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BIOLOGICAL AND CHEMICAL TREATMENT OF MONO-, POLYCYCLIC-, CHLORO-

AND NITRO-AROMATIC HYDROCARBON MIXTURES IN SEDIMENT AND SOIL

By

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and approved by

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ABSTRACT OF THE DISSERTATION

Biological and chemical treatment of mono-, polycyclic-, chloro- and nitro-aromatic hydrocarbon

mixtures in sediment and soil

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Hazardous waste composting is a demonstrated technology for many types of organic

contaminants. However, treatment outcome is often uncertain for hydrophobic compounds and

treatability studies are needed to predict performance. A composting pilot study was conducted

to determine the treatability of substituted aromatic hydrocarbons in two 15 m^3 samples of tar-like

dye manufacturing waste sludge. Performance over a 49-day period was characterized for

mixtures of 17 aromatic compounds using first-order kinetics. Half-life was generally lower (8-

18 days) for chlorobenzene, xylenes, nitrobenzene, aniline, toluene, and ethylbenzene when

initially present at relatively high mass (above 10%). Nitrobenzene did not degrade in material

with a carbon to nitrogen ratio of 18:1 but did degrade in material with a ratio of 98:1. Although

50% to 75% of the contaminant mass was degraded, benzene, nitrobenzene, *N*-

nitrodiphenylamine, naphthalene, and benzo(a)anthracene concentrations were above regulatory

standards in finished compost. A fugacity analysis showed that compounds partitioned

significantly (ρ<0.05) to the organic fraction and non-aqueous phase liquid of the composting

material. Application of Fenton reaction to improve treatment was more effective after bulking. No significant ($\rho < 0.05$) differences between biotreatment alone and biotreatment with pre and post Fenton oxidation were observed. Alkaline hydrolysis using 5% w/w $CaMg(OH)_4$ removed nearly all of 25,000 mg/kg tetryl in soil. Picric acid and *N*-methylpicramide were tentatively identified as treatment products. Biotransformation of the nitroaromatic compound RDX was determined by comparing kinetic meta-data obtained from 42 studies. Degradation rates were statistically faster for aerobic and anaerobic bacteria than for fungi. Results of this study show that biodegradability, bioavailability, and kinetics are critical elements of a treatability analysis and can identify the need for additional treatment processes. Processes can be integrated into biotreatment using cost effective reagents that enhance compound removal. Additional research is needed to define the chemical, biological, and physical interface of the integrated system to improve process outcome.

Acknowledgements

I am deeply grateful to my advisor Dr. Jerry Kukor for his guidance, patience and enthusiasm throughout this project. Without him this dissertation would not be possible. Special thanks to my committee members Drs. Joan Bennett, Peter Strom, Lily Young, and Gerben Zylstra for their advice and long-term encouragement. Dr. Bob Cowan was instrumental in my early work and provided invaluable suggestions for my research approach and data analysis. A U.S. Army, ARDEC Science Fellowship award provided support from 2010-2011.

I dedicate this dissertation to my mom

Vivian Lehto Sheehan 1927 - 2012

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List of Abbreviations

ANOVA   analysis of variance

C:N   carbon to nitrogen ratio

CERCLA   comprehensive environmental response and liability act

C_f final compound concentration at end of composting (day 49)

CFR   code of federal regulations

CFU   colony forming unit

C_o compound concentration at the start of composting

COD:N:P   chemical oxygen demand to nitrogen to phosphorus ratio

C_t compound concentration at time t.

DRO   diesel range organics, C_{10} to C_{28}

DNX   hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine

EPA   environmental protection agency

f   fugacity. Thermodynamic principle related to chemical potential that uses
               pressure rather than energy to describe the likely movement of a compound from
               a particular phase

H   Henry's law coefficient

HMX   Octa-hydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine

k   first order rate constant (d^{-1})

K_d soil-water partition coefficient

K_{oc} organic carbon partition coefficient (partition coefficient for adsorption)

K_{ow} octanol-water partition coefficient (partition coefficient for NAPL since NAPL is
               a miscible solvent and partitioning is by absorption)

MEDINA   methlenedinitramine

mFr   modified Fenton reaction. Iron is chelated to keep Fe^{+3} in solution at pH near
               neutrality.

MGP   manufactured gas plant

MHC   moisture holding capacity

MNX   hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine

NAPL	non aqueous phase liquid
ND	no evidence of degradation
NDAB	4-nitro-diazobutanol
N_i	biomass at time t
NJAC	New Jersey administrative code
NJDEP	New Jersey department of environmental protection
NPL	national priority list
NRTS	non-residential treatment standards
NS	no standard
PAH	polycyclic aromatic hydrocarbon
RCRA	resource conservation and recovery act
RDX	royal demolition explosive; 1,3,5-trinitro-1,3,5-triazacyclohexane
RI	risk investigation
SVOC	semi volatile organic compound
μ	Specific growth rate
t_d	microbial population doubling time
$t_{1/2}$	half-life (day)
TNT	2,4,6-trinitrotoluene
TNX	hexahydro-1,3,5-trinitroso-1,3,5-triazine
V_i	volume of compartment i
VOC	volatile organic compound
Z	fugacity capacity. Compound concentration ratio between two phases.

Preface

On April 22, 1970, twenty million people across the U.S. gathered to demand a cleaner environment. New York City alone rallied one million people. Organizers declared this first Earth Day "a commitment to make life better, not just bigger and faster, to provide real rather than rhetorical solutions…….April 22 seeks a future worth living."[1] It is possible that the thread leading to this dissertation began then.

It was this Earth Day combined with events like the near-to-home oil spill from the Exxon/Mobil refinery in Greenpoint Brooklyn into the Newtown Creek (still one of the worst oil spills in U.S. history) and the contamination of the Hudson River by GE produced PCBs as well as the further away Love Canal, the Amoco Cadiz oil spill, the Union Carbide gas leak in Bhopal (to name only a few) that inspired a deep commitment to the environment due to the tragic consequences of its abuse.

Over the years my environmental avocation transformed to academic majors in biology (BS), microbiology (MS), and engineering (MS) and a career of seeking real solutions for oil spills, toxic chemicals, and industrial waste. The cycle back to academics and to this dissertation occurred when the oil waste and toxic chemical problems that needed to be solved exceeded the state-of-the-art solutions. Thus, the work described in the following sections began as a professional remediation project for hazardous waste and transitioned from applied technology into research after treatment stalled, and further studies were needed to understand why.

This dissertation is written in manuscript format. Each chapter presents a stand-alone effort that describes multicomponent composting (Chapter 1), predicts or explains treatment

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[1] Hill, G. Nation set to observe Earth Day, New York Times April 21, 1970.
http://graphics8.nytimes.com/packages/pdf/topics/earthday.pdf

limitations (Chapter 2), investigates chemical methods for process improvements or alternatives (Chapter 3 and Chapter 5) or extends biodegradation to alternate but similar compounds (Chapter 4).

All work presented in this dissertation is original. The composting pilot test described in Chapter 1 was conducted under contract by site owners to a consulting engineering firm[2], but I retained responsibilities for developing the compost concept design, identifying operation and monitoring methods, and for on-site project management. Others performed system construction and physical process operation under my direction. The detailed analysis of the composting data was well beyond the scope of the pilot test and was conducted for research purposes only. Due to contract budgetary constraints, analyses used to monitor the compost process were minimized to conserve cost. This limitation is common in commercial field applications but is generally inconsistent with academic research. Bridging this divide is a significant aspect of data analysis and interpretation of composting results described in Chapter 1. Hydrolysis studies reported in Chapter 5 were conducted with the assistance of a laboratory technician, Mr. Sheng-Yih Lee.

This dissertation is submitted in partial fulfillment of the requirements for the Ph.D., Graduate School-New Brunswick, Rutgers University, and has been in progress for a long time. Unlike most students, I will not start my career at the conclusion of this dissertation. Instead, the process has paralleled my career and at every step has given me the training, research discipline, and academic challenges needed to stretch my professional capabilities and remain consistent to the values expressed on April 22, 1970.

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[2] The engineering firm is not named to prevent inference of the study site that is unidentified at the owner's request.

Introduction

This dissertation explores bioremediation of sediments and soils containing high concentrations of mixed or individual hydrophobic aromatic hydrocarbons. This is a challenging area of research due to matrix heterogeneities, potential substrate interactions, and mass transfer limitations that generally reduce the effectiveness and predictability of biodegradation processes. The goal of this dissertation research is to better understand treatment limitations and the potential for integrated chemical methods to overcome them. This document is organized into five chapters that explore biodegradability (Chapters 1 and 4), bioavailability (Chapter 2), and chemical treatment (Chapters 3 and 5) of mono-, polycyclic-, chloro-, and nitro-aromatic hydrocarbons (FIG 1). The style format follows guidelines of the Journal of The American Society for Microbiology. Each chapter contains separate sections for the abstract, introduction, materials and methods, discussion, conclusions and references.

The novelty of the research presented here is in the integrated systems approach used to link performance, prediction, and improvement of hazardous material composting. The foundation of this dissertation is a field-scale compost feasibility study for treatment of dye manufacturing waste sludge. This study began as a commercially funded project and was part of a remediation alternatives investigation (RI) for contaminated waste at a CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act or "Superfund") regulated facility. Although composting had never been demonstrated for this type of waste, other treatment alternatives such as incineration, site capping and solidification were less attractive choices due to higher implementation costs and negative public opinion.

Composting was thought to be the best design option for bioremediation of the dye manufacturing sludge since it includes self-heating and physical mixing components assumed beneficial to material processing and treatment. Because this was the first project of its kind there

were some uncertainties and therefore opportunities to explore the treatment process beyond the scope of the RI.

Results of the composting study and a discussion of the biodegradability of 17 compounds present in the waste sludge are presented in Chapter 1of this dissertation. Biodegradability is quantified using pseudo first-order kinetics derived from compound concentrations measured over a 49 day operating period. An explanation of why certain compounds degraded while other intrinsically degradable compounds did not, was needed to develop a data based approach for improving treatment. Chapter 2 is an assessment of fugacity as a screening tool to determine phase distribution, and by extension, bioavailability of individual compounds in multicomponent mixtures.

Chapter 3 addresses the logical next step of determining how compound availability can be improved to increase the effectiveness of composting. Fenton oxidation was investigated as a chemical method of enhancing compound availability since it has been used in both the classic form that requires a low pH, and a modified form that allows application at a pH more compatible with microorganisms. The hypothesis for integrating chemical oxidation into the compost treatment train is that application prior to composting would increase compound availability whereas application after composting would reduce concentrations of residual compounds.

Chapters 1 through 3 are concerned with treatment of multicomponent chemical waste from dye manufacturing. Among other compounds the waste contained relatively high concentrations of nitro-substituted aromatic compounds including aniline, nitrobenzene and *N*-nitrosodiphenylamine, all characteristic of the azo family of dyes. Interestingly, the first dye compound is believed to be picric acid, a highly nitrated phenol in the same chemical family as the nitroaromatic TNT. Chapter 4 investigates the biodegradability of the nitroamine explosive RDX (Royal Demolition Explosive- 1,3,5-trinitro-1,3,5-triazacyclohexane) by deriving kinetic constant meta-data from published studies. Half-life data were used to compare the degradation rates for aerobic bacteria, anaerobic bacteria, and fungi. The potential for chemical degradation

of the nitroamine explosive tetryl (2,4,6-trinitrophenylmethylnitramine) is reported in Chapter 5. The effectiveness of base hydrolysis was determined using several alkaline agents. Although this type of chemical treatment has been used to degrade TNT and RDX in soils it has not been demonstrated on high concentrations (approaching pure product) of tetryl.
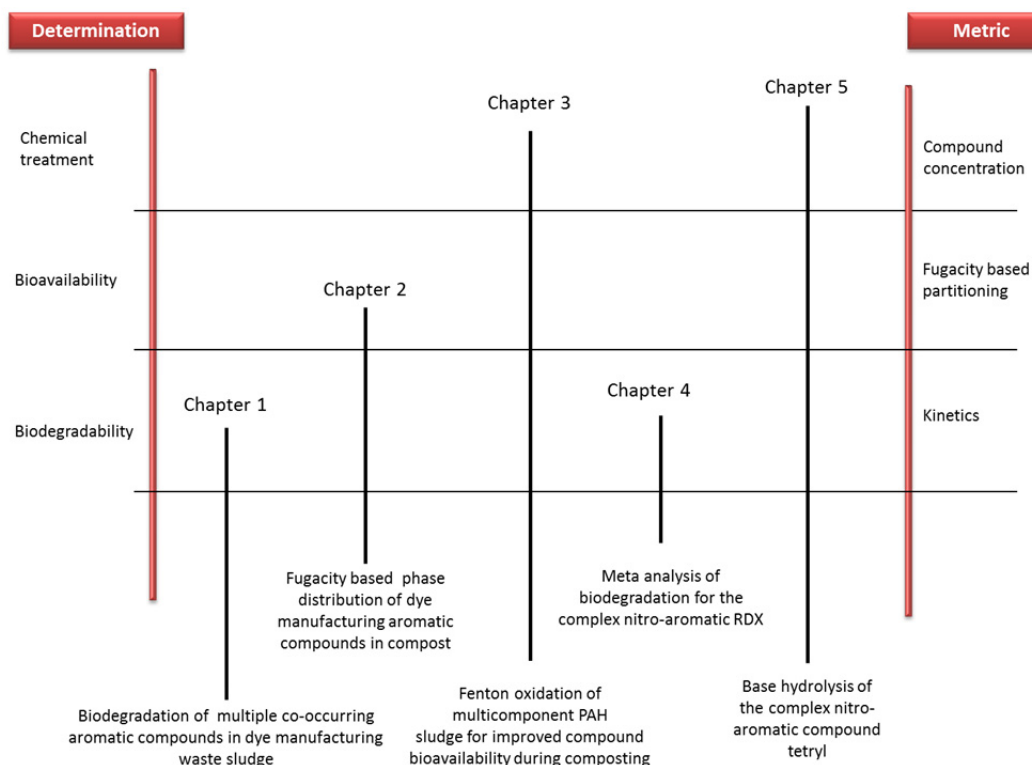
FIG 1 Organizational framework for dissertation research in *ex situ* biological and chemical treatment of mono-, polycyclic-, chloro-, and nitro-aromatic hydrocarbons in sediment and soil

Chapter 1

Biotransformation of multiple co-occurring substituted and unsubstituted aromatic compounds in

composted dye manufacturing waste sludge

ABSTRACT

Synthetic dyes are soluble organic compounds formed from coal tar and petroleum

distillates that add permanent color to textiles. Dye manufacturing surged in the U.S. after WWI

and was virtually unregulated until the Environmental Protection Agency (EPA) was established

in the 1970's. Many historic accounts of pollution caused by direct discharge of dye products and

chemical intermediates have been recorded. Retention of production waste in on-site disposal

pits was a common alternative to direct discharge until the early 1980's. Due to the toxicity of

chemicals in the sludge, many former dye manufacturing facilities are on the EPA National

Priority List (NPL) and dye waste disposal areas require remediation under the Comprehensive

Environmental Response Compensation and Liability Act (CERCLA, commonly known as

Superfund). The purpose of this study was to investigate the feasibility of composting as a

remedial strategy for waste sludge containing mixtures of substituted and un-substituted

monoaromatic and polycyclic aromatic hydrocarbons. Approximately 15 m^3 of contaminated

sludge were excavated from two separate disposal areas at a former manufacturing facility. The

sludge was a viscous, tar-like material containing moderate (100-1,000 mg/kg) to high (1,000-

4,000 mg/kg) concentrations of benzene, toluene, ethylbenzene, xylenes, chlorobenzene, 1,2,4-

trichlorobenzene, nitrobenzene, aniline, *N*-nitrosodiphenylamine and naphthalene. Other

compounds (1,2-dichlorobenzene, carbazole, dibenzofuran, 2-methylnaphthalene,

acenaphthalene, fluorene, and benzo(a)anthracene) were present but at lower concentrations (100

mg/kg or less) and could not be included in trend analyses. Excavated sludge was bulked with

woodshavings and amended with calcium carbonate and ammonium nitrate as needed to adjust pH and the carbon to nitrogen ratio. Moisture was managed to maintain 30% (v/v) of the moisture holding capacity. General heterotrophic and specific naphthalene degraders were estimated weekly using standard plate count techniques. Microbial growth increased with increasing temperature and reached stationary phase at the maximum compost temperature of 38 ^{o}C. A comparison of degradation trends in the sludge from two locations shows that aniline and nitrobenzene degraded faster when each compound represented a higher percent of the total contaminant mass, the waste material contained a relatively high carbon to nitrogen ratio, and a relatively high mass percent of toluene was present. Chlorobenzene and xylenes were more rapidly degraded when the initial mass of toluene, aniline and nitrobenzene were relatively low. The degradation rates of benzene and naphthalene appeared to be independent of co-occurring compounds. No evidence of degradation was obtained for 1,2,4-trichlorobenzene and *N*-nitrosodiphenylamine. Compound biotransformation rates are likely due to the metabolic characteristics of highly adapted microbial population sand are consistent with multisubstrate interactions observed by others. Thus, in addition to providing a source of nitrogen the rate of aniline and nitrobenzene degradation may be enhanced by a high concentration of toluene, and increased biomass of toluene degraders that also degrade nitrobenzene. The dominance of toluene degraders may negatively affect the degradation of chlorobenzene.

Degradation of benzene and naphthalene appear to be independent of co-occurring compounds. No evidence of degradation was obtained for 1,2,4-trichlorobenzene and *N*-nitrosodiphenylamine. Regulatory driven treatment objectives for the risk relevant compounds benzene, *N*-nitrosodiphenylamine, nitrobenzene, naphthalene, and benzo(a)anthracene were not met in this study despite the high mass removal of total contaminants. This exemplifies the difficulty of targeting biotransformation of select compounds in a multisubstrate mixture of

contaminants. More research is needed to confirm that observed degradation patterns are due to nutrient limitations and/or multisubstrate interactions and to improve biotransformation of risk relevant compounds.

INTRODUCTION

Synthetic dyes are soluble organic compounds formed from coal tar and petroleum distillates that add permanent color to textiles (1). The first synthetic dye was picric acid, discovered by Peter Woulfe in 1777 (2). After its explosive properties were discovered in 1871, picric acid production was diverted for military use and the dye industry shifted to other compounds (3). Early work by William Henry Perkin showed that mauve and other dyes could be manufactured by nitrating the benzene and toluene fractions of coal tar and reducing these fractions to produce aniline, *o*-toluidine and *p*-toluidine. These aromatic amines were then oxidized to produce the dye. It was later shown that chemical color and chemical structure were related and required an aromatic ring such as benzene, naphthalene, or anthracene as well as a side chain radical classified as azo (N=N-), carbonyl (C=O), methine (-CH), or nitro (NO_2) (4).

Prior to WWI much of the U.S. supply of synthetic dyes and intermediates was obtained from Germany. War embargos of foreign imports and post war acquisition of German dye patents by U.S. firms resulted in a highly profitable dye manufacturing industry that surged after WWI leading to development and production of a wide variety of dye colors and coal tar intermediates (5, 6).

The dye manufacturing industry in the U.S. was virtually unregulated until the 1970's when the Environmental Protection Agency was established. Prior to that time, waste disposal was determined by factory owners based on convenience and economics despite public concerns about possible health and environmental impacts. In 1866, for example, the Gowanus Canal in

New York City was nicknamed "Lavender Lake" due to the presence of untreated dye chemicals. By 1875 neighbors of other Brooklyn based dye factories were complaining about dust, noise and odors and in 1915 neighbors of the Cott-A-Lap Company in Somerville, NJ, known mostly for burlap wall coverings, began complaining about pollution from the additional manufacturing of aromatic intermediates and dye products. Robert C. Jeffcott, owner of the company, responded to complaints by creating a second company called Calco Chemical in more rural Bound Brook, NJ, specifically to manufacture dyes. Possibly the most egregious pollution report of its time was the discharge of dye effluent from the Brooklyn-based National Aniline & Chemical Company in 1920 that caused the closure of nearby Jamaica Bay to swimming and oyster harvesting eliminating a thriving industry that had supplied 300,000 bushels of oysters annually (6).

Waste management at dye facilities eventually evolved from direct discharge to on-site storage or disposal. This was the typical practice at many former dye manufacturing facilities across the country including the Nyanza Chemical Company in Massachusetts, Martin-Marietta/Sodyeco Inc.in North Carolina, American Cyanamid (former Calco Chemical) in New Jersey, the Beaunit Corp (former Circular Knit and Dye) in South Carolina, and the Krusowaty Farm in New Jersey (7). Environmental site investigations at these facilities have identified a complex mixture of volatile and semi-volatile polycyclic aromatic hydrocarbons, coal tar creosote, chlorinated solvents, and nitrated amines in waste sludge and soils although specific compounds vary depending on the age of the factory and products produced.

Waste disposal areas containing dye manufacturing chemicals are classified as hazardous. Remediation is required under the federal Comprehensive Environmental Response, Compensation and Liability Act (CERCLA, see list of abbreviations page xi), and remedies that permanently and substantially reduce the volume, toxicity, or mobility of the hazardous substance, are preferred over those that do not (42 USC §9621). Physical and chemical methods

designed to sequester contaminants in process waste sludge are the most common remedial

strategies used at former dye manufacturing facilities on the National Priority List (NPL) (7).

Although these methods meet CERCLA requirements, contaminants sequestered on site remain in

the ground resulting in permanent deed restrictions that limit future development and use.

Ex situ biological treatment of dye waste sludge is potentially an alternative to traditional

physical/chemical remediation strategies. If effective, the cost of treatment could be greatly

reduced and, since bioremediation can transform hazardous chemicals into non-toxic metabolic

end-products or intermediates, risks to public health and the environment may be eliminated.

Biological treatment of dye waste sludge is challenging, however, due to its complex organic

matrix and high concentration of multiple chemical constituents that make it difficult to predict

treatment rate and outcome. Research studies conducted on chemical mixtures of polycyclic

aromatic hydrocarbons (PAHs), for example, show conflicting results with both faster and slower

degradation rates demonstrated for mixed constituents compared to single components (8). In

some studies competitive substrate inhibition reduced the rate or prevented degradation of

otherwise rapidly degradable compounds (8-10). In other studies multiple component substrates

increased microbial biomass and provided co-substrates that improved degradation of normally

recalcitrant, high molecular weight compounds (11, 12). These apparently conflicting

observations are further confounded in materials containing high concentrations of organic

materials, especially when a separate, non-aqueous liquid (NAPL) phase is formed. Compounds

with a high molecular weight and octanol–water partitioning coefficient (log K_{ow}) typically

partition to these materials and are generally less bioavailable (13). Thus biotransformation of

compounds in highly organic materials depends on chemical and physical characteristics that

influence liquid-liquid partitioning in the NAPL as well as sorption and sequestration phenomena

of soils (14). Research by Pignatello and Xing (15) showed that these mass transfer limitations

can be minimized or reversed by physically manipulating the soil, increasing the temperature or adding chemicals.

Composting is a potential remediation strategy for dye manufacturing sludge because it incorporates the physical mixing and elevated temperatures shown to improve compound bioavailability. Antizar-Ladislao, et al. (16) for example, demonstrated improved degradation of PAHs during composting at an elevated temperature of 38 ^{o}C. Similar results were reported by Potter, et al. (17) for removal of PAHs in composting at mesophilic or low thermophilic temperatures. Thermophilic composting conditions, however, are generally unfavorable for treatment of mixed organic constituents in hazardous waste since very high temperatures tend to limit microbial community diversity (18, 19).

Although mixed windrow, static pile and in-vessel composting configurations are commonly available for bioremediation purposes, windrow composting has the highest throughput, lowest cost, and greatest operational flexibility (20). Windrow composting is an *ex situ* technology typically used to stabilize domestic sewage sludge, yard-waste, manure, and organic municipal waste in long rows of covered or uncovered material that are routinely mixed with automated tracked or manually driven turners. System design and process controls can vary and depend largely on material characteristics, treatment objectives and chemical constituents (21). A review of composting conditions for treatment of PAH contaminated soils conducted by Antizar-Ladislao, et al. (13) emphasized the importance of designs that maintain an aerobic environment, a constant moisture content and mesophilic temperatures. These conditions are consistent with more specific on-farm composting guidance that suggests a moisture content of 40-65%, at least 5% oxygen, pH between 5.5 and 9, and a C:N ratio of 20:1 to 40:1 (22). The configuration of the windrow, type and amount of bulking agent and frequency of turning are

selected based on maintaining a porosity of about 30%. Additional forced aeration may be needed to augment limited diffusion caused by compaction of dense material (20).

The most important consideration in hazardous waste composting is the intrinsic degradability of the compounds and presence of active microbial populations with diverse metabolic capabilities (23). In general, *ex situ* biotransformation systems operate by stimulating naturally occurring microorganisms, addition of exogenous organisms or both (24-26). Many researchers have shown, however, that biostimulation of indigenous microorganisms that have adapted over time to a particular set of chemical substrates and growth conditions, are superior to exogenous blends of microorganisms in both the rate and extent of chemical biotransformation (27-29). Aerobic degradation of dye chemicals by fungi and bacteria have been reviewed in detail (30). While many studies have demonstrated the feasibility of composting soils containing PAHs, gasoline, diesel fuel, waste oil, explosives, propellants and halogenated solvents (31-34), this is the first study to investigate the use of windrow composting to remediate dye manufacturing waste sludge. The research challenge was to overcome material handling, moisture management, phase partitioning, and multicompound biotransformation complexities that characterize this type of waste. Research was conducted at pilot-scale to more realistically assess process feasibility for site remediation.

MATERIALS AND METHODS

Sample collection. Source material for this study was obtained from two separate waste disposal areas (designated A and B) at a former dye manufacturing facility (site is not identified at the request of the owner). Each location is an unlined pit covered with water and used until the early 1980's to store dye manufacturing waste sludge.

Three grab samples were collected from each location using a long-reach backhoe. Samples were collected from material located in the central area of the backhoe bucket using clean, alcohol-washed spades and each sample placed into sterile, 500 mL glass jars. Composite samples were prepared for location A and location B by combining the three grab samples from each area and mixing thoroughly. This resulted in a single, three-point composite sample for each location.

Sample analysis. Composite samples were submitted to Accutest Labs in Dayton, NJ, for analysis of volatile organic compounds, semi-volatile organic compounds, nitrogen, phosphate, density, moisture, pH, and total organic carbon. The microbial community was examined by Envirogen, Inc., Lawrenceville, NJ. Moisture holding analysis was conducted by the project engineer in the field. A summary of analyses, methods, sample volumes, holding times, and preservatives is provided in Appendix 1-A.

Volatile organic compounds were identified using closed-system purge-and-trap extraction following EPA SW-846 Method 5035 with analysis by gas chromatography/mass spectrometry (GC/MS) according to EPA SW-846, Method 8260B. Semi-volatile organic compounds were determined using ultrasonic extraction using EPA SW-846, Method 3550B and GC/MS analysis by EPA SW-846 Method 8270C. The sample pH was measured by electrometric measurement of a 1:1 mixture of sample and deionized water (EPA SW-846 Method 9045D).

Enumeration of heterotrophic and naphthalene degrading microbes was made using a standard plate count technique. A 1 g sample of sludge was added to 10 mL of sterile, deionized water. The slurry was thoroughly mixed and supernatant removed to form a series of dilutions. A 0.1 mL inoculum from each dilution was added to R2A agar for heterotrophic growth and M9 minimal salts media (without additional carbon source) for enumeration of specific naphthalene

degraders). Naphthalene was added to the minimal salts media by placing 1 g of crystalline

flakes (CAS Number 91-20-3, Sigma-Aldrich, St. Louis, MO.) to the cover of the inverted (glass)

petri dish. Inoculated agar plates were incubated at 35^oC for 48 hrs. Enumerations were made by

counting the number of colony forming units in the dilution that produced between 30 and 300

colonies.

Moisture holding capacity (MHC) was determined volumetrically by placing an air-

dried sample into a 150 mL graduated cylinder with a screened perforated bottom. Water (100

mL) was added to the top of the sample, the cylinder was loosely covered to prevent evaporation,

and water was allowed to gravity drain through the sample. Drained water and remaining

headspace water were measured after 24 hours. MHC (% v/v) was determined to be the

difference between 100 mL and the sum of the drained and headspace water. Sample density was

obtained by placing between 1 and 5 cm^3 of the sample into a tared graduated cylinder and

weighing the cylinder. Material density (g/cm^3) was determined as the difference between the

tare weight and the final weight.

Total organic carbon was determined by the Lloyd Kahn Method (US EPA, 1988). In

this method, inorganic carbon from carbonates and bicarbonates is removed by acid treatment,

and organic compounds are oxidized to carbon dioxide. Carbon dioxide was measured using

Perkin Elmer Model 240C Elemental Analyzer. Ammonia-N was determined by distillation,

capture of released ammonia in dilute sulfuric acid, and colorimetric analysis (EPA Method

1690). Organic nitrogen was determined as Kjeldahl nitrogen (EPA Method 1688) by wet

oxidation, conversion of organic nitrogen to ammonium, and measuring the ammonia released by

distillation with sodium hydroxide. Sample extraction using potassium chloride followed by

colorimetric analysis was used to measure inorganic concentrations of nitrite-N, nitrate-N and

ammonium-N (Method 84-2, Methods of Soil Analysis, American Society of Agronomy, 1965).

The Mehlich 3 method commonly used for agricultural soils (35) was used to measure available phosphorus in compost. ASTM Method D4928 (Standard Method for Water in Crude Oils by Coulometric Karl Fischer Titration) was used to determine sample moisture.

Sludge Characterization. Sludge samples from locations A and B appeared as a sticky, tar-soil mixture. Both materials were highly organic with a TOC of 90% in sludge sample A and 57% in sample B. The intrinsic pH of location A sludge was near neutral (pH 7-8) while the pH of sludge from location B was acidic (pH 5). Triplicate samples, collected at each location were screened for eight organic compounds (TABLE 1.1 and TABLE 1.2).

TABLE1.1 Compound concentration (mg/kg) in waste sludge samples collected from Location A

Compound	Sample			Mean	SD
	A-1	A-2	A-3		
Benzene	929	350	1,500	926	575
Toluene	1,520	820	1,300	1,213	358
Xylenes	1,040	840	2,100	1,327	677
Naphthalene	8,830	36,000	32,600	25,810	14,803
Nitrobenzene	3,260	9,000	979	4,413	4,133
1,2-Dichlorobenzene	62	130	38	77	48
N-Nitrosodiphenylamine	6,620	21,000	6,020	11,213	8,481
2-Methylnaphthalene	837	2,200	368	1,135	952

TABLE 1.2 Compound concentration (mg/kg) in waste sludge samples collected from Location B

Compound	Sample			Mean	SD
	B-1	B-2	B-3		
Benzene	494	1,510	963	1,237	387
Toluene	2,100	1,860	769	1,315	771
Xylenes	1,720	2,180	246	1,213	1,368
Naphthalene	1,220	310	822	566	362
Nitrobenzene	38	534	1270	902	520
1,2-Dichlorobenzene	20	8	206	107	140
N-Nitrosodiphenylamine	302	1,860	903	1,382	677
2-Methylnaphthalene	147	8	17	13	6

Pilot system design and operation. A pilot-scale windrow composting system was used to evaluate treatment feasibility of dye-waste sludge. The pilot system consisted of three-sided concrete treatment bays with an operating capacity of approximately 15 m^3 (20 cu yd). Each bay was constructed to a height of 3 m (10 ft), a width of 3 m (10 ft) and a depth of 2.4 m (8 ft). These dimensions approximate the operating height and half the width of a full-scale windrow to a length of 3 m (10 ft). Target operating conditions were selected to encourage compound biotransformation by aerobic, mesophilic, naturally occurring bacteria (TABLE 1.3).

TABLE 1.3 Target operating conditions for compost pilot test

Parameter	Target Value
C:N ratio	30:1
moisture (%)	20-30
oxygen (%)	10-21
pH	6 to 8
porosity (%)	30
Temperature (max $^{\circ}$C)	40

Three materials were composted during the field pilot study: Material 1 (M1) was collected from location A; Material 2 (M2) contained sludge collected from location B; and Material 3 (M3) was a duplicate of Material 2 without pH adjustment or nutrient addition and was used as a negative control. Bulking agents were added at two loadings to determine if volume increase due to amendments could be minimized. Woodshavings were added to M1 sludge at a volumetric ratio of 1:4 while an equal volume of woodshavings and sludge were blended to create M2 and M3. The density of bulked M1, after final mixing, was approximately 1.2 g/cc (2,023 lb/cu yd) with a final pH of approximately 8.5. The bulk density of woodshaving amended M2 sludge was approximately 0.8 g/cc (1,348 lb/cu yd).

Due to the acidity of sludge collected from location B approximately 2.65 kg of calcium carbonate/m^3 material (about 5 lb/cu yd) was added to adjust the pH to near neutrality (7-8). M3 was prepared similarly to M2 but pH was not adjusted (TABLE 1.4). Because of differential bulking the total mass of M1 was 17,676 kg (47,194 lbs), and about 12,000 kg (27,391 lb) for M2 and M3.

TABLE 1.4 Material balance for compost amendments

Amendment	Material 1		Material 2		Material 3	
	cu yd	m^3	cu yd	m^3	cu yd	m^3
Waste-Sludge	16	12	0	0	0	0
Waste-Sludge	0	0	10	7.5	10	7.5
Wood-shavings	4	3	10	7.5	10	7.5
CaCO$_3$	0	0	45 lb	20 kg	0	0
Total Vol	20	15	20	15	20	15
pH (final)	8.5		7.5		5.6	

Note: Volumes are approximate +/- 2 m^3. Bulk density of M1 is 1.2 g/cm^3 (2,023 lb/cuyd). The bulk density of M2 and M3 is approximately 0.8 g/cm^3 (1,348 lb/cu yd).

Aerobic conditions were maintained in the composting material by continuous subfloor aeration using blowers operating in convergent flow at about 1 m^3/min (40 cfm). At this rate and assuming a 30% porosity, pore space air was exchanged about every 3.5 min. Oxygen transport within the compost was managed by maximizing diffusivity in the pile by bulking the material with woodshavings and routinely mixing to maintain a loosely packed, well-structured material. The addition of woodshavings also improved material mixing by coating the exposed tar and reducing self-adhesion.

Composting material was mixed in batches equal to about 1/3 of the volume and the pile was completely inverted during mixing. A rubber-tired loader equipped with a hydraulically operated screener/crusher bucket (ALLU SM3-Ideachip SM3-17) was used to blend the material. The ALLU bucket had a volume of 1.8 cu yd (1.4 m^3) and contained 3 crushing drums that

rotated in unison. Reversible hydraulics and high rotating speeds (300 rpm) allowed loosening of

wet, sticky, or bridged material. Hammers positioned between the discs crushed or shredded

agglomerated material. Particles with a diameter greater than about 3 in (8 cm) that were not

crushed (such as hard tars, rocks, metal fragments or wood) were excluded from the compost mix.

Compost was mixed weekly during treatment.

Fertilizer Addition. Commercial fertilizer was added to the material to augment base-

line nutrient conditions. The amount of fertilizer required was calculated based on the mass of

carbon present in the material and a target total C:N of 30:1 (TABLE 1.5). Base-line nitrogen

was estimated as the total amount of organic and inorganic nitrogen in the material. Organic

nitrogen was higher in Material 1 compared to Material 2 resulting in intrinsic C:N ratios of

approximately 18:1 and 98:1, respectively. The C:N ratio in M2 was adjusted to 30:1 by adding

6,000 mg/kg ammonium nitrate fertilizer (Scotts Turf Builder 29-3-4). Fertilizer was not added

to M3 to maintain a negative control. The C:N ratio in M1 was lower than the operating target of

30:1 due to the high concentration of organic nitrogen. No adjustment was made to this material

since the nitrogen concentration was already high and further increase would be

counterproductive. A phosphorus concentration in the range of 400 mg/kg was measured for both

materials.

TABLE 1.5 Nutrient characteristics of composting materials

Nutrient	Material 1	Material 2
Ammonium-N (mg/kg)	385	244
Kjeldahl- N (mg/kg)	12,700	2,460
Nitrate-N	<1,800	<140
Nitrite-N	<0.2	<0.2
Total-N (mg/kg)	13,085	2,704
Total organic carbon (mg/kg)	242,000	265,000
Unamended C:N	18:1	98:1
Amended C:N	18:1	30:1

Rate constants. Compound degradation during composting was characterized using a first-order rate coefficient (k). The first order rate law is defined as: $-\frac{d[C]}{dt} = k\,[C]$, where k is the first-order rate constant (1/time), C is concentration (mg/kg), and t is time (days). Integrating the rate law gives: $-kt + \ln[C]o$, and a plot of ln[C] vs. time gives a straight line with a slope of (–k). The linear regression coefficient (R^2) was used as an indicator of how well the rate law described the observed transformation. A threshold R^2 value of 0.7 or greater ($\rho < 0.05$) for eight samples collected weekly during the 49 day operation period was used as a positive indicator of compound degradation while data resulting in R^2 values less than 0.7 were considered ambiguous and not indicative of biotransformation. This value is considered high enough that over-prediction of performance is unlikely but low enough to be practical in field-scale applications. Half-life values, based on first order rate constants, were calculated using the equation $t_{1/2} = (\ln(2)/k$.

Microbial growth rate. The growth dynamics of the microbial populations in the composting material were estimated using standard plate count methods that estimate concentration based on colony forming units as described by Horwath and Paul (36). Relative biomass was determined at the start (time = 0) and at weekly intervals through day 49. Specific growth rates (μ) of heterotrophs and naphthalene degraders were calculated during exponential phase growth using the formula growth rate = $\mu = \mathrm{Ln}\,(N_2 / N_1) / (t_2 - t_1)$, where N_1 and N_2 are biomass at time 1 (t_1) and time 2 (t_2), respectively (37). The number of divisions per day and the population doubling time were calculated based on the specific growth and doubling time ($t_d = \ln2/\mu$

RESULTS

The 17 compounds identified in sludge collected from two waste disposal ponds are typical of dye manufacturing processes. The sludge contained four monoaromatic hydrocarbons, three chlorinated aromatic hydrocarbons, three nitrogen-substituted aromatic hydrocarbons, two heterocyclic hydrocarbons, and four polycyclic hydrocarbons (FIG 1.1).
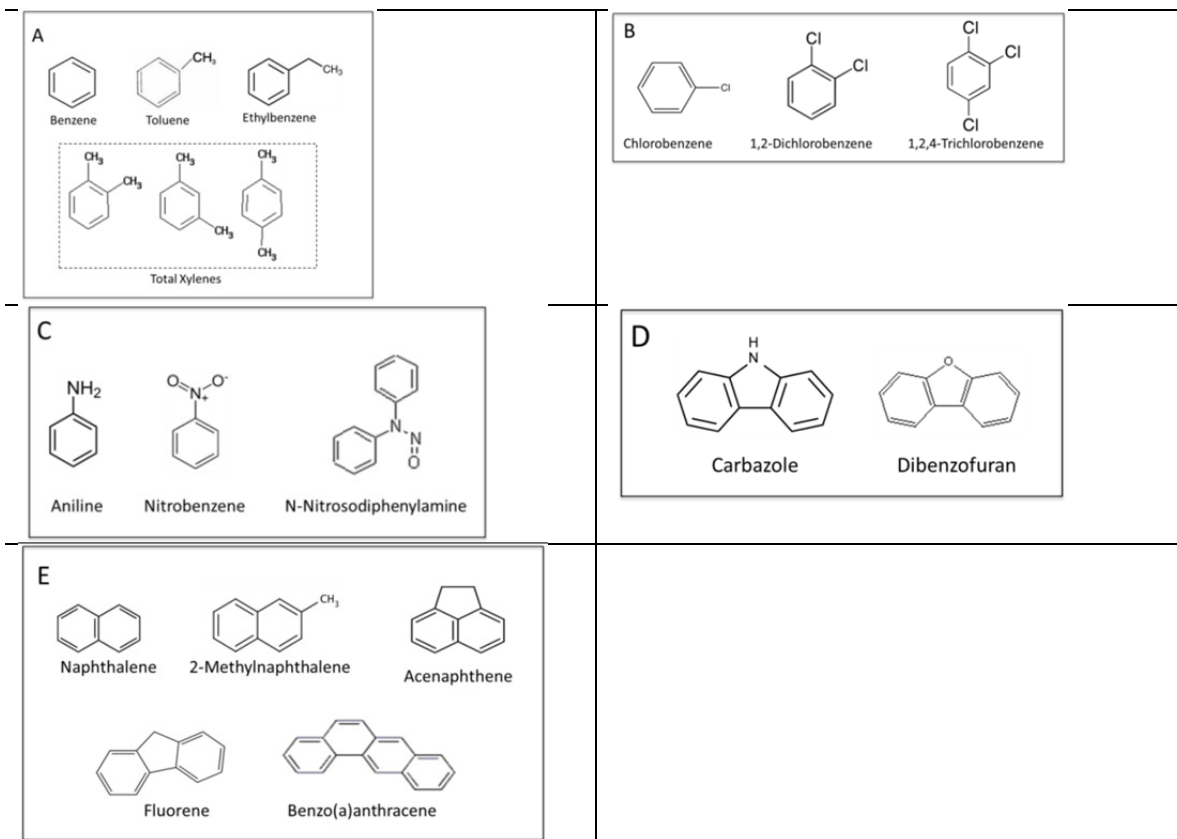
FIG 1.1 Chemical structures of monoaromatic (A), chloroaromatic (B), nitroaromatic (C), heterocyclic aromatic (D) and polycyclic aromatic hydrocarbons (E) identified in dye manufacturing waste sludge.

A chemical comparison of M1 and M2 compost shows that naphthalene and benzene are present at similar concentrations in both materials. Otherwise, chemical concentrations are different in each material with chlorobenzene, xylene, and *N*-nitrosodiphenylamine dominant in M1 and nitrobenzene, aniline and toluene dominant compounds in M2 (FIG 1.2).
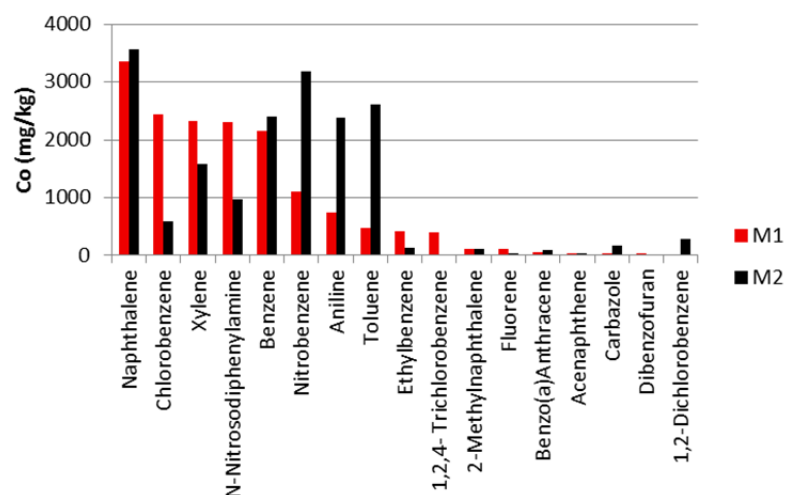
FIG 1.2 Initial concentration (C_o) of chemical compounds in M1 and M2 compost

Mass removal of chemical compounds.   The initial total mass of the 17 chemical

compounds was greatest in M1 (286 kg) relative to M2 (219 kg) while M3 contained the least

(125 kg). Although materials appeared visually and chemically similar, the degradation

characteristics of certain chemicals differed between materials (Table 1.6).

TABLE 1.6 Initial concentration (C_o) and final concentration (C_f) of compounds identified in
each test material

Compound	Concentration (mg/kg)								
	M1			M2			M3 (control)		
	C_o	C_f	SD [1]	C_o	C_f	SD	C_o	C_f	SD
Benzene	2,160	6	1	2,410	66	6	1,071	473	172
Toluene	468	14	6	2,610	239	1	913	699	114
Ethylbenzene	418	30	6	125	51	2	103	157	11
Xylenes	2,330	197	27	1,580	449	1	1,030	1,175	21
Chlorobenzene	2,440	241	26	584	235	21	489	834	12
1,2, Dichlorobenzene	25	30	5	278	129	22	226	71	33
1,2,4-Trichlorobenzene	406	276	25	3	6	0	51	135	34
Nitrobenzene	1,100	873	168	3,180	191	75	1,300	1,148	341
Aniline	741	207	136	2,390	140	144	1,430	730	862
N-Nitrosodiphenylamine	2,300	2,165	106	971	818	248	1,500	1,395	275
Carbazole	30	39	10	172	95	42	148	142	26
Dibenzofuran	30	29	6	3	36	12	41	37	11
Naphthalene	3,360	218	23	3,570	359	189	3,650	2,010	849
2-Methylnaphthalene	111	55	6	117	84	26	176	168	80
Acenaphthene	39	32	6	33	29	9	32	42	17
Fluorene	104	19	8	37	17	6	20	19	4
Benzo(a)anthracene	49	40	20	93	44	33	97	22	0.15
Total	16,111	4,471	585	18,156	2,988	837	12,277	9,257	2,862

[1] SD is standard deviation of C_f (n=3).

Compound concentration at the start of treatment was based on a single sample. Variability is not surprising given the heterogeneity of the high organic sludge and difficulty mixing the sludge with bulk amendments. Material mixing became easier as composting progressed resulting in a more homogeneous blend and decreased sample variability. The final concentration of each compound determined at the end of composting assumes that degradation from day 42 through 49 is asymptotic. Samples collected on these days were considered replicates for purposes of approximating the final average concentration and standard deviation (n=2). Progress samples collected during treatment were not replicated. This approach is consistent with sampling plans developed for the purpose of feasibility testing where compound concentration is compared only to initial concentrations and regulatory treatment objectives. A complete set of degradation data for the 49 day treatment period is provided in Appendix 1-B.

Average compound concentrations were normalized as weight percent of the total mass of the 17 chemicals identified at the start and end of composting. All three test materials have a relatively high initial mass percent of naphthalene (20 to 35%) and benzene (9 to 13%). Material 1 (FIG 1.3) is uniquely characterized by 10% or more (w/w) of *N*-nitrosodiphenylamine, chlorobenzene and xylenes while M2 (FIG 1.4) contains greater than 10% (w/w) each of toluene, nitrobenzene, and aniline. Aniline, nitrobenzene, and *N*-nitrosodiphenylamine exceeds 10% (w/w) in Material 3 (FIG 1.5). Approximately 72 and 54% of the initial mass of contaminants were removed in Materials 1 and 2, respectively, compared to 25% removal in M3 (Control) during the 49 day composting period. Chemical removal from the Control is assumed to be by volatilization during aeration or mixing however, biotransformation may have occurred. The 25% removal in M3 consisted of approximately 14% naphthalene, 6% aniline, and 5% benzene. It is notable that nitrobenzene, and *N*-nitrosodiphenylamine appear recalcitrant in M1 but only *N*-

nitrosodiphenylamine was recalcitrant in M2. Mass removal of naphthalene and aniline represent

most of the constituent loss in the control material during composting.
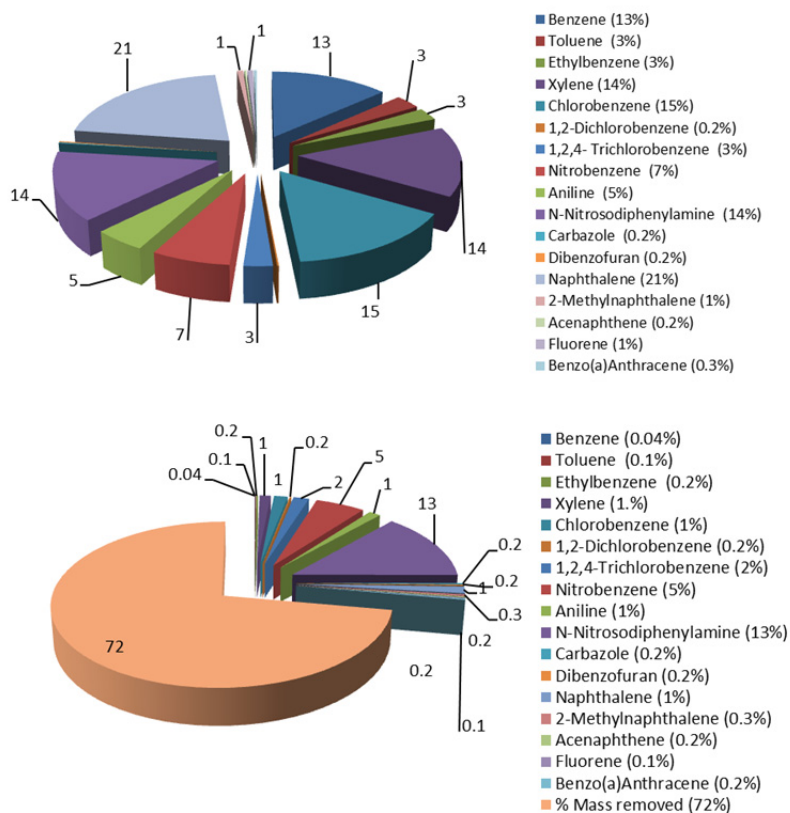
FIG 1.3 Percent distribution by mass of compounds identified in Material-1 at the start (top) and end
(bottom) of composting.

Top pie chart legend:
- Benzene (13%)
- Toluene (14%)
- Ethylbenzene (1%)
- Xylene (9%)
- Chlorobenzene (3%)
- 1,2-Dichlorobenzene (2%)
- 1,2,4- Trichlorobenzene (0.02%)
- Nitrobenzene (18%)
- Aniline (13%)
- N-Nitrosodiphenylamine (5%)
- Carbazole (1%)
- Dibenzofuran (0.02%)
- Naphthalene (20%)
- 2-Methylnaphthalene (1%)
- Acenaphthene (0.2%)
- Fluorene (0.2%)
- Benzo(a)Anthracene (0.5%)

Bottom pie chart legend:
- Benzene (6%)
- Toluene (7%)
- Ethylbenzene (0.3%)
- Xylene (4%)
- Chlorobenzene (1%)
- 1,2-Dichlorobenzene (0.07%)
- 1,2,4-Trichlorobenzene (0.01%)
- Nitrobenzene (8%)
- Aniline (6%)
- N-Nitrosodiphenylamine (2%)
- Carbazole (0.4%)
- Dibenzofuran (0.01%)
- Naphthalene (9%)
- 2-Methylnaphthalene (0.3%)
- Acenaphthene (0.1%)
- Fluorene (0.1%)
- Benzo(a)anthracene (0.2%)
- % Mass removed

FIG 1.4 Percent distribution by mass of compounds identified in Material 2 at the start (top) and end (bottom) of composting.
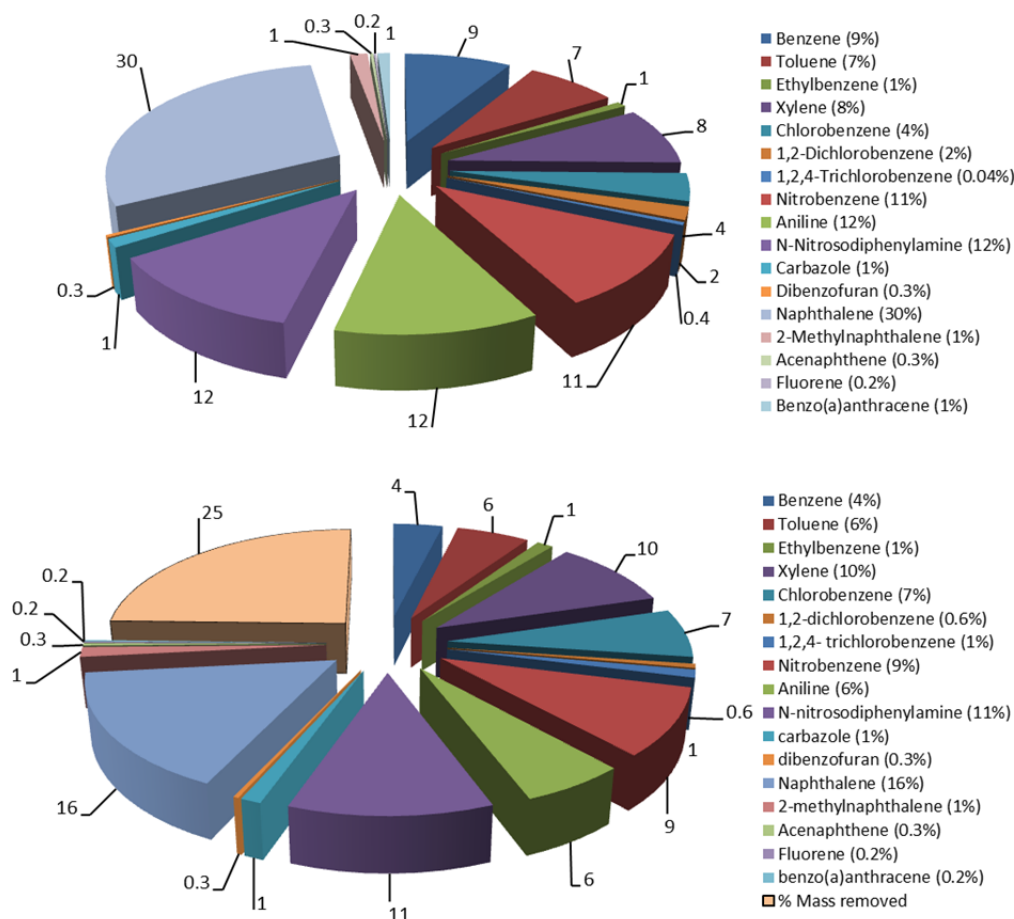
FIG 1.5 Percent distribution by mass of compounds identified in Material 3 at the start (top) and end (bottom) of composting.

Rate of degradation. The relative rate of degradation for each compound in test materials is quantified by half-life values based on pseudo first-order kinetics. Kinetic estimates were graphically derived from concentration data obtained weekly during the 49 day treatment period (analysis provided in Appendix 1-C). Half-life values ranged from 5 to 46 days depending on the compound and the Material (TABLE 1.7). Half-life values were calculated only when the corresponding correlation coefficient (R^2) for the linear regression used to derive the first order rate coefficient was greater than 0.7. This condition was used as a positive indication of

compound degradation. A correlation coefficient below 0.7 (determined for compounds with initial concentrations greater than 100 mg/kg) was used as a negative indication of degradation (ND, no evidence of degradation). Data trends for compounds present at initial concentrations ≤ 100 mg/kg could not be determined thus providing no evidence for, or against, degradation (indicated by a dash in Table 1.7) . With the exception of nitrobenzene and aniline, degradation rates were faster for compounds in M1compared to M2. Nitrobenzene appeared recalcitrant in M1 but was readily degraded in M2 and aniline degraded twice as fast in M2 compared to M1 compost.

TABLE 1.7 Summary of pseudo first order rate coefficient (-k, d^{-1}) and calculated half-life values (days) for chemical compounds identified in dye manufacturing waste sludge

Compound	Material 1			Material 2			Control		
	$(-k)\ d^{-1}$	(R^2)	Half-life	$(-k)\ d^{-1}$	(R^2)	Half-Life	$(-k)\ d^{-1}$	(R^2)	Half Life
Benzene	0.136	0.8803	5	0.0762	0.9651	9	0.0143	0.2309	ND
Toluene	0.0623	0.7691	11	0.048	0.9186	14	0.0149	0.4804	ND
Ethylbenzene	0.0857	0.9377	8	0.017	0.7072	41	0.0057	0.1632	ND
Xylenes (total)	0.0386	0.9156	18	0.0236	0.8539	29	0.0275	0.4475	ND
Chlorobenzene	0.0543	0.8409	13	0.0192	0.7758	36	0.0275	0.4475	ND
1,2-Dichlorobenzene	0.0021	0.0039	-	0.015	0.7224	46	0.0202	0.2023	ND
1,2,4-Trichlorobenzene	0.0089	0.2831	ND	0.0037	0.0047	-	0.0089	0.3946	-
Aniline	0.0352	0.7176	20	0.067	0.7554	10	0.0067	0.0101	ND
Nitrobenzene	0.0043	0.1726	ND	0.0696	0.0824	10	0.0126	0.0744	ND
N-Nitrosodiphenylamine	0.0016	0.0109	ND	0.0024	0.0398	ND	0.0046	0.0886	ND
Carbazole	0.0006	0.0004	-	0.015	0.6581	46	0.0108	0.0654	-
Dibenzofuran	0.0014	0.0064	-	0.0266	0.2761	-	0.0044	0.09	-
Naphthalene	0.067	0.7544	10	0.055	0.9491	13	0.0028	0.0049	ND
Fluorene	0.0211	0.2313	-	0.015	0.4755	-	0.0039	0.0188	-
Acenaphthene	0.004	0.0788	-	0.0023	0.0394	-	0.0056	0.185	-
Benzo(a)Anthracene	0.0069	0.114	-	0.0201	0.4553	-	0.0166	0.134	-
2-Methylnaphthalene	0.0233	0.3466	-	0.0131	0.5317	-	0.0021	0.006	-

[1] Dash (-) indicates that the initial compound concentration was too low to reliably provide evidence of degradation.
[2] No degradation (ND) is indicated for high concentration compounds with an R^2 below 0.7.

System operation. A comparison of operating conditions maintained over the 49 day compost period shows a lower C:N ratio in M1 compared to M2 (a full operational log is provided in Appendix 1-D). This is due to a typically higher organic nitrogen content (measured as Kjeldahl-

Nitrogen minus ammonium-Nitrogen) in Material 1 while total organic carbon is about the same

in both materials (FIG 1.6). The pH of both materials was maintained in the range of 6.5 to 8.5,

however, M2 was consistently closer to neutrality. Moisture management was moderately

successful with an average moisture content of about 37% in M1 and 24% in M2.
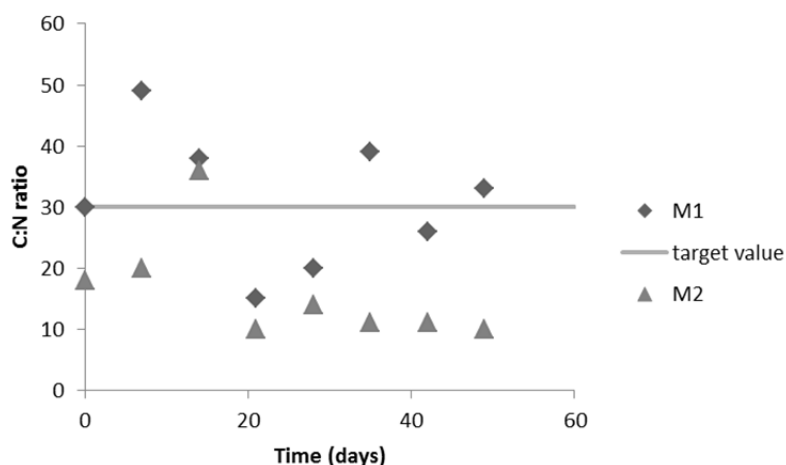
FIG 1.6 C:N ratio in compost materials during 49 day treatment period relative to target value.

Microbial growth. Composting temperatures between 30^oC and 38^oC were maintained

for about 15 to 20 days during the 49 day composting period. During this time, however, the

temperature of the un-amended control did not increase above the ambient 17^oC measured at the

start of testing in late October. The temperature in the control continuously decreased to a low of

4^oC as treatment continued into December at day 49. Temperatures in composting of Material 1

peaked on day 21 and on day 15 for Material 2. During this 49 day period, both heterotrophic

(FIG 1.7) and specific naphthalene degraders (FIG 1.8) increased and were sustained throughout

the test duration.
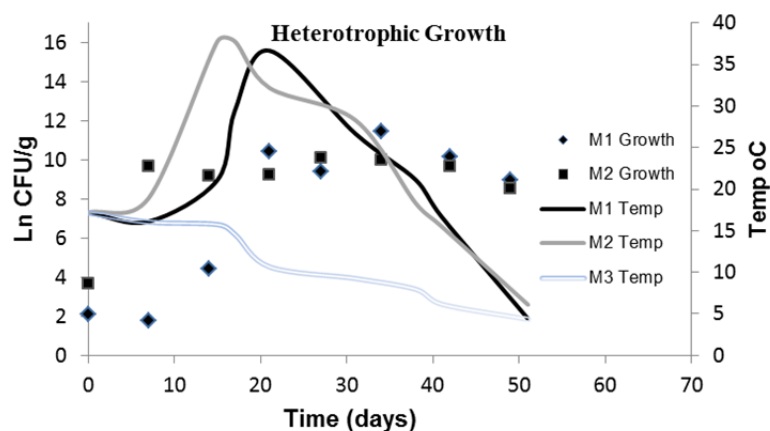
FIG 1.7 Temperature profile and growth curve for non-specific, heterotrophic bacteria (by plate count) in Material 1 and Material 2. Specific growth rates (μ d^{-1}) are estimated from exponential phase growth; $\mu =$ 0.615 in Material 1 and 0.85 in Material 2.
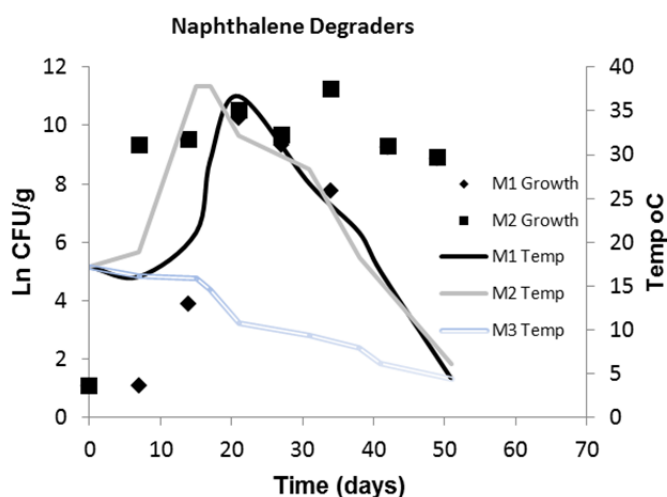
FIG 1.8 Temperature profile and growth of naphthalene degrading microorganisms in Material 1 and Material 2. Specific growth rates (μ d^{-1}) are estimated for exponential phase growth; $\mu =$ 0.653 in Material 1 and 1.176 in Material 2.

Microbial exponential growth of both heterotrophs and naphthalene degraders is observed at or shortly after start-up in Material 1 and had ended by about day . This period was followed by a stationary or possibly a declining phase that extends through day 49. Maximum biomass of about 20,000 CFU/g was reached by both groups of organisms in Material 1. Microbes in

Material 2, however, exhibit a lag period with exponential growth beginning a little before 7 days after test start-up. This period was followed by a stationary phase that extended through day 49. Similar to Material 1, maximum biomass of about 20,000 CFU/g was reached by both types of organisms in Material 2. A first order regression of exponential growth showed higher specific growth rates (μ d^{-1}) for organisms in Material 2 than in Material 1. Specific naphthalene degraders had a higher specific growth rate in M1 material relative to heterotrophs. In Material 1 however, the specific growth rate of heterotrophs and naphthalene degraders were about equal.

DISCUSSION

Sample variability. Sludge used in this study was collected from two separate disposal ponds at a former dye manufacturing site. Both samples contained the same chemical compounds but at different concentrations and relative distributions. Similar variability for compound concentration, total mass and composition of PAH contamination has been described at manufactured gas plants (MGPs) (38). The observed differences in compound concentrations between M1 (containing sludge from waste-pond A) and M2 (containing sludge from waste-pond B) is not unexpected since multiple product lines, disposal practices, and settling properties of the waste (among other factors) can lead to heterogeneous sludge that varies by depth and horizontal distribution. As a result, initial concentrations of chemical compounds in the dye manufacturing waste sludge were highly variable and inconsistent at the early stage of composting. The variability lessened as composting progressed and concentrations decreased. This pattern of convergence during the composting process has also been reported by researchers remediating highly contaminated "oil lakes" created after the Iraqi invasion of Kuwait (39). Some researchers have attributed high variability in PAH concentration in contaminated soils to spherical tar residues present in soil even after blending (40). Tar-balls and tar-soil aggregates were also

present in the early stages of dye-waste composting and these likely contributed to the wide range of early stage concentration data. Aggressive mixing was used in this pilot study in an attempt to break aggregates and form a more homogeneous blend. Effective mixing was limited by the thixotropic nature of the material due to the high organic tar-like sludge. As degradation progressed during the composting period the physical properties of the sludge material changed allowing more aggressive mixing and crushing of tar-compost aggregates. Thus unlike municipal composting, the primary importance of turning the compost pile in this study was to homogenize the material rather than to release heat (41).

Kinetic analysis. First order rate coefficients, determined by linear regression of all 8 data points (weekly chemical analysis for 49 days) characterized degradation trends and provided a metric for comparing compound degradability. Observed rates are a combination of biotransformation, mass transfer, dissolution and desorption. Surprisingly, half-life values observed in this study were generally consistent with those reported in the literature (TABLE 1.8), however, a wide range of values has been reported.

Derived biotransformation rates reflect activity by microbial consortia that have acclimated to the physical and chemical environment over several decades. The increased growth of indigenous heterotrophic bacteria evidenced an active consortia in the sludge composting that increased with temperature. Identification and characterization of the microbial consortium was not included in this project since objectives focused on combined degradation rates and not on individual contributions by specific organisms. Studies show that isolated cultures and exogenous blends of microorganisms do not improve degradation of crude oils (42) and, although individual species of bacteria have been isolated that contribute to compound degradation of PAHs, more complete degradation occurs by mixed consortia (43, 44). Early work by Atlas and Cerniglia (45) demonstrate the ubiquity and broad metabolic capabilities of microorganisms in

the environment and many studies document the benefits of biostimulation compared to

bioaugmentation (24, 27, 29, 46, 47). Thus, for this project knowledge of individual species

present in the dye manufacturing sludge was ancillary to stimulation of the adapted community.

TABLE 1.8 Comparison of half-life values experimentally determined in this
study, with values reported in the literature (48)

Compound	Material 1 half-life (days)	Material 2 half-life (days)	Literature half-life (days)
Benzene	5	9	5-16
Toluene	11	14	4-22
Ethylbenzene	8	41	3-10
Xylenes (total)	18	29	7-28
Chlorobenzene	13	36	68-150
Aniline	20	10	5-25
Naphthalene	10	13	17-48
1,2-Dichlorobenzene	1	46	68-150
Carbazole	—[1]	46	5-25
Nitrobenzene	ND[2]	10	17-48
1,2,4-Trichlorobenzene	ND	--	28-180
N-Nitrosodiphenylamine	ND	ND	10-34
Dibenzofuran	--	--	7-28
Fluorene	--	--	32-60
Acenaphthene	--	--	12-102
Benzo(a)Anthracene	--	--	52-100
2-Methylnaphthalene	--	--	No Value

[1] (---) denotes initial compound concentration ≤ 100 mg/kg and linear regression coefficient < 0.7.
[2] ND (No Evidence of Degradation) indicates compound concentration is initially greater than 100 mg/kg but linear regression coefficient is <0.7.

+

Four observations from the half-life results are:

- *N*-nitrosodiphenylamine appears recalcitrant in M1.

- Nitrobenzene appears recalcitrant in M1 but degrades relatively quickly in M2.

- Aniline degrades more slowly in M1 compared to M2.

- Ethylbenzene, xylenes and chlorobenzene degrade more rapidly in M1 compared to M2.

The reason for the recalcitrance of *N*-nitrosodiphenylamine is unclear. This compound is initially present at relatively high concentration. Although limited research has been conducted, existing data show cometabolic degradation of *N*-nitrosodiphenylamine under anaerobic conditions (49). Research also indicates that diphenylamine can be used as a carbon and energy source under aerobic conditions (50). It is possible that nitrated derivatives of diphenylamines are also aerobically degradable so degradation of *N*-nitrosodiphenylamine might be expected (51). It is interesting to note that aniline is a degradation product of both aerobic and anaerobic degradation of *N*-nitrosodiphenylamine (49, 51). The relatively high amount of aniline in the sludge may be a factor in the recalcitrance of *N*-nitrosodiphenylamine if end-product inhibition is a factor. The structure of *N*-nitrosodiphenylamine is markedly different than other nitro-aromatics in the waste sludge and this may also have an influence on its biodegradability (52).

Relative mass. Differences in compound degradation rates in M1 and M2 noted for chlorobenzene, xylenes, nitrobenzene, aniline and ethylbenzene may be explained by microbial adaptations to the relative mass of each compound, the presence of multisubstrates, and/or the nutrient conditions in the sludge. It appears that when the initial compound concentration is high, so is the rate of degradation (TABLE 1.7). This is not surprising since a microbial population acclimated to xenobiotic chemicals in the environment is expected, and has been observed in other ecosystems (28, 46, 53-55). Thus ethylbenzene degrades much more quickly in M1 where it is present at an initial mass of 3% than in M2 where it is initially present at less than 1% of the total mass of contaminants (TABLE 1.8). Similarly in M1 where chlorobenzene, and xylene are initially about 15% of the total mass of contaminants degradation half-lives are about 2-3 times faster than in M2 where the initial mass is only about 3% and 9% of the total. At the same time, aniline degrades slowly and nitrobenzene may not degrade at all in M1 where their initial mass is lower than for the more rapidly degraded compounds. These conditions are reversed in M2

compost. Here nitrobenzene and aniline represent more of the total mass (18% and 13%, respectively) and faster degradation of both compounds and slower degradation of xylene and chlorobenzene are observed.

TABLE 1.9 Key differences between degradation half -life and composting conditions in Material 1 and Material 2

		M1		M2	
		Mass (%)	Half-life (days)	Mass (%)	Half-life (days)
Compound	Chlorobenzene	15	13	3	36
	Xylenes	15	18	9	29
	Nitrobenzene	7	ND	18	10
	Aniline	5	20	13	10
	Toluene	3	11	14	14
	Ethylbenzene	3	8	<1	41
Condition	C:N	16 (ambient 18)		40 (ambient 98)	
	pH	7-8		8-9	
	Bulking agent (% sludge vol)	25		100	

[1] Average adjusted value (intrinsic value)

[2] ND is no evidence of degradation

Multisubstrate interactions. Degradation profiles observed in each composting material may also reflect substrate interactions. Research has shown that competition in multisubstrate environments by diverse microbial populations can slow down degradation of some easily degradable compounds while the resulting increase in biomass can improve degradation of typically less degradable compounds (8, 56). The relatively slow rate of chlorobenzene degradation in M2 (and reverse condition in M1) is consistent with the co-occurring high mass percent of toluene (TABLE 1.9). Research has also shown that when toluene is present the degradation of xylenes (and benzene) can be faster than in its absence (56). This is somewhat contrary to results of the present study that show faster degradation of xylenes at lower initial concentrations of toluene (M1) than at a higher ones (M2). However, toluene was present in both materials.

Seemingly contradictory degradation data exist for many compounds when present in mixtures of other organic compounds. Some studies, for example, show that aniline biotransformation is inhibited by co-substrates (57) while other studies show aniline degradation in the presence of organic substrates. This apparent conflict may be due to metabolic diversity in aniline degrading microbial populations. Haigler and Spain (58) presented evidence that nitrobenzene is degraded by toluene dioxygenase in *P. putida* F1 and other toluene degrading organisms due to the enzyme's broad substrate specificity. The mass of toluene is higher in M2 and may be an additive factor in nitrobenzene degradation in that material simply by a greater abundance of enzyme while substrate competition may be more defining at lower concentrations.

Compost conditions. Bulking agents in the form of woodshavings were added to sludge materials at two volumetric loading ratios. M1 contained a 1:4 ratio (woodshavings to sludge) and M2 contained a 1:1 ratio. Bulking agents are typically added to improve structure and porosity for composting (20, 21). Straw, woodchips or woodshavings are typically selected for this purpose (22, 59). Woodshavings have an average C:N ratio of 641:1 (22) contributing to the average C:N ratio in M1 of about 16 and about 40 in M2. These values are within a reasonable operating range for composting (22). It is possible that the faster degradation rate of nitrobenzene and aniline in M2 is related to the higher C:N ratio resulting from the greater addition of woodshavings in that material (and the intrinsically high C:N ratio of 98:1 for unamended material). Adaptation to growth on nitrobenzene and aniline as a nitrogen source could provide a competitive advantage to microbes in material with limited ambient nitrogen. Many organisms have been identified that can use nitrobenzene as a sole source of carbon and nitrogen (60, 61). Oxidative pathways that release nitrite and reductive pathways that release ammonia have been described (57, 61-63).

Regulatory treatment objectives. Waste sludge generated from dye manufacturing is a Resource Conservation and Recovery Act (RCRA) source listed hazardous waste (dye and pigment production code K181) and contains several toxic chemicals above EPA regulatory limits (40 CFR §261.24) including benzene, chlorobenzene, 1,4-dichlorobenzene and nitrobenzene. These compounds as well as other constituents of the dye manufacturing waste sludge are regulated by the New Jersey Department of Environmental Protection (NJDEP) Site Remediation Program through the NJ administrative code (N.J.A.C. 7:26D). Only benzene, nitrobenzene, N-nitrosodiphenylamine, naphthalene and benzo(a)anthracene exceeded the NJDEP remediation standards for non-residential direct contact soil remediation standards before composting. Although 72% and 54% of the total mass of contaminants in M1 and M2 respectively were removed from the compost, these risk relevant compounds remained above NJDEP regulatory remediation requirements for non-residential direct contact soils (TABLES 1.10 and 1.11).

Why residual concentrations of biodegradable, risk relevant compounds persisted after composting is uncertain but studies have shown that mass transfer, dissolution and desorption reactions can limit the bioavailability of hydrophobic organic compounds (64-67). Based on work by Pignatello and Xing (15) it was anticipated that the increased composting temperature would increase desorption and dissolution into the aqueous phase for more complete treatment. Although temperature of the composting material was increased to $38^{\circ}C$ it was not sustained. Temperature remained above $30\ ^{\circ}C$ for only 16 and 26 days in M1 and M2, respectively. Antizar-Ladislao, et al. (68) showed that a constant temperature of $38^{\circ}C$ over 56 days of in-vessel composting followed by thermophilic temperatures provided the best treatment for PAHs in coal-tar contaminated soil. Researchers have shown that the addition of readily degradable organic materials can be used to increase the temperature of compost (69). Therefore, addition of a

carbon substrate at day 15 and 21 may have improved overall treatment in each material. It is

possible, however, that exogenous substrate would result in population shifts that would decrease

degradation of risk relevant compounds rather than improve it. This possibility should be

considered in future research.

TABLE 1.10 Comparison of Treatment Results for Compounds identified in
Material 1with NJ Non-Residential Treatment Standards

Compound	Material 1				
	Co^1 (mg/kg)	Cf^2 (mg/kg)	$NRTS^3$ (mg/kg)	% Reduction Needed	% Reduction Achieved
Benzene [4]	2,160	5	5	100	100
Toluene	468	12	9,100	none	97
Ethylbenzene	418	26	11,000	none	94
Xylenes	2,330	178	170,000	none	92
Chlorobenzene	2,440	222	7,400	none	91
1,2-Dichlorobenzene	25	26	59,000	none	none
1,2,4- Trichlorobenzene	496	258	820	none	48
Nitrobenzene [4]	1,100	754	340	69	31
Aniline	741	111	NS^5	none	85
N-Nitrosodiphenylamine[4]	2,300	2,240	390	83	3
Carbazole	30	32	96	none	none
Dibenzofuran	30	25	NS	none	17
Naphthalene [4]	3,360	202	17	99	94
2-Methylnaphthalene	111	50	2,400	none	55
Acenaphthene	39	27	37,000	none	31
Fluorene	104	13	24,000	none	88
Benzo(a)anthracene [4]	49	26	2	96	47

[1] Co= initial compound concentration.

[2] Cf= final compound concentration at completion of 49 day composting period

[3] NRTS denotes Non-Residential Treatment Standards for New Jersey as of 2013
http://www.nj.gov/dep/srp/guidance.
[4] Compound concentration ≤ NRTS treatment standard

[5] Aniline and dibenzofuran do not have published soil treatment standards (NS).

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TABLE 1.11 Comparison of treatment results for compounds
identified in M2 with NJ Non-Residential Treatment Standards

Compound	Material 2				
	Co[1] (mg/kg)	Cf[2] (mg/kg)	NRTS[3] (mg/kg)	% Reduction Needed	% Reduction Achieved
Benzene[4]	2,410	62	5	99.79	97.43
Toluene	2,610	240	9,100	none	90.8
Ethylbenzene	125	52	11,000	none	58.4
Xylene	1,580	448	170,000	none	71.65
Chlorobenzene	584	249	7,400	none	57.36
1,2-Dichlorobenzene	278	113	59,000	none	59.35
1,2,4- Trichlorobenzene	3	6	820	none	none
Nitrobenzene	3,180	138	340	89.31	95.66
Aniline	2,390	38	NS[5]	none	98.41
N-Nitrosodiphenylamine[4]	971	642	390	59.84	33.88
Carbazole	172	65	96	none	62.21
Dibenzofuran	3	27	NS	none	none
Naphthalene [4]	3,570	225	17	99.52	93.7
2-Methylnaphthalene	117	66	2,400	none	43.59
Acenaphthene	33	22	37,000	none	33.33
Fluorene	37	12	24,000	none	67.57
Benzo(a)anthracene [4]	93	21	2	97.85	77.42

[1]Co= initial compound concentration.

[2]Cf=final compound concentration at completion of 49 day compost period.
(http://www.nj.gov/dep/srp/guidance/scc/).

3NRTS denotes Non-Residential Treatment Standards for New Jersey as of 2013.

[4]Compounds did not meet standards after composting.

[5]Aniline and Dibenzofuran do not have published soil treatment standards (NS).

CONCLUSIONS

1. Mass reductions of un-substituted, chloro-substituted, methyl-substituted and nitro-substituted-aromatic hydrocarbons, comprising multicomponent mixtures in dye manufacturing waste sludge, can be achieved through composting.

2. Regulatory driven treatment objectives for key risk relevant compounds including benzene, *N*-nitrosodiphenylamine, nitrobenzene, naphthalene, and benzo(a)anthracene were not met, however, in 49 days despite the high mass removal of total contaminants. This exemplifies the difficulty of targeting biotransformation of select compounds in a multisubstrate mixture of contaminants.

3. First-order rate coefficients and half-life values derived from composting together with the initial mass percent of each constituent compound characterize the degradation capabilities of the adapted indigenous microbial populations. The relationship between these values for nitrobenzene, aniline, chlorobenzene, and toluene are consistent with research describing multisubstrate interactions and the use of aniline and nitrobenzene as a nitrogen source.

4. The results of laboratory research can be observed in large-scale pilot studies for remediation of complex sludge. More research is needed to confirm these interactions and, from an application perspective, determine if interactions can be modified to manipulate the degradation of specific compounds.

5. Mesophilic composting temperatures were achieved but not sustained for the duration of the composting period. Modified operation to improve this condition may enhance treatment of risk relevant compounds assuming the higher temperatures influence compound bioavailability. Additional testing is recommended, however, since the common method for increasing temperature is to amend the compost with an easily degradable carbon source and this may change the microbial community dynamics and degradation potential of key compounds.

6. Other methods of improving the bioavailability and biotransformation of recalcitrant compounds should be considered.

Appendix 1-A

Analytical methods for key parameters

Parameter	Analytical Method	Sample Volume	Holding Time	Preservation
Organic Nitrogen	Kjeldahl Method (EPA Method 1688)	500 mL	28 days	pH<2 H_2SO_4
Inorganic Nitrate-N	Method 84-2 Method of Soil Analysis, American Society of Agronomy, 1965	100 mL	48 hours	Cool 4°C
Inorganic Nitrite-N				
Inorganic Ammonium-N				
Inorganic Phosphate	Olsen test (1954)	50 mL	28 days	pH<2 H_2SO_4
% Moisture	Karl Fischer ASTM Method D4928	50 mL	28 days	Cool 4°C
Moisture Holding Capacity	Gravimetric	50 mL	24 hrs	None- field analysis
pH	SW-846 method 9045D	100 mL	0 days	None- field analysis
Total Organic Carbon	EPA Lloyd Kahn Method 1988	50 mL	28 days	pH<2 H_2SO_4
Volatile Organic Compounds	SW-846 method 5035/8260B	5 g	14 days	Cool 4°C /4 drops HCl
Semi Volatile Organic Compounds	SW-846 Method 3550B/8270	1000 mL	7 days for extraction/40 days after extraction	Cool 4°C /4 drops HCl
Microbial Count	Plate count, Method 99-3, Method of Soil Analysis, American Society for Agronomy, 1965	10 g	24 hr	Cool 4°C

Appendix 1-B

Compound concentration at weekly intervals during the 49 day composting period

Compound	Sample Time (days)								Avge (n=2)	SD (n=2)
	t=0	t= 7	t= 14	t=21	t=28	t=35	t=42	t=49		
Material 1										
Benzene	2,160	1,970	877	69	438	20	7	5	6	1
Toluene	468	661	501	131	564	50	18	10	14	6
Ethylbenzene	418	537	266	136	155	82	34	26	30	6
Xylenes	2,330	298	1,600	911	1,030	550	216	178	197	27
Material 2										
Benzene	2,410	1,090	980	428	182	213	70	62	66	6
Toluene	2,610	1,180	1,290	754	368	504	238	240	239	1
Ethylbenzene	125	81	96	109	58	75	49	52	51	2
Xylenes	1,580	882	1,060	932	578	705	450	448	449	1
Material 3										
Benzene	1,071	619	1,240	1,570	3,560	1,360	594	351	473	172
Toluene	913	902	1,260	1,690	2,680	1,300	779	618	699	114
Ethylbenzene	103	94	92	173	179	262	149	165	157	11
Xylenes	1,030	1,020	1,100	1,600	1,630	1,910	1,160	1,190	1,175	21

Compound										
Material 1										
Chlorobenzene	2,440	3,270	1,610	865	1,810	551	259	222	241	26
1,2, Dichlorobenzene	25	16	81	72	28	20	33	26	30	5
1,2,4-Trichlorobenzene	406	282	320	461	241	186	293	258	276	25
Material 2										
Chlorobenzene	584	493	467	512	268	375	220	249	235	21
1,2 Dichlorobenzene	278	181	171	158	113	121	144	113	129	22
1,2,4-Trichlorobenzene	3	17	43	25	26	23	6	6	6	0
Material 3										
Chlorobenzene	489	282	151	480	2,400	1,480	842	825	834	12
1,2, Dichlorobenzene	226	146	30	295	80	72	94	47	71	33
1,2,4-Trichlorobenzene	51	75	26	36	66	107	159	111	135	34

	Sample Time (days)									
Compound	t=0	t= 7	t= 14	t=21	t=28	t=35	t=42	t=49	Avge n=2	SD n=2
Material 1										
Nitrobenzene	1,100	762	1,000	1,980	952	652	992	754	873	168
Aniline	741	498	527	1,500	329	103	304	111	207	136
N-Nitrosodiphenylamine	2,300	1,880	1,250	3,020	2,230	1,680	2,090	2,240	2,165	106
Material 2										
Nitrobenzene	3,180	2,200	2,360	1,380	226	305	244	138	191	75
Aniline	2,390	1,200	181	511	227	107	242	38	140	144
N-Nitrosodiphenylamine	971	1,030	1,030	900	632	932	994	642	818	248
Material 3										
Nitrobenzene	1,300	1,300	267	1,560	1,960	2,000	1,390	907	1,148	341
Aniline	1,430	584	213	608	2,820	2,730	1,340	121	730	862
N-Nitrosodiphenylamine	1,500	851	270	838	1,560	1,390	1,590	1,200	1,395	275

	Sample Time (days)									
Compound	t=0	t=7	t= 14	t=21	t=28	t=35	t=42	t=49	Avge n=2	SD n=2
Material 1										
Carbazole	30	20	68	97	41	22	46	32	39	10
Dibenzofuran	30	19	31	43	22	18	33	25	29	6
Material 2										
Carbazole	172	154	122	102	106	73	125	65	95	42
Dibenzofuran	3	35	40	31	30	30	44	27	36	12
Material 3										
Carbazole	148	91	17	113	132	79	160	124	142	26
Dibenzofuran	41	26	37	31	35	57	29	45	37	11

	Sample Time (days)									
Compound	t=0	t= 7	t= 14	t=21	t=28	t=35	t=42	t=49	t=49	Avge n=2	SD n=2
Material 1											
Naphthalene	3,360	1,218	2,300	1,910	4,540	1,030	183	234	202	218	23
2-Methylnaphthalene	111	572	72	222	191	53	33	59	50	55	6
Acenaphthene	39	236	25	30	41	24	21	36	27	32	6
Flurorene	104	80	11	16	22	12	11	24	13	19	8
Benzo(a)anthracene	49	50	34	46	78	36	31	54	26	40	20
Material 2											
Naphthalene	3,570	13,032	2,500	2,400	1,480	597	645	492	225	359	189
2-Methylnaphthalene	117	4,549	133	160	106	70	84	102	66	84	26
Acenaphthene	33	760	22	32	23	24	24	35	22	29	9
Flurorene	37	430	22	20	14	12	16	21	12	17	6
Benzo(a)anthracene	93	149	61	43	51	50	23	67	21	44	33
Material 3											
Naphthalene	3,650	131,480	1,660	466	2,910	3,150	3,420	2,610	1,410	2,010	849
2-methylnaphthalene	176	4,521	162	53	200	156	147	224	111	168	80
Acenaphthene	32	761	27	6	31	28	35	54	30	42	17
Flurorene	20	439	23	5	18	17	20	22	16	19	4
Benzo(a)anthracene	97	148	49	11	74	89	48	22	22	22	0

Appendix 1-C:

Graphic derivation of first order rate constant
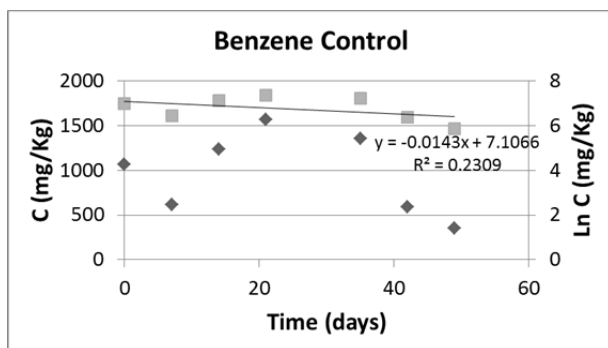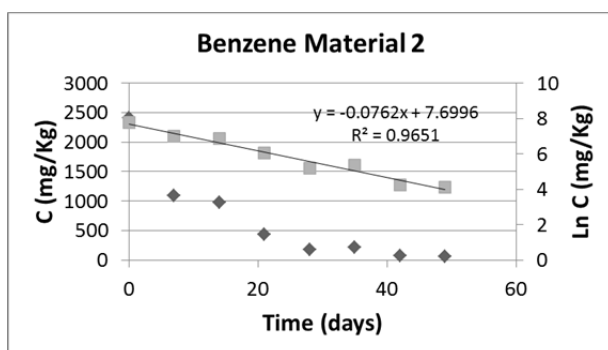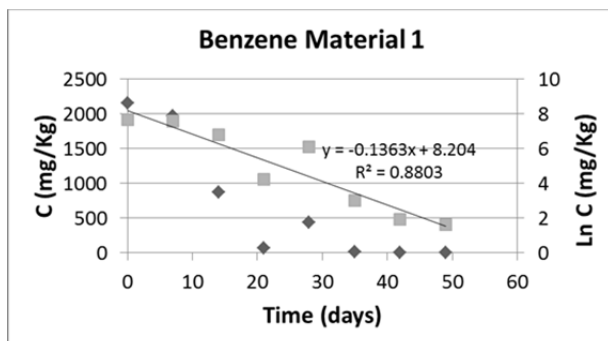
Rate constant derivation for the aromatic hydrocarbon benzene. Change in benzene concentration over time ($\blacklozenge$). A first order regression ($\blacksquare$) of concentration data results in a degradation rate constant (k d^{-1}) of -0.1363 in Material 1 with a regression coefficient (R^2) of 0.8803. A lower rate constant of -0.0762 is estimated for benzene in Material 2 with a regression coefficient of 0.9651. Benzene concentration in Control Material fluctuated and no clear degradation trend could be determined.
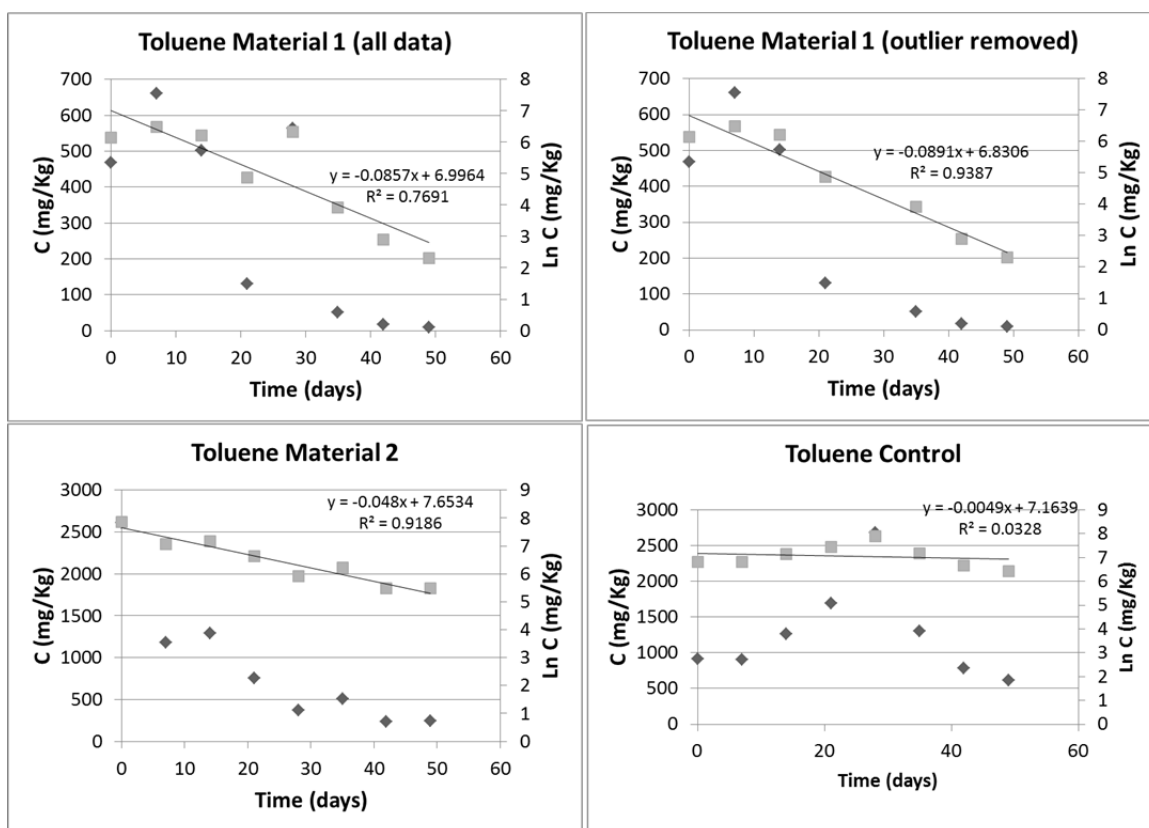
Rate constant derivation for the aromatic hydrocarbon toluene. Change in toluene concentration over time ($\blacklozenge$). A first order regression ($\blacksquare$) of concentration data results in a degradation rate constant ($k\ d^{-1}$) of -0.857 in Material 1 with a regression coefficient (R^2) of 0.7691. A second regression of concentration data in Material 1 is calculated with data from day 28 removed as a possible outlier. This improved the correlation coefficient to 0.9387 and the degradation rate constant to -0.0891. A lower rate constant of -0.048 is estimated for toluene in Material 2 with a regression coefficient of 0.9186. Toluene concentration in Control Material fluctuated and no degradation trend could be determined.

Ethylbenzene Material 1

$y = -0.0623x + 6.4082$
$R^2 = 0.9377$

Ethylbenzene Material 2

$y = -0.017x + 4.7551$
$R^2 = 0.7072$

Ethylbenzene Control

$y = 0.0149x + 4.5992$
$R^2 = 0.4807$
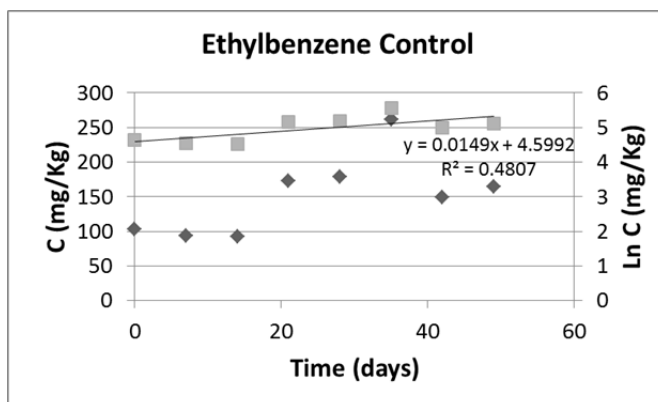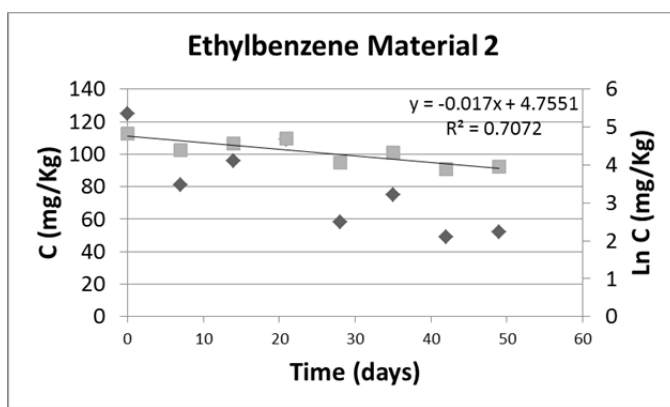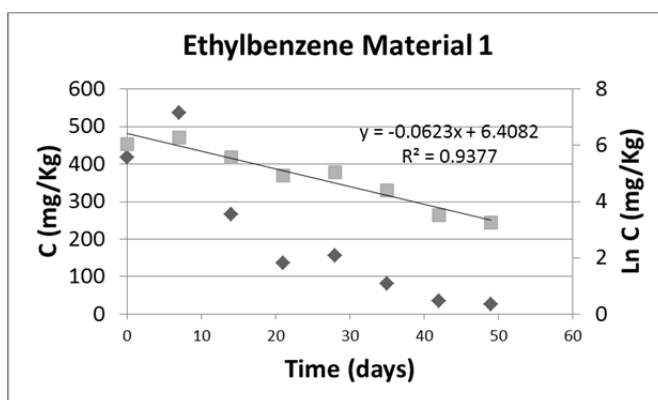
Rate constant derivation for the aromatic hydrocarbon ethylbenzene. Change in ethylbenzene concentration over time (♦). A first order regression (▪) of concentration data results in a degradation rate constant (k d^{-1}) of -0.623 in Material 1 with a regression coefficient (R^2) of 0.9377. A lower rate constant of -0.017 is estimated for ethylbenzene in Material 2 with a regression coefficient of 0.7072. Ethylbenzene concentration in Control Material fluctuated and no degradation trend could be determined.
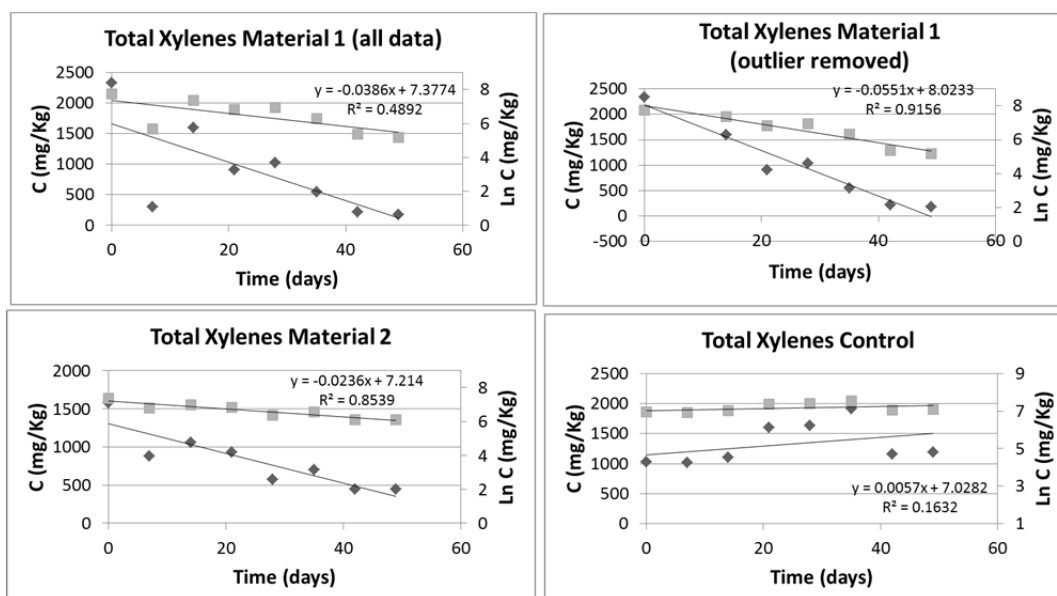
Rate constant derivation for the aromatic hydrocarbon xylene. Rate constant derivation for the aromatic hydrocarbon xylene. Change in total xylene concentration over time (♦). A first order regression (▪) of concentration data results in a degradation rate constant (k d^{-1}) of -0.0386 in Material 1 with a regression coefficient (R^2) of only 0.4892. A second regression of concentration data in Material 1 is calculated with data from day 7 removed as a possible outlier. This improved the correlation coefficient to 0.9156 and the degradation rate constant to -0.0551. A lower rate constant of -0.0236 is estimated for total xylenes in Material 2 with a regression coefficient of 0.8539. Total xylenes concentration in Control Material fluctuated and no degradation trend could be determined.

Rate constant derivation for the halogenated aromatic hydrocarbon chlorobenzene. Change in chlorobenzene concentration over time (♦). A first order regression (▪) of concentration data results in a degradation rate constant (k d[-1]) of -0.0543 in Material 1 with a regression coefficient R[2]) of 0.8409. A lower rate constant of -0.0192 is estimated for chlorobenzene in Material 2 with a regression coefficient of 0.7758. Chlorobenzene concentration in Control Material fluctuated and no degradation trend was determined.
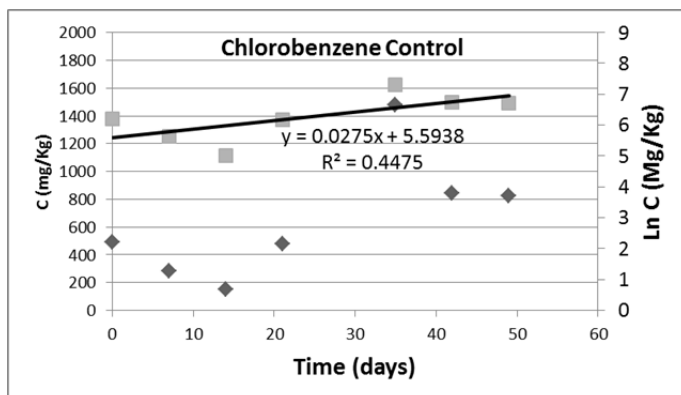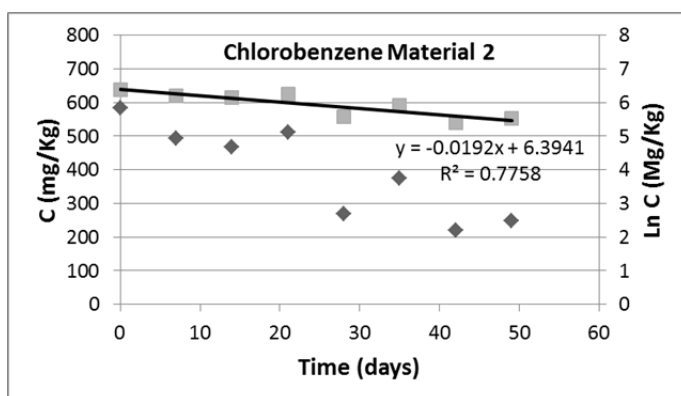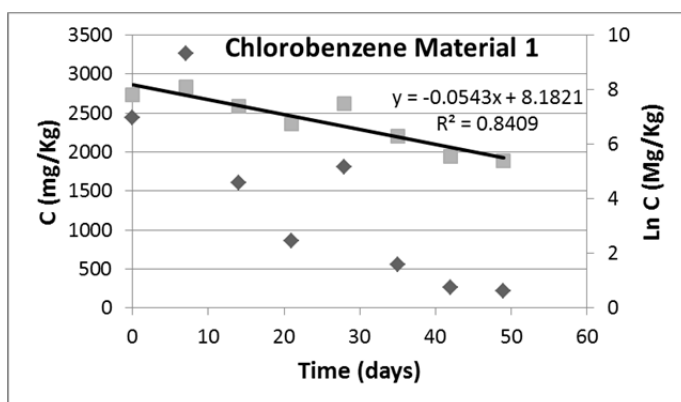
Rate constant derivation for the halogenated aromatic hydrocarbon 1,2,-dichlorobenzene. Change in 1,2-dichlorobenzene concentration over time (♦). A first order regression (▪) of concentration data results in a degradation rate constant (k d[-1]) of -0.0021 in Material 1 with a regression coefficient (R[2]) of 0.0039 (no discernable degradation). A rate constant of -0.015 is estimated for 1,2-dichlorobenzene in Material 2 with a regression coefficient of 0.7224. The concentration of 1,2-dichlorobenzene concentration in Control Material fluctuated and no degradation trend was determined (R[2]<0.700)

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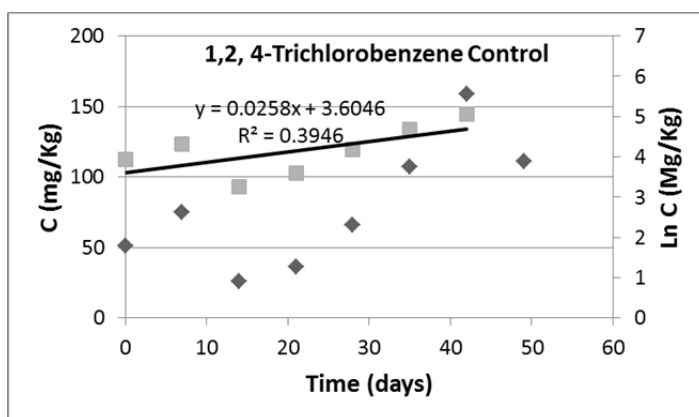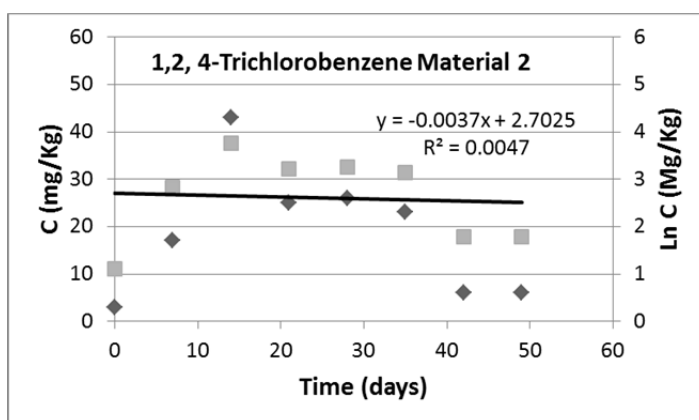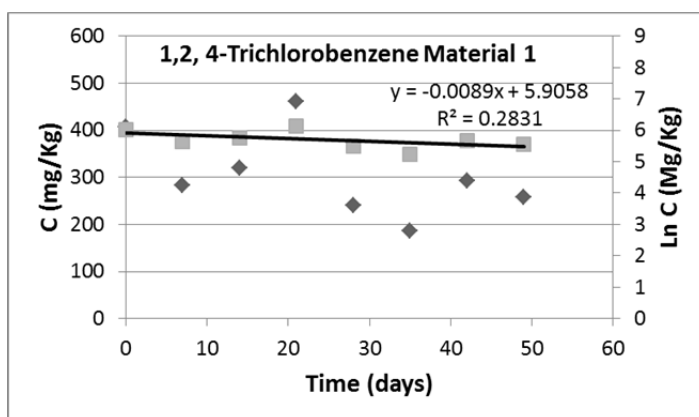
Rate constant derivation for the halogenated aromatic hydrocarbon 1,2,4-trichlorobenzene. Change in 1,2,4-trichlorobenzene concentration over time (♦). A first order regression (▪) of concentration data shows no discernable degradation in Material 1, Material 2 or the control (regression coefficients ($R^2<0.700$).
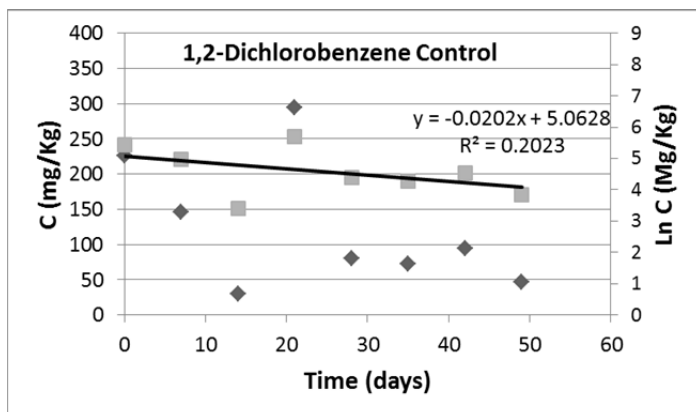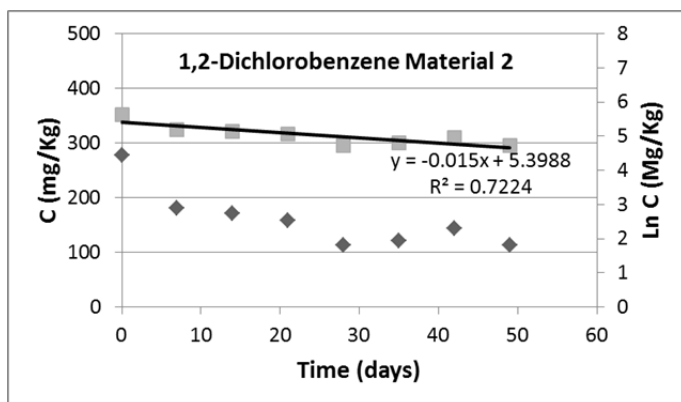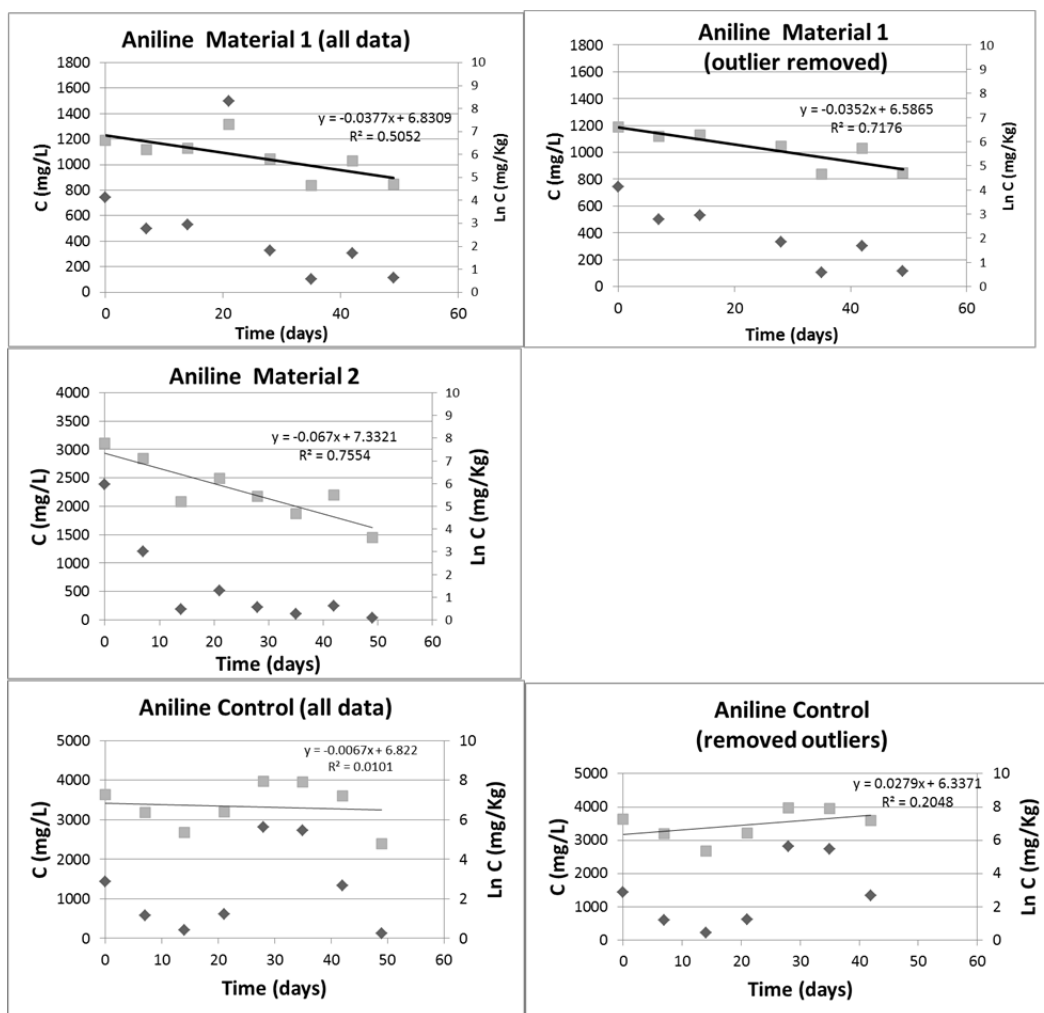
Rate constant derivation for the nitro- aromatic hydrocarbon aniline. Change in aniline concentration over time (♦). A first order regression (▪) of all concentration data in Material 1 results in regression coefficient (R^2) of 0.5052 suggesting limited confidence in the degradation tend. Confidence is improved (R^2=0.7176) by removal of the inconsistently low value obtained on day 14 resulting in a rate coefficient (k) of -0.0352 d^{-1} . A slightly higher rate constant (0.067 d^{-1}) is estimated for aniline in Material 2 (R^2 = 0.7554). Regression coefficients for aniline in Control Material (0.0101 using all data and 0.2048 with the inconsistently low value on day 14 removed) suggests lack of aniline degradation in Control Material.
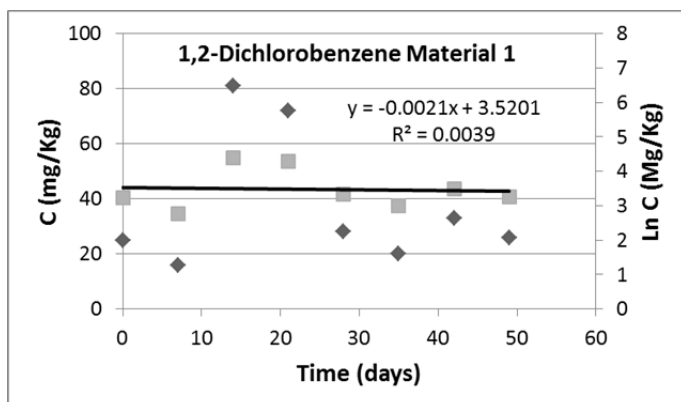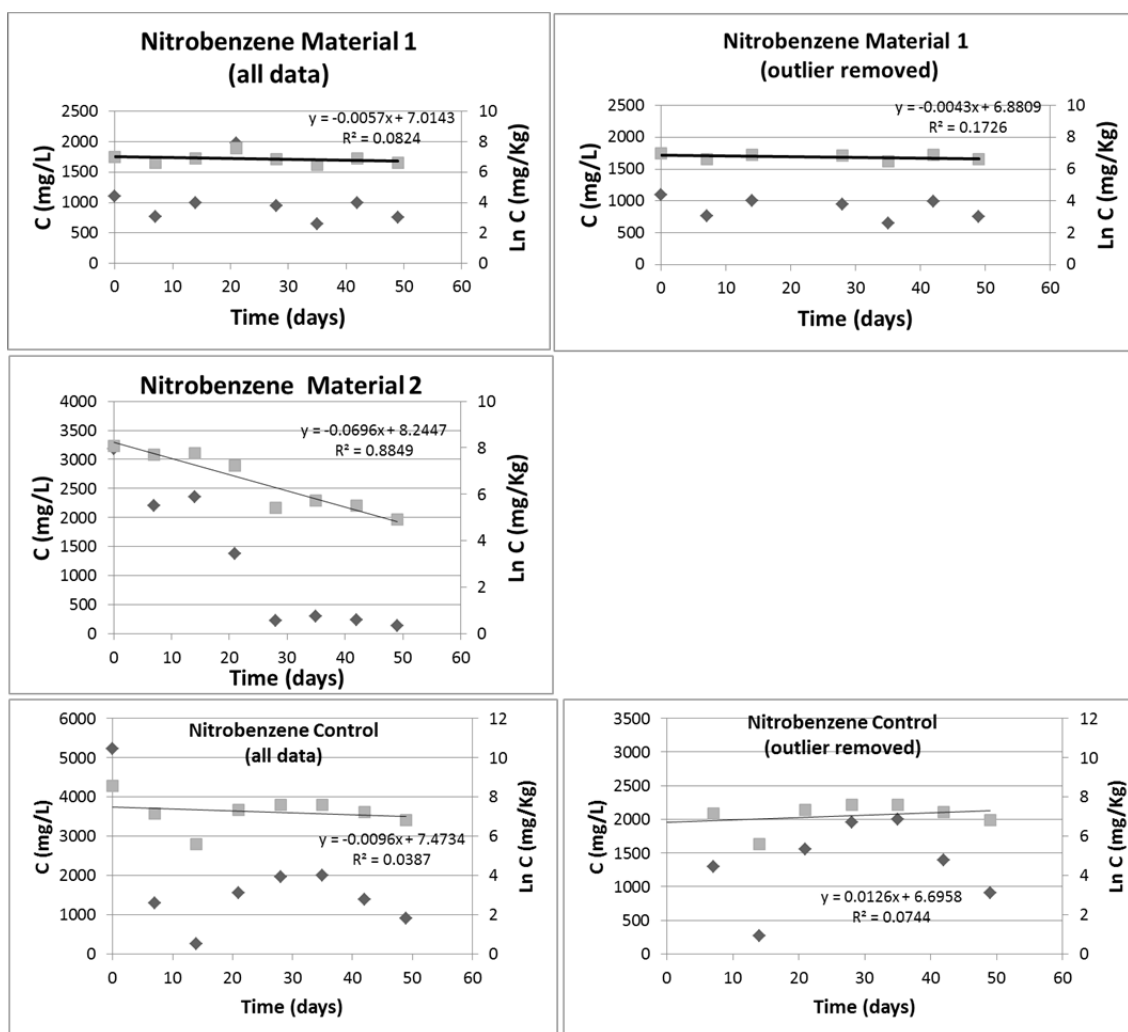
Rate constant derivation for the nitro- aromatic hydrocarbon nitrobenzene. Change in nitrobenzene concentration over time (♦). A first order regression (▪) of concentration data in Material 1 results in regression coefficients (R^2) of 0.0824 or 0.1726 with the inconsistently high value on day 21 excluded from the analysis. These values are similar to the control data (R^2 = 0.0387 or 0.0744) with the day 0 starting concentration excluded from analysis). R^2 values < 0.70 indicate a lack of discernable degradation. Nitrobenzene degradation is supported in Material 2 with a first order coefficient of -0.0696 and an R^2 value of 0.8849. No potential outliers were identified.
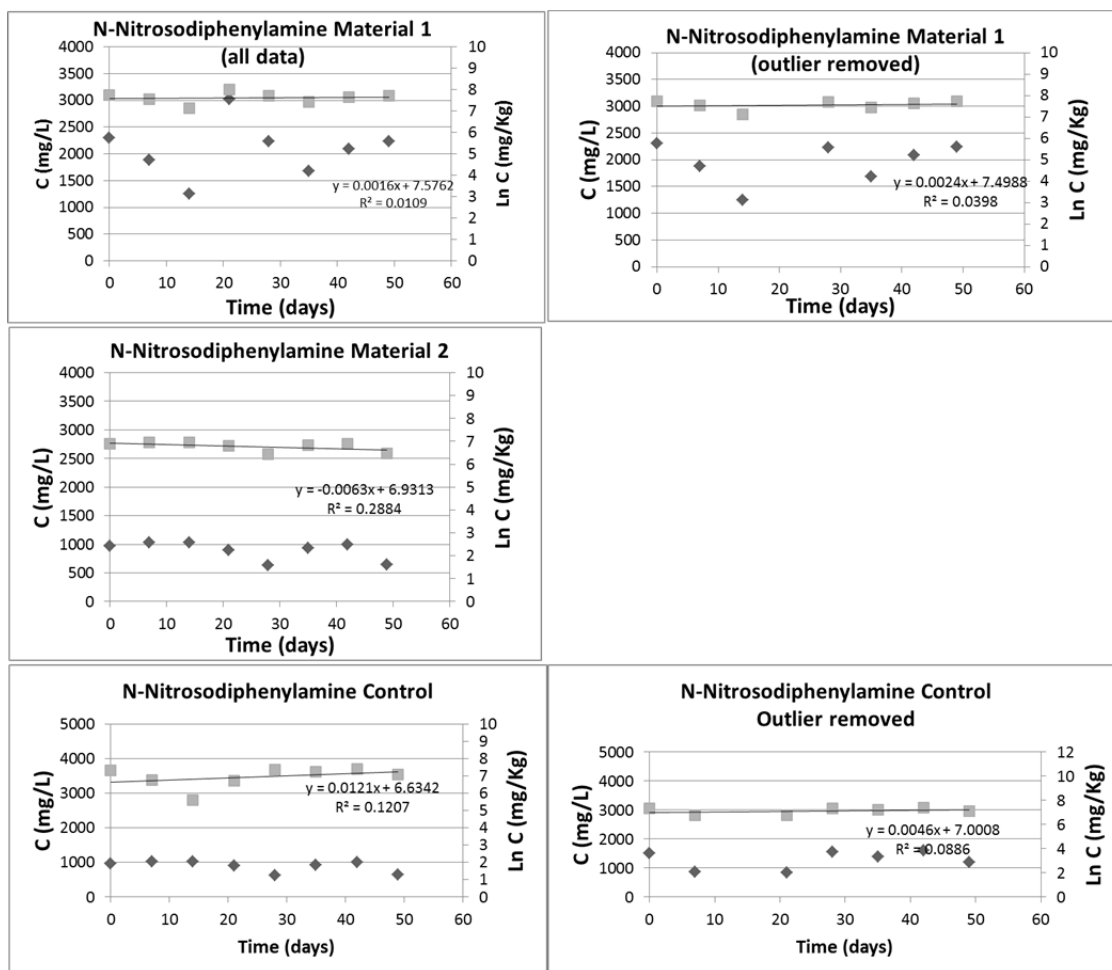
Rate constant derivation for the nitro- aromatic hydrocarbon *N*-nitrosodiphenylamine. Change in *N*-nitrosodiphenylamine concentration over time (♦). A first order regression (▪) of concentration data n Material 1 results in regression coefficients (R^2) of 0.0109 using all data or 0.398 with the inconsistently low value on day 14 excluded from the analysis. These values are similar to the results for Material 2 (R^2=0.2884) where no outliers were identified and control data (R^2 = 0.1207 using all data and 0.0886 with day 14 data excluded). R^2 values < 0.70 indicate a lack of discernable degradation.
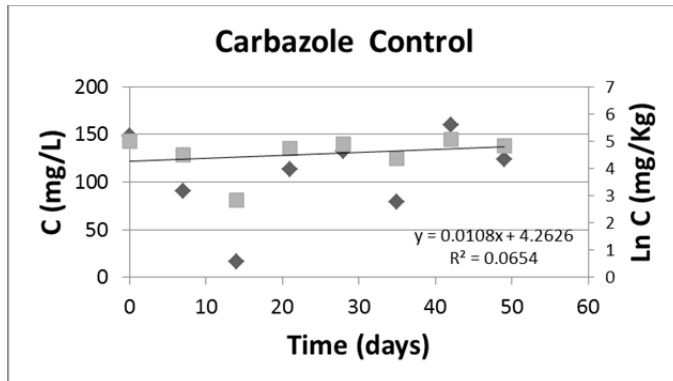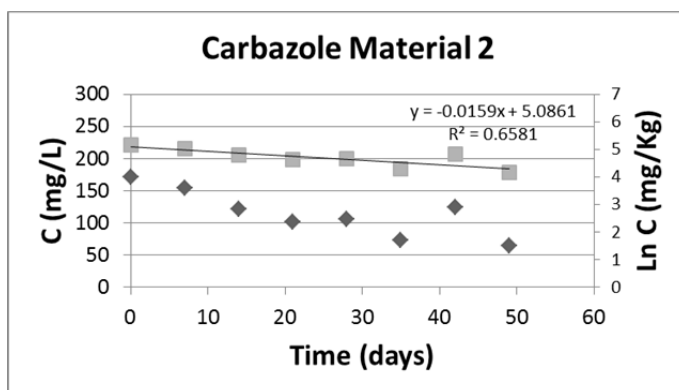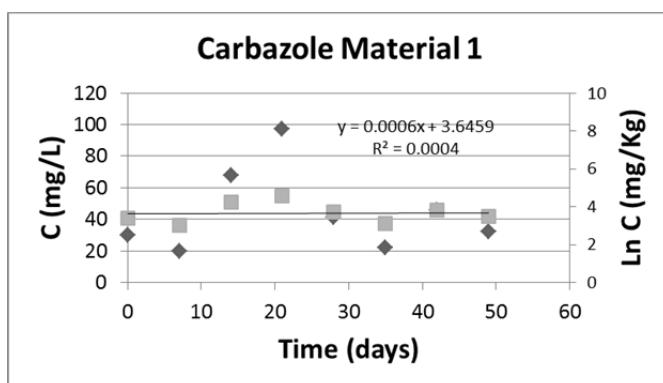
Rate constant derivation for the heterocyclic hydrocarbon carbazole. Change in carbazole concentration over time (♦). Degradation does not appear to occur in the Control or Test Material 1 but may occur in Test Material 2. A first order regression (▪) of concentration data from Material 1, 2 and the Control results in regression coefficients (R^2) of 0.0004, 0.6581 and 0.0654 respectively.

Dibenzofuran Material 1

$y = -0.0014x + 3.3137$
$R^2 = 0.0064$

Dibenzofuran Material 2

$y = -0.0032x + 3.5973$
$R^2 = 0.0746$

Dibenzofuran Control

$y = 0.0044x + 3.4896$
$R^2 = 0.09$
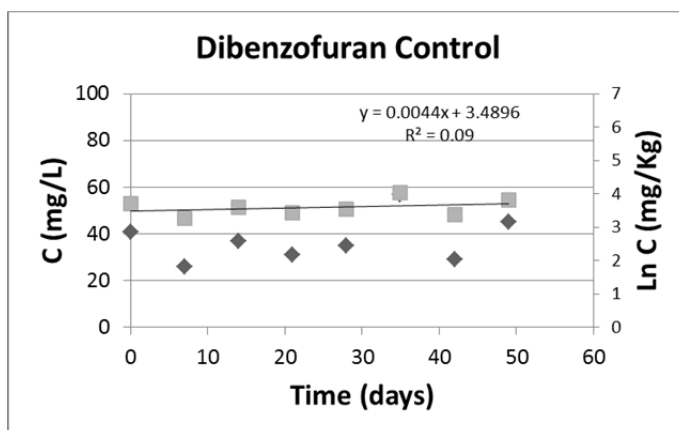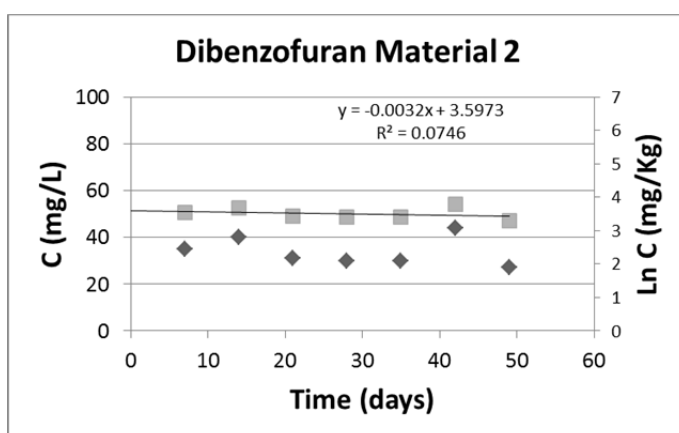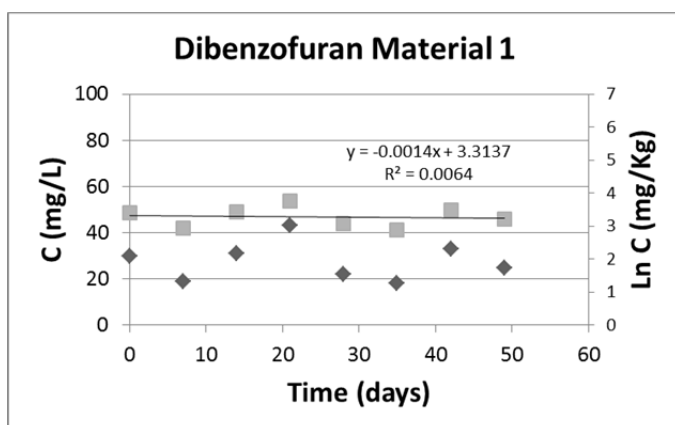
Rate constant derivation for the heterocyclic hydrocarbon dibenzofuran. Change in dibenzofuran concentration over time (♦). Degradation does not appear to occur in any of the test materials. A first order regression (▪) of concentration data from Material 1, 2 and the Control results in regression coefficients (R^2) of 0.0064, 0.0746 and 0.09 respectively.

Naphthalene Material 1 (all data)

y = -0.0674x + 8.4944
$R^2 = 0.7544$

Naphthalene Material 1 (outlier removed)

y = -0.0648x + 8.2371
$R^2 = 0.8981$

Naphthalene Material 2 (all data)

y = -0.055x + 8.2924
$R^2 = 0.9491$

Naphthalene Control (all data)

y = 0.0028x + 7.5617
$R^2 = 0.0049$
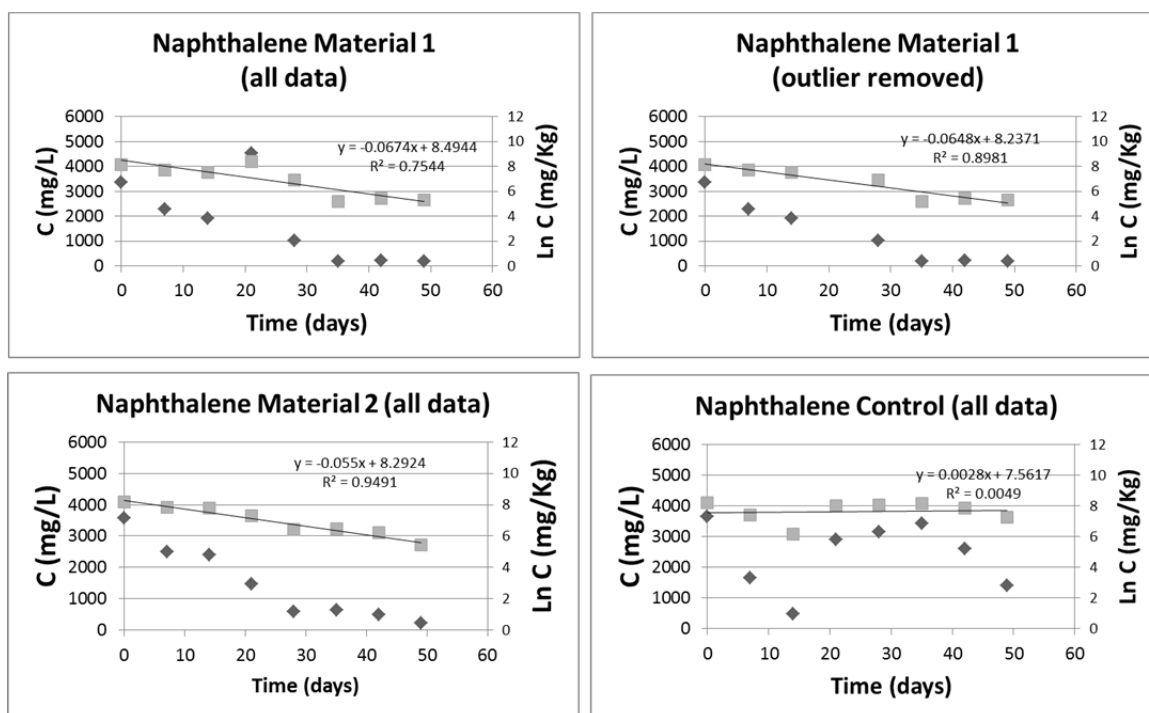
Rate constant derivation for the polycyclic aromatic hydrocarbon naphthalene. Change in naphthalene concentration over time (♦). A first order regression (▪) of concentration data in Material 1 results in a degradation rate constant (k d^{-1}) of -0.0674 (R^2=0.7544) or -0.0648 (R^2=0.8981) if the inconsistently high value on day 21 is removed from the analysis. The estimated k value in Material 2 is -0.055 with an R^2 of 0.9491. No degradation trend was observed in the control Material (R^2=0.0049).
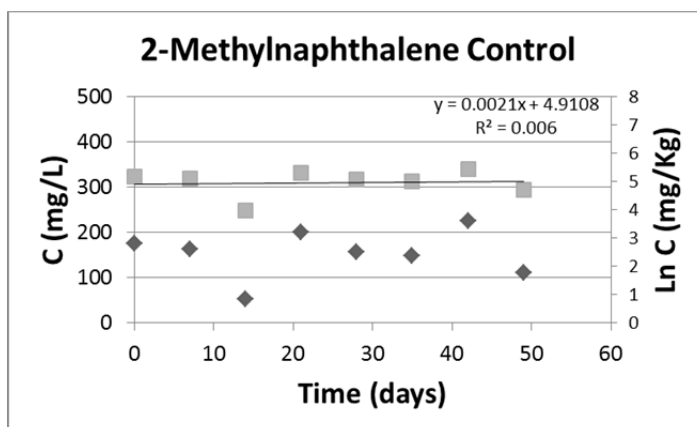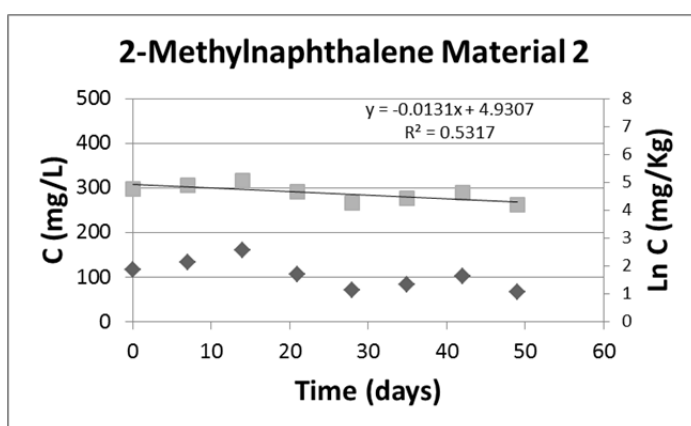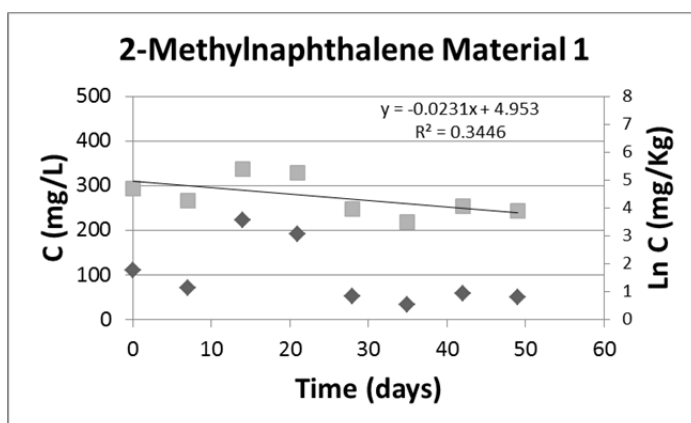
Rate constant derivation for the polycyclic aromatic hydrocarbon 2-methylnaphthalene. Change in 2-methylnaphthalene concentration over time (♦). No degradation of 2-methylnaphthalene was observed in Material 1, 2 or the control. A first order regression (▪) of concentration data in each test material resulted in regression coefficients (R^2) of 0.3446 and 0.5317 and 0.0049.

Fluorene Material 1

$y = -0.0211x + 3.4575$
$R^2 = 0.2313$

Fluorene Material 2

$y = -0.0152x + 3.2629$
$R^2 = 0.4755$

Fluorene Control

$y = 0.0039x + 2.694$
$R^2 = 0.0188$
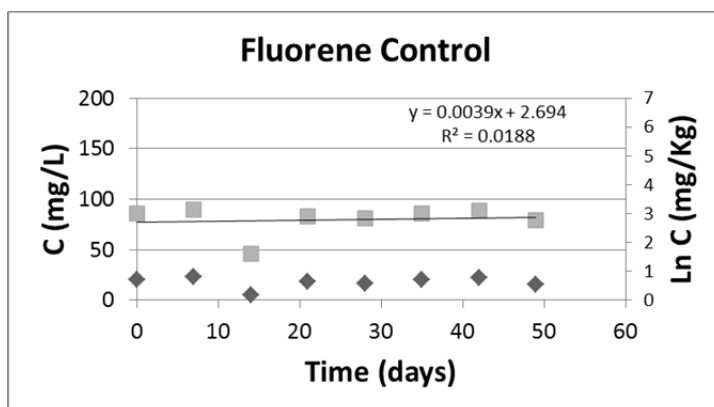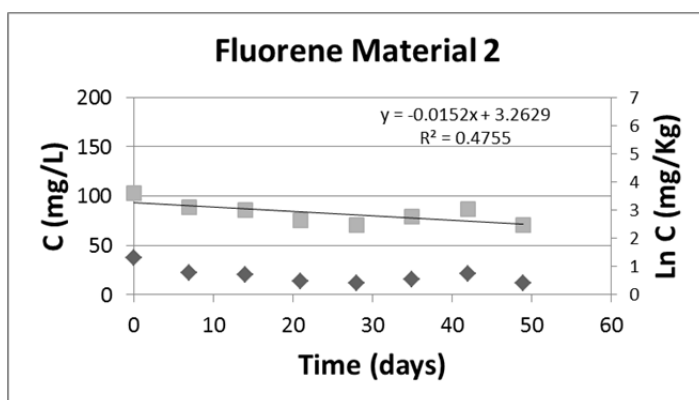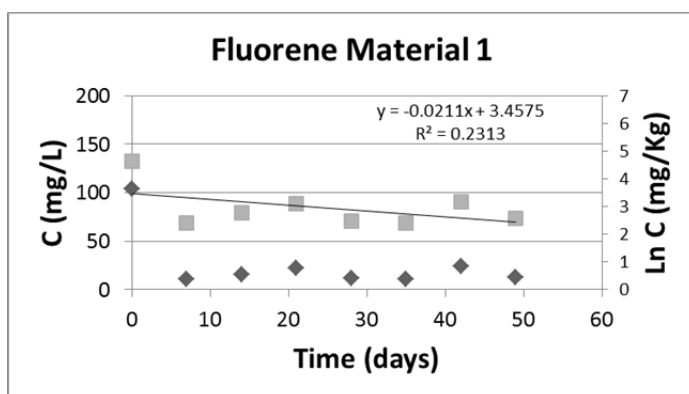
Rate constant derivation for the polycyclic aromatic hydrocarbon fluorene. Change in fluorene concentration over time (♦).   No degradation was observed in Material 1, 2 or the Control. A first order regression (▪) of concentration at weekly time intervals in each test material resulted in regression coefficients (R^2) of 0.2313, 0.4755 and 0.188.
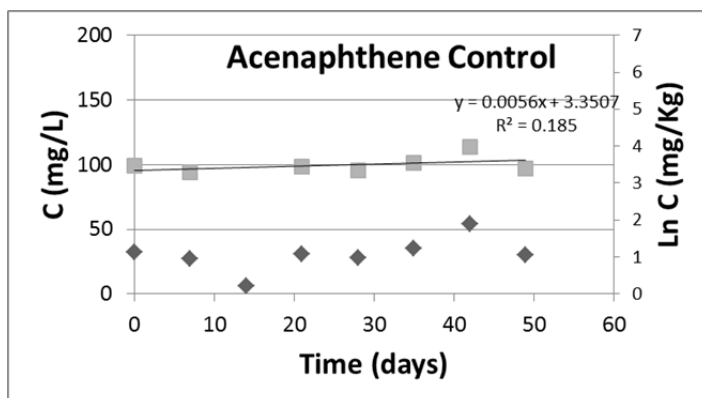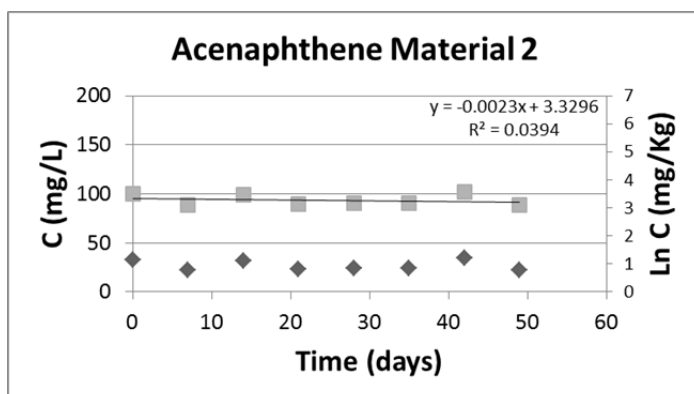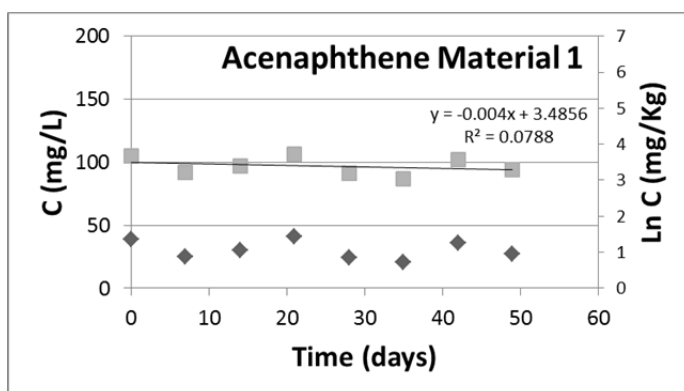
Rate constant derivation for the polycyclic aromatic hydrocarbon acenaphthene. Change in acenaphthene concentration over time (♦). No degradation was observed in Material 1, 2 or the Control. A first order regression (▪) of concentration at weekly time intervals in each test material resulted in regression coefficients (R^2) of 0.0788, 0.0394 and 0.0185.

Benzo(a)anthracene Material 1

$y = -0.0069x + 3.9035$
$R^2 = 0.114$

Benzo(a)anthracene Material 2

$y = -0.0201x + 4.3227$
$R^2 = 0.4553$

Benzo(a)anthracene Control

$y = -0.0166x + 4.1215$
$R^2 = 0.134$
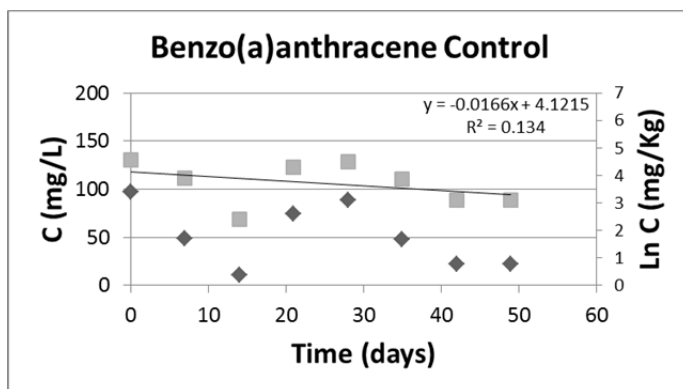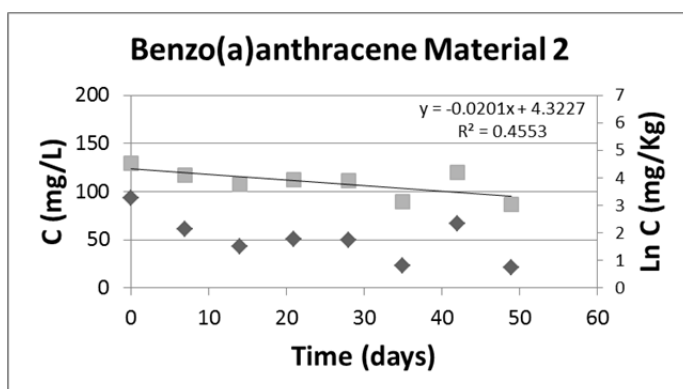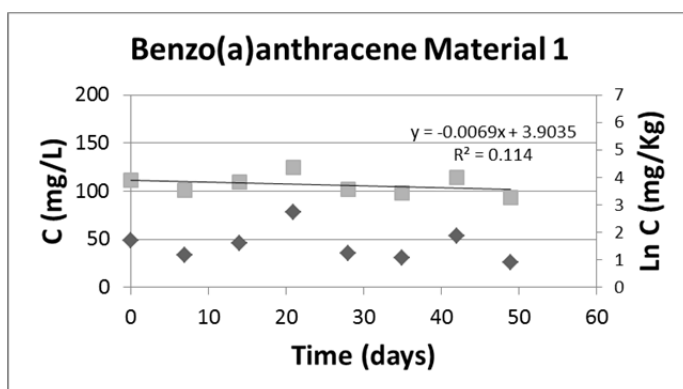
Rate constant derivation for the polycyclic aromatic hydrocarbon benzo(a)anthracene. Change in benzo(a)anthracene concentration over time (♦). No degradation was observed in Material 1, 2 or the Control. A first order regression (▪) of concentration at weekly time intervals in each test material resulted in regression coefficients (R2) of 0.0114, 0.4553, and 0.134.

Appendix 1-D:

Nutrient content in composting material during 49 day treatment period

Material 1	Time (days)							
Parameter	t=0	t= 7	t= 14	t=21	t=28	t=35	t=42	t=49
Nitrogen, Total Kjeldahl (mg/kg)	12,700	11,500	12,200	19,300	20,600	21,200	19,500	24,500
Nitrogen, Ammonia (mg/kg)	272	466	514	461	420	426	409	468
Nitrogen, Nitrate (mg/kg)	<1,800	<680	<600	<29	<30	<32	<29	<31
Phosphorous, total (mg/kg)	392	414	457	299	1,750	387	495	364
Total Organic Carbon (mg/kg)	242,000	245,000	461,000	190,000	286,000	237,000	220,000	238,000
pH	9	9	9	8	7	7	8	8
Nitrogen, Nitrite (mg/Kg)	<0.2	<1	<0.2	<0.49	<0.55	0	0	0
Moisture, Percent (wt/wt)	44	41	33	31	36	34	30	35
Total Nitrogen	21,272	11,966	12,714	19,761	21,020	21,626	19,909	24,968
C:N ratio	19	21	38	10	14	11	11	10

Material 2	Time (days							
Parameter	t=o	t= 7	t= 14	t=21	t=28	t=35	t=42	t=49
Nitrogen, Total Kjeldahl (mg/kg)	2,460	3,630	5,870	12,100	11,600	5,970	7,210	8,360
Nitrogen, Ammonia (mg/kg)	244	596	643	612	961	1,010	995	969
Nitrogen, Nitrate (mg/kg)	<140	<260	<130	<28	<27	<29	<25	<25
Phosphorous, total (mg/kg)	1,650	552	654	498	633	505	671	535
Total Organic Carbon (mg/kg)	265,000	206,000	245,000	197,000	251,000	273,000	210,000	309,000
pH	7.4	7.7	7.3	7.4	7.4	7.4	7.6	7.6
Nitrogen, Nitrite(mg/Kg)	<0.2	1.1	<0.2	<.51	<0.8	<0.2	0.29	0.22
Moisture, Percent (wt/wt)	26	22	23	25	24	28	20	21
Total Nitrogen	1,444	4,226	6,513	12,712	12,561	6,980	8,205	9,329
C:N ratio	184	49	38	15	20	39	26	33

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Chapter 2

Fugacity based distributions of mono- and polycyclic-aromatic hydrocarbons in dye

manufacturing waste sludge compost

ABSTRACT

Hazardous waste composting is a demonstrated technology for many types of organic

contaminants. Unfortunately, successful composting can be unpredictable for hydrophobic

compounds due to phase partitioning and mass transfer limitations that separate these compounds

from the microbial community. This physical separation reduces compound bioavailability and

can result in recalcitrant concentrations of risk relevant compounds above regulatory limits.

Empirical treatability studies seldom distinguish between residuals that occur due to

bioavailability limitations and those that are due to biodegradability limitations. The purpose of

this research is to determine if a fugacity Level 1 phase distribution model can be used as a

screening tool to assess the cause of degradation limits. Fugacity is the "escaping tendency" of

compounds out of a phase and is the same for all phases at equilibrium. Concentration (C) is

related to fugacity (f) by a capacity coefficient (Z) such that $C = Z f$, and is determined by

partition coefficients of the compound in each phase. Compound mass and degradation half-life

data for 17 dye manufacturing chemicals in two test materials from a previous composingt study

(Chapter 1) were used in the fugacity analysis. Concentrations and relative phase distributions

were calculated for each compound using the expression $f = M \Sigma (Vi\ Zi)$ where M is the total

molar mass of compounds in the composting material, Vi is the phase volume and Zi is the

corresponding fugacity capacity. The composting material contained 30% (w/w) organic carbon

in 15 m^3 of compost and a non-aqueous pahse liquid volume of 0.3 m^3 or 0.22 m^3 in two

materials. Non aqueous phase liquid (NAPL) was defined as the total mass of the 17 risk relevant

compounds in the compost. The NAPL volume decreased as compounds were degraded. At

equilibrium the distribution of hydrophobic organic compounds was mainly to compost organic

carbon and NAPL. The presence of a NAPL phase did not significantly affect the distribution of

chemicals to the pore water of the compost (df = 35, $\rho < 0.05$). Compound concentration in the

pore water is directly correlated to solubility (R^2 = 0.8 to 0.9 excluding aniline) but is not

correlated to biodegradation half-life (R^2 = 0.2 to 0.3). Compound concentration in the pore

water therefore may indicate bioavailability but it does not necessarily indicate rate of

biotransformation. A comparison of predicted changes in pore water concentrations before and

after treatment provides better evidence of biodegradation. The dual assessment of

bioavailability (based on pore water concentration) and biodegradability (based on differences in

pore water concentration before and after treatment) provides a basis for improving treatment of

residual compounds. Methods that improve bioavailability are indicated for compounds with low

pore water concentrations whereas strategies that support growth and specific metabolic activity

of the microbial populations are needed for degradation of available but undegraded compounds.

INTRODUCTION

Hazardous waste composting is a demonstrated treatment technology for soils

contaminated with gasoline, oil, coal tar, polychlorinated biphenyls and explosives (1-8).

Although this technology is based on the well-documented ability of naturally occurring soil

microbes to degrade hydrocarbons (9-11), the process is characteristically unpredictable, site

specific and often incomplete (12, 13). Recent composting studies for dye manufacturing waste

sludge (Chapter 1) showed residual concentrations of hydrophobic organic chemicals that

persisted through treatment even though a high mass of total contaminants was degraded. The

recalcitrance of hydrophobic compounds in biotreatment is not uncommon and is generally

attributed to multiphase partitioning that separates organic compounds from the degrading microbial community. Weissenfels, et al. (14) for example, demonstrated that recalcitrant polycyclic- aromatic hydrocarbons (PAHs, see list of abbreviations, page ix) were degraded after extraction and reinjection into the same soil thereby showing that biodegradation was limited by compound availability and not biodegradability. The biodegradability of compounds such as PAHs that partition to the organic or non-aqueous phase liquid (NAPL) fractions is controlled by mass transfer (15) at the NAPL- or organic carbon-water interface (12, 16). In this process the organic compound dissolves out of the NAPL and repartitions into the aqueous and soil phases where its bioavailability may be limited by sorptive reactions (17-25). Compound recalcitrance in biotreatment is expected to be high when the dissolution and desorption rates are slow. Composting is defined as a biological conversion process for organic materials and therefore has a high organic carbon content. As a result, partitioning of hydrophobic compounds is a concern and an estimate of bioavailability, especially for risk relevant compounds, is a critical factor in determining treatment feasibility.

Compound bioavailability has been measured using analytical methods based on chemical extraction limits (26, 27) and by theoretical mass transfer calculations based on sorption and diffusion (22, 28, 29). The mathematical approach has the advantage of predicting how much of a compound introduced into the environment partitions to the aqueous phase, where it is most likely to biotransform (16), and how much remains in the sediment or volatilizes to the atmosphere. Fugacity based mathematical models are commonly applied to predict the fate and transport of pollutants in the environment (17, 30, 31) and recently have been used to predict the biotreatment potential of hydrophobic compounds in biopile remediation (15). Four levels of the fugacity model are defined with model complexity increasing with each level (32). Level I assumes system equilibrium and that chemical loss due to biotic or abiotic transformation or

mineralization does not occur. Level II assumes equilibrium partitioning with steady-state input

and transformation losses while Level III incorporates non-equilibrium, steady-state conditions

and Level IV describes unsteady state distribution (Mackay, 1979).

This study uses the fugacity Level I model to identify the phase distribution and

concentration of dye manufacturing compounds in waste sludge composting at the start and at the

completion of composting when the material is in static condition and no (or minimal) losses

occur through volatilization or biodegradation. In this condition equilibrium is assumed and there

is no input or output to or from the system. Thus Level III and IV analyses are not appropriate.

Level II analysis incorporates Level 1 but is expanded to determine the dominant removal

process. This analysis is more appropriate for dynamic systems where leachate, groundwater and

volatilization from surface waters are potentially significant compound removal pathways.

Fugacity is a thermodynamic principle related to chemical potential that uses pressure

rather than energy to describe the likely movement of a compound out of a particular phase

("escaping tendency") (32). In this approach compounds move along a fugacity gradient, from

high to low, and equilibrium is achieved when the net escaping tendency between two phases is

zero (33). Fugacity (f) is related to concentration (C) by a fugacity capacity coefficient (Z) such

that $C = Zf$ (32). The fugacity capacity is a function of the partition coefficient (K) defined as

the compound concentration ratio between two phases. If $C = Zf$ then the partition coefficient

$K_{1,2}$ (between phase 1 and phase 2) is $Z_1 f / Z_2 f$ or $K_{1,2} = Z_1 / Z_2$, since at equilibrium fugacity is

constan (29). The fugacity capacity for air (Z_a) is a fixed value based on the ideal gas law and at

dilute compound concentrations $Z_a = 1/RT$. The fugacity capacity for water (Z_w) is an inverse of

the Henry's Law coefficient (H) determined as the compound liquid state vapor pressure divided

by the compound solubility (34). The fugacity capacity for soil, sediment, or sludge (Z_s) is

determined by the soil density (ρ_s), the soil-water partition coefficient (K_d) and Z_w such that $Z_s =$

$K_d * \rho_s * Z_w$ (35). The soil-water partition coefficient is used to predict soil adsorption and is determined as the fraction of organic carbon in the soil multiplied by the organic carbon partition coefficient (K_{oc}) of the compound ($K_d = f_{oc} * K_{oc}$) (35).   The soil-water partition coefficient is replaced by the octanol-water coefficient when calculating the fugacity capacity of NAPLs since the NAPL is a miscible solvent and partitioning is by absorption, not adsorption.

The equilibrium fugacity for all phases is derived by Mackay (33) as $f = M / \Sigma (V_i Z_i)$ where M is the total number of moles of a a constituent and V_i is the phase volume. The compound concentration in each phase is obtained by calculating the number of moles in each phase ($M_i = f * Z_i * V_i$) and, since molarity is mass divided by volume each phase concentration (C_i) is obtained by the expression $C_i = f * Z_i$ (35).

The objective of this study was to determine the relative phase distribution and concentration of dye waste chemicals in composting material using the Level 1 fugacity model. Results of the model analysis would augment kinetic evaluations and mass removal results from previous pilot studies.

MATERIALS AND METHODS

Model Data Biodegradation results from a compost pilot test for treatment of two dye manufacturing waste sludge materials (Chapter 1) were used in this study. Seventeen compounds ranging in mass and degradability were identified (TABLE 2.1). Degradation half-life values were reported for 8 of the 17 compounds (TABLE 2.2). Initial concentrations of the 9 other compounds were near 100 mg/kg and and compound degradation was not significant ($R^2 < 0.7$, $\rho < 0.05$). Although nitrobenzene concentrations were relatively high, degradation was only observed in one test material used in the study.

The physical-chemical properties used to calculate the fugacity of each compound are provided in TABLE 2.3.

TABLE 2.1 Initial mass and final mass of compounds after composting two materials collected from two locations at a former dye manufacturing facility

| | Mass (g) | | | |
| | Material 1 | | Material 2 | |
Compound	Initial Mass	Final Mass	Initial Mass	Final Mass
Benzene	38,880	108	28,920	792
Toluene	8,424	252	31,320	2,868
Ethylbenzene	7,524	540	1,500	612
Xylenes	41,940	3,546	18,960	5,388
Chlorobenzene	43,920	4,338	7,008	2,820
1,2, Dichlorobenzene	450	540	3,336	1,548
1,2,4-Trichlorobenzene	7,308	4,968	36	72
Nitrobenzene	19,800	15,714	38,160	2,292
Aniline	13,338	3,726	28,680	1,680
N-Nitrosodiphenylamine	41,400	38,970	11,652	9,816
Carbazole	540	702	2,064	1,140
Dibenzofuran	540	522	36	432
Naphthalene	60,480	3,924	42,840	4,308
2-Methylnaphthalene	1,998	990	1,404	1,008
Acenaphthene	702	576	396	348
Fluorene	1,872	342	444	204
Benzo(a)anthracene	882	720	1,116	528

TABLE 2.2 Half-life values for compounds in two composting materials (M1 and M2) composed of dye manufacturing waste sludge

| | Half-life[1] (d) | |
Compound	M1	M2
Benzene	5	9
Toluene	11	14
Ethylbenzene	8	41
Xylene	18	29
Chlorobenzene	13	36
Nitrobenzene	ND[2]	10
Naphthalene	10	13
Aniline	20	10

[1]Half-life (d) was graphically derived from weekly concentration data over a 49 day composting period (Chapter 1).

[2]ND indicates initial compound concentration was above 100 mg/kg but no degradation was evident

TABLE 2.3 Physical-chemical properties of compounds characterizing dye manufacturing waste sludge

	Compound								
Property	Benzene	Ethyl Benzene	Toluene	o-Xylene	Chlorobenzene	1,2-Dichlorobenzene	1,2,4-Trichlorobenzene	Nitrobenzene	Aniline
molecular weight (g/mole)	78.11	106.2	92.1	106.17	112.6	147	181.44	123	93.1
water solubility (mg/L)	1.78E+03	1.52E+02	5.15E+02	1.70E+02	4.90E+02	1.00E+02	3.00E+02	1.90E+03	3.50E+04
vapor pressure (mm Hg)	7.60E+01	7.00	2.20E+01	7.00	8.80	9.60E-01	2.90E-01	1.50E-01	6.70E-01
Henry's Law coefficient (atm-m^3/mol)	5.43E-03	7.90E-03	6.61E-03	4.94E-03	3.46E-03	1.88E-03	1.42E-03	2.40E-05	1.90E-06
Log K_{ow}	2.13	3.15	2.73	3.12	2.84	3.38	4.02	1.87	9.00E-01
Log K_{oc}	1.81	2.83	2.41	2.84	2.20	3.06	3.22	1.56	3.59

Property	N-Nitroso-diphenylamine	Carbazole	Dibenzofuran	Naphthalene	2-Methyl-naphthalene	Fluorene	Acenaphthene	Benzo(a)-anthracene
molecular weight (g/mole)	74.08	167.2	168.19	128.2	142.2	166.22	154.21	228.3
water solubility (mg/L)	3.51E+01	7.48	1.70	3.10E+01	2.54E+01	1.90	3.88	1.40E-02
vapor pressure (mm Hg)	1.00E-01	2.66E-04	8.30E-04	2.34E-01	6.80E-02	6.67E-04	2.31E-02	1.16E-09
Henry's Law constant (atm-m^3/mol)	5.00E-06	1.50E-08	1.01E-04	1.27E-03	5.06E-02	7.65E-05	1.20E-03	4.50E-06
Log K_{ow}	3.16	3.59	4.05	3.30	3.86	4.18	3.92	5.61
Log K_{oc}	3.11	3.50	4.05	3.11	3.93	3.90	3.70	6.14

Compost Model. A four-compartment model was used to describe separate but interconnected phases in the compost. Compartments are defined as pore space air, pore space water, compost solids, and NAPL (FIG 2.1).
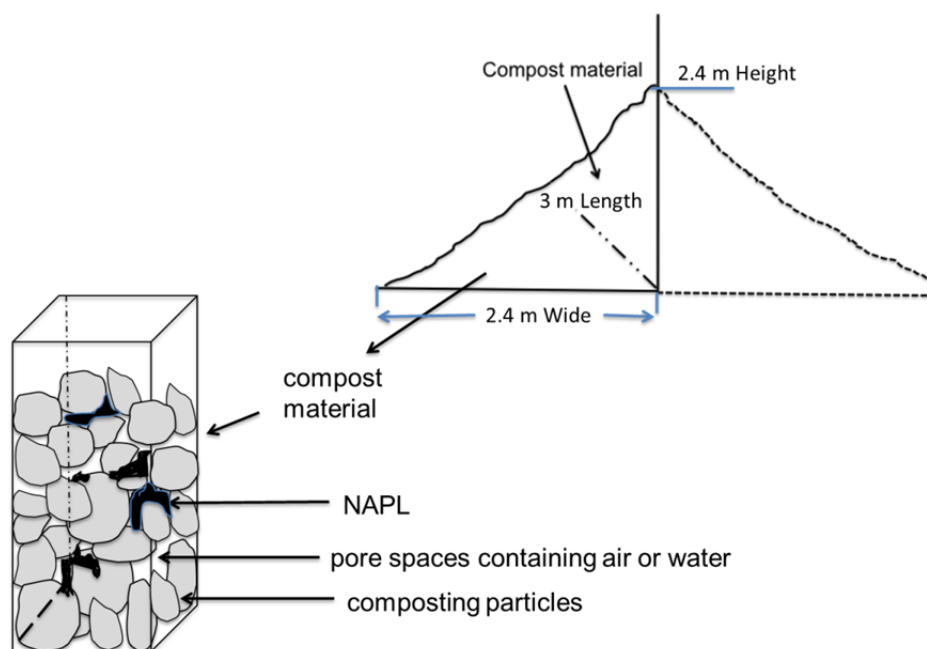
FIG 2.1 Phase compartment model for composting material

The composting matrix was a blend of woodshavings and dye manufacturing waste sludge resulting in a material with 30% organic carbon (w/w) and a multicomponent non-aqueous phase liquid interspersed among the particles. Phase compartment volume estimates were based on a total compost volume of 15 m^3 with a 30% v/v porosity (4.5 m^3 total pore space). Water volume assumes that 30% of the total pore space is filled with water (1.35 m^3) with the remaining 70% of the total pore space filled with air (3.15 m^3). The NAPL compartment is defined as the total mass of all 17 organic compounds identified in the dye waste sludge. NAPL mass was converted to volume using a density of 970 kg/m^3 consistent with work by Pollard, et al. (15). Model composing material characteristics are summarized in (TABLE 2.4). The level 1 fugacity model assumes equilibrium among all phase compartments and is valid only when the composingt pile is static and biodegradation has either not begun or has ended.

TABLE 2.4 Compost characteristics defining the four compartment fugacity model

Characteristic	Material 1	Material 2
Volume (m^3)	15	15
Bulk density (kg/m^3)	1,200	800
Porosity (%)	30	30
Volume pore air (m^3)	3.1	3.1
Volume pore water (m^3)	1.35	1.35
% Organic carbon (w/w)	30	30
Total mass of contaminants (kg)-initial	290	218
Total mass of contaminants (kg)-final	80	36
NAPL bulk density (kg/m^3)	970	970
NAPL volume (m^3)-initial	0.3	0.22
NAPL volume (m^3)-final	0.08	0.04

1 Value based on Pollard, et al. (15).

Fugacity Calculations. Fugacity based concentrations of the 17 chemicals in the dye manufacturing waste were calculated for pore space air, pore space water, composting material and NAPL phase compartments using the expression $f = M/\sum(V_i Z_i)$. Calculations were facilitated by an Excel spreadsheet made available by Nieman (35). Phase compartments in equilibrium have equal fugacities as determined by the fugacity capacity (FIG 2.2).
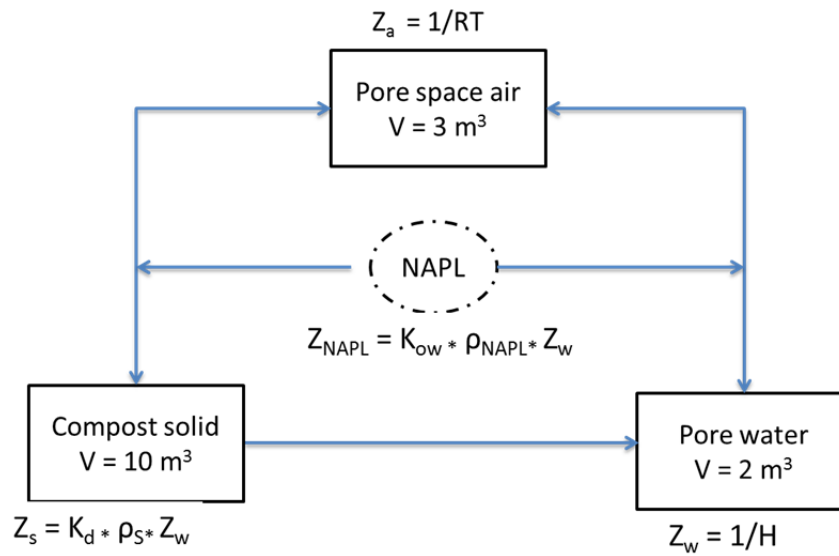
FIG 2.2 Volume and fugacity capacity of each model compartment

Fugacity was calculated for benzene (as an example) using parameters in provided in TABLE 2.5.

$Z_a = 1/RT = 1/(8.3145)(293\ ^oK) = 0.00041\ mol/m^3\text{-Pa}$

$Z_w = 1/H = 1/550 = 0.00182\ mol/m^3\text{-Pa}$

$Z_s = K_d*\rho_s*Z_w = (19.35)*(1.2)*(0.00182) = 0.042\ mol/m^3\text{-Pa}$

$Z_n = Z_w * K_{ow} = (0.00182)*(134) = 0.24\ mol/m^3\text{-Pa}$

The fugacity equation $f = M / \Sigma\ Z_iV_i$ for benzene between compartments was calculated

using fugacity capacity and compartment volumes.

$f = M / (Z_a*V_a) + (Z_w*V_w) + (Z_s*V_s) + (Z_n*V_n)$

$f = 497 / (0.00041 * 3.1) + (0.00182 * 1.6) + (0.042 * 10) + (0.24 * 0.3)$

$f = 497 / 0.0013 + 0.0029 + 0.42 + 0.072$

$f = 497 / 0.4962$

$f = 1{,}001.6\ Pa$

Benzene concentration in each compartment is calculated as:

$f = M / Z_i * V_i$ rearranged to:

$M = f * Z_i * V_i$ since M = concentration * volume:

$C_i = f * Z_i$

The fugacity of air, water, composting solids and NAPL is assumed to be equal for Level 1 fugacity calculations. Thus the concentration of benzene in each phase is given by:

M benzene = $f * Z_a * V_a$ = 1001.6 * 0.00041* 3.1 = 1.27 moles

$C_a = f * Z_a = (1001.6*0.0004) = 0.4812$ moles/m^3 * 78.18 g/mole = 32 mg/L in air

M benzene = $f * Z_w * V_w$ = 1001.6 * 0.00182 * 1.6 = 3.5 moles

$C_w = f * Z_w = (1001.6 * 0.00182) = 1.8$ moles/m3 * 78.1 g/mole = 142 mg/L in water

M benzene = $f * Z_s * V_s$ = 1001.6 * 0.042 * 10 = 420.7 moles

$C_c = f * Z_c = (1001.6 * 0.042) = 42$ moles/m3 * 78.18 g/mole = 3,285 mg/L in solids

M benzene = $f * Z_n * V_n$ = 1001.6 * 0.24 * 0.3 = 72 moles

$C_n = f * Z_n = (1001.6 * 0.24) = 240$ moles/m^3 * 78.18 g/mole = 18,774 mg/L in NAPL

The relative phase distribution of benzene to each compartment at equilibrium is therefore:

Mass distribution to air = (1.27 moles / 497 moles) * 100 = 0.26%

Mass distribution to water = (3.47 moles / 497 moles) * 100 = 0.70%

Mass distribution to composting solids = (421 moles / 497 moles) * 100 = 84.7%

Mass distribution to NAPL = (72 moles / 497 moles) * 100 = 14.5%

TABLE 2.5 Values used to parameterize fugacity equation

Chemical	Benzene
Molecular weight (MW)	78.18 g/mole
Mass in compost – Material 1	38,880 g
Total moles of compound in compost	(38,880 g) / (78.18 g/mole) = 497 moles benzene
Henry's Law coefficient (H)	550 m^3 Pa/mol
Organic carbon normalized coefficient (K_{oc})	64.5
Organic carbon in compost (f_{oc})	30%
Soil water partition coefficient (K_d)	$K_d = f_{oc} * k_{oc} = (0.3)*(64.5) = 19.35$
Octanol-water partition coefficient (K_{ow})	134
Composting density (ρ_s)- Material 1	1.2 kg/L
Total pore volume (30% of composting material v/v)	4.5 m^3
Volume air (V_a) = 30% of total pore volume	3.1 m^3
Volume water (Vw) = 30% of total pore volume	1.6 m^3
Volume composting solids (Vc) (15.3 m^3 - 4.5 m^3)	10 m^3
Volume NAPL in Material 1 (Vn)	0.3 m^3
Volume Total (Vt) 10 m^3 + 0.3 m^3 + 3. 1m^3 + 1.6 m^3	15 m^3

The relative importance of including a NAPL phase separate from the organic carbon content of composting solids was determined by calculating fugacity concentration for pore water in the absence and presence of a NAPL phase and comparing changes in distribution results.

RESULTS

Relative partitioning. Fugacity calculations show that the initial mass distribution of chemicals was predominantly to the organic composting solids and NAPL phase compartments with a minor distribution to the pore air and water (TABLE 2.6 and TABLE 2.7). Aniline and benzo(a)anthracene (and to a lesser extent *N*-nitrosodiphenylamine, carbazole, dibenzofuran, and 2-methylnaphthalene) are distinguished by a relatively low affinity for NAPL whereas chlorobenzene and 1,2,4-trichlorobenzene show a relatively high affinity for NAPL. These distributions generally reflect differences in physical properties and mass transfer coefficients. Compounds with relatively high K_{oc} values tend to partition to organic carbon while those with high K_{ow} coefficients tend to partition to NAPL. Although K_{oc} and K_{ow} are correlated, K_{oc} describes adsorption and K_{ow} describes absorption into a miscible liquid.

TABLE 2.6 Fugacity distribution by percent of initial and final compound mass in Material 1

| | Material 1 Phase Distribution (%) | | | | | | | |
| | Air | | Water | | Compost | | NAPL | |
Compound	initial	final	initial	final	initial	final	initial	final
Benzene	0.25	0.42	0.58	0.95	85	92	15	6
Toluene	0.08	0.09	0.15	0.16	85	95	15	4
Ethylbenzene	0.04	0.04	0.06	0.06	85	96	15	4
Xylenes	0.02	0.02	0.06	0.06	86	96	14	4
Chlorobenzene	0.06	0.07	0.21	0.25	73	91	27	9
1,2, Dichlorobenzene	< 0.01	0.01	0.03	0.04	85	96	15	4
1,2,4-Trichlorobenzene	< 0.01	< 0.01	0.02	0.02	66	88	35	12
Nitrobenzene	< 0.01	< 0.01	1.04	1.16	85	95	14	4
Aniline	< 0.01	< 0.01	0.01	0.01	99.7	99.9	0.02	0
N-Nitrosodiphenylamine	< 0.01	< 0.01	0.03	0.03	91	98	9	2
Carbazole	< 0.01	< 0.01	0.01	0.01	91	97	9	3
Dibenzofuran	< 0.01	< 0.01	< 0.01	< 0.01	92	98	8	2
Naphthalene	< 0.01	< 0.01	0.03	0.03	89	97	11	3
2-Methylnaphthalene	0.02	0.02	< 0.01	0.01	93	98	7	2
Acenaphthene	< 0.01	< 0.01	0.01	0.01	88	96	12	4
Fluorene	< 0.01	< 0.01	< 0.01	0.01	86	96	14	4
Benzo(a)anthracene	< 0.01	< 0.01	< 0.01	< 0.01	98	99.3	2	1

TABLE 2.7 Fugacity distribution by percent of initial and final compound mass in Material 2

| | Material 2 Phase Distribution | | | | | | | |
| Compound | Air | | Water | | Soil | | NAPL | |
	initial	final	initial	final	initial	final	initial	final
Benzene	0.37	0.43	0.86	0.98	83	95	16	3
Toluene	0.12	0.13	0.22	0.25	84	96	16	3
Ethylbenzene	0.05	0.06	0.08	0.1	84	97	16	3
Xylenes	0.03	0.04	0.08	0.08	85	97	15	3
Chlorobenzene	0.08	0.11	0.3	0.39	71	93	29	7
1,2, Dichlorobenzene	0.01	0.01	0.05	0.06	84	97	16	4
1,2,4-Trichlorobenzene	< 0.01	< 0.01	0.03	0.04	63	91	37	10
Nitrobenzene	< 0.01	< 0.01	1.52	1.74	83	95	16	3
Aniline	< 0.01	< 0.01	0.02	0.02	99.9	99.9	0.02	0
N-Nitrosodiphenylamine	< 0.01	< 0.01	0.05	0.05	91	98	9	9
Carbazole	< 0.01	< 0.01	0.02	0.02	90	98	10	10
Dibenzofuran	< 0.01	< 0.01	0.01	0.01	92	98	8	8
Naphthalene	< 0.01	< 0.01	0.05	0.05	88	97	12	12
2-Methylnaphthalene	0.03	0.03	0.01	0.01	93	98	7	7
Acenaphthene	< 0.01	< 0.01	0.01	0.01	87	97	13	13
Fluorene	< 0.01	< 0.01	0.01	0.01	85	97	15	15
Benzo(a)anthracene	< 0.01	< 0.01	< 0.01	< 0.01	97	99.5	3	0.49

Fugacity calculations show that at the completion of composting the measured residual concentration and the predicted compost organic carbon concentration are directly correlated (R^2 = 0.99) in both test materials (TABLE 2.8 and TABLE 2.9). Due to the relatively small NAPL volume remaining at the end of composting, compounds shown to partition to the NAPL phase are highly concentrated.

TABLE 2.8 Comparison of measured residual compound concentration with predicted concentrations in other compost phase compartments in test Material 1

| Compound | Measured residual (mg/kg) | Predicted Concentration | | |
		water (mg/L)	Composting solids (mg/kg)	NAPL (mg/kg)
Benzene	6	0.64	10	87
Toluene	14	0.26	24	139
Ethylbenzene	30	0.21	52	299
Xylenes	197	1.4	340	1800
Chlorobenzene	241	6.9	394	4,780
1,2, Dichlorobenzene	30	0.01	52	299
1,2,4-Trichlorobenzene	276	0.73	436	7,630
Nitrobenzene	873	114	1490	8,430
Aniline	207	0.26	373	2
N-Nitrosodiphenylamine	2,165	0.82	3,800	11,800
Carbazole	39	0.06	68	233
Dibenzofuran	29	0.01	51	142
Naphthalene	218	0.8	379	1,630
2-Methylnaphthalene	55	0.03	97	230
Acenaphthene	32	0.03	56	256
Fluorene	19	0.01	174	30,700
Benzo(a)anthracene	40	0.0001	71	59

TABLE 2.9 Comparison of measured residual compound concentration with predicted concentrations in other compost phase compartments in test Material 2

| Compound | Measured residual (mg/kg) | Predicted Concentration | | |
		Water (mg/L)	Composting solids (mg/kg)	NAPL (mg/kg)
Benzene	66	4.87	73	657
Toluene	239	4.45	275	2400
Ethylbenzene	51	0.36	59	514
Xylenes	449	3.14	522	4140
Chlorobenzene	235	6.88	262	4760
1,2, Dichlorobenzene	129	0.05	149	1300
1,2,4-Trichlorobenzene	6	0.02	7	171
Nitrobenzene	191	25	22	1850
Aniline	140	0.18	168	1
N-Nitrosodiphenylamine	818	3.11	963	4500
Carbazole	95	0.15	112	573
Dibenzofuran	36	0.02	43	177
Naphthalene	359	1.39	420	2710
2-Methylnaphthalene	84	0.05	99	352
Acenaphthene	29	0.03	34	234
Fluorene	17	0.01	20	157
Benzo(a)anthracene	44	0.0001	53	65

Bioavailability. The initial and residual mass of each compound in the dye manufacturing waste composting materials were used in fugacity calculations to predict compound concentrations in pore space water (TABLE 2.10). Solubility is a key factor in determining partition distribution to the water phase and although it was not used directly in calculating fugacity it was used (with vapor pressure) to calculate Henry's Law coefficient and the fugacity capacity of the water phase.

TABLE 2.10 Fugacity predicted concentration in pore space water at the beginning (C_o) and end (C_f) of composting

| | | Compound concentration in water phase (mg/L) | | | |
| | | M1 | M1 | M2 | M2 |
Compound	Solubility	C_o	C_f	C_o	C_f
Benzene[1]	1780	141	0.64	155	4.9
Toluene	515	8	0.26	42	4.4
Ethylbenzene	152	3	0.21	0.78	0.36
Xylenes	170	15	1.4	10	3.1
Chlorobenzene	490	56	6.9	13	6.9
1,2, Dichlorobenzene	100	0.09	0.01	1	0.05
1,2,4-Trichlorobenzene	300	0.8	0.73	<0.01	0.02
Nitrobenzene[1]	1700	128	114	360	25
Aniline	35,000	0.95	0.26	3	0.18
N-Nitrosodiphenylamine[1]	35	8	0.82	3	3.1
Carbazole	7.48	0.04	0.06	0.24	0.15
Dibenzofuran	1.7	0.012	0.01	<0.01	0.02
Naphthalene[1]	31	12	0.8	12	1.4
2-Methylnaphthalene	25	0.06	0.03	0.06	0.05
Acenaphthene	3.8	0.03	0.03	0.029	0.03
Fluorene	1.9	0.057	0.01	0.02	0.01
Benzo(a)anthracene[1]	0.015	<0.01	<0.01	<0.01	<0.01

[1] Final compost concentration is above regulatory threshold for non-residential soils.

Compound solubility and pore water concentration correlate ($R^2 = 0.83 – 0.93$) only if aniline is removed from the analysis (FIG 2.3). Including aniline in the analysis resulted in poor overall correlation between pore water concentration and compound solubility in both materials

($R^2 < 0.01$). Aniline is anomalous due its relatively high solubility but low predicted

concentration in the pore water. Unlike other compounds virtually all of the aniline (99.8%)

partitions to the compost organic carbon due to its relatively high K_{oc}.
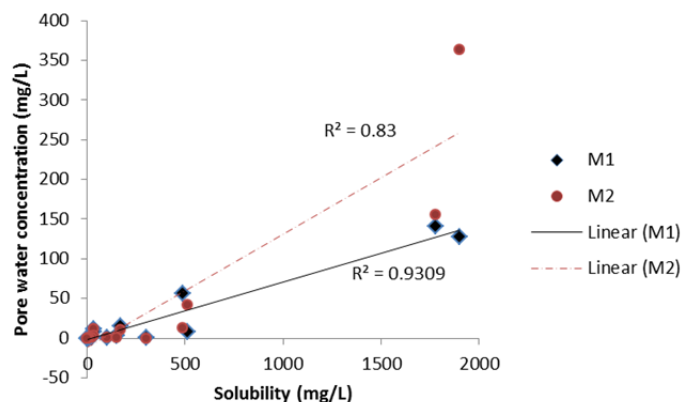
FIG 2.3 Correlation between compound solubility and predicted initial concentration in composting material pore water (aniline excluded from analysis)

Compounds are most available to microbes in soluble form and biodegradation is

expected to occur mainly in the water phase of the composting material. Compound

concentration in pore water however, did not correlate well ($p < 0.05$)with half-life data

(developed in Chapter 1) for Material 1 ($R^2 = 0.31$) or Material 2 ($R^2 = 0.22$) (FIG 2.4).
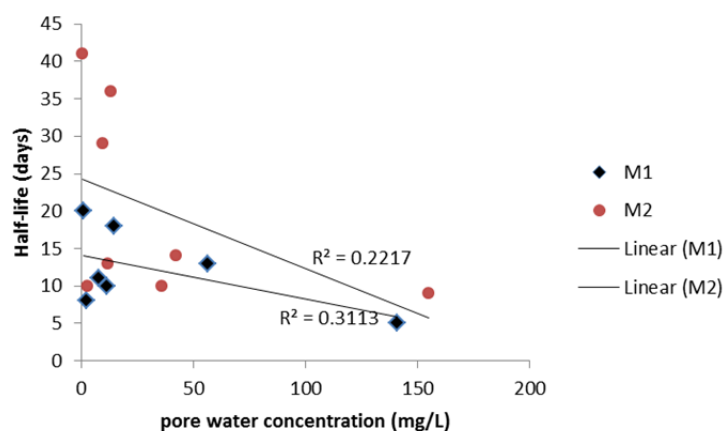
FIG 2.4 Correlation between pore water concentration and degradation half-life in Materials 1 and 2 (aniline excluded)

Fugacity calculations that excluded NAPL as a separate phase predicted an increase in pore water concentration (TABLE 2.11). An independent samples t-test was used to determine if including a NAPL phase in the fugacity model significantly ($p < 0.05$)influenced the pore water compound concentration. No significant difference is indicated between predicted concentrations when the model included or excluded NAPL df = 35, $p < 0.05$).

TABLE 2.11 Change in compound pore water concentration at the start of composting when NAPL is excluded (w/o) and included (w/) in the fugacity calculation for Material 1

	Concentration (mg/L)	
Compound	w/o NAPL	w/ NAPL
Benzene	166	141
Toluene	9	8
Ethylbenzene	3	3
Xylenes	17	15
Chlorobenzene	77	56
1,2, Dichlorobenzene	0.1	0.09
1,2,4-Trichlorobenzene	1.2	0.8
Nitrobenzene	150	128
Aniline	0.95	0.95
N-Nitrosodiphenylamine	9	8
Carbazole	0.047	0.04
Dibenzofuran	0.0134	0.012
Naphthalene	13	12
2-Methylnaphthalene	0.065	0.06
Acenaphthene	0.0389	0.03
Fluorene	0.066	0.057
Benzo(a)anthracene	0.0002	0.0002

DISCUSSION

The Level 1 fugacity model used in this analysis is a simple method for quantifying chemical partitioning in a heterogeneous matrix. Others have used fugacity to estimate the fate and transport of chemical pollutants, particularly hydrophobic organic compounds, in the

environment (36, 37). This is the first study to apply fugacity analysis to predict the phase distribution of hydrophobic dye manufacturing compounds in compost. This study modifies the basic environmental model defined by Mackay, et al. (38) to include a NAPL phase in addition to air, water and solids leaving out biota, sediment and aerosols.

An identified NAPL phase representing the total mass of contaminants and considered separately from the organic compound concentration is not common in *ex situ* treatment. Pollard, et al. (15) used this approach in a fugacity analysis of biopile treatment of benzene and PAHs. The results of the present study are consistent with the observations of Pollard et al. that NAPL and soil organic carbon are the main distribution phases with minor distribution to pore space air or water. Zemanek, et al. (19) demonstrated that 71% of PAHs (by weight) distributed to oil present at 2% of the soil mass. Results presented here predict that when the contaminant mass is approximately 2% of the total compost mass (Material 2), 85 to 98% of the PAHs distribute to the organic compost and only 3 to 13% to NAPL. Pollard, et al. (15) showed that as the percent carbon increased in biopiles from 9% to 15%, PAH distribution to NAPL decreased from 58% to 32% for benzene, 62% to 36% for anthracene and 13% to 5% for benzo(a)pyrene. The present study is consistent with these results showing by extension that a further increase in organic carbon to 30% results in only 16% distribution to NAPL for benzene, 13% for acenaphthene and 3% for benzo(a)anthracene. Thus identifying a NAPL phase separate from the organic carbon content becomes increasingly important as the percent carbon in the treatment material decreases. Residual concentrations partitioned to the NAPL phase are typically a long-term source of contamination and mass transfer from NAPL to the water phase is the rate limiting reaction for compound bioavailability. Thus, not including NAPL as a separate phase from organic carbon in highly contaminated materials may result in an overestimation of treatment potential.

Since the bulk of the compounds distribute to the NAPL and compost organic carbon, limited biodegradation might be expected. Guerin and Boyd (39), however, observed degradation rates and extents for naphthalene that exceeded predictions based on bioavailability assays and cautioned that bioavailability can be influenced by microorganisms not accounted for in phase distribution models. The opposite is also true and not all compounds with predicted bioavailability are biodegraded, since this also depends on the metabolic capacity of indigenous microorganisms. Results of the present study predict that nitrobenzene is available for biodegradation based on fugacity concentrations in the pore water of 128 mg/L in Material 1 and 360 mg/L in Material 2 at the start of composting. A final concentration of 114 mg/L in Material 1 pore water suggests compound availability but not biodegradability whereas a final concentration of 25 mg/L in Material 2 pore water suggests both bioavailability and biodegradability. This observation is consistent with biodegradation half-life data developed during the previous composting study (Chapter 1). The significance of this finding is that treatment improvement may require two different compost management strategies: one to improve growth and specific metabolic activity of indigenous microbes and the other to improve compound bioavailability.

CONLUSIONS

1. The fugacity Level 1 model is a useful tool to identify potential treatment limitations and focus corrective actions needed for improved treatment of hydrophobic organic compounds in compost.

2. Results show that NAPL and composting material organic carbon are the predominant partition phases for hydrophobic compounds. The importance of including a NAPL phase defined as the total concentration of contaminants in the

distribution model is increasingly important in materials with a low organic carbon content. Excluding a NAPL phase from the fugacity model may result in an underestimate of risk relevant compounds in residual materials.

3. The presence or absence of a NAPL phase in the fugacity distribution model did not significantly ($\rho<0.05$) influence the predicted pore water concentrations of dye manufacturing waste compounds. Water phase concentrations appear to correlate with compound solubility (excluding aniline) but not to biotransformation rates quantified by degradation half-life. Thus, compound bioavailability, indicated by the pore water concentration, does not necessarily indicate compound biodegradability.

4. A comparison of soluble compound concentrations of recalcitrant compounds before and after composting provides additional information for distinguishing between bioavailability and biodegradability limitations.

5. A fugacity Level 1 phase distribution model is therefore a useful screening tool for identifying composting limitations and improvements needed for a more complete treatment of hydrophobic chemicals.

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Chapter 3

Ex situ pre-treatment and post-treatment application of Fenton oxidation for enhanced

biotransformation of sediments containing hydrophobic organic compounds

ABSTRACT

Hydrophobic organic compounds are known to partition into organic fractions and non

aqueous phase liquids of contaminated soils and sediments. Slow mass transfer and desorption

rates to the aqueous phase have been shown to reduce the effectiveness of bioremediation and

sequestered compounds often remain in soils after treatment. Fenton oxidation was investigated

as a potential pre-treatment or post-treatment processing step to improve *ex situ* biotreatment by

direct oxidation of residual compounds and/or reduction of the organic matrix for improved

compound availability. Fenton oxidation is an exothermic reaction between hydrogen peroxide

and an iron catalyst (Fe^{2+}) that occurs at an optimal pH 3. It is therefore well suited as a

pretreatment oxidation method for intrinsically acidic materials such as dye manufacturing waste

sludge and coal-tar contaminated soil. The reaction produces hydroxyl radicals that are second

only to fluorine in oxidation potential. The Fenton reaction can be modified by the addition of

chelated iron to maintain soluble iron at near neutral pH. Modified Fenton oxidation is therefore

a preferred oxidation reaction for post-biotreatment since material pH is in a range that is

compatible with biological activity. Chemical oxidation and bioremediation studies were

conducted on dye manufacturing waste sludge containing volatile aromatics, aniline,

nitrobenzene, 2-methylnaphthalene, naphthalene, acenaphthene and benzo(a)anthracene and total

petroleum hydrocarbons, coal-tar contaminated soil from a former manufacturing gas plant

characterized by 2-, 3-, 4-, 5- and 6- ring polycyclic aromatic hydrocarbons and diesel range

organic (C_{10}-C_{28}) contaminated sediments deposited by receding flood waters after hurricane

Katrina. Fenton oxidation was achieved using 70 mM Fe^{2+} catalyst and various hydrogen

peroxide concentrations (0.5%, 1%, 3%, 5% or 10%). Fenton oxidation was modified (mFr) by substituting Fe^{3+} for Fe^{2+} and adding an equal molar ratio of chelator. Tests were conducted using gallic acid, citric acid, or ethylenediaminetetraacetic acid. Bioremediation samples were amended with bulking agent (1:1 v/v) and nutrients to achieve a chemical oxygen demand to nitrogen to phosphorus ratio of 100:5:1 to promote biodegradation, then transferred to aerated, temperature-controlled microcosms ($25\text{-}30^{o}C$) for 30 day incubation. Results show that Fenton oxidation of bulked material decreased volatile organic compounds and semi voloatile organic compound concentrations to a greater extent than oxidation of raw material. A 0.5% application of hydrogen peroxide (2:1 molar ratio of $H_2O_2{:}Fe^{2+}$) was as effective as higher concentrations in decreasing volatile compouns whereas a 3% hydrogen peroxide concentration (13:1 molar ratio) was more effective than lower concentrations for reducing semi volatile organic compounds. Headspace gas analysis suggests that volatilization is a primary removal mechanism for volatile compounds during Fenton oxidation. The concentration of total petroleum hydrocarbons in un-bulked samples decreased by 85% while specific sem volatile organic compounds remained constant and volatile compound degradation was incomplete. Conversely, total petroleum hydrocarbons were not reduced in bulked samples where specific volatile and semi volatile compounds were more completely degraded. Temperature profiles for oxidation reactions were similar for the chelators ethylenediaminetetraacetic acid, citric acid, and gallic acid, indicating ethylenediaminetetraacetic acid or citric acid could be substituted for the less available and more costly chelators. Reaction profiles showed an increase in temperature with increased peroxide concentration reaching a maximum change of $50\text{ - }70^{o}C$ for both types of reactions. A comparison of the modified Fenton reaction to the classic Fenton reaction shows that the modified reaction has a faster reaction rate and a lower temperature maximum. Temperature profiles suggest that Fenton oxidation occurs with ambient iron without addition of an exogenous catalyst. Integrated chemical oxidation and bioremediation demonstrates that bioremediation of

diesel range organics is more effective than Fenton oxidation and pretreatment does not improve

degradation. Although post-treatment chemical oxidation improves volatile compound removal

in bulked material by about 4%, oxidation was also shown to increase measurable concentrations

of volatiles in raw material and semi volatile compounds in post-treated material. These increases

are consistent with observations by others and are possibly attributable to enhanced desorption

and unmasking by direct oxidation of non-aqueous phase organics.   Biotransformation, and

Fenton oxidation significantly ($p<0.05$) reduced concentrations of polynuclear aromatic

hydrocarbons but no significant difference was found between treatments ($p<0.05$).

INTRODUCTION

The persistence of hydrophobic organic compounds in the environment has been

attributed to the sorption and partitioning of compounds into the organic fraction of the

surrounding matrix (1). This distribution reduces compound bioavailability and degradation is

controlled by the rate of mass transfer to the aqueous phase (2). Recent research (Chapter 1 and

2) demonstrates that the recalcitrance of compounds remaining after composting dye

manufacturing waste sludge, is due predominantly to the distribution of residuals to organic

carbon and the non-aqueous phase liquid in the treatment matrix. Composting is an attractive

remediation strategy for these types of highly organic materials since the intrinsic capacity of

composting material to self-heat should improve mass-transfer rates making organic compounds

more accessible for biodegradation. Although compound bioavailability is expected to increase

with increasing temperature, research has shown that this is true only for surface impacted

particles and not for particles with longer diffusional distances (3). Composting results from

previous research (Chapter 1) show that residual compounds remain despite reaching mesophilic

temperatures, although thermophilic temperatures may further increase mass transfer rates,

extremely high temperatures are generally incompatible with growth and activity of the

indigenous microbial community (4, 5). Thus, an increase in composting temperature alone insufficiently improves treatment of hydrophobic compounds and other methods are needed to increase compound bioavailability while not reducing biodegradability.

Watts, et al. (6) has suggested that advanced oxidation using the classic Fenton reaction can enhance desorption and improve bioavailability of a variety of contaminants in soil, and many investigators have reported successful oxidation of hydrophobic compounds in soils with high organic carbon content and non aqueous phase liquid (NAPL, see list of abbreviations, page xi) (7-12).

Fenton oxidation is a chemical reaction between hydrogen peroxide (H_2O_2) and ferrous iron (Fe^{2+}) that produces a hydroxyl radical ($OH\bullet$) according to the following reaction (13):

$$H_2O_2 + Fe^{2+} \rightarrow OH\bullet + OH^- + Fe^{3+}$$

Additional reactions between excess hydrogen peroxide and ferric iron (Fe^{3+}) produce a perhydroxyl radical ($HO_2\bullet$) and a superoxide radical ($O_2\bullet$).

$$H_2O_2 + Fe^{3+} \rightarrow HO_2\bullet + H^+ + Fe^{2+}$$

$$\bullet OH + Fe^{2+} \rightarrow OH^- + Fe^{3+}$$

$$HO_2\bullet + Fe^{3+} \rightarrow O_2\bullet + H^+ + Fe^{2+}$$

$$H_2O_2 + \bullet OH \rightarrow H_2O + HO_2\bullet$$
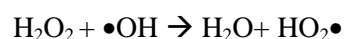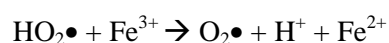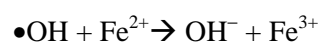
The hydroxyl radical formed by the Fenton reaction is an extremely strong oxidant, second only to fluorine in oxidation potential. Organic compounds are oxidized by hydroxyl radical addition as follows:

$RH + \bullet OH \rightarrow \bullet R + H_2O$

$\bullet R + Fe^{3+} \rightarrow Fe^{2+} +$ reaction by-products
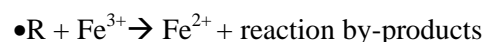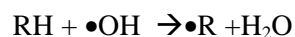
Where RH is a target organic contaminant, and $\bullet R$ is an organic radical.

Pignatello and Baehr (7) have shown that in the classic Fenton reaction, Fe^{2+} is required in stoichiometric amounts to H_2O_2 and a low pH (optimum of 3) is required to keep Fe^{2+} in solution. A substantial disadvantage is that Fe^{2+}, like the organic compound, is oxidized by the hydroxyl radical and this may result in incomplete oxidation of the organic compound. However, if the Fenton reagents H_2O_2 and Fe^{2+} are used in excess the Fe^{2+} is converted to Fe^{3+} at an almost instantaneous rate ($k = 53$ L mol^{-1} s^{-1}) and, after the initial surge of $OH\bullet$, Fe^{3+} catalyzes the oxidation of organic materials (7, 13).

Although Fenton oxidation may improve compound bioavailability by oxidation of NAPL and sorbed organics, the low pH required to maintain iron solubility is generally inhibitory or toxic to microorganisms. Increasing the pH to near neutrality results in precipitation of the iron catalyst needed to form the hydroxyl radical. Pignatello and Baehr (7) demonstrated that iron precipitation at pH above three can be minimized by chelating iron and forming a chemical complex that is water soluble at a near neutral pH. This approach has been used successfully to improve treatment of soluble petroleum compounds (14) and to enhance the desorption and transformation of chloroaliphatic compounds in contaminated soils (6). These studies showed that chelated Fe^{3+} improved degradation of organic compounds in groundwater at near neutral pH while an aggressive flux of $OH\bullet$ at the soil surface resulting from the vigorous oxidation reaction is thought to provide a mechanism for oxidizing sorbed organic contaminants.

Modification of the Fenton oxidation reaction for application at neutral pH allows the reaction to be easily integrated into a treatment train for bioremediation. Two key insertion points are before bioremediation as a pretreatment step or after bioremediation to further improve availability and direct oxidation of residual compounds. Pre-treatment can be further divided into pre- and post-applications to various material handling steps such as material bulking in composting. The goal of pretreatment is to improve the biodegradation potential of difficult-to-degrade compounds by making large, less soluble compounds into smaller, more soluble and more biodegradable ones. Alternatively, post-treatment oxidation is a polishing step for the further reduction of relatively low concentrations of biodegradable compounds or a contingency treatment for non-biodegradable compounds. Advantages and disadvantages of pre- and post-treatment integration opportunities for modified or un-modified Fenton oxidation are summarized in TABLE 3.1. The purpose of this study is to assess the added value of Fenton oxidation as a pre-treatment or post-treatment processing step integrated with bioremediation.

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TABLE 3.1Advantages and disadvantages of various bioremediation insertion points for Fenton or modified Fenton oxidation.

	Application Location of Chemical Oxidation in the Biotreatment Process		
Description	Before bulking	After bulking	After biotreatment
Purpose	Pretreatment	Pretreatment	Post treatment
Objective	Maximize bioavailable hydrocarbons prior to composting.	Create more surface area and improve mixing capability of sediment	Polishing step to improve extent of treatment beyond capability of biotreatment.
Advantage	Any adverse effect of chemical oxidation on microbial population can be mitigated prior to composting.	May improve reagent distribution	Any effect on microbial populations is irrelevant.
Disadvantage	High concentration of petroleum hydrocarbons may require higher concentration or volume of oxidation reagents.	Bulking agent may compete for oxidation reagents.	Further oxidation of organic matter may unmask specific compounds of concern at a point in the process when additional treatment is not an option.

MATERIALS AND METHODS

Analytical. All characterization and test samples were analyzed by Accutest

Laboratories, Dayton, NJ. Volatile organic compounds (VOCs) were quantified using closed-

system purge-and-trap extraction following EPA SW-846, Method 5035 with analysis by gas

chromatography/mass spectrometry (GC/MS) according to EPA SW-846, Method 8260B. Semi-

volatile organic compounds (SVOCs) were determined using ultrasonic extraction using EPA

SW-846, Method 3550B and GC/MS analysis by EPA SW-846 Method 8270C. Diesel range

organics (DRO, $C_{10} – C_{28}$) samples were extracted using pressurized fluid extraction (SW-846

method 3545) and analyzed by gas chromatography (SW-846 method 8015).   Sample pH was

determined by electrometric measurement of the aqueous solution resulting from mixing the

composting material sample 1:1 (v/v) with deionized water (EPA SW-846 Method 9045D).

Volatilization of aromatic hydrocarbons in headspace gas was measured using the MiniRae 2000

handheld instrument. The MiniRae is a photoionization detector with 9.8 ev, 10.6 ev, and 11.7 ev

internal lamps suitable for gas phase measurements of isobutylene, hexane, xylenes, benzene,

styrene, toluene and vinyl chloride. The detector was calibrated using fresh air for zero and

isobutylene at various concentrations. A two-part calibration spanned concentrations from 0-

15,000 ppm. Headspace gas measurements were made using the 0-2,000 ppm +/- 2 calibration

range .

Test Samples. Test samples used in this study were generally classified as dye

manufacturing waste sludge, coal-tar sediment, and diesel range organics (DRO) contaminated

sediment. Dye manufacturing waste sludge was collected from two water-covered waste disposal

locations at a former facility (undisclosed location). These samples contained differing

concentrations of volatile mono-aromatic compounds and 2-, 3-, and 4-ring PAHs (TABLE 3.2).

Samples had a sandy, tar-like consistency that made handling and mixing difficult. Sludges from

both locations were acidic with ambient pH 2.6 to 3.2.

Coal-tar samples were obtained from a former manufactured gas plant site on Front Street, Newark, NJ, and contained a variety of 2-, 3-, 4-, 5-, and 6-ring PAHs (TABLE 3.3). Samples were collected from a soil location 1-2 m below grade. Soil was an acidic (pH < 3.5) sandy-loam with visible chunks of hard, brittle coal-tar dispersed throughout the sample. Sample material containing diesel range organics was provided by the Army Corp of Engineers, Vicksburg, MS. This material contained sediments deposited by receding flood waters after hurricane Katrina and was collected from the general vicinity of the Murphy refinery in New Orleans, LA. Sediments were a composite of silt, clay and sands from various locations with an ambient pH of 4-5. The concentration of DRO in a single characterization sample was 726 mg/kg.

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TABLE 3.2 Chemical characteristics of dye manufacturing waste sludge sample

Compound	Concentration (mg/kg)	
	Sample 1	Sample 2
benzene	1,200	350
toluene	1,500	820
chlorobenzene	97	2,200
xylenes	1,070	840
ethylbenzene	80	< 30
1,2-dichlorobenzene	830	< 150
1,2,4-trichlorobenzene	< 30	3,200
aniline	6,500	< 150
nitrobenzene	6,400	9,000
2-methylnaphthalene	< 150	2,400
naphthalene	16,000	36,000
acenaphthalene	< 150	940
benzo(a)anthracene	< 150	270
TPH	not measured	73,000

TABLE 3.3 Chemical characteristics of Manufactured Gas Plant (MGP) sediment sample

Compound	Concentration (mg/kg)
Acenaphthene	274
Acenaphthalene	76
Anthracene	181
Benzo(a)pyrene	178
Benzo(b)fluoranthene	123
Benzo(g,h,i)perylene	75
Benzo(k)fluoranthene	166
Chrysene	212
Dibenzo(a,h)anthracene	30
Fluoranthene	464
Fluorene	175
Naphthalene	12
Phenanthrene	653
Pyrene	419

Reagents. Fenton's reagent was generated by mixing hydrogen peroxide (CAS # 7722-84-1, Fisher Scientific) with anhydrous ferrous sulfate (CAS # 7720-78-7 VWR) and 3,4,5-trihydroxybenzoic acid (gallic acid, CAS 149-91-7, Sigma-Aldrich), citric acid (CAS 77-92-9, Sigma-Aldrich) or ethylenediamine tetraacetic acid (EDTA, CAS # 60-00-04, Sigma-Aldrich). Ligand stability constants ranged between 11.85 and 14.73. Sample pH was adjusted using pulverized lime-$CaCO_3$ (Mallinchrodt-Baker).

Chemical Oxidation. Test materials were subsampled and 50 g transferred to a heat resistant container. Samples were hand mixed to break aggregated and agglomerated soil particles and to homogenize the material as much as possible. Deionized water (50 ml) was added to the 50 g samples to form a 1:1 (v/v) slurry. Fenton oxidation was applied by adding mL of 0.07 M Fe^{+2} as ferrous sulfate ($FeSO_4 \bullet 7H_2O$) followed by addition of H_2O_2 at 0.5%, 1%, 5%, and 10% (weight of peroxide to weight of solid sample) as needed for test conditions. The pH of acidic samples was adjusted by amending the sample with calcium carbonate ($CaCO_3$) to increase

pH to between 6 and 7 prior to application of mFr. An equal molar solution (0.07M) of chelator

(EDTA, gallic acid or citric acid) and Fe^{+3} as $Fe(ClO_4) \cdot 6\ H_2O$ was added to the slurry prior to the

addition of hydrogen peroxide at the concentration needed for test conditions. Peroxidation was

applied by adding hydrogen peroxide to the sample slurry at the test concentrations in the absence

of added chelator and iron catalyst. Temperature was recorded during all chemical oxidation

tests.

      Biotransformation.   Bioremediation of chemically oxidized or untreated samples was

determined in continuously aerated microcosms constructed from 32 oz (0.94 L) Kerr wide mouth

glass jars (FIG 3.1). An aquarium air diffuser stone (2 in long x 1-7/8 – in diameter) was placed

at the bottom of the microcosm and covered with 250 mL of 0.5-in diameter, washed pebbles. A

¼ in ID Tygon tube was attached to the diffuser and extended out of the microcosm to a

dedicated aeration pump (230 v/50 Hz with 150 mm Hg maximum pressure). Mesocosms were

incubated in a water bath at $30 – 35^oC$ and aerated continuously. Water was added to the bottom

of the microcosm so that the diffuser and stone were saturated. Water was replaced as necessary

to keep the diffuser covered and the air moist. A 0.25 mm mesh was placed on top of the stone

layer and 500 mL of sediment sample added to the microcosm.

      Fertilizer was added to the microcosm to provide a (chemical oxygen demand to nitrogen

to phosphorus (COD:N:P) ratio of 100:5:1. The pH was then adjusted using commercial

pulverized lime so that the material was at or near neutrality. Chelated iron using various ligands

was added to designated microcosms treated with hydrogen peroxide for modified Fenton

reactions. All microcosms with the exception of the killed control (0.1 mg/g $HgCl_2$,) were

amended with fertilizer and lime and incubated at $18\ ^oC$ to $25\ ^oF$ for 30 days. Samples were

collected for analysis on day 0 and day 30 to determine compound degradation.
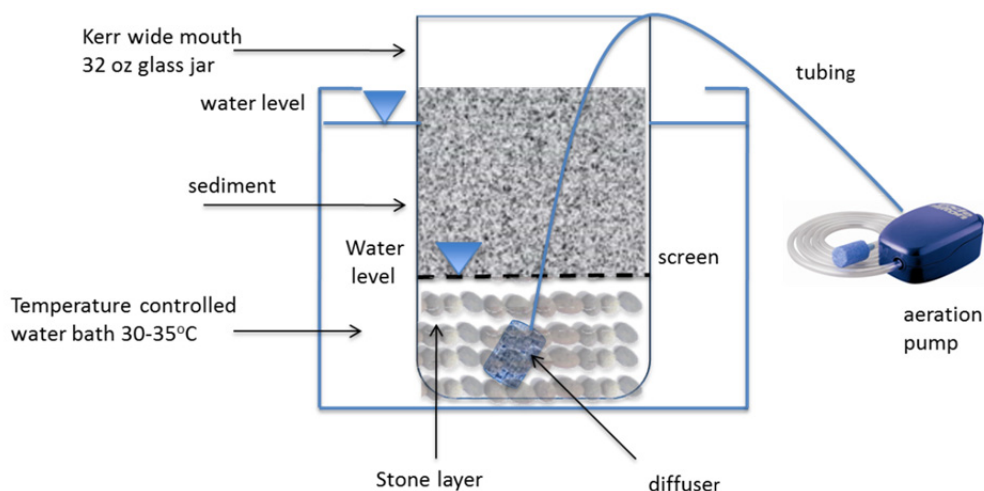
FIG 3.1 Mesocosm configuration for biodegradation tests

RESULTS

Fenton oxidation – pre-treatment of bulked and un-bulked contaminated sludge. The Fenton reagents hydrogen peroxide and ferrous sulfate ($FeSO_4 \cdot 7H_2O$) were added to un-amended dye manufacturing waste sludge (raw) and to sludge bulked 1:1 (v/v) with wood-shavings to determine if amendments typically used for composting would change the effectiveness of Fenton oxidation. With the exception of total petroleum hydrocarbons (TPH), chemical oxidation of bulked material generally resulted in lower compound concentrations than oxidation of the raw sludge. An application of 0.5% hydrogen peroxide (2:1 molar ratio of H_2O_2 to Fe^{2+}) was as effective as higher concentrations of 1% and 3% for decreasing benzene, toluene, chlorobenzene and xylenes for bulked sludge. A 3% application of hydrogen peroxide, however, (13:1 molar ratio of H_2O_2 to Fe^{2+}), was more effective than lower concentrations for reducing higher molecular weight, semi-volatile compounds (TABLE 3.4) with bulked sludge..

TABLE 3.4 Compound concentration in dye manufacturing waste sludge after treatment with hydrogen peroxide

Compound	Raw[1]	Raw[1] + F [2] (mg/kg)			Bulked [3] + F (mg/kg)		
		0.50%	1%	3%	0.50%	1%	3%
Benzene	350	580	140	190	21	26	14
Toluene	820	1,000	250	410	170	180	140
Chlorobenzene	2,200	2,000	530	840	850	860	810
Xylenes (total)	840	570	160	220	260	270	260
Nitrobenzene	9,000	7,100	7,200	10,000	3,300	2,900	2,000
1,2,4-Trichlorobenzene	3,200	850	1,800	3,000	950	860	620
Naphthalene	36,000	18,000	16,000	21,000	9,100	8,600	6,600
2-methylnaphthalene	2,400	2,600	2,400	1,700	1,200	1,200	840
acenaphthalene	940	700	630	710	380	400	310
dibenzofuran	780	760	700	830	370	370	270
Benzo(a)anthracene	270	180	180	220	120	110	63
TPH	73,000	75,000	52,000	11,000	85,000	79,000	77,000

[1] Raw material is defined as sludge without addition of bulking agent (wood shavings).
[2] F is Fenton oxidation.
[3] Bulked is raw sludge sample amended 1:1 v/v with wood shavings

Analysis of total petroleum hydrocarbons provided a more general measurement of organic compounds in the waste sludge. A temperature increase occurred as a result of Fenton oxidation of both the raw and bulked sludge (FIG 3.2). The rates of increase and temperature maxima were greater for the raw material compared to the bulked and coincided with a notable decrease in TPH but only a marginal decrease in the specific compounds. Temperature increase was accompanied by a frothing that lifted treated material and increased the reaction volume by a factor of three to four.
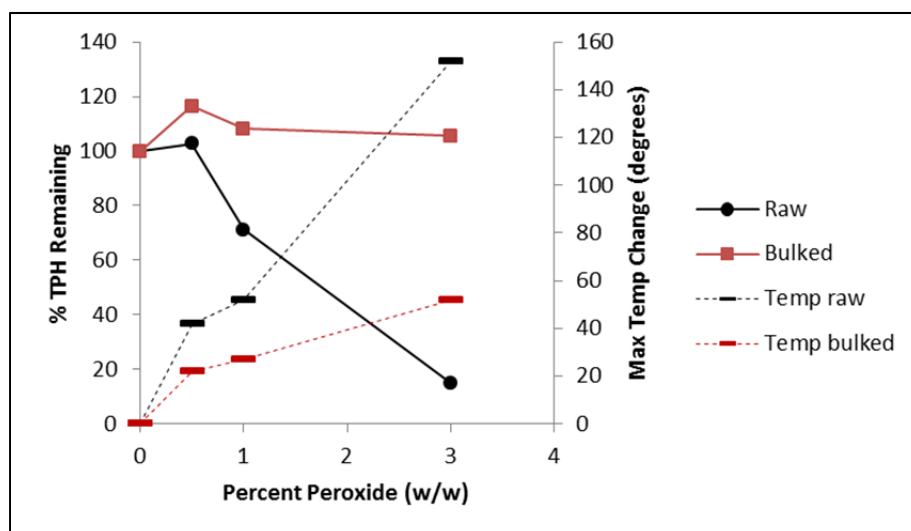
FIG 3.2 Total petroleum hydrocarbon (TPH) concentration and temperature (^{o}C) response
to Fenton Oxidation raw (as excavated) and bulked using 0.5%, 1%, and 3% hydrogen
peroxide

Modified Fenton Reaction – Comparison of Iron Chelators A solution of chelated

Fe^{+3} iron was used to modify the Fenton reaction and improve its effectiveness at near neutral pH.

The chelators gallic acid, EDTA and citric acid were compared by using a DRO contaminated

sediment sample containing diesel range organics and adjusting the pH to a near neutral range

before adding an equal molar solution of chelator and Fe^{+3} (0.07M:0.07M) to the sample creating

a 1:1 (v/v) slurry. Temperature increase following addition of 3% hydrogen peroxide was used

as a reaction indicator. The temperature profiles for all chelators were similar showing a

maximum increase of about 55° C compared to a temperature increase of 70° observed for

unmodified Fenton oxidation (addition of Fe^{+2} without chelator). Although Fenton oxidation had

a higher reaction temperature, the time to peak temperature was longer than for modified

reactions (Fig 3.3).
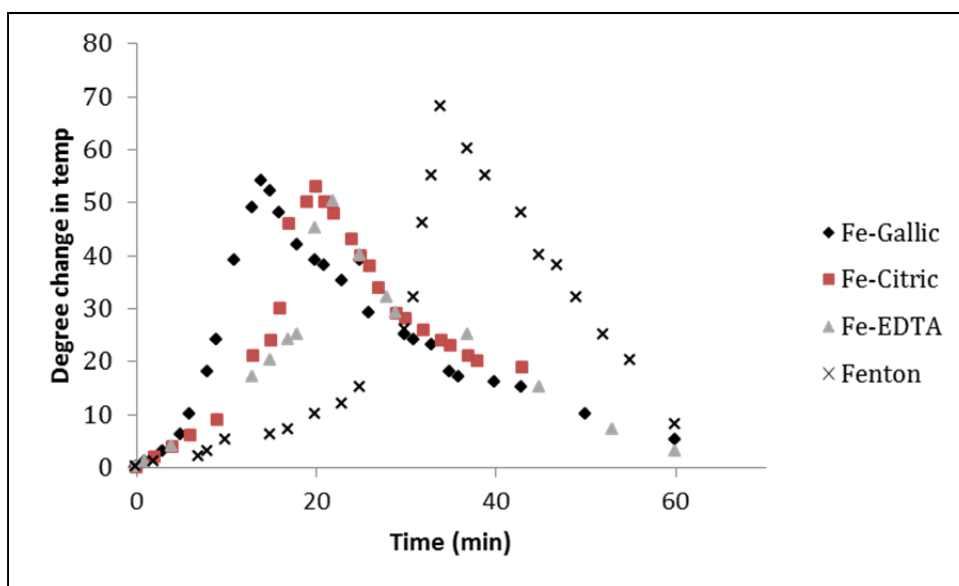
FIG 3.3 Temperature (^{o}C) profile for modified Fenton oxidation of diesel range organic contaminated sediments using various chelators for Fe^{3+} (70 mM:70mM) compared to non-chelated Fe^{2+} (70 mM) applied as classic Fenton reaction. Hydrogen peroxide was applied at 3% v/v.

Modified Fenton Reaction Effect of Peroxide Concentration Modified Fenton

oxidation using pH adjusted DRO sediments and Fe^{3+}-EDTA ligand shows a temperature increase

of 55°, 35° and 5° for peroxide concentrations of 10%, 5% and 1% respectively (Fig 3.4). Time to

reaction peak was shorter and reactions more vigorous for higher peroxide concentration.
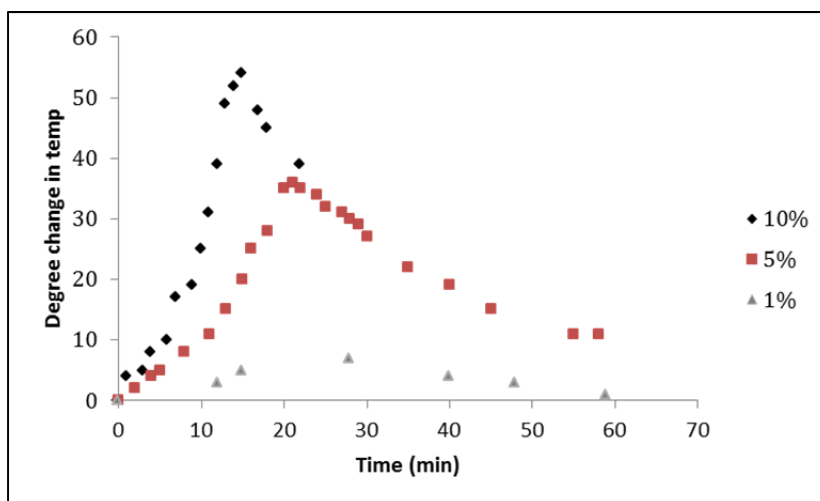
FIG 3.4 Temperature (^{o}C) profile for modified Fenton oxidation of DRO sediment using Fe^{3+}-EDTA at equal molar ratio (70 mM) and varied hydrogen peroxide concentrations

Comparison of Fenton reaction, modified Fenton reaction and peroxidation.

Reaction profiles resulting from various oxidation reactions applied to DRO sediments showed

reaction time similarities between mFr (neutral condition) and peroxidation under neutral

conditions (FIG 3.5). Although very similar, the peroxidation reaction had a lower peak

temperature. Under acidic conditions Fenton reaction and peroxidation also seemed similar

though peroxidation seemed slightly slower and reached a higher temperature max. The ambient

iron concentration in DRO sediment combined with the added hydrogen ion at acidic pH

essentially results in Fenton oxidation. Thus the reaction similarity between peroxidation and

Fenton reaction is not unexpected.
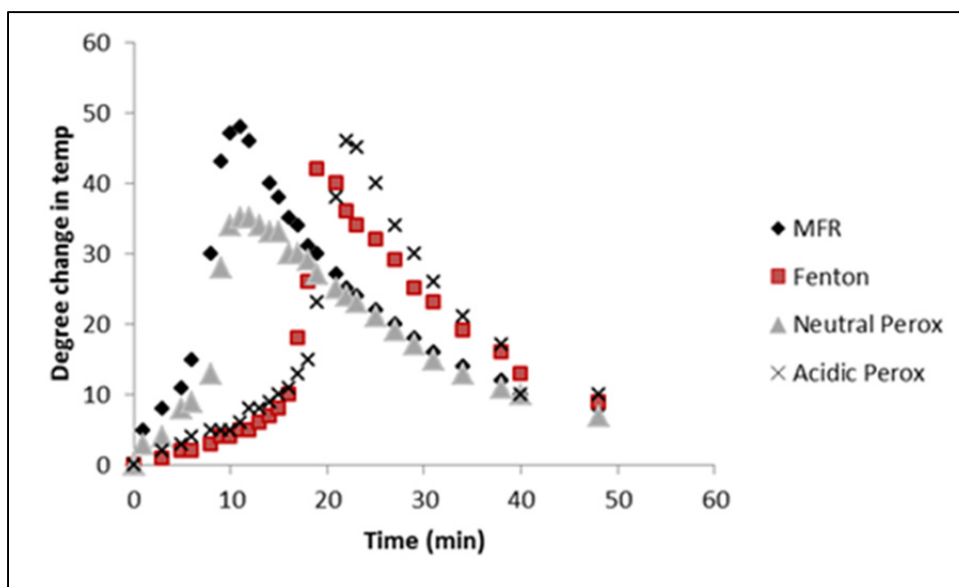
FIG 3.5 Reaction temperature (^{o}C) for mFr using Fe^{3+}-gallic acid (pH 6.56), Fenton Oxidation (pH 2.69) and peroxidation (6.76 and 2.72) by 10% (w/w) hydrogen peroxide.

Compound Volatilization. Headspace analysis during Fenton oxidation of bulked dye

manufacturing waste sludge using 3% hydrogen peroxide showed substantial volatilization that

increased with temperature and far exceeded volatilization resulting from material mixing alone

(FIG 3.6). Thus, differences observed in benzene, toluene, xylene, and chlorobenzene

concentrations after treatment with Fenton or modified Fenton oxidation may be due to thermal

stripping and not to chemical oxidation.
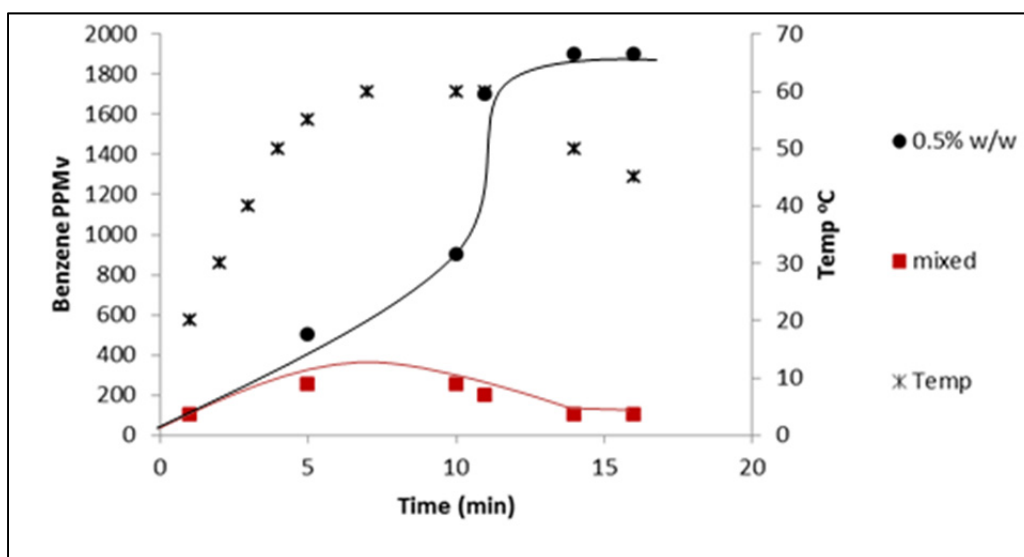
FIG 3.6 Temperature response (x) and concentration of volatile compounds (as benzene
equivalents) in headspace of reactor during Fenton Oxidation of dye waste sludge
using 0.5% w/w hydrogen peroxide (circle) compared to volatilization due to material mixing
without Fenton Oxidation (square).

Chemical Oxidation as Pre-treatment for Bioremediation. Chemical oxidation and

bioremediation were tested by pre-treating DRO sediment with Fenton reagents (Fe^{+2}) and 1% or

5% hydrogen peroxide followed by pH adjustment and bioremediation of the oxidized material

(FIG 3.7). Results show that oxidation decreased DRO concentrations at initial application (day

0) by about 50-60% but further improvement by biodegradation over the 30 day incubation period

was slight. By comparison, biodegradation in the absence of chemical oxidation decreased DRO

concentration by approximately 75%.
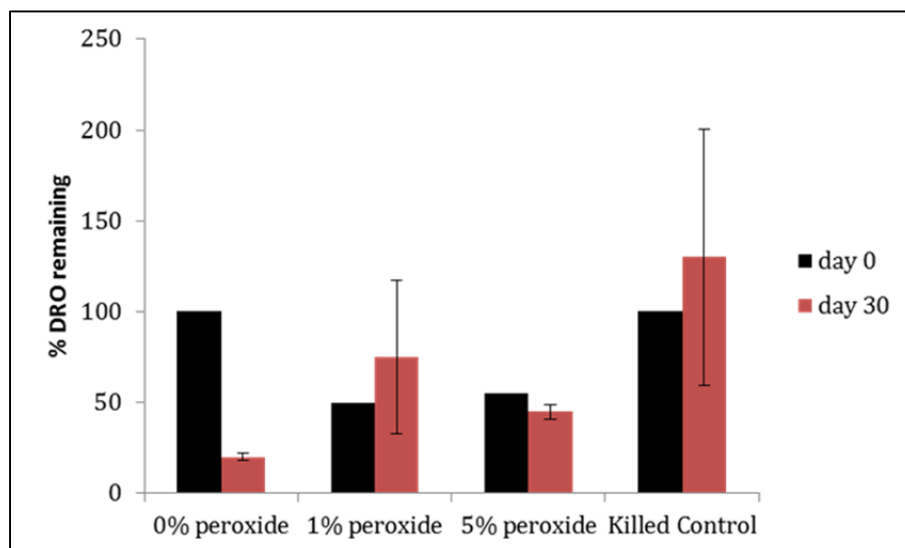
FIG 3.7 Chemical oxidation and bioremediation of DRO sediment after pre-treatment by Fenton oxidation.

Pretreatment of acidic dye manufacturing waste sludge by Fenton oxidation using 3% hydrogen peroxide resulted in a measured increase in concentrations of the volatile compounds (VOCs) benzene, toluene, ethylbenzene, xylenes, and chlorobenzene but a decrease in concentrations of semi-volatile compounds (SVOCs) 1,2-dichlorobenzene, aniline and naphthalene (TABLE 3.5).

Chemical Oxidation as Post-treatment for Bioremediation. Chemical oxidation applied as a post-treatment polishing step after bioremediation of dye manufacturing sludge was compared to bioremediation alone using mFr at near neutral pH and 3% hydrogen peroxide (TABLE 3.5). Biodegradation alone was effective in reducing volatile compounds (benzene, toluene, chlorobenzene, xylenes, ethylbenzene, and 1,2-dichlorobenzene). These compounds were further reduced (about 3 to 4%) after biodegradation by applying mFr (at near neutral pH) as a post treatment to biodegradation.

The benefit of post-treatment oxidation however appears limited to volatile compounds and may be the result of volatilization (as shown above). Fenton oxidation applied after

biotreatment did not significantly ($p < 0.05$) improve removal of semivolatile compounds aniline, nitrobenzene and naphthalene (compared to biotreatment alone).

TABLE 3.5 Comparison of pre- and post-biotreatment by Fenton oxidation using 3% hydrogen peroxide

Compound	Concentration (mg/kg)			
	Raw[1]	Raw + F[2] (pre-treatment)	Bio[3] only	Post Bio F[4] (post-treatment)
Benzene	1,200	4,400	81	29
Toluene	1,500	4,700	93	30
Chlorobenzene	97	550	<14	<9.4
Xylenes	1,070	2,640	44	13
Ethylbenzene	80	190	3	<9.4
1,2-dichlorobenzene	830	710	31	<75
Aniline	6,500	5,800	810	1,100
Nitrobenzene	6,400	3,800	810	1,200
Naphthalene	16,000	5,200	980	1,400

[1] Raw is defined as sludge sample without bulking agent
[2] Raw + F indicates that Fenton oxidation was applied to unbulked sludge sample before biodegradation.
[3] Bio refers to biological treatment in mesocosm reactor
[4] Post Bio F indicates Fenton oxidation was applied to biologically treated sample

Combined Pre-treatment and Post-treatment chemical oxidation of bioremediated PAH contaminated soil. Biotreatment of acidic coal-tar contaminated sediment containing 2-, 3-, 4-, 5-, and 6-ring PAHs was compared to biotreatment following pre-treatment by Fenton oxidation and to biotreatment followed by post-treatment mFr (TABLE 3.6). Only acenaphthene, fluroene, and phenanthrene concentrations were significantly decreased during treatment ($p < 0.05$) and there was no significant difference ($p < 0.05$) between biotreatment results with or without Fenton oxidation.

TABLE 3.6 Pretreatment and post-treatment of bioremediated coal tar contaminated soil.

| Compound | Concentration (mg/kg) [1] | | | | | | | |
	Day 0	SD	Bioremediation [3] Day 30	SD	Pre-treatment [4] (5%) Day 30	SD	Post Treatment [5] (5%)	SD
Acenaphthene [2]	274	43	75	8	87	1	56	15
Acenaphthylene	76	11	76	11	76	8	64	24
Anthracene	181	31	114	16	125	9	103	53
Benzo(a)anthracene	204	36	212	24	220	18	187	32
Benzo(a)pyrene	178	36	180	21	185	12	150	31
Benzo(b)fluoranthene	123	27	127	16	136	16	108	9
Benzo(g,h,i)perylene	75	12	90	11	96	9	77	50
Benzo(k)fluoranthene	166	51	134	13	133	26	86	43
Chrysene	212	41	201	21	213	21	176	32
Dibenzo(a,h)anthracene	30	6	36	4	36	3	25	12
Fluoranthene	464	113	467	53	459	33	416	85
Fluorene [2]	175	28	56	15	59	2	45	23
Indeno(1,2,3-cd)pyrene	76	6	86	9	91	8	73	44
Naphthalene	12	2	4	0	7	0	4	14
Phenanthrene [2]	653	122	284	48	310	8	243	65
Pyrene	419	107	481	57	441	40	409	89

[1] Concentration data are average values (n=2)

[2] Signifcant ($p < 0.05$) reduction of compound concentration occurs during treatment

[3] Bioremediation with out pre- or post-treatment Fenton oxidation

[4] Fenton oxidation using 5% H_2O_2 (w/w sludge) at day 0 followed by bioremediation for 30 days.

[5] 30 day biotreatment followed by mFr using 5% H_2O_2 (w/w sludge)

[3,4,5] no significant ($p < 0.05$) difference among treatments

DISCUSSION

This research investigates chemical oxidation as a pre- or post-treatment supplement to bioremediation. Results show that biotreatment is an effective method to reduce some volatile and semi-volatile compounds and is not improved by pre- or post- Fenton oxidation. Different groups of chemicals, however, respond differently to chemical oxidation depending on the ratio and concentration of applied reagents. The results show that lesser amounts of hydrogen peroxide (0.5% H_2O_2 and 70 mM Fe^{+2}) are needed to remove VOCs than for the semi-volatile compounds (3% H_2O_2 and 70 mM Fe^{+2}). This is similar to findings by Watts, et al. (15) that showed oxidation of aromatic compounds required less hydrogen peroxide than aliphatic compounds. His work demonstrated that 18%, 34%, and 38% of xylenes, toluene and benzene,

respectively desorbed during a 2 h oxidation reaction time. Desorption is reflected in the present study by the high rate of volatilization and total removal of 94%, 80%, 62% and 69% of benzene, toluene, chlorobenzene and xylenes, respectively, from bulked samples. This removal was greater than observed by Watts, et al. (15) and, since both studies used the same amount of hydrogen peroxide, observed differences may be due to the higher molar concentration of Fe^{+2} (70 mM vs 6.5 mM) used in the present study. Although 0.5% hydrogen peroxide concentration was effective in reducing VOCs, a higher concentration was needed to remove PAHs. Treatment was most efficient for PAHs using 3% hydrogen peroxide and 70 mM Fe^{2+} in bulked sediment (about a 13:1 molar ratio). Removal of PAHs was substantial but below 90% for all compounds. Naphthalene was reduced by 83% while other one- two-, three-, and 4- ring compounds were reduced to a lesser extent (65-78%). These results are consistent with work by others that report Fenton oxidation removal of 80% for two-ring compounds at a ratio of hydrogen peroxide to Fe^{2+} of 10:1 (14).

Fenton oxidation of raw and bulked dye manufacturing waste sludge using 0.5% to 3% hydrogen peroxide resulted in measured concentrations that were often higher than the analysis on the excavated (raw) sample. This is consistent with desorption mechanisms observed by others (6) but may also be due to direct oxidation of total petroleum hydrocarbons that, in high concentrations, function like a NAPL component in the treatment matrix (as discussed in Chapter 2). Oxidation of TPH in the dye manufacturing waste may result in desorption and/or unmasking of PAHs resulting in a measured increase in PAH concentration from improved extraction. This measured increase in concentration was not observed in bulked material possibly because the TPH coats the bulking material (in this case woodshavings) decreasing the diffusion pathway and increasing compound availability at the higher oxidation temperatures. This explanation is consistent with the mechanism described by Ghosh, et al. (3) for limited compound bioavailability related to particle morphology and elevated treatment temperatures. Vigorous

temperature increases resulting from Fenton reactions using hydrogen peroxide greater than 1%
have also been shown to enhance desorption, possibly due to the formation of a reducing species
and not to the hydroxyl radical (6). Some researchers suggest controlling reaction temperature by
a step-wise or graded addition of peroxide (10, 14, 16). This study was conducted in batch
reactions with peroxide addition in a single dose resulting in increased temperatures of up to $60^{o}C$
degrees and an increase in reaction volume by a factor of 4. Although no direct evidence of
oxidation was obtained it is not unreasonable to conclude that elevated temperature and volume
expansion are indicators of oxidation reactions. These conditions resulting from uncontrolled
reactions are reported to favor desorption as well as the direct oxidation of sorbed compounds
through increased activity of the hydroxyl radical at the soil surface (6).

Based on reaction intensity, modified Fenton oxidation using a chelated-Fe^{3+} complex
soluble at near neutral pH was equivalent to the classic Fenton reaction and to oxidation by
hydrogen peroxide in the absence of added iron (peroxidation). Peroxidation is essentially a
Fenton reaction at pH 2-3 if there is ambient iron in the treatment material. Watts, et al. (9)
demonstrated that treatment efficiencies for pentachlorophenol were actually faster in soils with
natural iron content than for soils with added iron and attributed this slower reaction to adjusting
the pH but a detailed mechanism was not provided. Modified Fenton reaction and unmodified
Fenton reaction comparisons were made using gallic acid to chelate Fe^{3+} although Pignatello and
Baehr (7) have shown that Fe^{3+}-nitrilotriacetate (NTA) and Fe^{3+}-hydroxyethyleniminodiacetate
(HEIDA) were more effective than Fe^{3+}-gallic acid in catalyzing Fenton oxidation of various
herbicides. Commercial use of gallic acid, NTA and HEIDA in remediation is not practical due
to limited availability and high cost of bulk quantities. Modified Fenton reactions using the more
available and less costly chelator compounds citric acid and EDTA were tested as substitutes and
were shown to have similar reaction profiles to gallic acid. EDTA was selected for use in
subsequent oxidation tests that applied a 1:1 molar ratio of Fe^{3+}:EDTA (70 mM) and various

concentrations of hydrogen peroxide. Modified Fenton reaction intensity increased with increasing peroxide concentrations. This is consistent with results of an oxidation kinetic model developed by Tang and Huang (17) that shows Fenton oxidation is first order with respect to organic concentration and the extent of compound degradation is determined by the applied concentration of hydrogen peroxide and Fe^{2+}.

Modified Fenton oxidation as a pretreatment for bioremediation of diesel range organics did not improve treatment. Bioremediation removed 75% of the total DRO and pretreatment with hydrogen peroxide removed 40-50%. Biodegradation of pretreated samples did not reduce DRO concentrations further. These oxidation results are lower than the 70-80% diesel removal achieved by Watts and Dilly (18) using the same molar concentration of hydrogen peroxide and similar reaction times as the present study. Removal efficiency may be due to sorption differences between the soils tested by Watts and Dilly and the DRO sediments used in this study.

Pre-treatment of PAH contaminated sediments prior to biotreatment did not improve removal efficiencies observed for biodegradation alone. This is consistent with work by Nam, et al. (14) that demonstrated 30-39% recalcitrance of fluorene and phenanthrene and 62-86% recalcitrance of pyrene and chrysene in soil treated by mFr followed by biodegradation. Koolivand, et al. (19) showed an 8% improvement in crude oil treatment by applying Fenton oxidation after composting. Results from the present study, however, showed insignificant removal of SVOC compounds by either pre-or post biotreatment by Fenton oxidation. Post biotreatment Fenton oxidiation appears to improve removal of VOCs by up to 4% due to volatilization.

CONCLUSIONS

    1. The effectiveness of Fenton oxidation as a pre-treatment or post-treatment supplement to biodegradation is variable and compound specific.

2. Volatile organic compounds are removed by relatively low hydrogen peroxide concentration (0.5%) if applied to bulked material.

3. Reduction of VOCs may be due to volatilization and not to direct oxidation. A higher concentration of hydrogen peroxide (3%) is needed for removal of SVOCs.

4. Fenton reaction is more effective in bulked material. This suggests that surface area is an important consideration in chemical oxidation.

5. Petroleum residues measured as non-specific TPH are oxidized in un-bulked material apparently at the expense of specific compounds.

6. Reaction profiles for modified Fenton oxidation are similar for gallic acid, citric acid and EDTA chelators. Therefore the more cost effective and available chelators citric acid or EDTA can be used for remediation applications.

7. Biotreatment of DRO is more effective than chemical oxidation. Post-treatment does improve removal of VOCs by about 4% above bioremediation but does not improve biotreatment of the SVOCs aniline, nitrobenzene, and naphthalene.

8. Observed increases in compound concentration after chemical oxidation suggests desorption and un-masking of contaminants dissolved in the organic phase. This increased availability however did not translate into significantly improved biotreatment ($\rho < 0.05$).

9. Future studies focused on matching the desorption of VOCs and SVOCs to well acclimated biodegradation are needed to better integrate these processes.

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Chapter 4

Meta-analysis of RDX Biotransformation rate by bacteria and fungi

ABSTRACT

Royal Demolition Explosive (RDX; hexaydro-1,3,5-trinitro-1,3,5-triazine) is a highly oxidized compound commonly used in military and industrial explosives. Many researchers have shown that bacteria and fungi have the metabolic capability to transform RDX into smaller, non-cyclic and de-nitrated compounds. Although much is known about the biodegradation pathways used by bacteria and fungi to transform RDX, far less is known about the kinetics. This meta-analysis uses published data from metabolic and feasibility studies to calculate a first-order rate coefficient and half-life ($t_{1/2}$, days) value as a common metric for the comparison of RDX degradation rates by three microbial groups: aerobic bacteria ($t_{1/2}$ = 1.08 days, SD = 1.38), anaerobic bacteria ($t_{1/2}$ = 5.62 days, SD = 6.25), and fungi ($t_{1/2}$ = 13.32, SD = 11.72) . Forty-two independent research studies in 25 publications with 14 studies describing each group provided the primary data for the meta-analysis. All studies were conducted in laboratory conditions. The Shapiro-Wilk test for normality showed half-life data were not normally distributed and were positively skewed for each group. A logarithmic transformation of the data was successfully applied. Levine's test of the transformed data showed equal variances ($F_{(2,39)}$ = 0.130, $\rho < 0.05$), thus the parametric one-way ANOVA test was used to describe distribution. Results showed that the distribution of half-life across the groups is not the same ($F_{(2,39)}$ = 23.2, $\rho < 0.05$). Post hoc analysis using the Tukey's Honestly Significant Difference (HSD) test identified significant differences in RDX half-life values between aerobic and anaerobic bacteria ($\rho < 0.05$), aerobic bacteria and fungi ($\rho < 0.05$) and anaerobic bacteria and fungi ($\rho < 0.05$). This study is the first attempt to broadly characterize and compare RDX degradation kinetics by bacteria and fungi using a meta-analysis approach.

INTRODUCTION

Royal demolition explosive (RDX) or hexaydro-1,3,5-trinitro-1,3,5-triazine is a large, dense, highly oxidized cyclic nitroamine used as an explosive fill in many military munitions. The manufacture, loading, use and disposal of these munitions has resulted in the need for new technologies that remove RDX from waste streams, soil and groundwater to reduce toxicity and protect human health and the environment. New technologies for the destruction or transformation of RDX are also needed to improve the safety, effectiveness and efficiency of processes that deactivate aging or unserviceable munitions, and render-safe unexploded ordnance.

Biological transformation of RDX is an emerging technology with demonstrated use in site remediation (1) and wastewater treatment (2). By extension, biological treatment may have potential as an enabling technology for destruction of explosive weapons. The optimism for application of biotechnology for this purpose is based on the robustness and versatility of RDX metabolic pathways that are fairly widespread among aerobic bacteria, anaerobic bacteria and fungi (3-5). Under anaerobic conditions for example, bacteria can use RDX co-metabolically (6-9) or as a source of carbon and energy for growth (10-12). In the latter situation RDX serves as the terminal electron acceptor for growth (13). The mechanism for co-metabolic degradation is through the transfer of two electrons sequentially reducing $N-NO_2$ bonds to form the nitroso derivatives MNX (hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine), DNX (hexahydro-1,3-dinitroso-5-nitro-1,35-triazine) and TNX (hexahydro-1,3,5-trinitroso-1,3,5-triazine) (4). Under reducing conditions these nitroso products are then transformed to transient hydrazines and finally to formaldehyde and methanol (4). Other researchers (14) suggest a single electron transfer for denitration of RDX under anaerobic conditions resulting in an anion radical that spontaneously fragments into methylenedinitramine (MEDINA), and ultimately into formaldehyde (HCOH), methanol (CH_3OH) and CO_2. A third anaerobic pathway has been

described (15) that involves direct enzymatic ring cleavage of RDX resulting in the formation of the transient compounds MEDINA and bis(hydroxymethyl)nitramine. This enzymatic ring cleavage pathway is a key mechanism for RDX assimilation by bacteria in the absence of exogenous carbon and nitrogen.

In aerobic conditions bacteria generally metabolize RDX as a source of nitrogen (3, 16-18). In this case denitration of RDX occurs through cytochrome P-450 enzyme catalyzed ring cleavage forming the products nitrite, nitrous oxide, ammonia, formaldehyde and CO_2 as well as the dead-end product 4-nitro-2,4-diazabutanol (NDAB) (19-21). Aerobic soil fungi also degrade RDX using cytochrome P-450 enzymes but the explosive does not provide a source of carbon and nitrogen needed for growth (22). Products formed by this process include MNX, HCHO, N_2O, MEDINA and small amounts of TNX and DNX (hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine) (23).

New technologies that exploit these pathways have the potential to advance military and civilian capabilities for explosive ordnance disposal, munitions demilitarization, waste treatment, and site remediation. To explore this possibility more fully, however, kinetic parameters for RDX transformation are needed. Although RDX degradation kinetics are generally described as first-order based on evaluation of microbial isolates (9, 24, 25) or cell-free extracts (26) the predominant research has been on degradation feasibility of RDX and the majority of studies present time-course data without providing a kinetic analysis. In addition, direct comparisons of biodegradation kinetics by anaerobic bacteria, aerobic bacteria and fungi have simply not been conducted. The purpose of this study is to fill this data gap by transforming published data through a secondary analysis that calculates a first-order rate constant and half-life estimate. These values serve as a metric for comparing multiple studies identified through a systematic review of the literature and meta-analysis of the data. The resulting unified framework provides

the context needed for guiding future research strategies and investments and for evaluating the role of biotechnology in the disposal of explosives.

MATERIALS AND METHODS

PrimaryData. Published research describing RDX biodegradation by aerobic bacteria, anaerobic bacteria, and fungi were reviewed to identify studies reporting quantitative data for initial and final concentrations of RDX over a defined time period. Only independent studies conducted in aqueous media in laboratory conditions were selected. The studies include degradation by mixed cultures from soil and sludge as well as specific isolates identified by the primary authors either before or after the study. Secondary analysis of rate excludes lag time and acclimation periods. Growth conditions were noted for all studies used in the meta-analysis but the type of metabolism was not used as a criterion for either including or excluding the research in the analysis.

The literature review was conducted using a key-word search of the Rutgers University Libraries indexes and databases in the subjects of science, technology, engineering and math and core or related indexes and data-bases in environmental science. Key search areas included Environmental Engineering Abstracts; Pollution Abstracts; ProQuest Environmental Sciences Collection; Applied Science and Technology Full Text (H.W. Wilson); Biotechnology Research Abstracts; Environment Abstracts; Marine Biotechnology Abstracts (ASFA); ProQuest Deep Indexing in Environmental Sciences; ProQuest Environmental Science Journals; SciFinder; and Water Resources Abstracts; Industrial and Applied Microbiology and Bacteriology Abstracts. Forty-two primary research studies from 25 published reports were selected for the meta-analysis. Studies were equally divided among three groups: aerobic bacteria (n=14); anaerobic bacteria (n=14); and fungi (n=14).

Secondary Data Analysis. The initial and final concentrations of RDX reported for a defined treatment period in each primary study were used to calculate a first-order kinetic coefficient (k, d^{-1}) and half-life ($t_{1/2}$, d) value. The calculated rate, in either form, is a secondary metric and dependent variable within the independent variable of aerobic bacteria, anaerobic bacteria and fungi. The kinetic coefficient was determined using the integrated form of the first-order rate equation (Equations 1 and 2) where $[A]_O$ is the initial concentration and $[A]$ represents the final concentration at time (t) . A rearrangement of the integrated rate law (Equation 3) allows a linear plot of ln[A] vs. (t) with the resulting slope equal to the rate constant. The half-life value for RDX was determined based on the calculated first-order rate constant using the relationship in Equation 4.

Equation 1. First order rate law. Where k is the first order rate constant (d-1), and [A] represents the final concentration at time (t) .

$$-\frac{d[A]}{dt} = k[A]$$

Equation 2. Integrated rate law. $[A]_O$ is the initial concentration.

$$[A] = [A]_0 e^{-kt}$$

Equation 3 Rearrangement of the integrated rate law.

$$\ln[A] = -kt + \ln[A]_o$$

Equation 4 Relationship of half-life ($t_{1/2}$, d) value to first-order rate constant (k).

$$t_{1/2} = \frac{\ln(2)}{k}$$

Statistics Three groups (aerobic bacteria, anaerobic bacteria, and fungi) of independent and equal size samples (n=14) were statistically analyzed using SPSS Statistics 21 software (IBM, New York, U.S.A). The Shapiro-Wilk test, using logarithmically transformed and untransformed data was used to assess distribution. Differences between groups were identified using a One-Way Analysis of Variance (ANOVA) on logarithmically transformed half-life data

and the Post Hoc Tukey's HSD test was used to determine which groups were statistically different from the others. A comparison of variance among groups was made using the Levine test for homogeneity. A summary of test objectives and hypotheses is provided in TABLE 4.1.

TABLE 4.1 Summary of statistics used to determine data distribution and differences among groups

Objective	Test	Null hypothesis (H_o)	Criteria to Reject H_o
Test for Normal distribution	Shapiro-Wilk	Data come from a normally distributed population	$p < 0.05$
Identify statistical differences among groups of data that are distributed	One-Way Analysis of Variance (ANOVA)	The distribution of half-life is the same across all groups	$p < 0.05$
Post-hoc analysis to identify groups that significantly differ from each other	Tukey's HSD	The distribution of the half-life is the same for each comparison	$p < 0.05$
Determine if variances in each group are equal	Levine Test	Variances are equal between groups.	$p < 0.05$

RESULTS

First-order kinetic coefficients and half-life values calculated from data provided in primary published literature for RDX biodegradation by anaerobic bacteria, aerobic bacteria, and fungi are provided in Tables 4.2, 4.3, and 4.4, respectively.

TABLE 4.2 Anaerobic Bacteria Group. Calculated rate constant and half-life values derived from studies using anaerobic bacteria. Source of inoculum and culture condition described by primary authors are provided for reference. The abbreviation (C) and (N) stand for carbon and nitrogen respectively.

Inoculum	k (d^{-1})	Half-Life (d)	Culture Condition	Source
Mixed culture	0.03	22.8	RDX sole source of C and N	(11)
Desulfovibrio desulfuricans	0.04	15.8	RDX sole source of C and N	(12)
Sulfate reducing enrichment culture	0.112	6.2	Exogenous Carbon (RDX-N source)	(10)
Desulfovibrio desulfuricans	0.12	5.6	RDX sole source of nitrogen	(12)
Anaerobic sludge	0.14	4.88	RDX sole source of C and N	(27)
Soil	0.2	3.47	RDX sole source of C and N	(28)
Sludge (*Klebsiella pneumoniae* strain SCZ-1	0.23	2.97	RDX sole source of C and N	(14)
Acetobacterium malicum (Haap-1)	0.24	2.85	H_2 provided	(9)
Pseudomonas putida [1]	0.33	2.11	Exogenous Carbon (RDX-N source)	(24)
Soil	0.33	2.11	RDX sole source of C and N	(29)
Mesophilic sludge	0.35	2	RDX sole source of C and N	(30)
Consortium mainly *Geobacter* sp. (78%) *Acetobacterium* sp.(14%)	0.72	0.96	Sulfate amended	(31)
Anaerobic sewage sludge	0.77	0.9	RDX sole source of C and N	(27)
Pseudomonas fluorescens [2]	0.84	0.82	Exogenous Carbon (RDX-N	(24)

[1] *P. putida* II-B -Xen A, whole cell biotransformation in succinate amended basal salts medium

[2] *P. fluorescens* I-C -XenB, whole cell biotransformation in succinate amended basal salts medium

TABLE 4.3 Aerobic Bacteria Group. Calculated rate coefficient and half-life value derived from studies using aerobic bacteria. Source of inoculum and culture condition described by primary authors are provided for reference.

Inoculum	k (d^{-1})	Half-Life (d)	Culture condition	Source
Rhodococcus opacus	0.13	5.33	Co-metabolic	(32)
Rhodococcus opacus	0.28	2.5	Co-metabolic	(32)
soil-unidentified consortium	0.47	1.46	Exogenous carbon RDX-N source	(33)
Gordonia sp,	0.66	1.05	Exogenous carbon RDX-N source	(34)
Stenotrophomonas malophilia PB1	0.73	0.95	Exogenous carbon RDX-N source	(17)
Williamsia sp. KTR4	0.78	0.89	Exogenous carbon RDX-N source	(34)
Stenotrophomonas malophilia PB1	0.79	0.75	Exogenous carbon RDX-N source	(17)
Stenotrophomonas malophilia PB1	0.81	0.85	Exogenous carbon RDX-N source	(17)
Rhodococcus YH1	1.04	0.67	Exogenous carbon RDX-N source	(35)
Gordonia KTR9	1.1	0.63	Exogenous carbon RDX-N source	(34)
Rhodococcus YH1	1.57	0.44	Exogenous carbon RDX-N source	(32)
Rhodococcus DN22	6.13	0.11	Exogenous carbon RDX-N source	(18)
Rhodococcus DN22	6.19	0.11	Exogenous carbon RDX-N source	(19)
Rhodococcus rhodochrous 11Y	6.31	0.11	Exogenous carbon RDX-N source	(36)

TABLE 4.4 Aerobic Fungi Group. Calculated rate ccoefficient and half-life value derived from studies using aerobic fungi. Source of inoculum and culture condition described by primary authors are provided for reference.

Inoculum	$K\ d^{-1}$	Half-life (d)	Culture condition	Half-life (d)	Source
Penicillium janczewskii strain AK9613	0.33	2.11	Co-metabolic	2.11	(11)
Penicillium strain AK96151	0.33	2.11	Co-metabolic	2.11	(11)
Phanerochaete chrysosporium	0.23	3.08	Co-metabolic (lignolytic condition)	3.08	(23)
Penicillium janczewskii strain AK9664	0.22	3.16	Co-metabolic	3.16	(22)
Acremonium strain CSSF-1	0.09	7.75	Co-metabolic	7.75	(23)
Hypholoma fasciculare	0.08	8.89	Co-metabolic	8.89	(22)
Phanerochaete chrysosporium	0.08	8.35	Co-metabolic	8.35	(37)
Leucopaxillus candidus	0.06	11.29	Co-metabolic	11.29	(22)
Agrocybe semiorbicularis	0.05	12.98	Co-metabolic	12.98	(22)
Rhodotorula sp.	0.03	24.41	Co-metabolic	24.41	(23)
Penicillium Chrysogenum	0.03	24.41	Co-metabolic	24.41	(23)
Coprinus comatus	0.03	27.4	Co-metabolic	27.4	(22)
Clitocybe nebularis	0.02	33.81	Co-metabolic	33.81	(22)
Bulleria sp.	0.02	35	Co-metabolic	35	(23)

Half-life values calculated for each group suggest that the relative rate of RDX degradation is fastest by aerobic bacteria (mean =1.05 d, SD = 1.37 d) and slowest by fungi (mean = 14.2, SD = 11.8) with the degradation rate by anaerobic bacteria falling in-between (mean = 4.64, SD = 6.38). This is illustrated (FIG 4.1) by using Equation 1 to calculate the theoretical degradation curve for aerobic bacteria, anaerobic bacteria and fungi to degrade 100 g of RDX (assuming aqueous media, nutrients in excess, total availability of RDX, and no toxicity or inhibition).
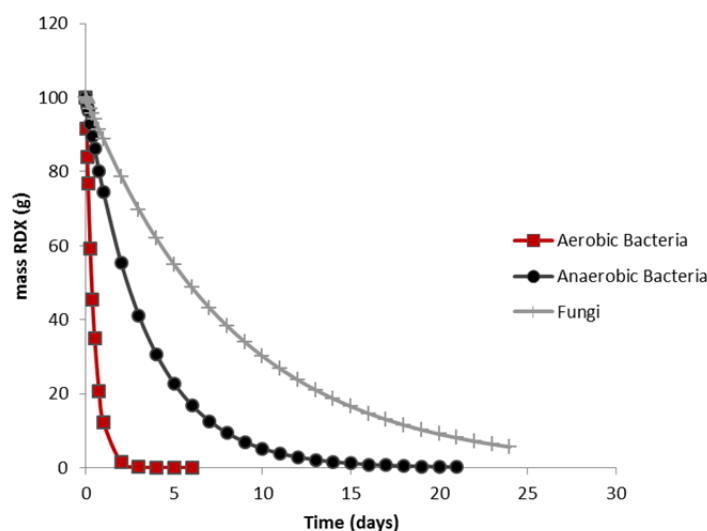
FIG 4.1 RDX degradation rate comparison for bacteria and fungi. The comparison is based on calculated first-order rate constants derived from primary literature and assumes an available initial concentration of 100 g RDX without inhibition or toxicity

Further comparison of the rate data was conducted to determine if observed differences were significant. The Shapiro-Wilk test for normality showed data were not normally distributed and were positively skewed (TABLE 4.5) based on Skewness and Kurtosis values generally greater than 1. After logarithmic transformation the data normally distributed (TABLE 4.6). Levine's test of the transformed data showed equal variances (F (2,39) = 0.130, ρ = <0.05) thus the parametric one-way ANOVA test was used to describe distribution. Results showed that the

distribution of half-life across the groups is not the same at the 0.05 level ($F(2,39) = 23.2$, $\rho <$

0.05).

TABLE 4.5 Results of Shapiro-Wilk test for normality using untransformed half-life values. Null hypothesis that data come from a normally distributed population is rejected ($\rho < 0.05$). Data appear to be generally skewed to the right based on large positive numbers calculated for Skewness and Kurtosis.

Group	Shapiro-Wilk			Descriptive Parameter	
	Statistic	df	Sig.	Skewness	Kurtosis
Aerobic Bacteria	0.678	14	0	2.6	7.56
Anaerobic Bacteria	0.639	14	0	2.29	4.758
Fungi	0.868	14	0.04	0.622	-1.162

TABLE 4.6 . Results of Shapiro-Wilk test for normality using logarithmically transformed half-life values. Null hypothesis that data come from a normally distributed population is accepted ($\rho > 0.05$). Skewness and Kurtosis values generally between 1 and -1 indicating a balanced distribution.

Group	Transformed- Shapiro-Wilk			Descriptive Parameter	
	Statistic	df	Sig.	Skewness	Kurtosis
Aerobic Bacteria	0.909	14	0.153	-0.288	-0.051
Anaerobic bacteria	0.919	14	0.21	0.795	0.387
Fungi	0.905	14	0.131	-0.298	-1.31

Post hoc analysis using the Tukey's HSD test identified significant differences in RDX

half-life values between aerobic and anaerobic bacteria ($\rho < .001$), aerobic bacteria and fungi ($\rho <$

0.05) and anaerobic bacteria and fungi ($\rho = .012$).

DISCUSSION

Few studies have directly compared the biodegradation rate of RDX by aerobic and

anaerobic bacteria and fungi. A study by Fuller, et al. (24) showed that degradation of RDX by

Pseudomonas fluorescens and *Pseudomonas putida* was faster under anaerobic conditions

compared to aerobic conditions. But, since aerobic metabolism of RDX by bacteria results in

rapid cleavage of the heterocyclic ring while anaerobic metabolism results in rapid reduction of the nitro groups followed by slower ring cleavage (19, 38), it might be inferred that more complete degradation is faster under aerobic conditions. While research has shown that degradation of RDX by white rot fungi in lignolytic conditions is slow with $t_{1/2}$ values of weeks (37), other studies show that filamentous fungi growing in non-lignolytic conditions degrade RDX with $t_{1/2}$ values of days (39). The combination of results from many studies using meta-analysis is a much stronger predictor of degradation rates than predictors based on individual studies, and may be a means for reconciling seemingly contradictory data. Based on this study, the degradation rate of RDX by aerobic bacteria is statistically faster than for anaerobic bacteria and fungi, while anaerobic bacteria degrade RDX faster than fungi.

This study is the first attempt to broadly characterize and compare RDX biodegradation kinetics by bacteria and fungi using a meta-analysis approach. Ma, et al. (40) built quantitative structure activity relationship (QSAR) models for dissipation of 16 PAHs in the rhizosphere using meta-analysis to estimate the effect of size. He found it to be a powerful method to group individual studies for a more robust prediction of the true effect size. Meta-analysis has also been used to attribute microbial diversity (based on functional gene response) to environmental conditions and oil concentration in contaminated soils (41). Gan, et al. (42) combined phenotype data for *Novosphingobium* strains from the literature with meta-analysis of the complete genome and identified genes associated with industrial processes and bioremediation.

Currently, published research tends to focus on the feasibility of RDX biodegradation and on the details of metabolic pathways. Due to the emerging nature of research in this area, development of comparative kinetics may be premature. An estimate of biodegradation rate however, even a preliminary one, is important to consider at an early stage when evaluating the general feasibility of biotechnology as a remediation or explosive disposal solution. A completely viable and understood metabolic pathway for RDX biodegradation may have little

practical value if the time required by the mitigating organism is greatly extended. The relative rate comparison made here however, is likely to be obscured by other factors influencing degradation rate such as mass-transport, toxicity, and availability.

The kinetic values derived in this study through the graphic analysis of initial and final concentrations reported in primary treatability studies are of course less accurate than multipoint degradation studies conducted for the sole purpose of determining rate. The use of 42 data sets however allows statistical conclusions that are based on a larger sample size than typically practical in individual laboratory studies. Although the sample set reflects current publishing activity on this topic, the total number of cases may still be insufficient to accurately reflect a true distribution. Confidence in the data characterization is increased by the Shapiro-Wilk test, which by design has sufficient power at low sample sizes to avoid incorrect rejection of the hypothesis that the distribution of half-life is the same across all groups (i.e., Type I error). The analysis uses the broad category of aerobic bacteria, anaerobic bacteria and fungi as independent variables and the calculated half-life of RDX as the dependent variable. How the organisms used the RDX (co-metabolically, as a carbon source, or as a carbon and nitrogen source) was not a factor in the study. Future work is needed to better understand the effect of the specific metabolic pathway on data distribution and on the resultant degradation trends described for anaerobic and aerobic bacteria and fungi.

CONCLUSIONS

1. Meta-analysis of published research data for RDX biodegradation is a useful tool to identify trends otherwise obscured or possibly misleading at the individual study level.

2. Based on a sample of 42 studies there is a significant difference ($p<0.05$) in the rate of RDX degradation between aerobic, anaerobic bacteria and fungi.

3. Relative degradation rates are fastest for aerobic bacteria and slowest for fungi among the three groups.

4. This is the first study to broadly characterize and compare degradation rates of explosives using a meta-analysis approach. It is therefore, only a preliminary assessment of trends derived from a larger set of data. Additional research is needed to increase the number of studies included in the meta-analysis and to better understand the effect of the metabolic pathway on degradation rate.

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Chapter 5

Alkaline hydrolysis of tetryl contaminated soil

ABSTRACT

The historic use of the explosive 2,4,6-trinitrophenylmethylnitramine (tetryl) has resulted in soil contamination at a variety of military facilities. Due to its toxicity, remediation is required to reduce or remove tetryl from the environment. Alkaline hydrolysis is a demonstrated remediation method for soils containing explosive compounds. Although substantial research exists for alkaline hydrolysis of the explosives RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), HMX (octa-hydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine), and TNT (2,4,6-trinitrotoluene) few studies have been conducted on tetryl contaminated soil. This study is the first to compare the effectiveness of alkaline agents in hydrolyzing high (granular) concentrations of tetryl (2,000 mg/kg and 25,000 mg/kg) from soil. Gravelly, sandy-loam soils were collected from two contaminated disposal pits at a former manufacturing facility. Tetryl hydrolysis by the alkaline agents calcium peroxide, calcium hydroxide, calcium magnesium hydroxide, sodium hydroxide and potassium hydroxide were compared at a mass loading of 2.5% , and the effect of mass loading was determined by applying calcium hydroxide to soil at 1%, 2.5% and 5% by weight. Test samples were mixed and agitated in the dark at 25 $^{\circ}$C for 72 hours prior to analysis. Complete hydrolysis of 25,000 mg/kg tetryl occurred when the molar concentration of hydroxyl ions is equal to or greater than that of tetryl. This threshold occurs at pH 13. Partial hydrolysis of tetryl occurs at lower pH values. Thus calcium oxide (pH< 12) is less effective than calcium hydroxide and calcium magnesium hydroxide (12<pH< 13) in hydrolyzing 25,000 mg/kg tetryl whereas the most effective alkaline agents are sodium and potassium hydroxide (pH>13). Incomplete hydrolysis of tetryl at pH <13 resulted in a maximum removal of 20,000 mg/kg. Picric acid, methylnitramine, and N-methylpicramide are possible organic products produced

during hydrolysis of tetryl. Additional work however is needed to analytically identify products produced by this process.

INTRODUCTION

Tetryl (2,4,6-trinitrophenylmethylnitramine) is an explosive compound used extensively by the U.S. Military in booster charges and detonators from about 1916 to 1979. Due to its toxicity, tetryl has been replaced by the explosive compound RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) and is no-longer used or manufactured in the United States (1). Historical use of tetryl however has resulted in many reports of soil contamination at military manufacturing, loading, and training facilities and remediation is often required to protect human health and the environment (2).

Many researchers have demonstrated the successful remediation of soils impacted by the explosive compounds tetryl, RDX, HMX (octa-hydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine), and TNT (2,4,6-trinitrotoluene) using alkaline hydrolysis (3-7). Alkaline hydrolysis is highly effective for degrading nitramine and nitroaromatic explosives because electron-withdrawing nitro groups make the explosive molecule electron deficient and susceptible to nucleophilic attack by hydroxide ions (8). Although substantial research exists for alkaline hydrolysis of TNT, RDX and HMX, few studies describe the process for tetryl.

A variety of alkaline agents have been used to increase the pH of soil and water to levels favorable for explosive hydrolysis. Arienzo (9) compared the effectiveness of the alkaline agents sodium hydroxide (NaOH), calcium peroxide (CaO_2) and calcium hydroxide ($CaOH_2$) on TNT hydrolysis in water and soil slurry systems at 25^oC. Although all agents were effective, $Ca(OH)_2$ removed TNT the fastest. Furthermore, removal of TNT improved as mass loading of both $Ca(OH)_2$ and CaO_2 increased from 0.1% to 2% and pH increased from 8.0 to 12.5. Unfortunately, no further discussion of this correlation was provided. Other researchers have investigated the

use of commercial lime products containing $Ca(OH)_2$ for the purpose of remediating and

managing military training range soils contaminated by RDX, HMX and TNT (10). These

studies also note that an increase in pH, resulting from higher mass additions of (1%, 3%, and

5%) hydrated lime ($Ca(OH)_2$), correlate to faster reaction kinetics. The efficiency of hydrolysis

as a result of liming, however, depends on the direct contact of the explosive compound and the

hydroxyl ion, and is assumed to take place in the soil pore water (11). The optimal soil moisture

for hydrolysis of TNT is approximately 25%-30% w/w (12).

The relationship between pH and explosive concentration is suggested by titration studies

that indicate a pH between 10 and 11 is needed for degradation of 20 mg/kg TNT wheras pH 12

is needed to degrade 86 mg/kg (12). An analysis by Heilmann, et al. (6) shows that hydrolysis of

RDX is faster and more complete as temperature and/or pH increase. The "completeness" of

hydrolysis is defined stoichiometrically for the purpose of estimating alkali consumption and

assumes that, in the case of TNT, hydroxide ions substitute for the nitro groups. Thus, one to

three moles of OH ion would be needed per mole of TNT. In fact, work by Emmrich (13)

demonstrated the release of two nitro groups per TNT molecule at pH 13 while only 1.5 nitro

groups were released at pH 12.

It is generally assumed that alkaline hydrolysis occurs according to pseudo-first order

kinetics where the concentration of hydroxide is constant (3, 6, 10, 13) and may occur only if the

hydroxyl ion concentration exceeds that of the explosive (13, 14). Thus, relatively low

concentrations of explosives (64 mg/kg RDX and 17 mg/kg TNT) may be completely degraded,

while hydrolysis of higher concentrations (1,853 mg/kg RDX and 58 mg/kg HMX) may be

incomplete at the same pH and treatment duration (10). This is consistent with findings that show

products formed at pH greater than 11 are generally small molecular weight molecules suggesting

more complete degradation of intermediates while larger molecules form at a lower pH (4)

suggesting less complete degradation. The specific products of alkaline hydrolysis depend on the

explosive target and extent of degradation. It is generally understood that prior to elimination of the nitro group on the TNT molecule the hydroxyl ion and the nitro group both remain attached to the same carbon forming a Meisenheimer complex that gives the intense color typical of TNT "pink water" (15, 16). Except for small amounts of nitroanilines and methylanilines the major end products of TNT hydrolysis are nitrite, nitrous oxide, ammonia, formaldehyde and formate (17). Similarly, tetryl hydrolysis reportedly occurs through a Meisenheimer complex releasing nitrate and nitrite and the organic compounds methylnitramine, *N*-methylnitramine, and picric acid (18).

The purpose of this study is to investigate the role of pH in tetryl hydrolysis through a comparison of the effectiveness of the alkaline agents calcium hydroxide, calcium-magnesium hydroxide, sodium hydroxide, potassium hydroxide and calcium peroxide in the treatment of soil containing 25,000 mg/kg and 2,000 mg/kg tetryl.

MATERIALS AND METHODS

Sample Collection. Soil samples were collected from 2 waste-discharge pits located at a former munitions manufacturing site. Site locations were discovered and marked during a remedial investigation unrelated to this work. Pits were unlined and records indicate a dimension of approximately 0.3 m to 1.5 m deep and about 3 m wide. Soils in both locations contained weathered bedrock and rubble surrounded by what appeared to be a greyish-brown gravelly sandy-loam. A composite soil sample was made for each disposal pit by collecting equal volumes of soil (about 500 mL) from each of three locations representing the surficial, middle and deep layers of the pit. Soils from each layer were mixed together in equal proportions to form a 1.5 L composite sample from pit-1 and a 1.5 L composite sample from pit-2.

Analytical. The pH of each sample was determined using Environmental Protection Agency (EPA) method SW-846 method 9645C. Measurements were made by electrode (Fisher Scientific, Fairlawn, NJ) in the aqueous phase of a 1:1 water sample mixture (v/v). Moisture content was measured gravimetrically after drying to constant weight at 105 ^{o}C (19). Tetryl concentration was determined using an acetonitrile and ultrasonic extraction method (20). The extract was analyzed by reversed-phase high performance liquid chromatography (HPLC) with a photodiode detector. A Waters Nova-Pak C18 column (30 cm x 3.9 mm, 4 µm particle size) with a 70% to 30% (v/v) Baker HPLC grade acetonitrile and water mobile phase at a flow rate of 1 mL per min was used for the analysis. Water used to prepare the mobile phase was purified using a Milli-Q Type I Reagent Grade Water System (Millipore Corporation). Chromatograms were examined at 254 nm using Empower data acquisition software. Tetryl concentrations were determined in comparison with known standard solutions (Restek Chromatography Products and Solutions).

Sample Characterization. Soils from each composite were classified into textural fractions based on standard methods for sieve analysis described in ASTM D 422-Standard Test Method for Particle-Size Analysis of Soils. A set of 6 stainless-steel U.S. Standard Sieves (Nos. 5, 10, 35, 60, 120, and 230) were weighed and stacked upwards from highest number (finest) sieve to the lowest. Air-dried composite soil (500 g) was placed in the top sieve, covered and the sieve stack shaken for 10 min. Particles with diameters greater than 2 mm were excluded from the study. Soils from both pits appeared visually similar except that soils from pit-1 contained a yellowish powder-like substance consistent with product grade tetryl. The concentration of tetryl was determined to be more than 10 times higher in pit-1 (25,000 mg/kg) than in pit-2 (2,000 mg/kg). Soils were relatively dry with a neutral pH (TABLE 5.1) and are described as a granular sandy-loam based on particle size classification (TABLE 5.2).

TABLE 5.1 Sample characterization

Characteristic	Soil (pit 1)	Soil (pit 2)
Moisture (% by wt)	5.7+/- 0.63	8.18 +/- 0.72
pH	7.11+/- 0.15	6.99 +/- 0.08
Tetryl Concentration	25,000 mg/kg	2,000 mg/kg

TABLE 5.2 Particle size distribution for composite soil from
 pit 1 and pit 2

Fraction identification	Size (mm)	U.S. Sieve size	Fraction percent (w/w)
Pebbles-very fine granules	4-2	5-10	20
Sand- coarse- very coarse	1 - 0.5	10-35	13
Sand- medium	0.24 – 0.5	33-60	25
Sand-fine	0.125 – 0.24	60-120	15
Sand – very fine	0.625 – 0.125	120-230	11
Clay and Silt	<0.125	<230	16

Hydrolysis Tests. Hydrolysis tests were conducted by adding 2.5% (w/w) of the

alkaline agent to 30 g soil samples containing 2,000 mg/kg and 25,000 mg/kg tetryl. Tests were

conducted in triplicate at each concentration using sodium hydroxide and potassium hydroxide-

KOH (Fisher Scientific, Fairlawn, NJ); calcium peroxide (Alfa Aesar, Ward Hill, MA); calcium

hydroxide -$Ca(OH)_2$, (Sigma, St. Louis, MO); and calcium magnesium hydroxide -$CaMg(OH)_4$

(Super Limoid Type SA, Graymont Dolime Inc., Genoa OH). The effectiveness of loading rate

on tetryl hydrolysis was determined by adding 1%, 2.5%, and 5% (w/w) of the lime product

CaO_2, $Ca(OH)_2$ and $CaMg(OH)_4$ to 30 g soil samples containing 2,000 mg/kg and 25,000 mg/kg

tetryl. The mass loadings selected for this study provide excess molar ratio of hydroxyl ion to

tetryl molecule (TABLE 5.3). A sample containing 30 g of tetryl contaminated soil at each

concentration but no alkaline agent was included as a test control.

TABLE 5.3 Molar ratio of hydroxyl ions (OH-) to tetryl at three mass loadings of alkaline agent.

Alkaline Agent	OH equivalents	MW g/mol	Tetryl Concentration	Mass Loading of Alkaline Agent			Tetryl Concentration	Mass Loading of Alkaline Agent		
				1%	2.50%	5%		1%	2.50%	5%
$Ca(OH)_2$	2	74		3	7.5	15		38	96	192
$CaMg(OH)_4$	4	132	25,000 mg/kg (0.08 M)	3.5	8.7	17	2,000 mg/kg (0.007 M)	43	109	218
NaOH	1	40		not tested	7.2	not tested		not tested	90	not tested
KOH	1	57		not tested	5	not tested		not tested	63	not tested
CaO_2 [1]	2	72		not tested	7.5	not tested		not tested	96	not tested

[1] assumes CaO_2 reacts with water to form $Ca(OH)_2$ + H_2O_2

Water was added to each sample (10 mL) to bring total soil moisture to approximately 35% (w/w). All test samples were mixed after addition of the alkaline agent and agitated in the dark at room temperature (25 ^{o}C) at 160 circular rpm for 72 hours (Model C25, New Brunswick Scientific, New Brunswick, NJ). Samples were air dried in the dark for 7 days prior to analysis.

RESULTS

Alkaline agents compared in this study are not equally effective in hydrolyzing tetryl in soil (FIG 5.1). When compared at a mass loading of 2.5%, calcium peroxide was the least effective alkaline agent resulting in incomplete removal of both the lower (2,000 mg/kg) and higher (25,000 mg/kg) concentrations of tetryl. Sodium and potassium hydroxide, however, completely removed both concentrations of tetryl compared to a maximum of 20,000 mg/kg tetryl removed by $CaMg(OH)_4$ and 17,500 mg/kg tetryl removed by $Ca(OH)_2$. Both $Ca(OH)_2$ and $CaMg(OH)_4$ were less effective at the reduced loading of 1% while increasing the mass addition to 5% did not improve removal of tetryl (FIG 5.2).
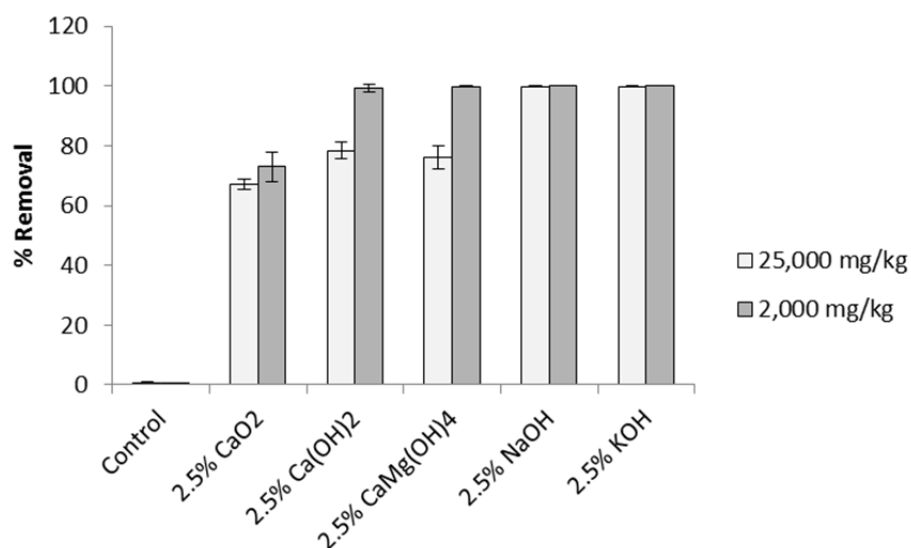
FIG 5.1 Mean (of triplicate samples) percent removal of tetryl from soil initially containing 25,000 and 2,000 mg/kg tetryl. Bars represent standard deviation.
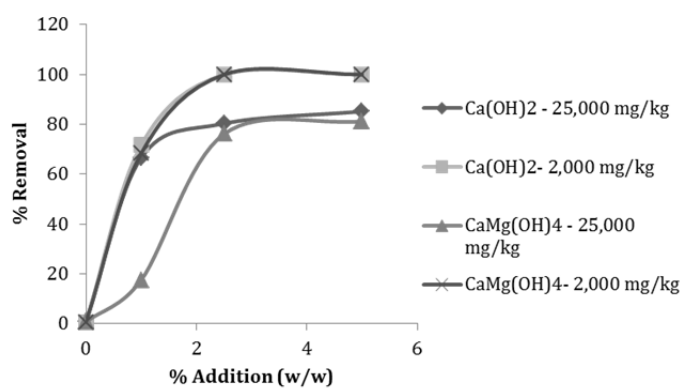

FIG 5.2 Percent removal of 25,000 and 2,000 mg/kg tetryl by alkaline agents applied at 0%, 1%, 2.5% and 5% mass loading. Removal of tetryl increases with addition of $Ca(OH)_2$ and $CaMg(OH)_4$ up to a mass loading of 2.5%.

Reaction pH of soils amended with alkaline agents at a mass loading of 2.5% ranged from a low of 11.19 for CaO_2 to a high of about 13 for NaOH and KOH. Amendments using $Ca(OH)_2$ and $CaMg(OH)_4$ resulted in soil pH of approximately 12 (TABLE 5.4). The pH remained relatively constant (+/- 0.2) for each treatment throughout the reaction period.

TABLE 5.4 Measured pH of test soil after 2.5% mass addition of alkaline agent

	Tetryl Concentration (mg/kg)	
Alkaline Agent	25,000	2,000
None	6.99	7.22
2.5% CaO_2	11.21	11.19
2.5% $Ca(OH)_2$	12.32	12.33
2.5% $CaMg(OH)_4$	12.75	12.68
2.5% NaOH	13.11	13.15
2.5% KOH	13.01	13.06

A chromatographic analysis of soils containing 25,000 mg/kg tetryl and treated with NaOH, KOH, $CaMg(OH)_4$ and $Ca(OH)_2$ at a mass loading of 2.5% presented emerging peaks corresponding to retention times of 1.75 min and 1.98 min (FIG 5.3). These peaks are present in all treatments. Compound identification was not included in this study.
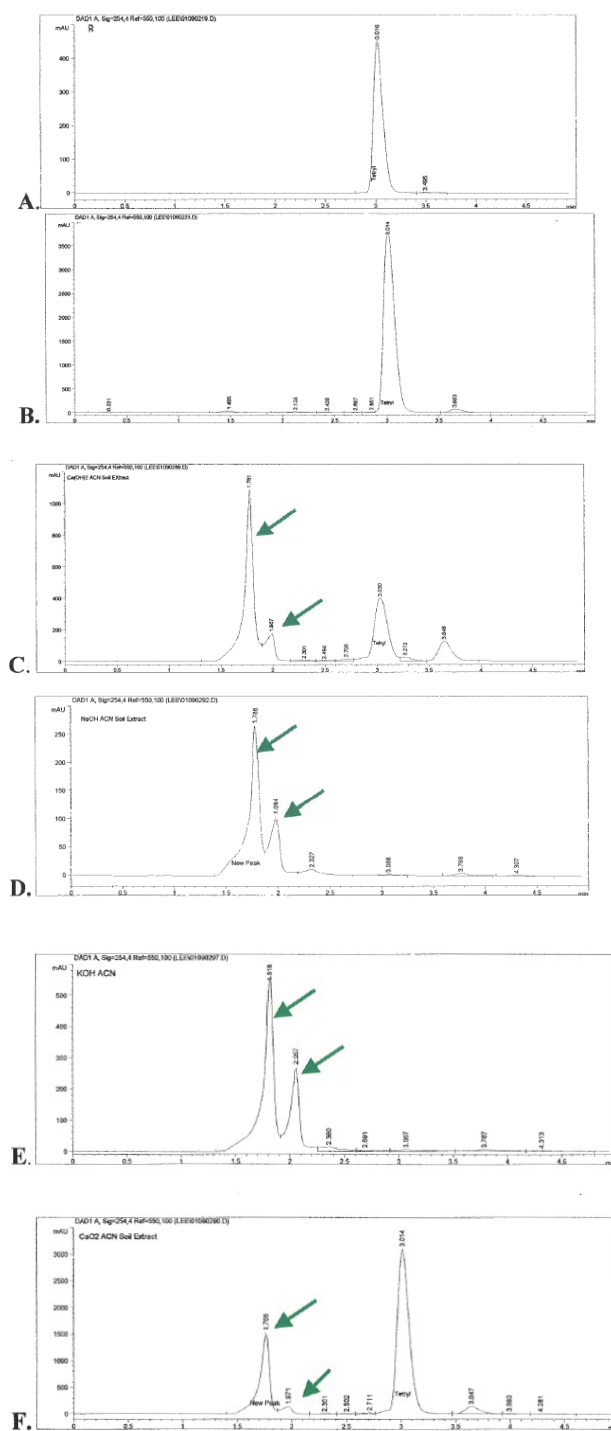
FIG 5.3. Chromatographic analysis of soil from sample 1 (2.5% tetryl) treated with 2.5% alkaline reagent: (A) tetryl standard; (B) untreated control; (C) $Ca(OH)_2$; (D) NaOH; (E) KOH; (F) $CaMg(OH)_4$ Arrows indicate emerging peaks. Identification of compounds associated with emerging peaks was not included in this study.

DISCUSSION

This is the first study to compare the effectiveness of alternative alkaline agents on the hydrolysis of high concentrations of tetryl in soil. Results show that higher concentrations of tetryl are degraded as pH is increased. Thus the alkaline agents sodium hydroxide and potassium hydroxide, with a reaction pH above 13, degraded 25,000 mg/kg tetryl while the lime products calcium hydroxide and calcium magnesium hydroxide degraded 20,000 mg/kg at a reaction pH at around 12. Research by Emmrich (13) shows that alkaline hydrolysis of TNT in solution occurs only if the hydroxyl ion molar concentration exceeds the molar concentration of TNT. If this observation is true for tetryl, complete hydrolysis of 25,000 mg/kg would require at least 0.087 M of OH⁻ and 2,000 mg/kg tetryl would need 0.007 M OH⁻. A mass loading of 2.5% alkaline agent delivers a molar ratio of OH⁻ to tetryl of about 5:1 for KOH; 7:1 for NaOH; 8:1 for $Ca(OH)_2$ and 9:1 for $CaMg(OH)_4$. Since each alkaline agent delivers an excess of OH⁻ , complete hydrolysis by all agents would be expected. Complete hydrolysis of 0.087 M tetryl occurred, however, only when using sodium and potassium hydroxide, which, by comparison to the lime products, delivered a lower molar ratio of hydroxide ions. Bajpai, et al. (14) studied the stoichiometry of OH⁻ consumption and found that about 13 mole of OH⁻ per mole of TNT was needed for complete degradation. Complete degradation of 25,000 mg/kg tetryl in this study, however, was observed when potassium and sodium hydroxide were supplied at molar concentrations well below 13. Furthermore, increasing the mass loading of $Ca(OH)_2$ and $CaMg(OH)_4$ to 5% did not improve degradation above that observed at a mass loading of 2.5%. These results are consistent with kinetics that are independent of hydroxyl ion concentration (6). The hydrolysis of TNT as a function of hydroxide ion concentration at various pH values is discussed by VanEngelen (21). Her work demonstrates that the degradation of TNT is a function of a constant concentration of OH⁻ at a measured pH. Complete hydrolysis of 25,000 mg/kg tetryl in the present study was observed at pH 13 and pH 12 for tetryl concentration of 2,000 mg/kg. At these pH values the

molar ratio of OH⁻ to tetryl is approximately 1:1 while below this threshold the molar

concentration of OH⁻ is less than the molar concentration of tetryl (TABLE 5.5).

TABLE 5.5 Molar ratio of hydroxyl ion to tetryl at 2,000 and 25,000 mg/kg as a
function of pH

| | | [OH⁻]:Tetryl Molar Ratio | |
pH	OH⁻ M	Tetryl = 25,000 mg/kg	Tetryl = 2,000 mg/kg
7	1.00E-07	1.15E-06	1.44E-05
8	1.00E-06	1.15E-05	1.44E-04
9	1.00E-05	1.15E-04	1.44E-03
10	1.00E-04	1.15E-03	1.44E-02
11	0.001	0.0115	0.1435
12	0.01	0.1148	1.435
13	0.1	1.148	14.35

Thus, the pH resulting from the mass addition of the selected alkaline agent is the

limiting factor for hydrolysis of a given concentration of tetryl. Although some research suggests

that hydrolysis does not occur if, at a given pH, the molar concentration of OH- is less than the

molar concentration of the explosive,VanEngelen (21) showed that as long as pH was constant,

hydrolysis occurred, but at a slower rate. This is consistent with results of the present study

where test concentrations of tetryl are incompletely hydrolyzed by the alkaline agent CaO_2 at pH

11 while the lime products $Ca(OH)_2$ and $CaMg(OH)_4$ result in a soil pH 12 (2.5% mass loading)

and completely hydrolyze tetryl at 2,000 mg/kg but incompletely hydrolyze tetryl at 25,000

mg/kg. Improved treatment is not expected by doubling the mass loading to 5% since increased

lime does not raise the pH to the threshold level of 13. Hydrolysis of the higher concentration is

complete only at pH 13 or above resulting from the application of NaOH and KOH. More

complete removal of tetryl at a lower pH value may have been achieved by extending the

treatment period beyond the 72 hr shaking and 7 day drying period. This possibility should be

considered in future analyses.

Tetryl hydrolysis by the alkaline agents used in this study, is accompanied by the

emergence of products seen as three emerging peaks on the HPLC chromatogram (FIG 5.3).

Although direct identification of these compounds was not included in this study, results are

consistent with findings by Kayser, et al. (18) that demonstrate the formation of two peaks with

retention times (RT) of approximately 1.75 and 1.98 min, and one peak with a retention time

approximately 3.6 min as a result of alkaline hydrolysis. By comparison to the work by Kayser,

et al. (18) the peaks formed during the current study may be provisionally identified as picric acid

(1.75 min RT), methylnitramine/NO_2/NO_3 (combined peak area at 1.98 min RT) and 2,4,6-

trinitro-n-methylamine, commonly known as *N*-methyl picramide (3.6 min RT) (Fig 5.4). It is

interesting to note that the peak emerging at 3.6 min is evident only in chromatograms

documenting incomplete hydrolysis of tetryl by lime products FIG 5.3), C and F) and not when

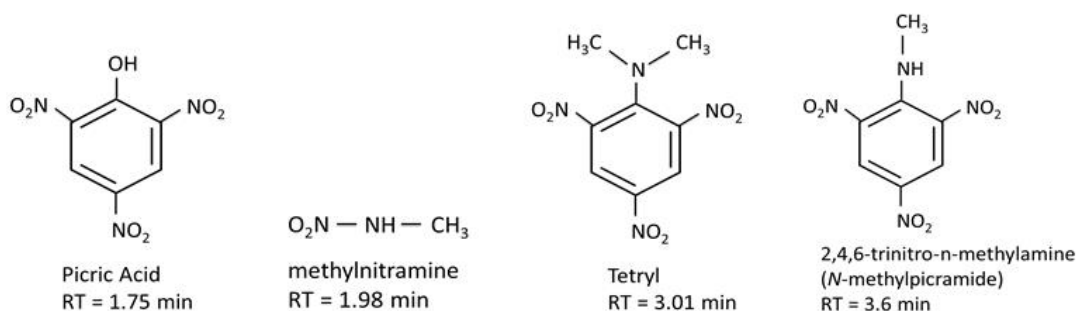tetryl is completely hydrolyzed by sodium or potassium hydroxide (FIG 5.3, D and E).

FIG 5.4 Alkaline Hydrolysis products of tetryl postulated by comparison to work by Kayser, et al. (18).
Compounds are listed in order of shortest to longest retention time (RT) by HPLC.

The postulated products are consistent with work by others who have shown alkaline

hydrolysis of explosives to occur by reduction of nitro groups through a Meisenheimer charge

complex resulting in picric acid and ring cleavage producing methylated nitramines (5, 15, 22-

24).

CONCLUSIONS

1. High soil pH (high OH^- concentration) resulting from the addition of an alkaline

     agent is believed to be the key driver for effective hydrolysis of tetryl.

2. An equal molar concentration of tetryl and hydroxyl ions are needed for complete hydrolysis of tetryl while incomplete hydrolysis occurs when the molar concentration of OH^- is less than that of tetryl. Successful application of alkaline agents as a remedial approach for tetryl contaminated soil depends on the maximum reaction pH imposed by the selected agent as an indicator of OH^- concentration and balance it against the concentration of tetryl.

3. The mass addition of alkaline agent is determined by the amount of agent required to raise the soil pH to the level needed to provide the molar equivalent or excess of hydroxyl ion.

4. Picric acid, methylnitramine, and 2,4,6- trinitro-n-methylamine (*N*-methylpicramide) are proposed as the organic products produced during hydrolysis of tetryl. Additional work is needed to analytically confirm product identification.

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Chapter 6

Synthesis and follow-on research

RESEARCH SIGNIFICANCE

The goal of field-scale bioremediation research is to bridge the technology gap between basic research and commercial application. This gap exists because, although laboratory data are used to inform the design of field systems, remediation results from field studies are seldom published. Diplock, et al. (1) conducted treatability studies on 20 hydrocarbon contaminated materials and 6 field scale systems and concluded that laboratory derived degradation rates "significantly overestimated" rates obtained in the field. Kumar, et al. (2) also describe a disconnect between laboratory research and commercial application of bioremediation and suggested that poor prediction of field scale treatment is most likely due to complex soil heterogeneity, and the unpredictability of microorganisms due to variability in populations. The effect of material variability on treatability can be reduced by increasing the size of the test system. Large scale field pilot tests are therefore better than laboratory studies for predicting performance of full scale bioremediation systems.

Performance in commercial remediation application, is generally defined by three questions: how fast does the contaminant degrade, how far (extent of degradation), and how much will it cost? Treatment feasibility in this context is, therefore determined by the probability that the system will meet regulatory objectives in an economically defined duration. Results from this study frame the major decision points in a bioremediation feasibility assessment: degradability, bioavailability, and degradation rate (FIG 6.1).
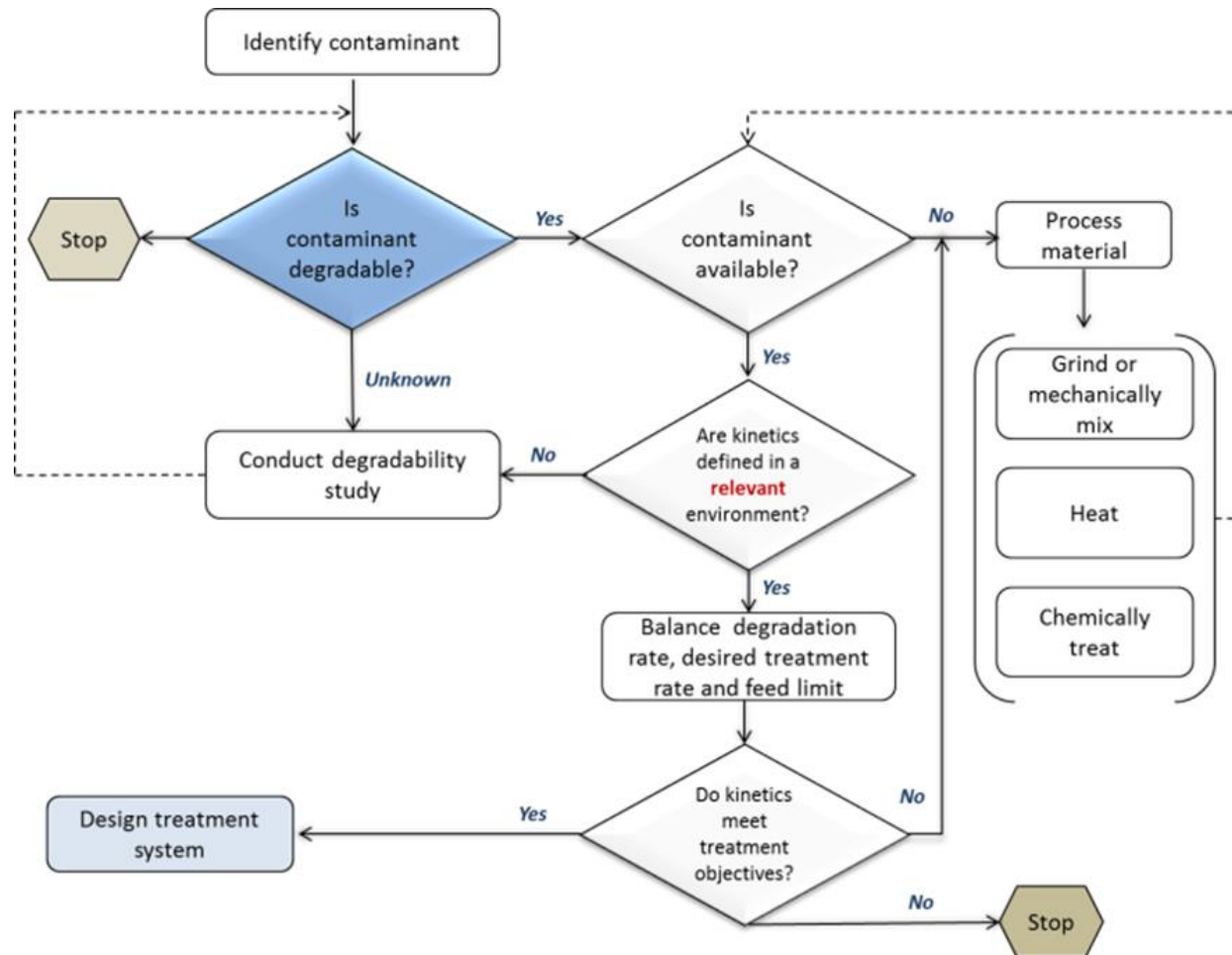
FIG 6.1 Decision flow chart for preliminary feasibility determination for bioremediation

Field scale treatability tests were conducted on dye manufacturing waste sludge to determine the biodegradability of mixtures of chemical compounds. Compound degradation patterns observed during treatment were interpreted by extrapolating results reported in the literature for multi-substrate interactions in mixtures similar to the dye waste. The resulting conclusions were that the carbon to nitrogen (C:N, see list of abbreviations, page xi) ratio, compound concentration ratios, and toluene concentration were potential controlling factors for treatment. A follow-on laboratory study designed to reproduce degradation patterns observed in the field for nitrobenzene, chlorobenzene, and xylene, by adjusting the C:N and toluene concentration, would be a logical next step to validate the extrapolated interpretation. Identifying the organisms responsible for the degradation patterns would also be interesting. Detailed microbial analyses are not normally done in field scale projects since evaluation is performance based and community activity, rather than make up, is considered more relevant. Heterotrophic plate count estimates are, however, sometimes used by industry to confirm the presence of active microbial populations. Population estimates for heterotrophic bacteria in the range of 240 to 5.8 x 10^7 CFU g^{-1} (colony forming units) compared to a density of 6,400 to 4.1 x 10^8 CFU g^{-1} for specific hydrocarbon degraders, have been reported for fuel oil contaminated soil, with low microbial densities correlated to low degradation rates (1). Specific identification of microbes has been used, in some circumstances, to create seed cultures for augmentation of bioremediation systems. The literature is consistent, however, in reports of biostimulated indigenous organisms out performing systems augmented with exogenous microbes (3-5). Knowledge of the specific make-up of the microbial populations in this study could improve future treatment since degradation patterns appear to be caused by specific metabolic capabilities.

Although specific microorganisms were not identified in this study, nitroaromatic degradation rate meta-data were established for broad groups of microorganisms. Meta-analysis showed that aerobic bacteria, anaerobic bacteria, and fungi degrade the nitroaromatic compound

RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) at different rates. Degradation rates by aerobic bacteria were faster than rates by anaerobic bacteria and fungi. Thus, treatment duration is expected to be minimized in systems designed and operated to assure air supply meets demand. Mechanical aeration of large soil systems is often difficult due to material heterogeneity and anaerobic pockets, though undesirable, may be unavoidable. This may reduce the overall treatment rate. The management of moisture and pH in the material is also critical since dry or acidic conditions favor fungal growths, which are relatively slow degraders of RDX. Fungi have broad degradative capabilities (6-8) and although degradation may be slower, exogenous enzymes characteristic of fungi have the potential to improve treatment. Relatively little research has been conducted using fungi as the primary organism in remediation. Future studies that compare the role of bacteria and fungi in nitroaromatic degradation would contribute greatly to expanding the treatment capabilities in a variety of treatment environments.

Degradation Rate. First order biodegradation rates are a simple framework for comparing degradation of various substrate mixtures by indigenous microbial consortia. The linearized form (Equation 2) of the first order rate equation (Equation 1) can be used in feasibility testing to roughly calculate treatment duration or extent of treatment under optimal conditions: It is therefore an estimate of the theoretical best the system can achieve. Actual treatment would be lower, however, due to constraints of bioavailability and substrate interactions.

Equation 1 First order rate equation: $dC/dt = -kC_t$

Equation 2 Linearized form of first order rate equation: $LnC_o = LnC_t + kt$

Where:

- C_o = initial concentration of contaminant

- C_t = concentration of contaminant at time t

- k = kinetic constant (day^{-1})

- t = time (days) = treatment duration

Degradation rate data derived in Chapter 1 result in calculated treatment durations (for achieving regulatory compliance concentrations) of 45, 273, and 78 days or 81, 32, and 97 days for benzene, nitrobenzene and naphthalene, respectively, depending on the test sample. Rate calculations assume the compound is continuously available over the extended treatment period. Even if compounds are not limited by availability, and successfully degraded in the projected time period, a duration of 81 to 273 days may not be practical based on non-technical program objectives such as short-term property sale, planned site use or the negative impact of extended costs.

Bioavailability. Degradable compounds that are not bioavailable are unlikely to degrade to full potential, resulting in a risk that treated material will not reach remediation objectives. Although mass transfer limitations are intuitive for tar-like material, the effect of lower concentrations of organic compounds on mass transfer may not be clear. Fugacity analysis can focus a project to the limiting treatment step- biodegradability or bioavailability. Compounds with a fugacity distribution to organic carbon or non aqueous phase liquid (NAPL) are likely to require additional processing steps that are often costly. Every cost component included in a full scale treatment plan requires justification. The fugacity distribution model provides a simple and transparent basis for treatment steps that increase project costs. Composting is a useful treatment design if enhanced bioavailability is required since intrinsic self-heating is an economical way to

improve desorption and diffusion rates. This may be true, however, only if particle size is reduced. Ghosh, et al. (9) observed polycyclic aromatic hydrocarbons (PAH) adsorption on the inner surfaces of soil particles. Adsorption to near surface regions and shallow diffusional distance are factors that lead to faster desorption rates relative to larger particles with longer diffusional paths. Thus, physical grinding and shredding during composting mixing was a means of improving compound availability without introducing additional processing steps. The literature is clear on difficulties encountered in biodtransforming sequestered contaminants (10-12). Field scale grinders and mixers are designed to physically break aggregates. While sheet or large tar-aggregates were broken up during mixing, small tar balls slipped through mechanical devices and remained through treatment. Pug mills designed to smash soil aggregates are not effective on tar since small tar aggregates are actually combined during milling. Mixing and grinding compost material was not sufficient for assuring compound availability.

Integrating chemical treatment for oxidation of tar-aggregates or for direct oxidation of residual chemical compounds was evaluated as a method to increase compound bioavailability and overall treatment. The bench-scale effectiveness of the Fenton and modified Fenton oxidation approach is well documented in the literature (13-16) . Transitioning this approach to commercial application, however, required an assessment of alternative chelating reagents since the common laboratory reagents were not available in bulk quantities or too expensive for the quantities needed for field application. EDTA (ethylenediaminetetraacetic acid) and citric acid were identified as alternative chelators to laboratory grade gallic acid. The integration concept for Fenton oxidation and composting assumed application as a pretreatment to composting either before or after bulking or as a post-treatment polishing step applied after composting was complete (FIG 6.2).
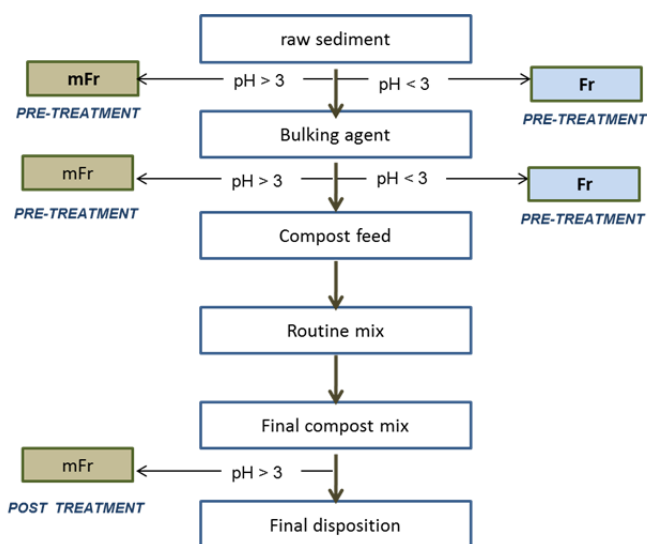
FIG 6.2. Potential insertion points for Fenton or modified Fenton oxidation for enhanced compound availability in composting

Both pre- and post- treatment applications were shown to increase the available concentration of contaminants at certain peroxide additions. The increased availability, however, did not result in biodegradability. Toxicity of hydroxyl radicals to *Xanthobacter flavus* FB71 was shown by Buyuksonmez, et al. (17) using organisms acclimated to hydrogen peroxide. Microbes responded metabolically to Fenton oxidation with an increase in catalase activity prior to complete inactivity. This suggests that even if the treatment soil is not completely sterilized by Fenton reagents, microbial degradative activity may be reduced. An interesting area for follow-on research is to determine if microbial populations can self-seed from soil areas not directly oxidized and if so, how long of a re-acclimation period is needed. A revised conceptual model for integrating Fenton oxidation and composting (FIG 6.3) based on these research results, simplifies Fenton oxidation insertion points to post treatment only. Modified Fenton oxidation (mFr) was used in this model since material is near neutral pH after composting. A material recycle back to composting (after an acclimation period) would be needed to decrease residual compound concentrations made available by mFr to complete treatment.

Dye manufacturing waste sludge

bulked

pH adjusted to
Near neutrality

acclimate

Compost

VOC

SVOC

13:1 (3% H_2O_2)
removal = 93-96%

13:1 (3% H_2O_2)
removal = 87-94%

mFr   Post-treatment

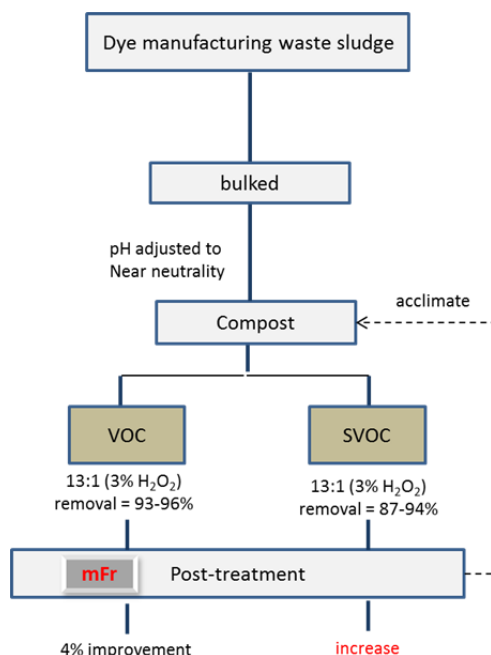4% improvement   increase

FIG 6.3 Revised integration concept for Fenton oxidation and composting

Biodegradation of contaminants, prior to post treatment by mFr, is a cost effective method of contaminant reduction. Biological degradation of high concentrations of contaminants would reduce the amount of chemical reagents used in treatment. Laboratory experiments are needed to determine if recycle of chemically oxidized compost is effective in completing treatment. This concept could be evaluated by comparing microbial activity (heterotrophic plate counts) before post treatment by modified Fenton oxidation and after various periods of acclimation following post treatment. Acclimated material could then be composted and residual concentrations of contaminants measured in the final composted material.

Unlike Fenton oxidation, base hydrolysis is an effective chemical treatment for complete or near complete removal of high tetryl concentrations from soil. Residual break-down products were identified, however, and additional treatment by biodegradation may be necessary to complete treatment. Thus, pretreatment by base hydrolysis would be a preferred treatment configuration. Hydrolysis of a range of tetryl concentrations can be accomplished by applying construction grade hydrated lime (Dolomite- $CaMg(OH)_4$) in dry form to treatment material. The

effectiveness of base hydrolysis dramatically reduces the risk of residual explosives remaining after treatment.

CONCLUSIONS

1. Biodegradability, bioavailability and degradative kinetics are critical components of treatability studies. Degradation patterns for components of complex chemical mixtures can indirectly identify conditions that control degradative outcome. When the degradability of compounds are known, and residual concentrations partition to the organic fraction of the treatment material , additional treatment steps to improve compound bioavailability are warranted.

2. Biotreatment methods that include vigorous material handling steps can help to reduce the particle size and associated diffusion pathways for hydrophobic compounds, thereby improving compound bioavailability. Field scale mechanical mixers and grinders are designed to break large particles into smaller ones but small diameter contaminated materials can remain intact through the mixing or grinding process. Improved equipment is needed to break small diameter tar aggregates during treatment.

3. Chemical methods are alternatives to mechanical processes for improving compound bioavailability in contaminated materials and can also directly reduce contaminant concentrations.

4. Modified Fenton oxidation can be integrated with composting as a post treatment application to remove or improve availability of residual compounds. Increased bioavailability at this stage would require material recycle back to composting for further treatment.

5. Chemical treatment by base hydrolysis can be integrated as a pretreatment to bioremediation, since organic compounds produced by tetryl hydrolysis may require additional treatment.

6. Biodegradation of residual explosive compounds can be accomplished by broad groups of microorganisms. Degradation rates appear to be group specific with faster rates of RDX biodegradation reported for aerobic and anaerobic bacteria than for fungi. This is important because engineered conditions that favor one group over another may offer a future method for controlling treatment duration and efficiency of explosives as well as other hydrophobic organic compounds.

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