroscopy of solvent extracts under base/neutral conditions (B/Ns, EPA Method 625/ SW 846 8270). B/Ns are a subset of TPH. The results are shown in Table 1. Blank spaces in the table indicate that the concentration of the chemical was below the detection limit shown on the last line. The standard set of B/Ns is 43 indicator compounds which were selected by EPA in the 1970s. For the B&M samples, CDM extended the analyses to identify and estimate the concentration of the compounds creating the 10 largest peaks in chromatogram, in addition to the standard set. The "Others" line in Table 1 is the sum of the concentration of compounds which could not be fully identified

by gas chromatography/mass spectroscopy or which were present only in some samples.

The data show the concentration of B/Ns declined to almost non-detectable limits after 9 months in both the fertilized and unfertilized experiments. This 12-month sample confirmed this result. The TPH concentrations were less consistent, although the final sample was below the detection limit.

CDM concluded from the pilot experiments that the TPH, B/N and dye carrier compounds in the test samples could be degraded biologically under the test conditions and that B&M should proceed with full scale implementation of process.

CURRENT STATUS OF SOILS FROM THE LAGOONS

In August 1990, CDM analyzed composite samples from the soils excavated from the trench in 1986 and Lagoon #1 in 1988. Samples were collected at a depth of 12" below the soil surface and composited from at least four locations for each sample. **Table 2** shows the B/N results. The estimated soil volumes, the TPH concentrations, and the estimated mass of TPH are shown in **Table 3.**

The TPH and B/N concentrations were higher in the soils excavated from Lagoon #1 in 1988 than in a 1986 sample (see Table 1) because the 1988 sample was collected primarily from soils containing the thin blue-pigmented layer, where the TPH compounds have concentrated. The concentrations in the drummed soils excavated in 1988 were higher than those in the piled soils from 1988 most likely because the latter have been exposed to aerobic conditions and suitable to biodegradation. Conversely, the drummed material has remained anaerobic and little or no degradation was observed.

The TPH in the soils from the trench were likely of similar concentration and composition as those from the lagoon at the time of excavation. By September 1990, the TPH concentration in the trench soils

was less than 20% of the concentration in the Lagoon #1 material piled in 1988 and 10% of the concentration of the anaerobically preserved material in the drums. Apparently, the TPH in the soil from the trench decreased to the concentration measured in September 1990 because it had been exposed to aerobic conditions for two years, compared to one year for the soils piled from Lagoon #1.

REVISED LAGOON CLOSURE PLAN

The pilot tests have shown the feasibility to biochemically degrade the TPH and specific dye carrier compounds. CDM recommends that B&M proceed with treat all of the soils excavated in 1987 and 1988 by this method. The biological degradation should be allowed to continue until the TPH has been reduce to 300 mg/kg. CDM recommends this target concentration based on comparison to the Department of Environmental Protection guideline for on-site reuse of similar soils, as described in Policy #WSC-89-001, "Management Procedures for Excavated Soils Contaminated with Virgin Petroleum Oils".

CDM also recommends that B&M test the subsoils in Lagoons #1 and #2 for TPH. Additional soil should be excavated for treatment if the TPH is above the 300 mg/kg limit.

FINAL DISPOSAL OF SOILS

In its 1988 Site Assignment, the Norfolk Board of Health stipulated that "the composted residue [from lagoon sludges] shall be removed to a commercial landfill outside of the Town of Norfolk that has been designated as approved for that purpose by the Massachusetts Department of Environmental Quality Engineering" [now the DEP]. CDM believes that the Board's concern at that time was that the material proposed for disposal would resemble sludge and that it might contain significant concentrations of TPH and B/N dye carrier compounds. CDM recommends that the Board reconsider the disposal issue in light of the fact that the material is not sludge, but rather sand with less than 0.5% organic material. Furthermore, the proposed treatment will

biodegrade the TPH and B/N compounds. The treated material will have characteristics similar to sand recovered from street sweeping and would provide excellent daily cover in the Town's landfill. Local disposal would provide a safe, economical solution to site closure for B&M.

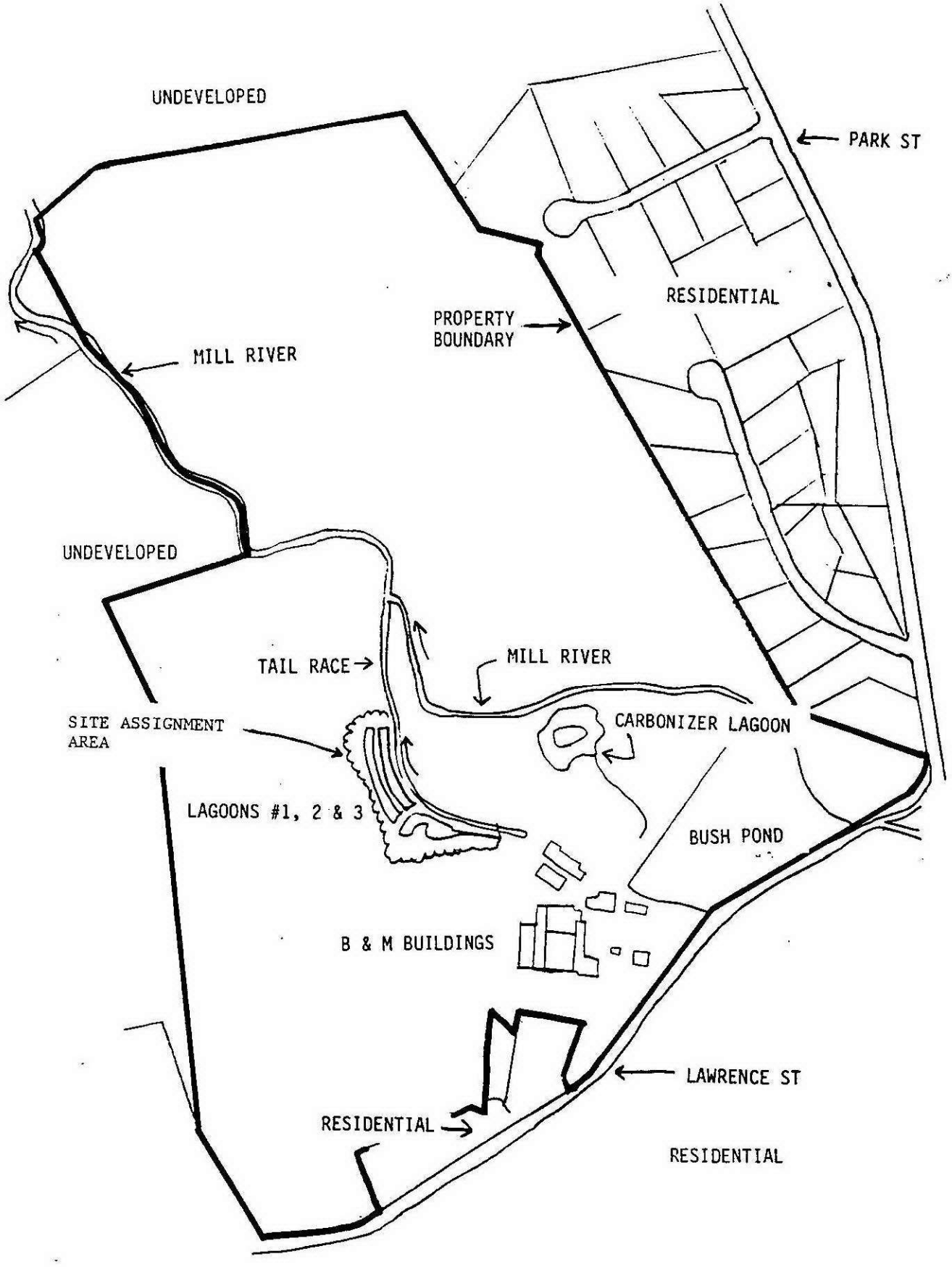


FIGURE 1
BUCKLEY & MANN PROPERTY BOUNDARY
AND ADJACENT LAND USE

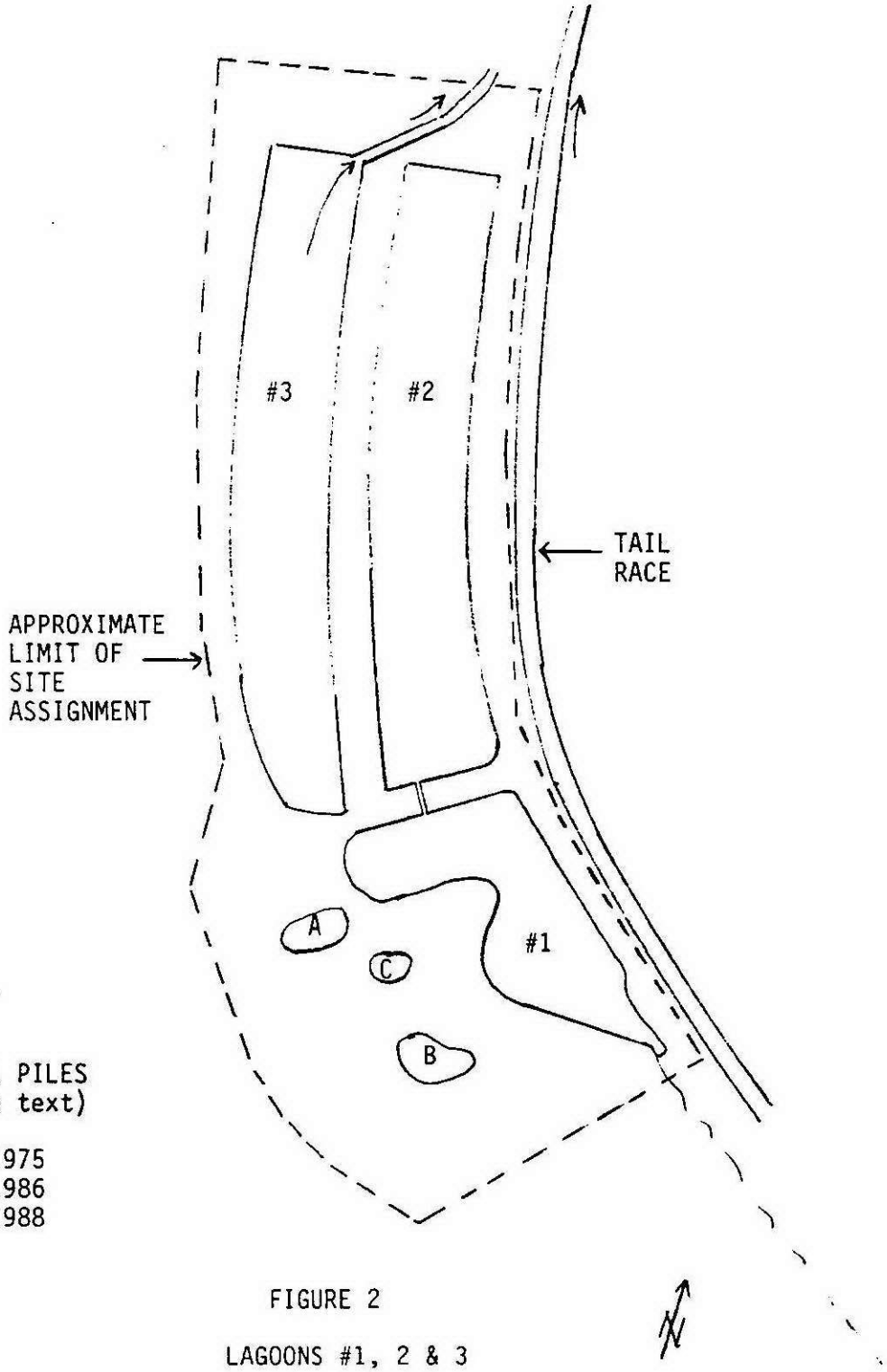


FIGURE 2

LAGOONS #1, 2 & 3

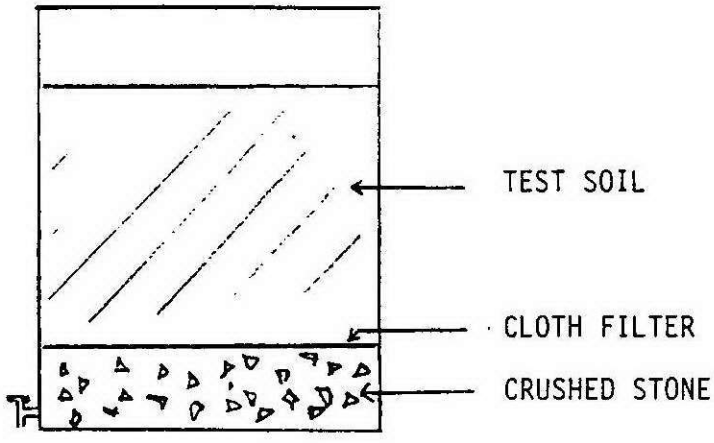


FIGURE 3

BENCH SCALE TEST

TABLE 1
PILOT EXPERIMENT
BASE NEUTRALS, mg/kg

	LAGOON FIELD SAMPLES		PILOT START	NO. FERTILIZER PILOT 3 MONTHS	NO. FERTILIZER PILOT 9 MONTHS	FERTILIZED PILOT 9 MONTHS	FERTILIZED PILOT 12 MONTHS
PARAMETER	5/86	5/86	5/89	8/89	3/90	3/90	5/90
bis(2-Chloroethyl)ether							
1,3-Dichlorobenzene		3.2					
1,4-Dichlorobenzene		9.1					
1,2-Dichlorobenzene		5.7					
bis(2-Chloroisopropyl)ether							
N-Nitroso-di-n-propylamine							
Hexachloroethane							
Nitrobenzene							
Isophorone							
bis(2-Chloroethoxy)methane							
1,2,4-Trichlorobenzene	16	61	1.1				
Napthalene	10	8.7	1.4	1.9			
Hexachlorobutadiene							
Hexachlorocyclopentadiene							
2-Chloronapthalene							
Dimethyl phthalate							
Acenaphthylene				2			
Acenapththene	8.6	5.3	3.9	2.4	0.19	0.25	
2,4-Dinitrotoluene							
2,6-Dinitrotoluene							
Diethyl phthalate							
4-Chlorophenyl phenyl ether							
Fluorene	3.4		2.1	2.3			
N-Nitrosodiphenylamine							
4-Bromophenyl phenyl ether							
Hexachlorobenzene							
Phenanthrene			1	8.7			
Anthracene				2.3			
Di-n-butyl phthalate							
Fluoranthene				4			
Pyrene				7.9			
Butyl benzyl phthalate							
3,3'-Dichlorobenzidine							
Benzo(a)anthracene				2.7			
bis(2-Ethylhexyl)phthalate					0.95	0.21	0.11
Chrysene				3.2			
Di-n-octyl phthalate							
Benzo(b)fluoranthene				5.1			
Benzo(k)fluoranthene							
Benzo(a)pyrene				5			
Indeno(1,2,3-c,d)pyrene							
Dibenz(a,h)anthracene							
Benzo(g,h,i)perylene				1.9			
Benzene, 2-ethyl-1,4-dimethyl	11	3.8					
Methyl napthalene	16	17	3.3				
Dimethyl napthalene	2		1.8	5			
4-Nonyl phenol	10	11			0.78	0.8	0.5
Heptadecane	7	7		5(?)			
1,1'- Biphenyl	23	29					
Isoquinoline	1.4		2.8				
Ethenyl napthalene			8.1				
Others	7	40	10	13	1	1	1.5
Detection limit	3.3	1.7	0.4	0.83	0.16	0.16	0.08

TABLE 2
CURRENT SOILS DATA
BASE NEUTRALS, mg/kg

	TRENCH SOILS (PILED)	LAGOON #1 SUBSOILS (PILED)	LAGOON #1 SUBSOILS (DRUMMED)
PARAMETER	8/90	8/90	8/90
bis(2-Chloroethyl)ether			
1,3-Dichlorobenzene			0.79
1,4-Dichlorobenzene			0.79
1,2-Dichlorobenzene			
bis(2-Chloroisopropyl)ether			
N-Nitroso-di-n-propylamine			
Hexachloroethane			
Nitrobenzene			
Isophorone			
bis(2-Chloroethoxy)methane			
1,2,4-Trichlorobenzene	0.34	1.5	22
Napthalene			10
Hexachlorobutadiene			
Hexachlorocyclopentadiene			
2-Chloronapthalene			
Dimethyl phthalate			
Acenaphthylene			1.4
Acenapththene	0.65	2.4	45
2,4-Dinitrotoluene			
2,6-Dinitrotoluene			2.6
Diethyl phthalate			
4-Chlorophenyl phenyl ether			1.4
Fluorene			
N-Nitrosodiphenylamine			
4-Bromophenyl phenyl ether			
Hexachlorobenzene			
Phenanthrene		0.72	10
Anthracene		0.69	2.9
Di-n-butyl phthalate			
Fluoranthene		0.7	1.4
Pyrene		0.77	0.9
Butyl benzyl phthalate			
3,3'-Dichlorobenzidine			
Benzo(a)anthracene			
bis(2-Ethylhexyl)phthalate		2.3	3.4
Chrysene			
Di-n-octyl phthalate			
Benzo(b)fluoranthene	0.33		
Benzo(k)fluoranthene			
Benzo(a)pyrene			
Indeno(1,2,3-c,d)pyrene			
Dibenz(a,h)anthracene			
Benzo(g,h,i)perylene			
Benzene, 2-ethyl-1,4-dimethyl			
Methyl napthalene			139
Dimethyl napthalene			
4-Nonyl phenol	0.72	23	
Heptadecane			45
1,1'- Biphenyl	0.47		56
Isoquinoline			
Ethenyl napthalene			
Others	7	100	300
Detection limit	0.3	0.6	0.7

TABLE 3

TOTAL PETROLEUM HYDROCARBONS IN SOILS NEAR LAGOON #1

Source	Volume Ft^3	TPH mg/kg or ppm	TPH* Pounds
Lagoon #1 soils place in drums 1988	94	4,800	45
Lagoon #1 soils piled on plastic 1988	540	2,600	140
Trench soils excavated 1987	5400	440	240
Lagoon #1 soils excavated pre 1975	2700	0**	0

* Based on sand at 100 pounds per cubic foot
**CDM estimate based on pilot data

APPENDIX

* LIST OF PREVIOUS REPORTS

* SITE ASSIGNMENT AND COMMENTS

* EXCERPTS FROM THE LITERATURE ON BIOCHEMICAL DEGREDATION OF BASE/NEUTRAL COMPOUNDS

LIST OF PREVIOUS REPORTS

BUCKLEY & MANN SITE INVESTIGATION AND LAGOON CLOSURE

1. July 1986, CDM report on an Environmental Site Assessment
2. July 1986, Final Judgment, Superior Court Civil Action, Division of Water Pollution Control vs. Buckley & Mann, Inc.
3. February 1987, CDM request for site assignment
4. May 1987, CDM description of Proposed Pilot and Full Scale Composting of Lagoon Sludges
5. July 1988, Site Assignment from the Norfolk Board of Health
6. September 1988, CDM Comments on Site Assignment
7. December 1988, CDM Interim Report on Lagoon Closure

LEGAL NOTICE
NORFOLK BOARD OF HEALTH
SITE ASSIGNMENT

The Town of Norfolk Board of Health, acting in accordance with Section 150A of Chapter 111 of the General Laws, hereby assigns as a site for use for composting of sludge, property located on the northerly side of Lawrence Street in Norfolk, Massachusetts as follows:

1. The site is assigned for the purpose of conducting a pilot scale operation for the on-site drying and composting of chemical containing sludge as removed from the existing lagoons #1 and #2 on the subject site. The lagoons have been receiving effluent from the textile dyeing facility which has been discontinued in 1986.

2. The area assigned by the Board of Health is bounded and described as shown on a plan entitled "SITE ASSIGNMENT IN NORFOLK, MASS., Scale: 1" = 30', September 21, 1987, Stavinski Engineering Associates, Inc., Reg. Prof. Land Surveyors & Prof. Engineers, 78 South Street, Wrentham, Mass." a copy of which is on file in the Norfolk Board of Health office.

3. The composted residue shall be removed to a commercial or municipal landfill outside of the Town of Norfolk that has been designated as approved for that purpose by the Massachusetts Department of Environmental Quality Engineering.

4. The operations shall be generally as described in a report prepared by Camp, Dresser, and McKee, dated May 1987, Section entitled "Phase 1: Pilot composting", except as specified herein. A copy of this report is on file in the Board of Health office. Prior to any construction or operation of the pilot plant, detailed plans and specifications shall be submitted to the the Massachusetts Department of Environmental Quality Engineering and the Town of Norfolk Board of Health for review and written approval from those agencies. All construction and operations shall be subject to the following conditions:

A. ODOR CONTROL

Provisions shall be taken to ensure that no unpleasant or obnoxious odor, as determined by the Board of Health, shall be generated that will be a nuisance or a hazard to the public health. In the event of any such odor, Buckely and Mann shall immediately cease such operations upon verbal or written order from the Board of Health or its authorized agent.

B. PORE WATER AND LEACHATE CONTROL

All pore water and leachate release from the composting shall be collected. It shall be sampled and tested for Base/Neutral extractable compounds and Volatile Organic Compounds. If, in the opinion of the Board of Health, such water is found to be contaminated, it shall be collected, dried, and mixed with the composting residue.

C. PORE WATER FROM SLUDGE DEWATERING

Pore water from sludge dewatering shall be collected. It shall be sampled and tested for Base/Neutral extractable compounds and Volatile Organic Compounds. If in the opinion of the Board of Health, such water is found to be contaminated, it shall not be allowed to be discharged back to the surface waters or ground waters. It shall be the responsibility of the applicant to dispose of such waters offsite at a location approved by the Massachusetts Department of Environmental Quality Engineering, or to cause such contaminated waters to be treated to a degree suitable for discharge to the ground or surface waters as determined by the Massachusetts Department of Environmental Quality Engineering and the Norfolk Board of Health.

D. UNSUCCESSFUL PILOT PLANT OPERATION

Prior to any disposal of pilot plant sludge, it shall be tested for effectiveness in contaminant removal. If, in the opinion of the Board of Health, the residual contaminant level indicates the process to be ineffective, no further or additional operation shall be allowed, all operations shall be terminated, and this site assignment shall be null and void.

Any person aggrieved by this action of the Board of Health may, within sixty (60) days of this publication, appeal to the Commissioner of the Department of Environmental Quality Engineering, c/o Docket Clerk, 20th Floor, 100 Cambridge Street, Boston, MA 02101

NORFOLK BOARD OF HEALTH
Laurence Magner, Chairman
Thomas R. Gilbert, Clerk
Albert G. Andersen, Member

Issued about 7/25/88

CDM
environmental engineers, scientists, planners, & management consultants

CAMP DRESSER & McKEE INC.
One Center Plaza
Boston, Massachusetts 02108
617 742-5151

September 16, 1988

Mr. Richard Mann
Buckley & Mann, Inc.
P.O. Box 409
Franklin, MA 02038

Re: Norfolk Board of Health
Site Assessment Conditions

Dear Dick:

Camp Dresser & McKee Inc. (CDM), has reviewed the Site Assessment issued for public comment by the Norfolk Board of Health in late July, 1988 relative to sludge management and closure of the former wastewater treatment lagoons at Buckley & Mann. CDM has the following comments and the Board's conditons in the Assignment.

Item 3

The Board states that the residue shall be removed to a commercial or municipal landfill outside of Norfolk. CDM believes that a decision on whether or not to allow the dried and/or composted sludge into the Norfolk landfill should be made only after the residue has been characterized at the time of disposal. Norfolk should keep the option of local disposal open.

Item 4

While the water level in Lagoons 1 and 2 fell considerably in the summer of 1988, they did not dry out. This was surprising because rainfall for the year (as measured in Boston) through mid September was 15% below the long term average, drying conditons were better than average due to the hot summer, and groundwater levels in southeast Massachusetts are low. CDM concludes that the bottom of the lagoons are at the groundwater level and that the sludge will have to be removed in a wet condition, without the benefit of in-place drying.

CDM anticipates that it will be necessary to pump water from Lagoon #1 to #2 and remove the wet sludge from #1 with a front end loader or dredge. Then water would be pumped from #2 to #1 to access sludge in #2. The wet sludge would be stockpiled to dry up hill of Lagoon #1, so that free water would drain back toward that lagoon. This water should be substantially the same as the water which has been in the lagoons for many years, and the impact of the proposed dewatering on groundwater and the Mill-River would be the same as leaving the sludge in the lagoons.

CDM concludes that collecting the water during excavation and initial drying would not be possible. The Board's stipulation that the water be stored and tested prior to disposition is neither practical nor necessary as the area is not a groundwater recharge zone (see CDM's July 1986 report for further discussion on the local hydrology). For similar reasons, CDM believes it would be unnecessary to collect pore water from any subsequent soils aeration/drying beds which would be built on the site of Lagoon #2, as described in our February and May 1987 submittals. We agree with the Board that any leachate from composting operations should be collected and tested prior to disposal, or mixed into the compost, as the elevated temperatures in composting may cause unforseen leachate characteristics.

CDM also recommends that Buckley and Mann ask the Board to provide for comment its criteria for water found to be contaminated, as sited in Items 4B and 4C.

If you have any questions, please do not hesitate to contact us.

Very truly yours,

CAMP DRESSER & McKEE INC.

Robert A. Dangel

Approved by:

James T. O'Rourke, Ph.D., P.E.
Senior Vice President

ENVIRONMENTAL FATE OF SELECTED SEDIMENT POLLUTANTS

Final Report

By

Christopher P. Loreti
Alec W. Naugle
Warren J. Lyman
Susan F. Coons

Arthur D. Little, Inc.
Cambridge, MA 02140

Contract No. 68-01-6951, Task 24

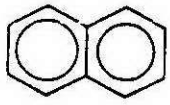
Charles Delos, EPA Task Manager

MONITORING AND DATA SUPPORT DIVISION
OFFICE OF WATER REGULATIONS AND STANDARDS
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC

September, 1987

POLYCYCLIC AROMATIC HYDROCARBONS

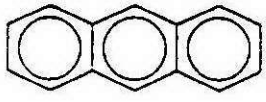
Structures:



Naphthalene

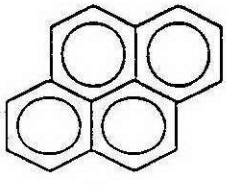
Common Synonyms:

Naphthene
White tar
Tar camphor



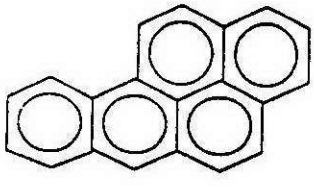
Anthracene

Paranaphthalene
Green oil
Tetra olive NZG



Pyrene

Benzo(def)phenanthrene



Benzo(a)pyrene

3,4-Benzopyrene
BaP

I. STATEMENT OF PROBABLE FATE

Polycyclic aromatic hydrocarbons (PAHs) are considered as a class in this section. Four examples are illustrated above and property data for them are given throughout the text.

Table 5-1 summarizes the important environmental fate processes for PAHs. In aquatic sediment environments, PAHs are expected to partition strongly to the sediment particles. This is especially true for the higher molecular weight PAHs (three or more rings) and in sediments rich in organic matter. Sorption to dissolved organic matter is also expected to occur, increasing the mobile fraction of the more hydrophobic PAHs to a far larger value than would be expected based on their aqueous solubility. Bioaccumulation is also expected to occur to some extent in sediment species, but since PAHs tend to be metabolized, depuration occurs rapidly once exposure ceases.

Neither hydrolysis nor abiotic oxidation or reduction is expected to be significant in sediments. Photolysis is also unlikely to be important, but may occur at the surface of sediments in very shallow water or on sediments resuspended near the water surface. Aerboic biodegradation is expected to be significant especilly where microbes have had a chance to adapt. In anoxic environments typical of many sediments, biodegradation is expected to be minimal.

3. Biodegradation

The biodegradation of PAHs depends largely on the specific compound being investigated and the environment in which it is present. In general, the rate of biodegradation decreases with the number of rings in the structure (27,28) and can be expected to be more rapid in aerobic environments than in anaerobic ones (29).

The smallest PAH, naphthalene is readily biodegraded in both soil (30) and aquatic environments (22,28,31). The degradation of larger PAHs has been found to be less rapid, however. The biodegradation rate of radiolabeled PAHs in aquatic sediments were found to decrease in the order naphthalene, anthracene, benzo(a)anthracene, benzo(a)pyrene (32), and a similar rate relationship was found between naphthalene and anthracene biodegradation in estuarine sediments (33).

PAHs are also degraded by fungi. Cerniglia et al. (34) found that the fungus Cunninghamella elegans, an estuarine isolate, is able to oxidize naphthalene, benz(a)anthracene, and benzo(a)pyrene.

The specific environment in which PAHs are present has a large effect on their degradation rate. Gardner et al. (35) studied the degradation of anthracene, fluoranthene, benz(a)anthracene, and benzo(a)pyrene in coastal sediments in a laboratory flowing-water system. All four PAHs decreased significantly with time (-2%/week) in fine sand and medium sand sediments; decreases in a marsh sediment were not significant at the 95% confidence level. The authors suggest this slower rate may have been due to stronger sorption of the PAHs to the marsh sediment than to the sands. Microbial degradation was also found to be slower at the bottom of sediments, where less nutrients and oxygen were present, but the rate of degradation was enhanced by the addition of the benthic polychaete worm, Capitella capitata, which presumably metabolized the PAHs and/or mixed the sediments and nutrients, thus better allowing microbial degradation to occur.

There is little literature on the anaerobic degradation of PAHs. In a recent review of anaerobic transformation processes, Rogers (29) noted

that aromatic hydrocarbons are generally considered resistant to degradation under anoxic conditions. Although he lists a number of compounds found to undergo anaerobic transformations, including benzene and some substituted benzenes, benzo(a)pyrene is the only PAH given. In dilute sediment, it was found to degrade to CH_4 and CO_2. Mihelcic and Luthy (36) have conducted experiments on the degradation of naphthalene and acenaphthylene in anaerobic soil solutions under denitrifying conditions. Both chemicals were found to be degraded, naphthalene much more rapidly so.

The results of biodegradation rate studies for several PAHs are given in Table 5-7. The environment in which the tests were carried out should be noted, as well as whether a period of acclimation was required, since the rate of biodegradation is often found to be more rapid in natural sediments that have received large quantities of PAHs. Rate constants given in Table 5-7 can be converted to half-lives by dividing them into 0.693.

The results in Table 5-7 illustrate the dependence of PAH biodegradation on environmental conditions. Most bacteria species cannot use PAHs as sole sources of carbon, but marine and freshwater sediments often contain sufficient alternative sources of organic carbon, and microbial degradation of PAHs may be significant as long as the sediments remain oxygenated. In anaerobic sediments, degradation is expected to be slow.

4. Oxidation/Reduction (abiotic)

Much of the literature on the abiotic oxidation of PAHs deals with atmospheric reactions. PAHs also undergo oxidation by ozone in aqueous solutions, with alkylation enhancing the rate (19). Butkovic et al. (37) examined the reaction rates of pyrene, phenanthrene and benzo(a)-pyrene with ozone at 25°C and found the rates to decrease roughly in the ratio 5:2:1. The corresponding half-lives in the presence of 10^{-4} M ozone at pH 7 were 0.18, 0.44, and 1.00 seconds respectively; pH had negligible effect on the rate. The authors note that these rates are much faster than those commonly reported in the literature (e.g. 19),

VI. LABORATORY AND FIELD FATE STUDIES

Much of the work involving the study of the behavior of PAH's in the natural environment or in model ecosystems has focused on the behavior of crude oil, of which PAHs are major components. Other studies such as those of Geisy et al. (10) and Dickson et al. (54) have examined the fate of individual compounds in model ecosystems of various scales. Several of these studies are described below.

Lee and coworkers have reported a number of studies (e.g. 15,45,46) on the behavior of PAHs in enclosed ecosystems. In one study (25), Prudhoe crude oil was enriched with naphthalene, 1-methylnaphthalene, 2,3-dimethylnaphthalene, anthracene, fluoranthene, benz(a)anthracene, and benzo(a)pyrene. The results indicated rapid removal from the water column. For the lower molecular weight compounds such as naphthalenes, anthracenes, and phenanthrenes, microbial degradation and evaporation were the primary removal process. Higher-weight compounds were primarily affected by photolysis and the sorption to and sedimentation with suspended particles. Once sedimentation occurred, biological degradation became an important removal process.

In another study by Lee (46) on the fate of heavy fuel oil spilled in a Georgia salt marsh, the accumulation of phenanthrene, chrysene and fluoranthene in sediments and benthic biota were investigated. The time required for these compounds to reach half of their maximum values were roughly 100, 70, and 30 days in sediment, mussels, and oysters, respectively; no phenanthene was detected in the sediment 150 days after the spill.

Hinga and Pilson (47) observed the persistence of radiolabeled benz(a)-anthracene in the same type of ecosystem as Lee et al. (25) over a 200-day period. The total amount of benz(a)anthracene decreased to about 6% of the total added between days 37 and 68. After that time, however, no further degradation was observed, suggesting that some portion of the compound may remain in the sediments indefinitely.

A number of reports on the fate of hydrocarbons in Puget Sound and off the coast of Washington have been made by Carpenter and Prahl (48-53). Much of this work has focused on the types, amounts and origins of PAHs in sediments, rather than on fate processes. Their work has shown the variability of zooplankton fecal pellets in the vertical transport of hydrocarbons (51) and the importance of different types of particles in sorption of PAHs (53).

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Effect of Environmental Parameters on the Biodegradation of Oil Sludge†

J. T. DIBBLE‡ AND R. BARTHA*

Department of Biochemistry and Microbiology, Cook College, Rutgers-The State University of New Jersey, New Brunswick, New Jersey 08903

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A laboratory study was conducted with the aim of evaluating and optimizing the environmental parameters of "landfarming", i.e., the disposal by biodegradation in soil of oily sludges generated in the refining of crude oil and related operations. Oil sludge biodegradation was monitored by CO_2 evolution and by periodic analysis of residual hydrocarbons. The parameters studied were soil moisture, pH, mineral nutrients, micronutrients, organic supplements, treatment rate, treatment frequency, and incubation temperature. Oil sludge biodegradation was optimal at a soil water-holding capacity of 30 to 90%, a pH of 7.5 to 7.8, C:N and C:P ratios of 60:1 and 800:1, respectively, and a temperature of 20°C or above. Addition of micronutrients and organic supplements was not beneficial; sewage sludge interfered with hydrocarbon biodegradation. Breakdown of the saturated hydrocarbon (alkane and cycloalkane) fraction was the highest at low application rates, but higher application rates favored the biodegradation of the aromatic and asphaltic fractions. An application rate of 5% (wt/wt) oil sludge hydrocarbon to the soil (100,000 liters/hectare) achieved a good compromise between high biodegradation rates and efficient land use and resulted in the best overall biodegradation rate of all hydrocarbon classes. Frequent small applications resulted in higher biodegradation than single large applications. Two 100,000-liter/hectare (255 barrels per acre) or four 50,000-liter/hectare oil sludge hydrocarbon applications per growing season seem appropriate for most temperate zone disposal sites.

Petroleum refining unavoidably generates considerable volumes of oil sludges. Common sources of these sludges are storage tank bottoms, oil-water separators, flotation and biological wastewater treatment units, cleaning of processing equipment, and soil from occasional minor spills on refinery grounds. The composition of these sludges varies according to their origin, storage, and treatment history. In a typical case hydrocarbons, water, and mineral solids are present in roughly equal proportions. Oily sludges constitute a disposal problem and, among other options, biodegradation in soil or "landfarming" offers a cost-effective yet environmentally acceptable alternative. In contrast to burial in anaerobic landfills, this mode of disposal leads to the relatively rapid biodegradation of the hydrocarbons by soil microorganisms, thus reducing the danger of groundwater contamination.

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‡ Present address: Research and Technical Department, Texaco, Inc., Port Arthur, TX 77640.

The biodegradation of accidentally spilled or waste oils in aquatic and terrestrial environments has been the subject of several recent reviews (3, 5, 7). A careful and extensive study of oil biodegradation in soil by Raymond et al. (20), as well as previous work reviewed in this paper, were all conducted in the field under local weather and precipitation conditions. To complement this work, we conducted a laboratory study on the effects of controlled environmental parameters on oil hydrocarbon biodegradation under simulated landfarming conditions. We expected this study to clarify the feasibility and efficiency of the landfarming practice under a variety of climatic and soil conditions. We were also seeking to optimize those parameters of the landfarming practice that can be readily controlled. Using an actual refinery sludge and soil from a prospective disposal site, we evaluated the effects of soil moisture content, soil pH, levels of inorganic fertilizer, micronutrients, and organic supplements, sludge loading rates, application frequencies, and temperature on hydrocarbon degradation in the landfarming process.

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Naphthalene Biodegradation in Environmental Microcosms: Estimates of Degradation Rates and Characterization of Metabolites

MICHAEL A. HEITKAMP, JAMES P. FREEMAN, AND CARL E. CERNIGLIA*

National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas 72079

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Naphthalene biodegradation was investigated in microcosms containing sediment and water collected from three ecosystems which varied in past exposure to anthropogenic and petrogenic chemicals. Mineralization half-lives for naphthalene in microcosms ranged from 2.4 weeks in sediment chronically exposed to petroleum hydrocarbons to 4.4 weeks in sediment from a pristine environment. Microbiological analysis of sediments indicated that hydrocarbon-utilizing microbial populations also varied among ecosystems and were 5 to 12 times greater in sediment after chronic petrogenic chemical exposure than in sediment from an uncontaminated ecosystem. Sediment from an ecosystem exposed to agricultural chemicals had a mineralization half-life of 3.2 weeks for naphthalene and showed about a 30-fold increase in heterotrophic bacterial populations in comparison to uncontaminated sediments, but only a 2- to 3-fold increase in hydrocarbon-degrading bacteria. Analysis of organic solvent-extractable residues from the microcosms by high-pressure liquid chromatography detected polar metabolites which accounted for 1 to 3% of the total radioactivity. Purification of these residues by thin-layer chromatography and further analysis by gas chromatography-mass spectrometry indicated that *cis*-1,2-dihydroxy-1,2-dihydronaphthalene, 1-naphthol, salicylic acid, and catechol were metabolites of naphthalene. These results provide useful estimates for the rates of naphthalene mineralization in different natural ecosystems and on the degradative pathway for microbial metabolism of naphthalene in freshwater and estuarine environments.

Polycyclic aromatic hydrocarbons (PAHs) are compounds of environmental and human health concern since some PAHs have been shown to be toxic, mutagenic, or carcinogenic (30, 39, 40). Human exposure to PAHs may occur from emissions during the incomplete combustion of fossil fuels or due to accidental discharge into aquatic and terrestrial environments during the transport, use, and disposal of petroleum products (30, 35, 40, 41). Naphthalene, a dicyclic aromatic hydrocarbon, and its methylated derivatives are considered some of the most acutely toxic compounds in the water-soluble fraction of petroleum (1). Exposure to naphthalene has caused a decrease in hemoglobin concentration and inhibited oxygen consumption in various experimental organisms (15, 46).

The microbial metabolism of naphthalene has been studied extensively, and the rate, metabolic pathway, enzymatic reactions, and genetic regulations of naphthalene catabolism have been well documented (4–13, 16–18, 20, 31, 32, 34, 38, 47). However, there are few reports showing the rate and metabolism of naphthalene in experimental systems designed to model natural ecosystems (3, 26, 27, 36, 41). The degradation of chemicals in the environment can be affected by several factors which may differ among ecosystems, such as organic and inorganic nutrient levels, temperature, pH, previous chemical exposure, microbial adaptations, and oxygen tension (2, 22, 33, 45). In addition, many species of bacteria, cyanobacteria, filamentous fungi, and yeasts coexist in natural ecosystems and may act independently or in concert to metabolize aromatic hydrocarbons (5, 19, 20). The purpose of this investigation was to determine the rates of mineralization, the metabolic pathway, and the initial oxidation reactions for naphthalene catabolism in microcosms containing sediment and water collected from three well-characterized ecosystems which differed in past exposure to petrogenic and anthropogenic chemicals.

MATERIALS AND METHODS

Chemicals. Naphthalene and phenanthrene were purchased from Aldrich Chemical Co., Milwaukee, Wis. [1,4,5,8-^{14}C]naphthalene with a specific activity of 5.10 mCi/mmol was purchased from Amersham Corp., Arlington Heights, Ill. *cis*-1,2-Dihydroxy-1,2-dihydronaphthalene (*cis*-naphthalene dihydrodiol) and *trans*-1,2,-dihydroxy-1,2-dihydronaphthalene (*trans*-naphthalene dihydrodiol) were obtained from David T. Gibson, University of Texas at Austin. Catechol, hexadecane, 1-naphthol, and salicylic acid were purchased from Chemical Service, Media, Pa. Solvents for high-pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC) were purchased from Burdick and Jackson Laboratories, Muskegon, Mich. Solvents and chemical standards used in this study were of the highest purity available.

Design of naphthalene biodegradation studies. Biodegradation of naphthalene was monitored in a flowthrough microcosm test system which enabled monitoring of naphthalene mineralization (complete degradation to CO_2) and extraction and recovery of both volatile and nonvolatile metabolites as well as undegraded naphthalene (23, 29). Microcosms consisted of 0.5-liter glass minitanks (Foxboro/Analabs, North Haven, Conn.) containing 20 g of homogenized moist sediment and 180 ml of water collected from three different ecosystems which varied in their watershed development, nutrient levels, and history of previous exposure to chemical contaminants. The pH and sediment organic carbon concentrations for these ecosystems are as follows: DeGray Reservoir, a man-made impoundment in southwestern Arkansas, pH 7.2, 3.2%; Lake Chicot, a natural oxbow lake in southeastern Arkansas, pH 7.6, 6.6%; and Redfish Bay, a natural estuary near Port Aransas, Tex., pH 7.8, 3.5%. Microcosms

* Corresponding author.

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Role of Dissolution Rate and Solubility in Biodegradation of Aromatic Compounds

GERHARD STUCKI† AND MARTIN ALEXANDER*

Laboratory of Soil Microbiology, Department of Agronomy, Cornell University, Ithaca, New York 14853

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Strains of *Moraxella* sp., *Pseudomonas* sp., and *Flavobacterium* sp. able to grow on biphenyl were isolated from sewage. The bacteria produced 2.3 to 4.5 g of protein per mol of biphenyl carbon, and similar protein yields were obtained when the isolates were grown on succinate. Mineralization of biphenyl was exponential during the phase of exponential growth of *Moraxella* sp. and *Pseudomonas* sp. In biphenyl-supplemented media, *Flavobacterium* sp. had one exponential phase of growth apparently at the expense of contaminating dissolved carbon in the solution and a second exponential phase during which it mineralized the hydrocarbon. Phase-contrast microscopy did not show significant numbers of cells of these three species on the surface of the solid substrate as it underwent decomposition. *Pseudomonas* sp. did not form products that affected the solubility of biphenyl, although its excretions did increase the dissolution rate. It was calculated that *Pseudomonas* sp. consumed 29 nmol of biphenyl per ml in the 1 h after the end of the exponential phase of growth, but 32 nmol of substrate per ml went into solution in that period when the growth rate had declined. In a medium with anthracene as the sole added carbon source, *Flavobacterium* sp. converted 90% of the substrate to water-soluble products, and a slow mineralization was detected when the cell numbers were not increasing. *Flavobacterium* sp. and *Beijerinckia* sp. initially grew exponentially and then arithmetically in media with phenanthrene as the sole carbon source. Calculations based on the growth rates of these bacteria and the rates of dissolution of phenanthrene suggest that the dissolution rate of the hydrocarbon may limit the rate of its biodegradation.

In the routine testing for biodegradation, organic chemicals are added to aqueous solutions at concentrations of 2 to 100 μg/ml. Because these concentrations exceed the water solubilities of many organic compounds, the validity of such tests has been questioned (2, 6). The metabolism of several organic substrates with water solubilities below 10 μg/ml at 25°C is well characterized (7, 10).

To mineralize or grow on substrates having low solubilities in water, microorganisms may require some physiological adaptation. Particular attention has been given to the growth and utilization of aliphatic hydrocarbons by bacteria (3, 14). Several mechanisms to facilitate the uptake of aliphatic hydrocarbons are known, for example, the formation of emulsifiers (9, 16) or the modification of the cell surface to increase its affinity for hydrophobic substrates and thus facilitate their absorption (11, 12). Aromatic compounds are of special interest because many are significant environmental pollutants, and millions of tons of such chemicals are used each year. Concern with the possible ecological effects of some of the aromatic compounds that are poorly soluble in water has resulted in a request by the U.S. Environmental Protection Agency for information on their biodegradation, as in the cases of anthraquinone, cumene, and biphenyl (20). The bacterial utilization of several aromatic hydrocarbons with low water solubilities has been investigated (14, 21). It has also been reported recently that the rate of mineralization at the end of the active phase of biodegradation by a mixed culture of microorganisms was less than the rate of spontaneous dissolution of palmitic acid but greater than the rate of spontaneous dissolution of octadecane (18).

The present study was designed to relate the kinetics of bacterial growth on several poorly soluble aromatic compounds to their rates of dissolution and solubilities in water.

MATERIALS AND METHODS

Medium. The inorganic salts solution contained (per liter of deionized water) 775 mg of K_2HPO_4, 350 mg of KH_2PO_4, 200 mg of $(NH_4)_2SO_4$, and 100 mg of $MgSO_4 \cdot 7H_2O$. One milliliter of a trace element solution (17) was added after the salts solution was sterilized. The final pH of the medium was 7.2. Glucose and succinate were autoclaved separately and added aseptically to the autoclaved medium. Stock solutions of aromatic hydrocarbons prepared in dichloromethane (5 and 50 g per liter) were added to empty sterile 250-ml incubation bottles by means of positive displacement pipettes (Scientific Manufacturing Industries, Emeryville, Calif.). The inorganic salts solution was added to these bottles after complete evaporation of the solvent. All glassware was cleaned in Nochromix (Godax Laboratories, Inc., New York, N.Y.).

Isolation of microorganisms. Sewage samples from the settling tanks of the Ithaca, N.Y., and Marathon, N.Y., sewage treatment plants were passed through Whatman no. 1 filter papers to remove particulate matter. Portions (1.0 ml) of the filtrate were added to screw-capped 30-ml test tubes containing 1 to 5 mg of an insoluble carbon source and 10 ml of the inorganic salts solution. The test tubes were incubated for 7 days at 29°C on a rotary shaker operating at 120 rpm, and 100-μl portions of the enrichment cultures were transferred to new medium and incubated for another 7 days. Portions (0.1 ml) of 10-fold dilutions of the second enrichment cultures were plated on a medium containing 13 g of Bacto-Agar (Difco Laboratories, Detroit, Mich.) and 3.5 g of Trypticase soy broth (BBL Microbiology Systems, Cockseville, Md.) per liter of deionized water. After incubation of

* Corresponding author.
† Present address: Ciba-Geigy, CH-4002 Basel, Switzerland.

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Degradation of 1,4-Dichlorobenzene by a *Pseudomonas* sp.

JIM C. SPAIN* AND SHIRLEY F. NISHINO

Air Force Engineering and Services Center, Tyndall Air Force Base, Florida 32403

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A *Pseudomonas* species able to degrade *p*-dichlorobenzene as the sole source of carbon and energy was isolated by selective enrichment from activated sludge. The organism also grew well on chlorobenzene and benzene. Washed cells released chloride in stoichiometric amounts from *o*-, *m*-, and *p*-dichlorobenzene, 2,5-dichlorophenol, 4-chlorophenol, 3-chlorocatechol, 4-chlorocatechol, and 3,6-dichlorocatechol. Initial steps in the pathway for *p*-dichlorobenzene degradation were determined by isolation of metabolites, simultaneous adaptation studies, and assay of enzymes in cell extracts. Results indicate that *p*-dichlorobenzene was initially converted by a dioxygenase to 3,6-dichloro-*cis*-1,2-dihydroxycyclohexa-3,5-diene, which was converted to 3,6-dichlorocatechol by an NAD^+-dependent dehydrogenase. Ring cleavage of 3,6-dichlorocatechol was by a 1,2-oxygenase to form 2,5-dichloro-*cis*, *cis*-muconate. Enzymes for degradation of haloaromatic compounds were induced in cells grown on chlorobenzene or *p*-dichlorobenzene, but not in cells grown on benzene, succinate, or yeast extract. Enzymes of the *ortho* pathway induced in cells grown on benzene did not attack chlorobenzenes or chlorocatechols.

Chlorobenzenes are widely used as solvents, degreasers, and intermediates in the synthesis of dyes and pesticides (14, 37). They are released into the environment both by accident and through routine disposal in waste treatment facilities. The fate of chlorobenzenes in the environment has been investigated, but the importance of biodegradation is still not clear, partly because biodegradation studies are hampered by the volatility of the chlorobenzenes. Bouwer and McCarty (3) reported mineralization of chlorobenzene, 1,4-dichlorobenzene (*p*-DCB), 1,2-dichlorobenzene, and 1,2,4-trichlorobenzene in fixed-film columns when acetate was supplied as the primary carbon source. Strong evidence for biodegradation of *p*-DCB in groundwater (33) and soil columns (23) was obtained in recent studies in Switzerland. A pure culture able to use chlorobenzene as the sole carbon source was isolated from soil and sewage by chemostat enrichment (29). Very recently, an *Alcaligenes* sp. able to grow on 1,3-dichlorobenzene was isolated from soil (6). In each of these studies biodegradation was preceded by an extended acclimation period. In contrast, *p*-DCB was not biodegraded in soil columns flooded with primary wastewater (17), and *p*-DCB and 1,2,4-trichlorobenzene were not biodegraded in activated sludge (12). Chlorobenzene biodegradation rates in microcosms containing aquifer material were very low and variable (38), and dichlorobenzenes and chlorobenzene were not biodegraded in an aquifer during groundwater recharge (31).

Bacterial metabolism of halogenated aromatic compounds has been the subject of recent reviews (22, 27). Biodegradation pathways of chlorobenzoic acids (27–29), chlorophenols (10, 11, 36), chlorobenzene (30), and 1,3-dichlorobenzene (6) have been thoroughly studied in pure cultures of bacteria. The most common catabolic pathways involve conversion of the parent molecules to chlorocatechols by the action of a monooxygenase (11, 27) or a dioxygenase (27, 30). Cleavage of the resultant chlorocatechols to form chloromuconic acids is catalyzed by nonspecific 1,2-dioxygenases termed type II pyrocatechases by Dorn and Knackmuss (7). Cleavage by a 2,3-oxygenase is unproductive and results in inactivation of the enzyme (21, 28). Chloride is eliminated by lactonization of chloromuconic acids and subsequent reactions to yield β-ketoadipic acid (27).

Biodegradation of *p*-DCB has not been studied in detail to our knowledge. We report in this paper the isolation, characterization, and metabolic activities of a bacterium able to degrade *p*-DCB as the sole source of carbon and energy.

MATERIALS AND METHODS

Isolation and growth of bacteria. The organism used in these studies was isolated from a mixture of sewage samples collected at Tyndall Air Force Base and Panama City, Fla. Inocula were suspended in 1.5 liters of minimal salts medium (MSB; see below) supplemented with yeast extract (10 mg/liter) and a few crystals of *p*-DCB (40 to 60 mg) in a 2-liter flask. The resultant suspensions were stirred continuously with a magnetic stirrer at room temperature. Half of the suspension was removed and replaced with fresh medium at 3- to 5-day intervals. *p*-DCB crystals were added as necessary to compensate for volatilization. When growth on *p*-DCB was evidenced by an increase in turbidity and a drop in pH, 250 ml of the suspension was transferred to a 250-ml chemostat. The suspension was stirred with a magnetic stirrer and incubated at 30°C. Fresh MSB was supplied by a pump (FMI, Oyster Bay, N.Y.) at a flow rate of 20 ml/h. Inclusion of *p*-DCB crystals in the medium gave unpredictable results because high concentrations of *p*-DCB were toxic. Therefore, *p*-DCB was supplied in the vapor phase. Air containing *p*-DCB vapor was provided to the culture via flow meters as described below. The initial *p*-DCB concentration was 0.5 mg/liter in the air and was gradually increased to 5 mg/liter over a period of 6 weeks. Samples of the enrichment culture were spread on MSB agar plates and incubated in a desiccator that contained crystals of *p*-DCB (100 to 200 mg) as the sole carbon source. Isolated colonies were tested for ability to grow at the expense of *p*-DCB. Isolates that grew well on MSB agar incubated in the presence of *p*-DCB, but not on control plates incubated without *p*-DCB, were tested for production of acid from *p*-DCB. Isolates were grown on MSB agar plates containing yeast extract (100 mg/liter) and bromthymol blue (0.04 g/liter). All of the cultures grew on yeast extract in the

* Corresponding author.

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