

**EFFECTS OF REDOX POTENTIAL AND pH ON THE FATE OF
NITROGLYCERIN IN A SURFACE AND AQUIFER SOIL**

A Thesis

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LIST OF ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Disease Registry
GDN	Glycerol Dinitrate
GMN	Glycerol Mononitrate
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
NG	Nitroglycerin
NIOSH	National Institute for Occupational Safety and Health
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
TNT	2,4,6-trinitrotoluene
USDHEW	United States Department of Health, Education and Welfare
USEPA	United States Environmental Protection Agency

ABSTRACT

Nitroglycerin, used in propellant formulations and ignition cartridges in military weapons, has been found as a surface soil contaminant on military installations. Concerns have arisen since the fate and mobility of nitroglycerin is not understood in these natural environments. The objective of this study was to determine the fate of nitroglycerin in natural field soils. Nitroglycerin degradation was examined using a surface soil and an aquifer soil under aerobic and anaerobic conditions at three pH levels. Studies were also performed to determine the influence of a supplemental carbon source on degradation. Radiolabeled ^{14}C -nitroglycerin was used to trace the partitioning between aqueous and soil phases. The only nitroglycerin remaining in solution appeared at pH 6 under aerobic conditions in both test soils. The addition of glucose as a carbon co-substrate did not exert a substantial effect on the rate of nitroglycerin degradation. Mass balance studies revealed partitioning differences between the two test soils with unidentified derivatives in both water and soil phases. Results from this study demonstrate nitroglycerin will not remain in parent form in anaerobic or aerobic environments (except in acidic locations), with carbon content having little influence on degradation rates.

CHAPTER 1

INTRODUCTION

Overview

Nitroglycerin (NG) is a powerful explosive widely used by the military in weapon ignitions and propellant formulations. The Environmental Protection Agency's Toxic Release Inventory Report revealed that 156,305 pounds of nitroglycerin were released to the environment or transferred by Army facilities in 2001 (United States Environmental Protection Agency (USEPA), 2001). Use of nitroglycerin in military activities has resulted in detections of nitroglycerin soil residues at manufacturing sites and military installation test ranges (Jenkins et al., 2001, 2002; Pennington et al., 2001, 2002, 2003). Concerns have developed that releases and residues may pose a threat for nitroglycerin to move through soil to contaminate groundwater and surrounding environments, resulting in adverse environmental and health impacts.

Little is known concerning nitroglycerin fate and mobility in contaminated field soils and sediments. Fate and transport studies demonstrate microbial degradation of nitroglycerin under aerobic and anaerobic conditions, with and without the need for an additional energy source, but these studies have utilized test media other than natural field soils (Bhaumik et al., 1997; Accashian et al., 1998, 2000; Christodoulatos et al., 1997; Smith et al., 1983; Marshall and White, 2001; Blehert et al., 1997). Toxicity data have focused on occupational safety in manufacturing plants and on the medicinal value of nitroglycerin as a cardiovascular vasodilator. Information on environmental toxicity from nitroglycerin contamination is limited, conflicting and dated. Therefore, to understand the fate of nitroglycerin in these natural environments is essential to the evaluation of the potential threat NG holds in the environment and to the development of remediation processes to eliminate any harmful effects.

Objectives

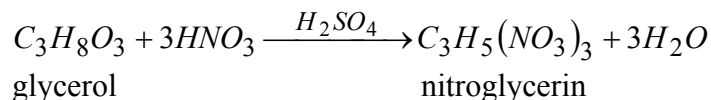
The objectives of this thesis are to compare the fate of nitroglycerin in natural field soils. Specifically, this study was designed to

- Determine the effects of redox potential (Eh) and pH on NG degradation potential using an oxidized system and a reduced system under a range of pH values from acidic to basic
- Compare the behavior of NG in a surface soil with that in an aquifer soil to determine what extent organic carbon exerts on nitroglycerin reduction
- Determine the influence of a supplemental carbon source on the disappearance rate of NG
- Determine the mobility and partitioning of nitroglycerin between aqueous and soil phases using ^{14}C -NG

CHAPTER 2 REVIEW OF LITERATURE

Discovery and Chemical Properties of Nitroglycerin

Nitroglycerin was discovered in 1846 by Italian chemist, Ascanio Sobrero (Columbia Electronic Encyclopedia, 2003). Sobrero synthesized nitroglycerin through direct nitration of glycerol with highly concentrated nitric acid in the presence of sulfuric acid:



The resulting pale yellow, viscous liquid was extremely sensitive to explosive initiation by shock creating an extremely dangerous and unpredictable compound. The three nitrate groups act as oxidizing agents coupled with hydrocarbon molecule fuel sources to produce an enormous amount of heat upon decomposition. When detonated, nitroglycerin produces about 10,000 times its own volume of gas (Microsoft Encarta, 2001). Nitroglycerin crystallizes in two forms, a labile (unstable) form with a melting point of 2.8°C and a stable form with a melting point of 13.5°C (Table 1). Even in crystallized form, the explosive shock hazard still remains. The sensitivity and highly explosive nature deemed nitroglycerin useless.

Table 1. Chemical Properties of Nitroglycerin *	
Property	Value
Chemical Formula	C ₃ H ₅ N ₃ O ₉
Molecular Structure	CH ₂ -O-NO ₂ CH-O-NO ₂ CH ₂ -O-NO ₂
Molecular Weight	227.09
Density	1.59 g/cm ³
Vapor Pressure	0.00026 mm of Hg at 20°C
Solubility in Water	1.95 g/L (Rosenblatt) 1.25 g/L (Windholz)
Heat of Detonation	218°C
Melting Point	2.8°C (¹ labile form) 13.5°C (stable form)
* References obtained from Rosenblatt et al., 1991; Windholz 1976 ¹ Unstable crystalline form	

Productive Use of Nitroglycerin

Although discovered by Ascanio Sobrero, productive use of nitroglycerin is credited to Alfred Nobel (Imperial College London, 2004). Through countless experiments and lab explosions, including one that killed his brother Emil, Alfred Nobel absorbed the liquid nitroglycerin in diatomaceous earth creating a safely handled paste. Nobel shaped the paste into rods to be used as explosive devices in the mining, quarrying and demolition industries. The product became known as dynamite. Military forces adapted the use of nitroglycerin in weapons of war and other military activity. When a newspaper mistakenly published Nobel's obituary before he actually died, Nobel was deeply disturbed to read that he would be remembered as the man who created the explosive that contributed to the carnage and destruction caused by war. To redeem his reputation, Alfred Nobel used the fortune he had made from nitroglycerin research to establish the Nobel Prize for achievements in science, medicine and literature that promote international peace.

Military Use of Nitroglycerin

Nitroglycerin alone (also known as glycerol trinitrate and 1,2,3-propanetriol trinitrate) is too unstable for practical use as a high explosive. However, safely combined with other compounds NG provides the thrust and power needed for propulsion. Nitroglycerin is used as a principal ingredient of double and triple-based propellants, blasting gelatins and ignition cartridges in various military projectiles, rockets, missiles and small arm ammunition. A common ignition cartridge is composed of 57.75% nitrocellulose, 40% nitroglycerin, 1.5% potassium nitrate and 0.75% diphenylamine (Pennington et al., 2002). Most double-based propellants are composed of nitrocellulose and nitroglycerin. Cordite is a double-based propellant containing 30-40% nitroglycerin and petroleum jelly as a stabilizer (UXOINFO, 2003). Triple-based propellants are commonly composed of nitrocellulose, nitroglycerin and nitroguanidine. Rocket propellant produced at Badger Army Ammunition Plant (Sauk County, WI) contained 50% nitrocellulose, 35% nitroglycerin, 10% diethylphthalate, 2% 2-nitrodiphenylamine and various lead fillers (United States Department of Health, Education and Welfare (USDHEW), 1978). An example of a small arms propellant contains 84% nitrocellulose, 10% nitroglycerin and 6% filler compounds (USDHEW, 1978).

Nitroglycerin Contamination on Military Installations

Concerns over potential environmental contamination from explosives residues on military installations have led to numerous range characterization studies in which explosive contaminants were identified and quantified (Jenkins et al., 2001, 2002; Pennington et al., 2001, 2002, 2003). Nitroglycerin residue contamination has been found on sampled military installations in surface soils to a depth of approximately 0.5 cm (Table 2). While representing only a small fraction of military ranges, the high residue concentrations indicate that nitroglycerin is a contaminant of concern and may be present on other ranges as well. Of further concern is the fact that the fate of nitroglycerin in these soils and the migration potential into groundwater systems is not yet fully understood.

Table 2. Nitroglycerin Contamination in Surface Soils on Military Installations (surface to 0.5 cm depth)

Facility	Maximum Concentration (ug/kg)	Source	Reference
Fort Lewis, WA	344	105-mm Howitzer	Jenkins et al., 2001
Massachusetts Military Reservation, MA	130,000	Gun firing positioning	Pennington et al., 2001
Yakima Training Facility, WA	13,000	Antitank (¹ LAW rockets)	Pennington et al., 2002
Yakima Training Facility, WA	17,000	120-mm tank	Pennington et al., 2002
Yakima Training Facility, WA	25,700	155-mm Howitzer firing position	Pennington et al., 2002
Yakima Training Facility, WA	246	81-mm mortar firing position	Pennington et al., 2002
Yakima Training Facility, WA	1,340	Claymore mine	Pennington et al., 2002
Fort Bliss - Dona Ana Range, NM	1,060	155-mm Howitzer range	Pennington et al., 2003
Fort Bliss- Dona Ana Range, NM	1,370	Artillery range	Pennington et al., 2003
Fort Bliss- Dona Ana Range, NM	1,160	Antitank (¹ LAW rockets)	Pennington et al., 2003
Fort Bliss- Dona Ana Range, NM	20,000	Crater of 155-mm Howitzer	Pennington et al., 2003
CFB Shilo, Manitoba, Canada	18.1	Gun propellants	Pennington et al., 2003
CFB Shilo, Manitoba, Canada	408	² MILAN missile	Pennington et al., 2003
Fort Drum, NY	0.5 ug/m ²	60-mm mortar	Jenkins et al., 2002
Camp Ethan Allen, VT	1,593 ug/m ²	81-mm mortar	Jenkins et al., 2002

¹LAW rockets – light anti-tank weapon

²MILAN – Missile d'Infanterie Leger Antichar, anti-tank missile

Nitroglycerin Toxicology Assessment

Human Toxicity

Sources of nitroglycerin exposure range from clinical usage as a coronary vasodilator, occupational exposure through inhalation and dermal contact, and as an environmental contaminant from military use or in wastewater discharges from commercial manufactures of dynamite. The National Institute for Occupational Safety and Health (NIOSH) reported approximately 250 million pounds of dynamite were produced by US manufacturers in 1976, with estimates of 8,000 people being exposed to nitroglycerin by inhalation and dermal absorption (USDHEW, 1978). Human toxicity studies have been conducted primarily for medicinal purposes and occupational health.

Common symptoms of human nitroglycerin exposure include headaches, dizziness, rapid pulse rate and respiratory distress. Severe cases report circulatory collapse, convulsions and death due to respiratory failure. A study at Badger Army Ammunition Plant reported 12% of 266 potentially exposed workers suffered symptoms due to nitroglycerin exposure (USDHEW, 1987). Air sampling at Radford Army Ammunition Plant concluded that nearly all sampled areas had nitroglycerin concentration exceeding the occupational safety levels of 0.2 ppm (USDHEW, 1987). The term “dynamite headache” was used to describe the violent throbbing pain produced by sudden reduction in blood pressure when exposed to nitroglycerin. After repeated exposures, workers tended to develop a tolerance to nitroglycerin and showed no symptoms. However, sudden severe symptoms developed upon re-exposure. This condition was termed “Monday Disease”, due to workers experiencing this after a weekend away from the plant.

Mammalian and Aquatic Toxicity

Whereas thorough toxicology information is available on human toxicity to NG as a medicinal product and in industrial environments, information regarding NG toxicity in other areas is sporadic. No teratogenic effects were noted in pregnant rabbits intravenously administered daily doses of nitroglycerin (Oketani et al., 1981). Genetic material did not seem to be affected in studies using in vivo and in vitro mammalian and bacterial cell systems (Lee et al., 1976) or in rats administered NG in feed (Ellis, 1978a, b), but weak mutagenic activity was noted in Ames assays (Ellis, 1978c). Reproductive material was affected by formation of carcinogenic tumors of the testes in lifetime feeding studies using rats (Ellis et al., 1978a, 1984), while Takayamam (1975) reported no reproductive abnormalities in rats and formation of non-carcinogenic tumors in mice subjected to NG contaminated drinking solutions. The available data lacks conclusive confidence as to the toxicological effects of NG.

Methemoglobin formation was a common condition found in nitroglycerin exposed test subjects. This condition prevents hemoglobin in red blood cells from carrying and delivering adequate amounts of oxygen to tissues throughout the body. Dogs dosed with daily nitroglycerin concentrations reached peak methemoglobin levels within 1-4 hours (Lee et al., 1976). Methemoglobin levels disappeared from the blood within 8-24 hours after dosing. The lack of oxygen to tissues can be observed by cyanosis, a slate gray to bluish discoloration in tissues.

Nitroglycerin is considered quite toxic to aquatic organisms (Bentley et al., 1978). Bentley exposed three species of algae to nitroglycerin concentrations up to 10 mg/L for 96 hours. *Anabaena flos-aquae* and *Microcystis aeruginosa* were unaffected by nitroglycerin, while *Navicula pelliculosa* showed a reduction in growth and reduced chlorophyll *a* values at low nitroglycerin exposure concentrations. Static and flow through test using the aquatic invertebrates *Chironomus tentans* and *Daphnia magna* over a 48-hour exposure period yielded median concentration at which 50% of the test population showed an effect (EC₅₀) at 3.1 mg/L and 12.5 mg/L, respectively. Both species showed significant generational effects.

Rainbow trout, channel catfish, fathead minnows and bluegills were used in 96-hour toxicity tests and 30-day exposure studies (Bentley et al., 1978). The 96-hour toxicity test resulted in median lethal concentrations (LC₅₀) ranging from 1.38-5.5 mg/L, with fathead minnows and channel catfish being the least susceptible in both flow through studies and static tests, respectively. Bluegill was the most sensitive species in both flow through and static tests. The 30-day tests focused on critical life stage studies, with egg hatchability being the most susceptible stage affected by nitroglycerin toxicity. Fathead minnows and channel catfish showed lowest significant response levels at 0.06 and 0.31 mg/L, respectively. Additional studies using ¹⁴C-NG showed little bioconcentration potential in rainbow trout, channel catfish, fathead minnows or bluegills.

Comparison of Regulatory Levels of Common Military Explosives

Nitroglycerin regulatory levels (Table 3) are slightly higher in comparison to the widely used explosives 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). The Agency for Toxic Substances and Disease Registry (ATSDR) reported air safety levels of 0.5 mg/m³ for TNT (ATSDR, 1996a) and 1.5 mg/m³ for RDX (ATSDR, 1996b). By comparison, air safety levels for NG fall just slightly higher than RDX at 2.0 mg/m³ (USEPA, 1987). The water lifetime health advisory (HA) level for NG is 5 µg/L, higher than TNT and

ACGIH TLV TWA ⁽²⁾		OSHA/NIOSH TWA ⁽³⁾		PEL/REL STEL ⁽⁴⁾	HA DWGL ⁽⁵⁾	Potency Factor ⁽⁶⁾	USEPA Carcinogen Class ⁽⁷⁾
ppm	mg/m ³	ppm	mg/m ³	mg/m ³	mg/L	mg/kg/day	
0.05	0.5	0.2	2.0	0.1	0.005	0.0166	C

¹ Literature Reference Rosenblatt et al, 1991 and USEPA, 1987
² American Conference of Governmental Industrial Hygienists, Threshold Limit Value, Time Weighted Average 8 hr/day, 5 day work week
³ Occupational Safety and Health Administration, permissible exposure limit/ National Institute of Occupational Safety and Health recommended exposure limit, Time Weighted Average 8 hr/day, 5 day work week
⁴ Permissible Exposure Limit, Recommended Exposure Limit Short Term Exposure Limit, 15 minutes
⁵ Health Advisory Drinking Water Equivalent Level
⁶ Carcinogenic Potency Factor calculated by Office of Drinking Water
⁷ USEPA Estimated Carcinogen Class: C = Possible human carcinogen, limited evidence of carcinogenicity in animals and insufficient data in humans

RDX at 2 µg/L. Nitroglycerin, TNT and RDX share a class C USEPA carcinogen classification as possible human carcinogens. Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), another commonly used military explosive, has HA levels of 400 µg/L, with a class D USEPA carcinogen classification as not being a human carcinogen (ATSDR, 1997). Nitroglycerin appears to be just slightly less toxic than TNT and RDX based on regulatory levels, but more toxic than HMX.

Numerous databases are available that provide extensive information on the toxicological effects of TNT, RDX and HMX in a wide variety of environmental settings. These databases can provide useful tools in evaluating the risks associated with exposure. Nitroglycerin lacks information to support a strong database, leaving the risks associated with exposure uncertain.

Nitroglycerin Metabolic Products

Figure 1 demonstrates nitroglycerin metabolism follows successive denitration to produce the dinitrate isomers glycerol 1,2- and 1,3-dinitrate (1,2-GDN and 1,3-GDN), and mononitrate isomers glycerol 1- and 2-mononitrate (1-GMN and 2-GMN). The GDN isomers have explosive properties similar to the NG parent compound (Urbanski, 1965), while the GMN isomers are considered weak explosives (Ellis et al., 1978c). GDN and GMN are more water soluble and volatile than NG, enabling them to migrate faster through environments (Christodoulatos et al., 1997). The GDN and GMN isomers further break down to glycerol and finally to carbon dioxide (Dacre and Rosenblatt, 1974).

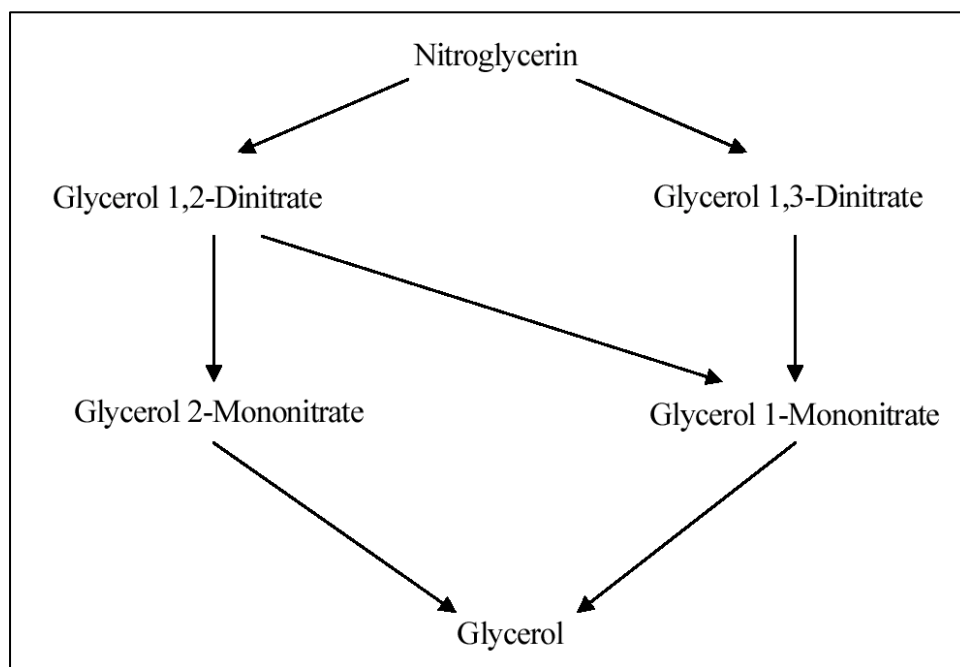


Figure 1. Nitroglycerin Metabolic Pathway

Lee et al. (1975) reported the isomeric 1,2- and 1,3-GDN to be less toxic than NG, 1-GMN more toxic and 2-GMN relatively nontoxic in rats and mice exposed to oral doses of GDN and GMN. These results differ from reported Ames assay tests revealing no mutagenic activity with 1,3-GDN and 1-GMN, weak mutagenic activity with 2-GMN and significant

increases in frameshift mutations without activation with 1,2-GDN (Ellis et al., 1978b). Radiotracer studies using rats fed ^{14}C -NG showed no remaining NG parent compound, but considerable amounts of GMN and glycerol in urine (DiCarlo et al., 1968; Lee et al., 1975). No environmental data was found on GDN and GMN isomers.

The lack of environmental data and inconsistency of toxicological data makes the risks associated with exposure to GDN and GMN isomers unapparent. With GDN and GMN isomers being more water soluble than NG, higher concentrations may be more readily transported and persist for longer periods of time in aquatic environments. With NG known as a toxicant in aquatic environments (Bentley et al., 1978), the GDN and/or GMN isomers could have potentially worse effects on aquatic organisms.

Treatment Methods for Nitroglycerin Contaminated Systems

Chemical and physiochemical methods of NG removal have reduced NG concentrations in wastewaters, but yield decomposition products that are undesirable to the environment. Digesting NG with bases or strong acids forms glycidol and various glycidyl-nitrite and nitrate compounds (Kaplan et al., 1982). Nitroglycerin adsorbed onto activated carbon requires further treatment with sodium sulfate or alkaline hydrolysis, and still yields undesirable nitrite and nitrate compounds (Accashian et al., 2000). Secondary treatments were needed to eliminate harsh end products formed. Oxidation processes using potassium permanganate or ozone effectively decompose NG, but require substantial amounts of substrate (Smith et al., 1983). While oxidation processes successfully denitrated NG, the treatments were economically inefficient due to high operating costs.

Research shows that biodegradation using microbial cultures is a promising treatment method for NG reduction. *Pseudomonas putida* and *P. fluorescens* isolated from NG contaminated soils were able to sequentially degrade toxic levels of NG to GDN and GMN isomers, but could not denitrate GMN isomers (Blehert et al., 1997). Marshall and White (2001) isolated the bacteria *Arthrobacter ureafaciens*, *Klebsiella oxytoca*, and a *Rhodococcus* species from soil samples acquired from a wastewater disposal lagoon at a formerly used NG manufacturing plant. All of the bacteria were able to degrade GTN, producing GDN and GMN isomers with *Rhodococcus* achieving complete removal of all nitrate esters. *Penicillium corylophilum* Dierckx is the only single fungi culture reported to achieve complete denitration of all NG esters to glycerol (Marshall and White, 2001; Blehert et al., 1997). Meng reported complete conversion of NG to glycerol using *Bacillus thuringiensis/cereus* cell extracts (Meng 1995). Even though complete biotransformation was achieved, concerns arose regarding the use of *B. thuringiensis* insect and mammalian pathogens in the environment.

Bhaumik et al. (1997) and Accashian et al. (1998, 2000) used sequential batch and packed bed reactors to determine NG degradation under aerobic conditions. Bhaumik examined the bioconversion of NG using mixed bacterial cultures and the fungus, *Phanerochaete chrysosporium*. The mixed cultures and *P. chrysosporium* completely denitrified NG, forming GDN and GMN isomers which remained in solution. Accashain et al. (2000) reported that mixed microbial cultures required an inoculum from a wastewater treatment plant to initiate complete NG mineralization. Complete denitration of NG by mixed microbial cultures from

aeration tank sludge showed a 10-fold faster removal rate under aerobic than under anaerobic conditions (Accashian et al., 1998).

In anaerobic experiments, Christodoulates et al. (1997) used bacterial consortia from an anaerobic digester to completely remove all nitrite and nitrate compounds of NG. Bhaumik et al. (1997) also found anaerobic digester sludge completely capable of denitrating NG.

Through most of the experiments cited, a question has been raised as to whether NG can be used as a sole energy source for degradation. *Pseudomonas putida* and *P. fluorescens* could not use NG as a sole source to denitrate the intermediate GMN (Blehert et al., 1997). Wendt et al. (1978) saw little to no reduction of GTN concentrations in control samples without a carbon supplement. The bacteria *Arthrobacter ureafaciens*, *Klebsiella oxytoca*, and a *Rhodococcus* species were able to use NG as a sole energy source (Marshall and White, 2001). Other mixed bacterial cultures from an aerated tank sludge rapidly degraded NG in the absence of a supplemental carbon source (Accashian et al., 1998). *Phanerochaete chrysosporium* was capable of denitrifying GTN under aerobic conditions without an added carbon source, but conversion improved substantially when a source was added (Bhaumik et al., 1997). Christodoulates et al. (1997) reported that mixed bacterial cultures in anaerobic microcosms with NG as the sole carbon source completely mineralized NG in 114 days compared to 26 days with an addition of 2000 mg/L of glucose.

Nitroglycerin degradation has been successful using natural and inoculated organisms, under aerobic and anaerobic conditions, with and without the aide of supplemental carbon sources. Although some microbes used in the studies mentioned in this section were isolated from contaminated army ammunition plant soil (Blehert et al., 1997) and wastewater lagoon soil (Marshall and White, 2001), no field soils were used as test media. The test media used included laboratory culture media (Accashian et al., 1998, 2000; Marshall and White, 2001; Blehert et al., 1997), digester sludge (Christodoulatos et al., 1997; Bhaumik et al., 1997) and wastewater from NG manufacturing plants (Smith et al., 1983). Investigations of NG degradation in field soils have not been reported.

CHAPTER 3 MATERIALS AND METHODS

Soils

The soils were chosen primarily for the difference in organic carbon content (Table 4). A surface soil, designated as Yokena Clay, was obtained from the Mississippi River floodplain near Vicksburg, MS. The soil was characterized as a silty clay loam, with 2.4 % total organic carbon. An aquifer soil, designated as LAAP-D, was obtained from Louisiana Army Ammunition Plant in Minden, LA. The aquifer soil was characterized as a clay loam with 0.20% total organic carbon. Prior to use, the soils were air-dried, ground and sieved through a 2 and 3-mm mesh sieve, respectively.

A supplemental energy source was added at the onset of incubation to increase the rate of reduction in the soils by the native microbial community. Yokena Clay reactors received a 0.75-g aliquot of an air-dried, high organic, ground marsh plant material from the Atchafalaya Basin, LA. LAAP-D reactors received a 1.0-g aliquot of glucose at initial time of incubation and a supplemental dose every four days during the experiment to maintain reduced conditions. Glucose was chosen as a more natural carbon source for an aquifer soil since it is a soluble form of organic carbon that does not act as a sorption source. Experiments were also conducted using the LAAP-D aquifer soil without the periodic supplemental glucose to compare the effects of additional carbon on the fate of nitroglycerin.

Soil	Particle Size Distribution			Cation Exchange Capacity (CEC) meq/100g	% Total Organic Carbon (TOC)
	Sand %	Silt %	Clay %		
Surface Soil, Yokena Clay	2	64	34	38.9	2.4
Aquifer Soil, LAAP-D	27	41	32	15.5	0.20

Eh-pH Reactors

Laboratory investigations were conducted in stirred reactors consisting of 2800-ml Fernbach glass culture flasks containing an 18:1 distilled-deionized water to soil ratio based on 150-g dry soil (Figure 2). Slurries were kept in suspension by continuous stirring with a magnetic stirrer to produce a homogenous medium. Experimental design followed the protocol developed by Patrick et al. (1973) with some modifications by Brannon (1983).

The study consisted of triplicate tests maintained at two redox potentials (+500 mV for oxidized conditions and -150 mV for reduced conditions) and three pH levels (pH 6, 7 and 8). Experiments comparing NG degradation rates with and without additional carbon energy sources were conducted at pH 8 under aerobic conditions (+500 mV) since it is difficult to attain a reduced potential (-150 mV) in the absence of an added energy source.

Redox levels (Eh) were obtained using platinum electrodes connected to a pH-millivolt meter. The Eh electrodes response was checked with a pH 7 buffer solution saturated with quinhydrone prior to placement in the stirred reactors. Meter relays were used to sustain desired Eh levels by turning on air pumps to maintain preset aerobic levels and nitrogen gas to maintain preset anaerobic levels. A pH electrode connected to a separate pH-millivolt meter was used to monitor pH. The pH electrodes were calibrated using pH 4 and pH 7 buffer solutions prior to insertion into the reactors. Slurry pH was adjusted by injection of 1N solutions of HCl or KOH via a syringe through a serum cap. The slurry suspensions were allowed to incubate at the chosen Eh/pH levels for approximately 14 days prior to addition of nitroglycerin.

Each reactor was spiked with one ml of ethanol containing 4 mg nitroglycerin (AccuStandard, Inc., New Haven, CT) to yield a total concentration of 1.5 mg/L. Slurries were sampled at intervals on day one (10 and 30 minutes, 1, 3, 4, 6, 8, 10 and 12 hours), then daily through an additional seven days. Aqueous sampling consisted of withdrawing 10 mls of suspended slurry and centrifuging at 1,000 relative centrifugal force (RCF) for 30 minutes to separate the aqueous phase. A 5-ml aliquot of the aqueous phase was diluted 1:1 with HPLC grade acetonitrile and filtered through a 10-ml disposable syringe equipped with a 0.45 μ Teflon filter. Soil samples were obtained at the completion of each test by centrifugation, removing the aqueous phase and retaining the soil phase. All samples were placed in amber glass vials with Teflon coated caps and frozen until analyzed.

Analytical Procedures

Nitroglycerin analyses was performed at the Environmental Chemistry Branch, Engineer Research and Development Center Waterways Experiment Center, using a modified version of EPA SW846 Method 8332 (USEPA, 1996). Chemical analyses were performed on a Waters High Performance Liquid Chromatograph (HPLC) with a model 486 tunable ultraviolet absorbance detector set at 220 nm. Separation was achieved using a Supelco LC-18 reverse phase HPLC column (25 cm x 4.6 mm) with a pore size of 5 μ m. A Supelco LC-CN reverse phase HPLC column (25 cm x 4.6 mm) was used as a confirmation column. A filtered 1:1 mixture of methanol to water solution was used as an eluent. Instrument and sampling parameters were set for a 50 μ L injection volume at a flow rate of 1.2 mL/min for a run time of 21 minutes. Column temperature and pressure were set at 3500 psi and 30°C, respectively.

A 1000 mg/L NG standard was obtained from Restek Corporation (State College, PA). A standard curve based on UV absorbance at 220 nm was developed using calibration standards prepared at concentrations of 100, 20, 10, 4, 0.4 and 0.1 ppm by dilution with acetonitrile. A mid-range standard was run interspersed among the test samples to verify the calibration and serve as a check on the column and detector performance. HPLC data were processed on a Waters Millennium Chromatography Workstation (Milford, MA).

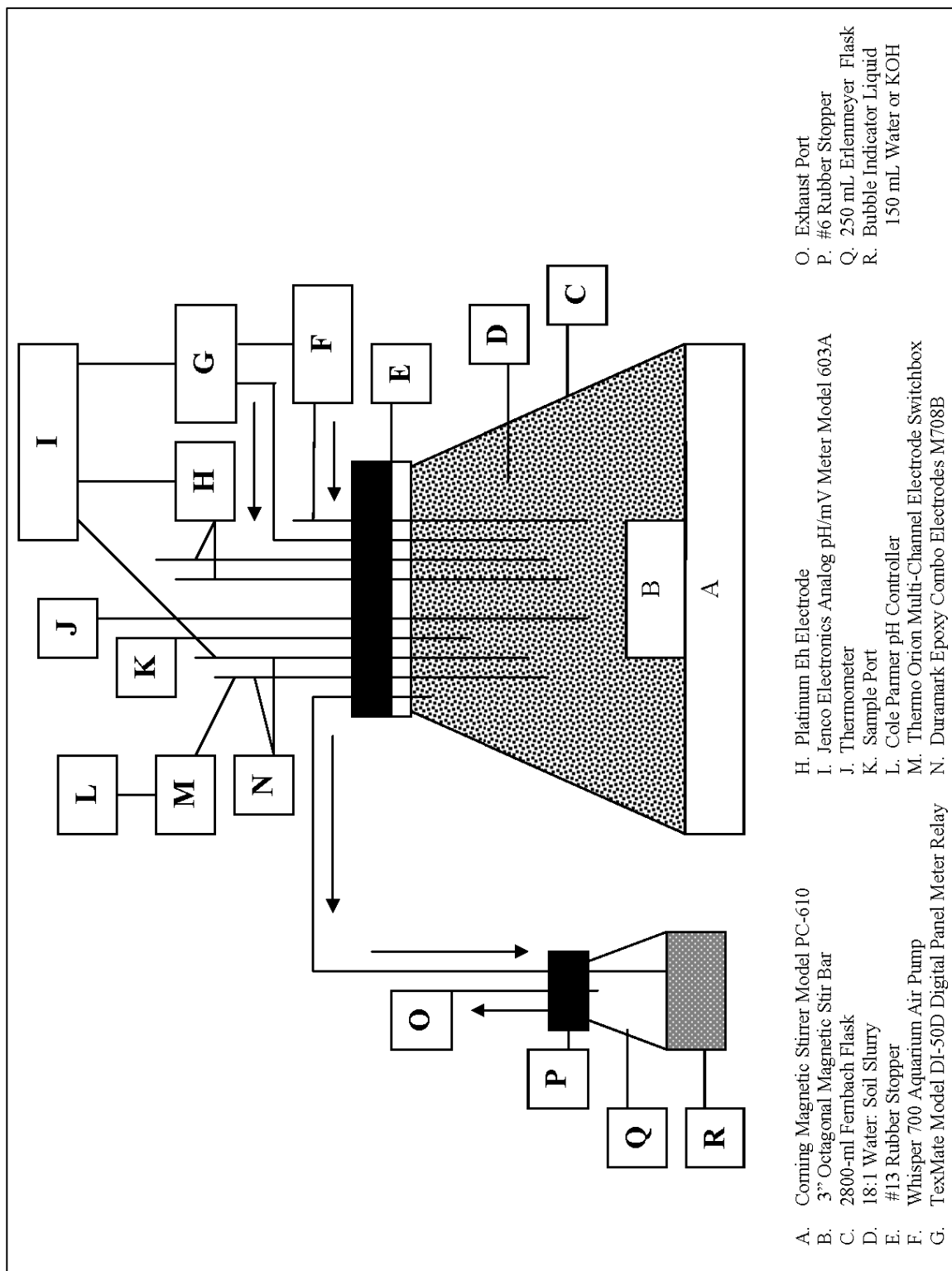


Figure 2. Nitroglycerin Reactor Diagram

Mass Balance Experiment

Mass balance was performed using uniformly labeled ^{14}C -NG with a specific activity of 53.5 mCi/mmol and radiochemical purity of 99% (PerkinElmer Life Sciences, Inc., Boston, MA). The spiking solution was a 99:1 unlabeled NG solution to radiolabeled NG solution. Tests were conducted at pH 7 using the same test soils, apparatus and sampling schedule previously described. A bubble trap containing 150-mls of 1N KOH was attached to the exit port to capture CO_2 . Sampling consisted of removing 5-mls of slurry, centrifuging at 1,000 RCF for 30 minutes and collecting a 1-ml aliquot of the aqueous phase. The KOH traps were sampled at the same times by removing 1-ml of KOH solution from the bubble flask. The KOH flask was replaced with 150-mls of fresh solution at each sample time. The aqueous phase and KOH samples were placed into 15 mls of Ultima Gold Liquid Scintillation Cocktail (Packard Bioscience Co., Meriden, CT) and counted on a Packard Tricarb 2500 TR Liquid Scintillation Counter (Packard Co., Meriden, CT). Approximately 10-g of the soil phase, collected from the centrifugation procedure that generated aqueous samples, was analyzed throughout the experiment for radioactivity. Radioactivity was released from soil samples by complete combustion using a Model 307 Packard Sample Oxidizer (Packard Co., Meriden, CT). Water and soil samples were also periodically analyzed for nitroglycerin using HPLC analysis.

Data Analyses

Analysis of concentration data was performed using Excel software statistics for averages, standard deviations and standard errors following Strum and Kirk's Principles of Discrete Systems and Digital Signal Processing (Addison-Wesley Publishing, Co., Boston, MA). Simple linear regressions were conducted using Sigma Plot software statistics following the Cholesky decomposition to invert the $X'Y$ matrix (SIAM, Philadelphia, PA). Regression analysis was used for determining first order rate coefficients and half-life calculations.

CHAPTER 4 RESULTS AND DISCUSSION

Effects of Eh

Nitroglycerin was unstable in both oxidized and reduced environments; however, redox potential exerted a marked impact on the rate of NG degradation. In aerobic tests (+500 mV), NG concentrations remained relatively stable through 10 hours, then dropped gradually until low to undetectable concentrations were reached (Figure 3). In oxidized systems, NG reaction rates would be expected to proceed slowly since electron activity is low. Fewer electrons would be available within the system to act as donors to assist in chemical reactions. The continual supply of oxygen also allows the nitrate groups to remain oxidized, preventing breakdown to reduced forms.

An immediate drop in NG concentrations was observed under anaerobic test conditions (-150 mV), with complete disappearance from aqueous solution within 24 hours (Figure 4). In reduced systems, electron activity is high. This environment provides free energy to drive chemical reactions. Oxygen depleted environments also create a favorable environment for denitrifying facultative and anaerobic microbes. In these conditions, chemical degradation of nitroglycerin would be expected to proceed at a faster rate.

Disappearance rates can be more accurately expressed by using the pseudo first order kinetic model

$$\ln(c_0/c) = kt$$

where c_0 = initial nitroglycerin concentration at time zero, c = nitroglycerin concentration over time, and k = pseudo first-order reaction constant. Once a value of k is determined, a half-life period ($t_{1/2}$) can be calculated using the equation

$$t_{1/2} = 0.693/k$$

Nitroglycerin removal from the aqueous solutions was most rapid under anaerobic conditions with pseudo first-order rate coefficients as high as 0.509 h^{-1} (Table 5.). Under aerobic conditions the removal rate was an order of magnitude lower with disappearance coefficients ranging from 0.015 to 0.035 h^{-1} . Average half-life determinations differed significantly under anaerobic conditions at 2.5 hours compared to aerobic conditions at 38.2 hours.

Effects of pH

Under anaerobic conditions, pH levels did not exert a strong effect on NG degradation, with all pH levels obtaining undetectable NG concentrations within 24 hours (Figure 5). The only NG that remained in solution at test completion was at pH 6 in both Yokena Clay surface soil and LAAP-D aquifer soil under oxidized conditions (Figure 6). Although remaining amounts were relatively small, 0.091 and 0.14 ppm respectively, results indicate that NG does not decompose as rapidly in acidic environments. These results were similar to those of Price et al. (1997, 2001), who found that the explosive compound TNT remained stable at very low

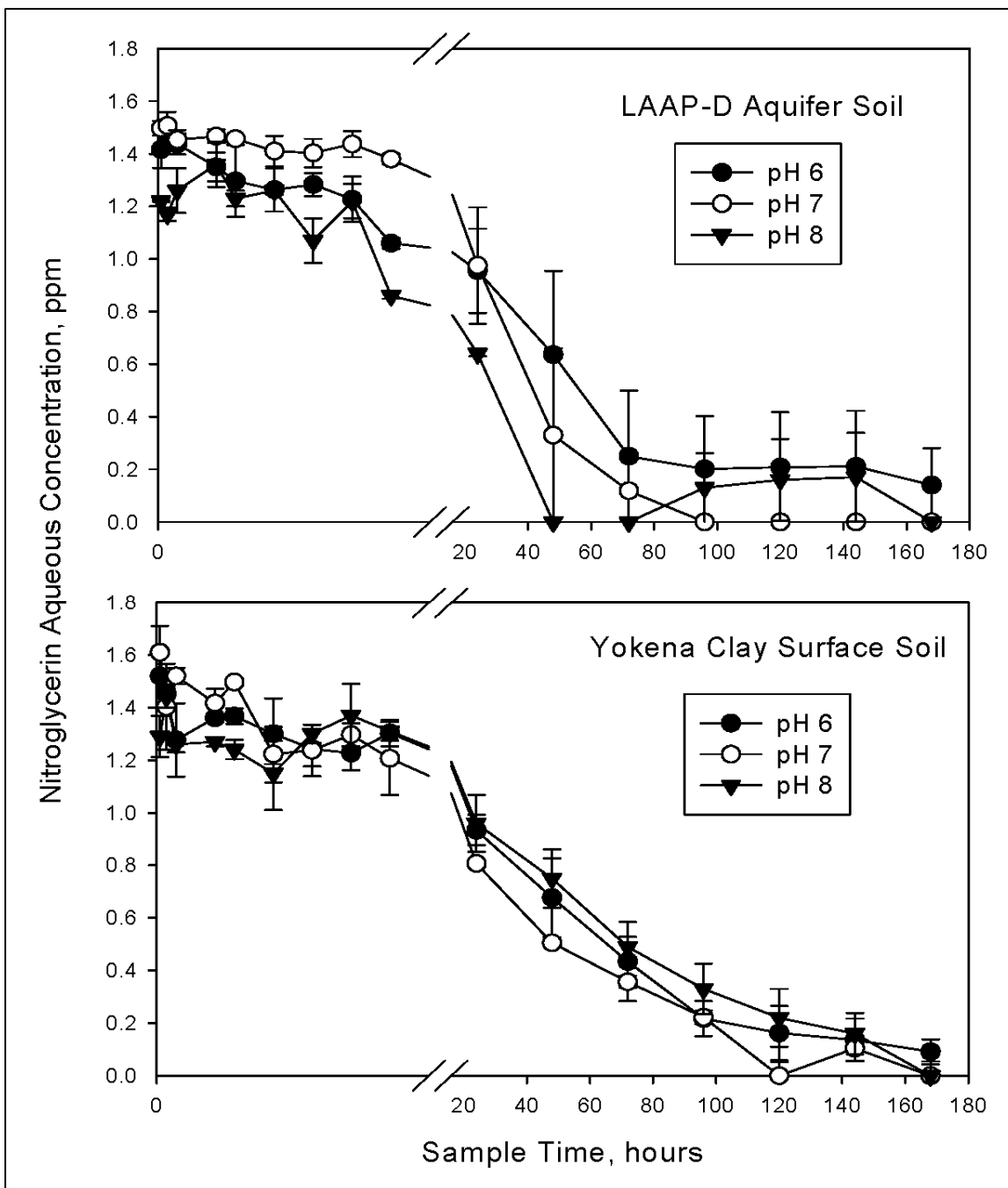


Figure 3. Nitroglycerin Aqueous Concentrations Over Time in Aerobic Reactors (+500 mV) at Three pH Levels in an Aquifer and a Surface Soil

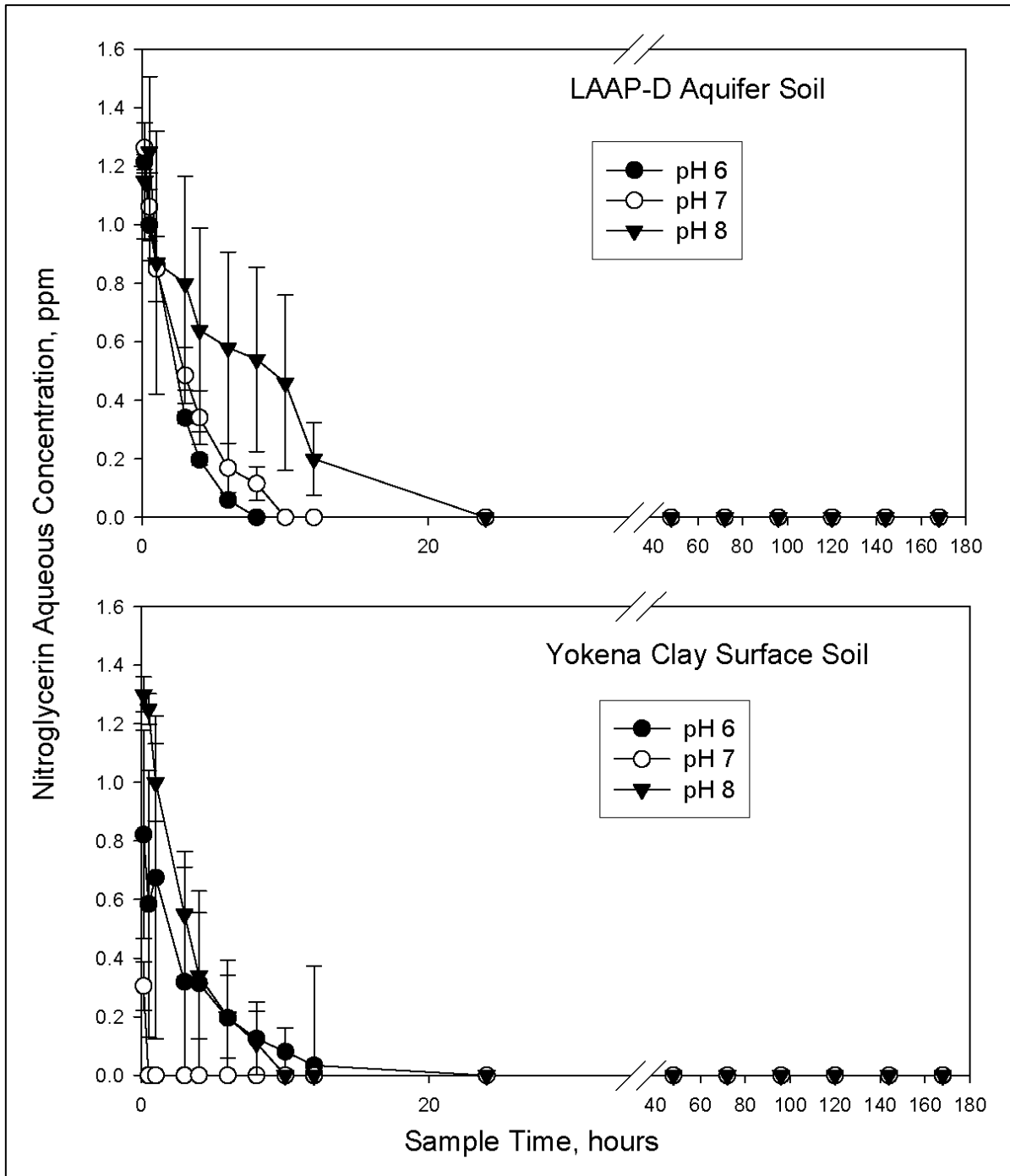


Figure 4. Nitroglycerin Aqueous Concentrations Over Time in Anaerobic Reactors (-150 mV) at Three pH Levels in an Aquifer and a Surface Soil

Table 5. Regression Coefficient (r^2), First-Order Rate Coefficient (k) and Half Life ($t_{1/2}$) for NG in Aerobic and Anaerobic Reactors at Three pH Levels in an Aquifer and a Surface Soil				
Soil	pH	r^2	k, hr⁻¹	$t_{1/2}$, hr
Aerobic (+500 mV)				
Aquifer Soil	6	0.93	0.015	46.2
Aquifer Soil	7	0.97	0.035	19.6
Aquifer Soil	8	0.92	0.017	41.3
Aquifer Soil ¹	8	0.86	0.015	46.2
Surface Soil	6	0.99	0.017	40.3
Surface Soil	7	0.98	0.021	33.2
Surface Soil	8	0.98	0.017	40.8
Anaerobic (-150 mV)				
Aquifer Soil	6	0.99	0.509	1.4
Aquifer Soil	7	0.99	0.309	2.2
Aquifer Soil	8	0.83	0.113	6.1
Surface Soil	6	0.98	0.227	3.1
Surface Soil	7	NA ²	NA	NA
Surface Soil	8	0.99	0.299	2.3
¹ This reactor received 1-g of glucose at initial incubation compared to the other LAAP-D aquifer soil reactors that received the initial inoculum plus a supplemental dose every 4 days. NA – Parameters unattainable due to fast reaction rate.				

levels under aerobic conditions only at pH 6 and below, and RDX only at pH 6 under anaerobic conditions.

One explanation to the persistence of NG in pH 6 reactors could be the effect pH has on cation exchange capacity. Lower pH levels result in decreased cation exchange capacities from the maximum potential allowed by a given soil. Although NG is considered to be a neutral compound, cation exchange capacity could play a minimal role in NG binding ability. Under these conditions, less chemical compound will be adsorbed by the soil where the degradation process is favored, permitting concentrations to remain in solution. A more logical explanation would be the inhibition of denitrifiers in the lower pH reactors. Yu and Patrick (2003) reported an inhibition of the denitrification process in rice soils at low pH levels, resulting in higher nitrite concentrations at pH 5.5 soils than at 7 and 8.5. Poorly drained soils with higher pH levels provide a more favorable environment for denitrifying bacteria to flourish, allowing for higher degradation rates of nitrate compounds.

Behavior in Surface Soil vs. Aquifer Soil

Cation exchange capacity and organic carbon content are two characteristics that differentiate Yokena Clay surface soil and LAAP-D aquifer soil. Yokena Clay has a higher

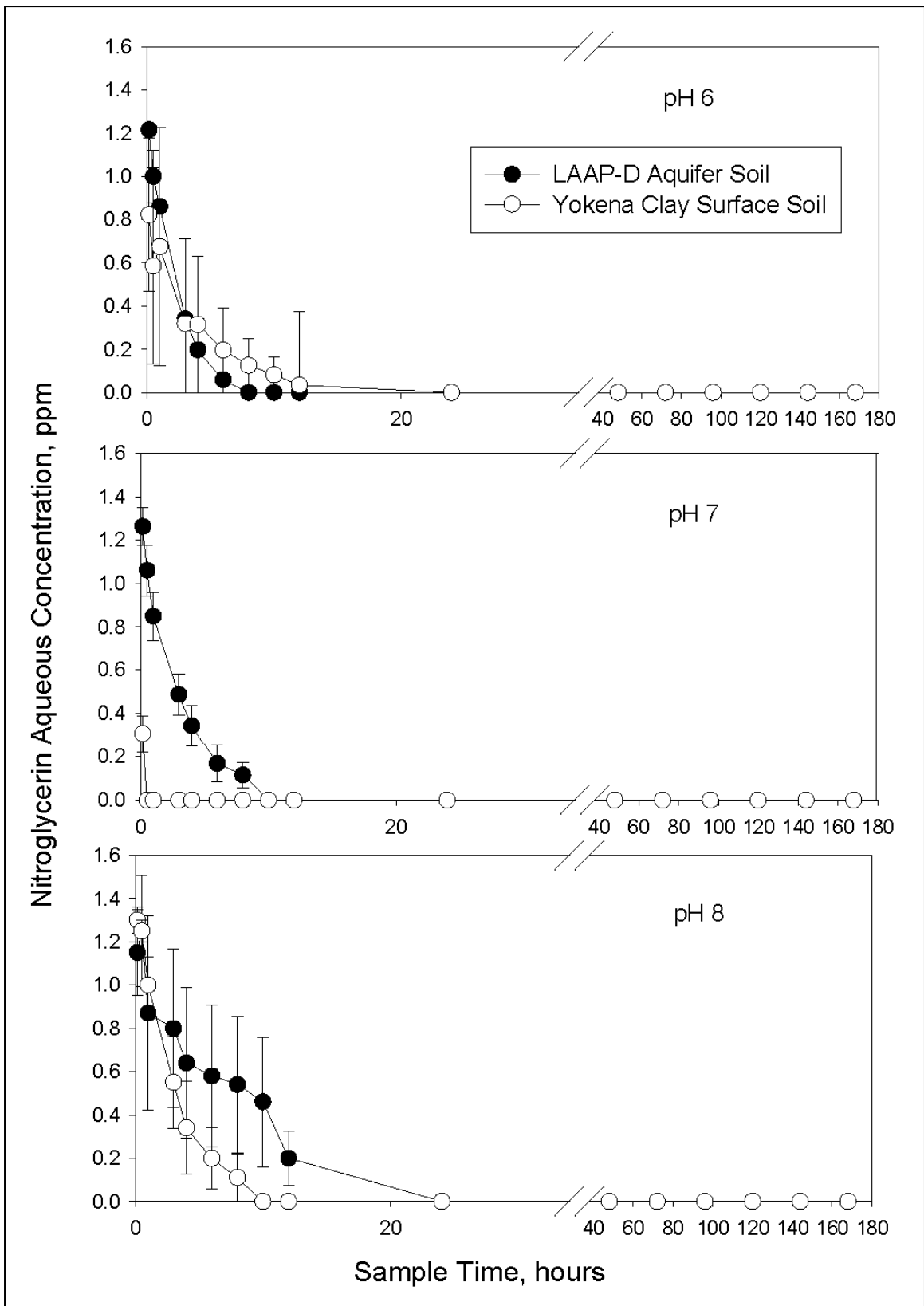


Figure 5. Nitroglycerin Aqueous Concentrations Over Time at Three pH Levels Under Anaerobic Conditions (-150 mV) in an Aquifer and a Surface Soil

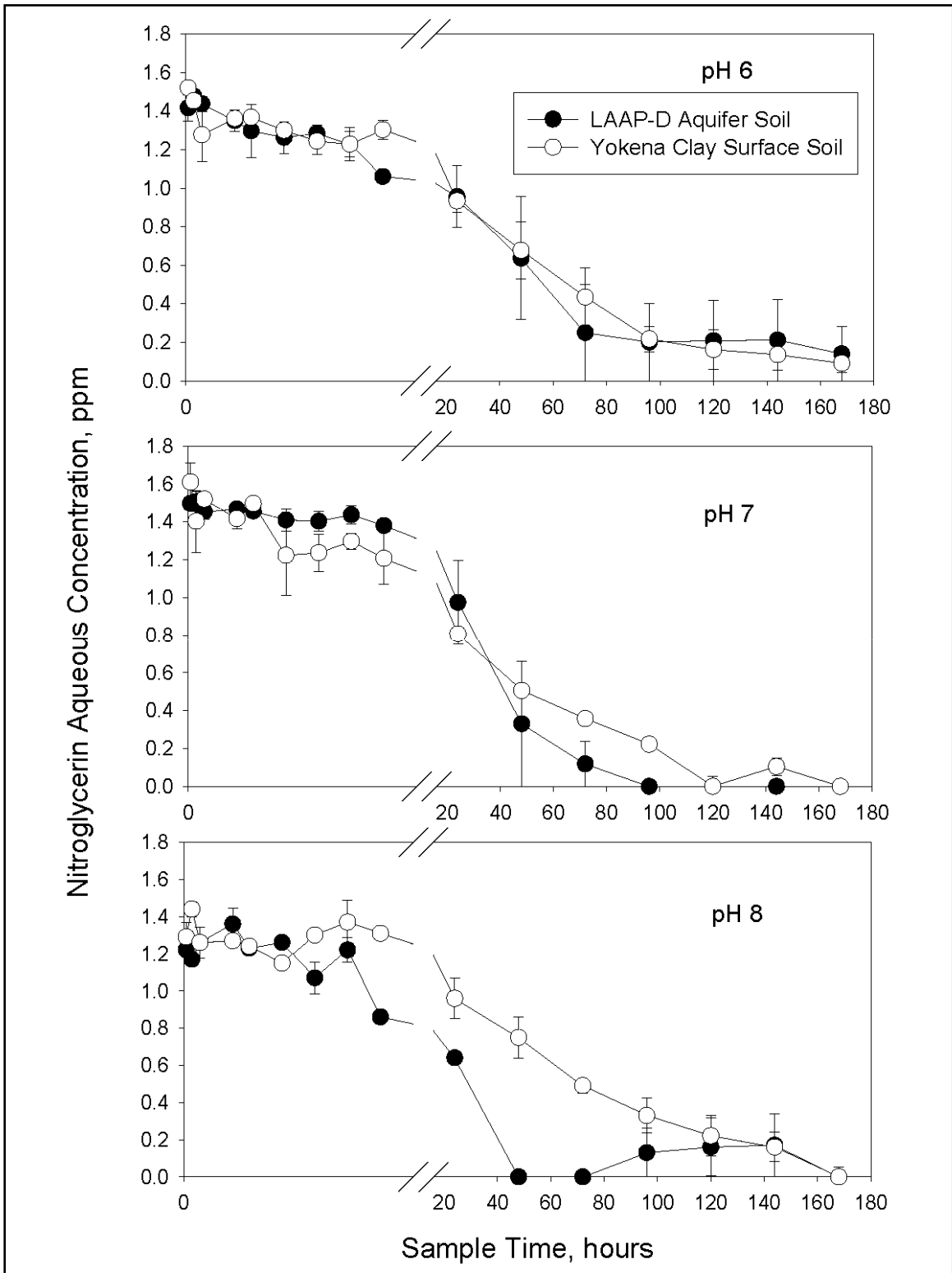


Figure 6. Nitroglycerin Aqueous Concentrations Over Time at Three pH Levels Under Aerobic Conditions (+500 mV) in an Aquifer and a Surface Soil

total organic carbon content (2.4% vs. 0.2%) and a larger cation exchange capacity (38.9 meq/100g vs. 15.5 meq/100g) compared to LAAP-D aquifer soil. Since nitroglycerin is a neutral compound, cation exchange capacity may only exert a small effect on adsorption as compared to the ability of organic carbon to bind NG. The higher organic carbon content of Yokena Clay reactors also provides a more favorable environment for NG degradation due to the organic carbon acting as an energy source to support microbial communities. Larsen and Aamand (2001) found herbicide degradation to be slower in deep sandy soils than in surface soils due to the low organic content of sandy soils and a low occurrence of bacteria capable of transforming nitrate compounds at deeper depths.

Surprisingly, organic carbon had little impact on nitroglycerin removal. At +500 mV the reduction trend was comparable in both test soils throughout the 168-hour test period (Figure 3). The average degradation coefficient was 0.018 h^{-1} in the surface soil compared to 0.022 h^{-1} in the aquifer soil. The general degradation rate profile appeared similar at -150 mV (Figure 4). Average disappearance rates at -150 mV were 0.175 h^{-1} in the surface soil and 0.310 h^{-1} in the aquifer soil. At test completion, no NG was present in either test soil.

Effects of Glucose Supplement

Half-life calculations revealed minimal differences in disappearance rates between the reactors receiving only initial carbon supplement ($t_{1/2} = 46.2 \text{ hrs}$) compared to those receiving aliquots throughout the test ($t_{1/2} = 41.3 \text{ hrs}$). This indicates that a constant input of organic carbon was not needed for degradation. The slightly enhanced rate in reactors receiving supplemental carbon supports other studies in which NG degradation improved with an additional carbon source (Bhaumik et al., 1997; Christodoularas et al., 1997; Accashian et al., 1998). Price et al. (2000) reported similar findings with the explosive compound TNT where conversion rates showed an increase when glucose was added to a low organic carbon aquifer soil.

Mass Balance

The closer examination of ^{14}C -NG in the test systems indicates the fate of NG is quite different in aquifer and surface soils. Most of the radioactivity with the low organic carbon aquifer soil reactors was recovered from the aqueous phase under both oxidized and reduced conditions (Figure 7). Amounts recovered in the aerobic soil phase were over three times that of anaerobic reactors, 36.7 % and 11.8%, respectively. Since reduced systems provide a favorable environment for denitrifying bacteria and chemical reactions to degrade NG, more NG would be expected to remain in oxidized environments. Cumulative mineralization amounts were equivalent in oxidized and reduced aquifer soil reactors (Figure 8). Total percent recovery of added radioactive material was high with 99.0% recovered in anaerobic reactors and 96.1% in aerobic reactors.

Partitioning of the radioactivity in the surface soil reactors was split between the soil phase and CO_2 (Figure 7). Percent recovery of CO_2 was greater in surface soil reactors compared to that of aquifer soil reactors with average recoveries of 38.6% and 7.9%, respectively. Cumulative mineralization rates reached higher values in anaerobic surface soil reactors than in the aerobic reactors (Figure 8). The surface soil was more capable of

complete denitrification of NG to CO₂. Total percent recovery is less than those of LAAP-D reactors with 89.4% in anaerobic reactors and 79.6% in aerobic reactors.

Results of the HPLC analysis of water and soil samples collected from the radiolabeled reactors at the end of the test period revealed no nitroglycerin concentrations. Therefore, the radioactivity was not attributable to the parent compound, but may be that of a degradation product such as GDN, GMN or even glycerol.

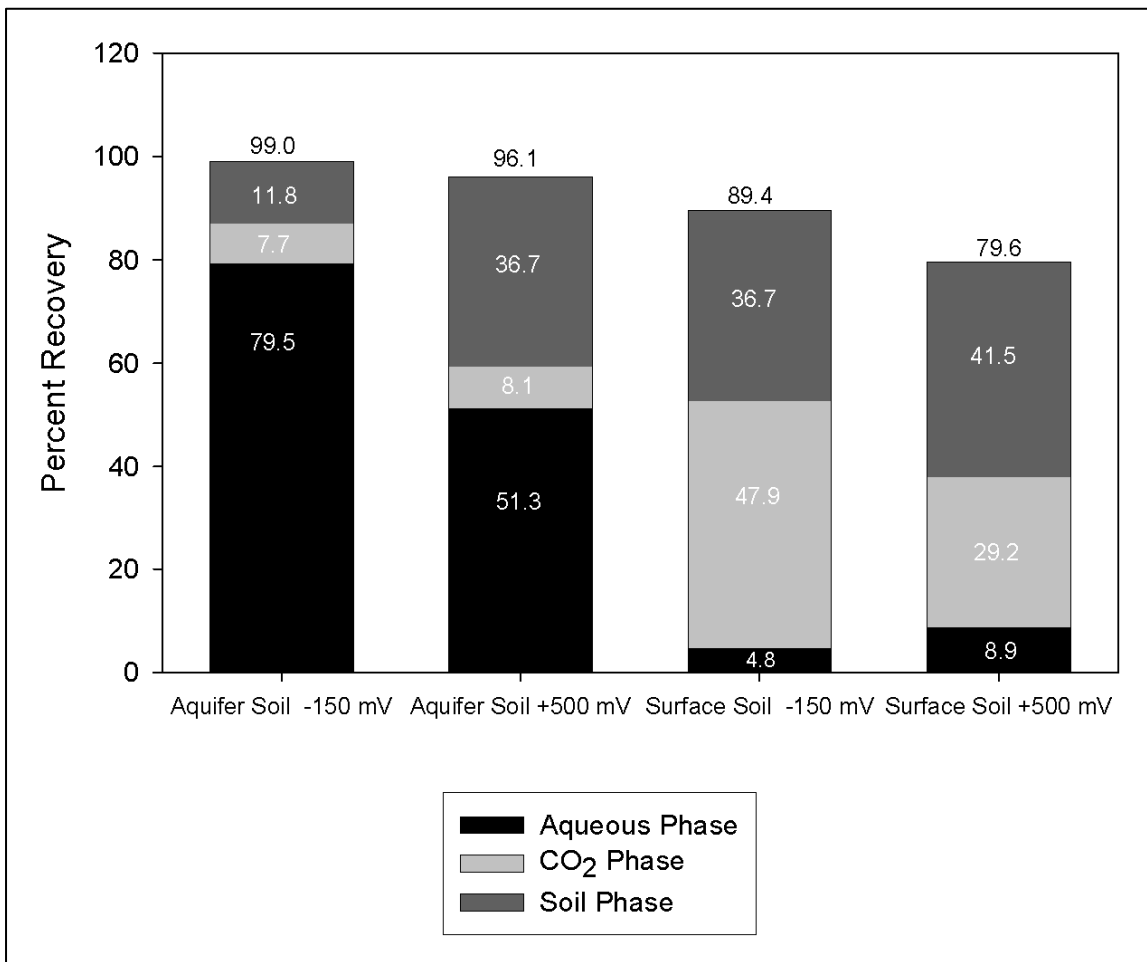


Figure 7. Partitioning and Percent Recoveries of ¹⁴C-NG Radioactivity Under Aerobic (+500 mV) and Anaerobic (-150 mV) Conditions at pH 7 in an Aquifer and a Surface Soil at Test Completion

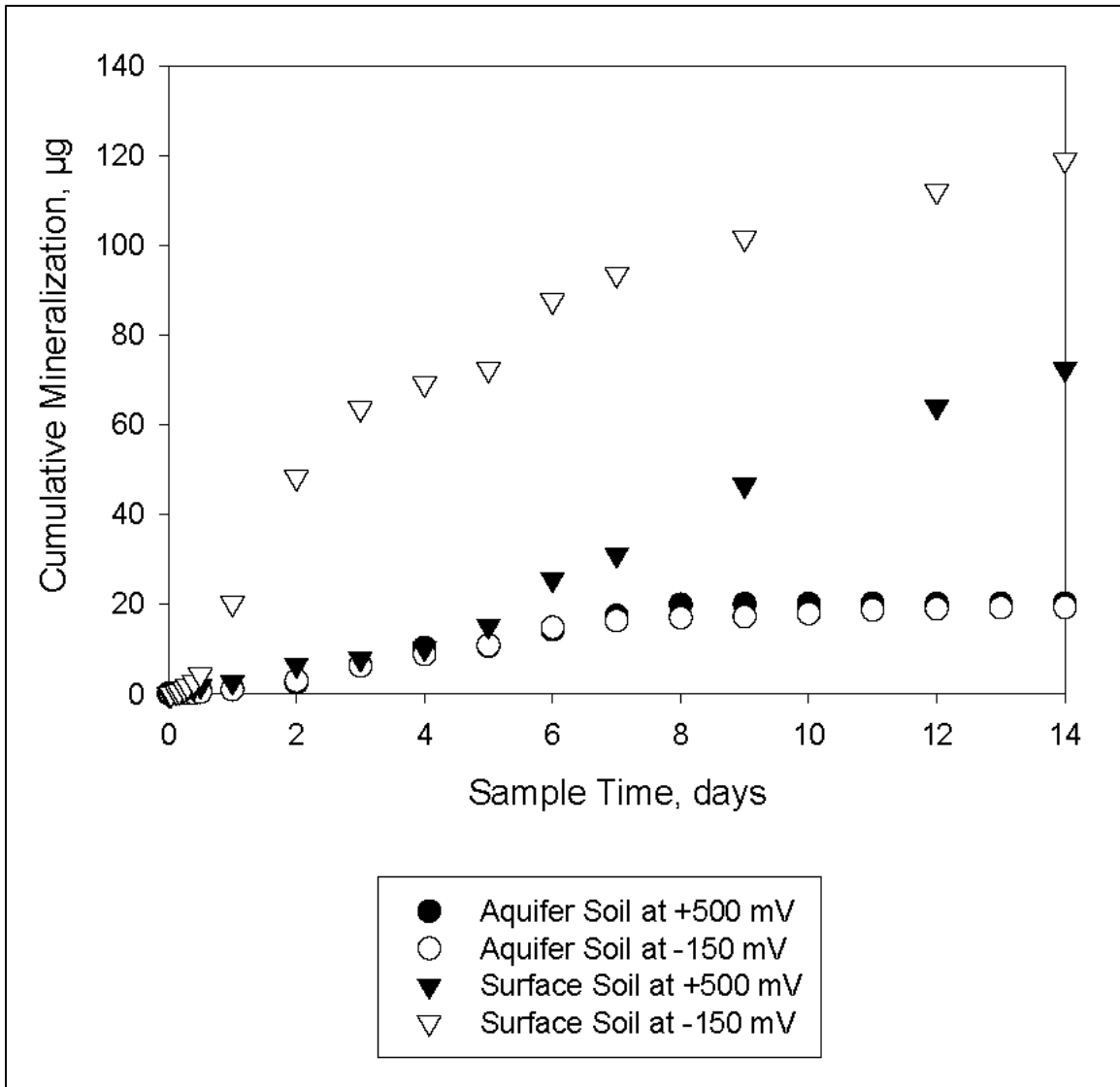


Figure 8. Cumulative Mineralization Rates for NG Under Aerobic (+500 mV) and Anaerobic (-150 mV) Conditions at pH 7 in an Aquifer and a Surface Soil

CHAPTER 5 SUMMARY

The fate of nitroglycerin in a surface soil and an aquifer soils was determined under both oxidized and reduced conditions at pH 6, 7, and 8. Nitroglycerin was unstable under all test condition. Degradation rates were faster and more complete under anaerobic than under aerobic conditions, with concentrations dropping to undetectable levels within 24 hours. Nitroglycerin remained in solution phase of both soils in the acidic environment of pH 6 under aerobic conditions.

Organic carbon content did not seem to influence the rate of NG degradation. Reduction trends were similar between the surface soil and the aquifer soil under aerobic and anaerobic conditions and at each pH level. The minimal effect of organic carbon on NG degradation was more noted in the supplemental carbon experiments. Average half-life values reflected little influence on reaction rates between reactors receiving only initial glucose aliquot to those receiving a supplemental dose throughout the experiment.

Mass balance results using uniformly labeled ^{14}C -NG demonstrated significant differences in the partitioning of radioactivity between the surface and aquifer soils. The surface soil mineralized more NG to CO_2 , while the aquifer soil left a significant portion of the radioactivity in the aqueous phase. Analysis of the aqueous and soil phases by HPLC revealed no NG. Mass balance results demonstrated that some other form of nitroglycerin persisted in these systems. Since this study focused on the parent compound NG, no analyses for NG breakdown products was conducted. It can only be speculated that the identity of the compound or compounds persisting are the breakdown products GDN, GMN or glycerol.

CHAPTER 6 CONCLUSIONS

The following conclusions are drawn as they relate to the research performed to meet the study objectives:

- Nitroglycerin will persist longer in oxidized environments than in reduced environments, but will degrade to undetectable levels under both conditions. This indicates that NG is capable of denitrification in aerobic and anaerobic environments.
- Nitroglycerin may remain in solution in acidic environments under aerobic conditions. This result illustrates a cause for concerns with the persistence of NG concentrations in exposed soils of acidic regions such as the Pacific Coastal, Central Midwest, Atlantic and Southern states.
- Nitroglycerin degradation is independent of soil carbon content and supplemental carbon inputs.
- Radiolabeled ^{14}C -NG studies indicate persistence of unidentified NG constituents. If the unidentified products are GDN and/or GMN, both are known to be more water soluble than NG, and can migrate faster with a higher potential to reach groundwater. Toxicity information on these degradation products is insufficient for evaluation of their risks to the health of the environment.
- The research performed for this thesis concentrated on the fate of nitroglycerin alone in solution form. By the conclusions presented, the nitroglycerin contamination found on military installations is most likely not nitroglycerin alone in solution form, but nitroglycerin in a propellant formulation. Combined with propellant chemicals, the composition of nitroglycerin would be altered, allowing for a more complex, persistent compound.

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Sally was born in Grand Rapids, Michigan, and raised in Kokomo, Indiana. She currently resides in Vicksburg, Mississippi, with her fiancé and their two dogs. She enjoys fishing in waters from her backyard lake to the Gulf of Mexico.