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BIODEGRADATION OF 2,4-DINITROTOLUENE AND 2,6-DINITROTOLUENE IN A PILOT-SCALE AEROBIC SLURRY REACTOR SYSTEM

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14. ABSTRACT

A pilot-scale study was conducted to evaluate the use of slurry reactors to treat 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT) contaminated soils from two former Army ammunition plants, the Volunteer Army Ammunition Plant (VAAP, Chattanooga, TN) and the Badger Army Ammunition Plant (BAAP, Baraboo, WI). Two identical 70-L Eimco bioreactors were augmented with aerobic DNT-mineralizing cultures and operated in series for a period of 3 months. The first reactor was fed soils wash after the removal of large particulates using soil washing. The effluent from the first reactor was fed to the second reactor after each feeding cycle.

Stable performance and complete degradation of 2,4-DNT (within ~ 2 days) was demonstrated at a loading rate of up to 20% (17,000 µM) for VAAP soil, and 40% (11,230 µM) for BAAP soil. The degradation of 2,6-DNT was minor in the first reactor, but was achieved in the second reactor in both cases. Studies indicate that the presence of high 2,4-DNT concentrations prevented sustained 2,6-DNT degradation. This effect was mitigated by the sequential mode of reactor operation. Results also demonstrated stoichiometric release of nitrite and NaOH consumption from the degradation of dinitrotoluene, implying the applications of these inexpensive measures for routine process control.

15. SUBJECT TERMS
explosives compounds, trinitroloene (TNT), 2,4-dinitrotoluene, 2,6-dinitrotoluene, bioremediation, slurry reactor

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PREFACE

This report documents research activities conducted by Dr. Chunlong Zhang of Rice University in collaboration with personnel at Air Force Research Laboratory, Tyndall Air Force Base, Florida. Most of the test data evaluated in this report was collected by Dr. Chunlong Zhang from February 17 through June 27, 1999, with the remainder collected till July 2, 1999 by Ms. Shirley Nishino.

Funding for this work was provided in part by the Strategic Environmental Research and Development Program (SERDP) through the Applied Research Associates (ARA), Inc. located at 4300 San Mateo Blvd. NE, Suite A-220, Albuquerque, NM. This project was conducted under the supervision of Dr. Joseph Hughes of Rice University and Dr. Jim Spain of Air Force Research Laboratory. Correspondence regarding technical issues should be addressed to:

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EXECUTIVE SUMMARY

A pilot-scale study was conducted to evaluate the use of slurry reactors to treat 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT) contaminated soils from two former army ammunition plants, the Volunteer Army Ammunition Plant (VAAP, Chattanooga, TN) and the Badger Army Ammunition Plant (BAAP, Baraboo, WI). Experiments were performed in 70-L Eimco bioreactor systems augmented with aerobic DNT-mineralizing cultures. The rate and extent of DNT biodegradation were determined to obtain optimal operating parameters in a sustained fill-and-draw feeding mode.

Two identical reactors were operated in series for a period of 3 months. To minimize operational problems associated with large particulates, soils were subject to a soil washing pretreatment prior to introduction into the reactors. The first reactor was fed the soil wash directly. The effluent from the first reactor was fed to the second reactor after each feeding cycle.

VAAP soil was fed at 5, 20 and 30% nominal solids loading rates (mass of soil used in the soil washing procedures per unit volume of reactor) and operated in a 10:90 (v/v) draw and fill mode. System performance for the BAAP soil was examined at five nominal solids loading rates (5, 10, 20, 30, and 40%). Stable performance and complete degradation of 2,4-DNT (within ~ 2-day period) was demonstrated at a loading rate of up to 20% (17,000 μM 2,4-DNT) for VAAP soil, and up to 40% (11,230 μM 2,4-DNT) for BAAP soil. The degradation of 2,6-DNT was minor in the first reactor for either soil, but was achieved in the second reactor in both cases. Studies indicate that the presence of high 2,4-DNT concentrations prevented sustained 2,6-DNT degradation. This effect was mitigated by a sequential mode of reactor operation.

Results also demonstrated a stoichiometric nitrite release from the degradation of dinitrotoluene. Moreover, oxygen uptake rates and NaOH consumption provided accurate assessments of microbial activity. These results suggest that cost-effective monitoring can be achieved through the use of these inexpensive measures for routine process control.

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1 INTRODUCTION

This draft report documents research activities conducted at Tyndall Air Force Base by Dr. Chunlong Zhang with the collaboration of personnel at Air Force Research Laboratory, Tyndall Air Force Base, FL. Funding for this work was provided in part by Strategic Environmental Research and Development Program (SERDP) through the Applied Research Associates, Inc., Albuquerque, NM under the Contract Number F08637-9B-C-6002. This project is a part of the overall project — Discovery of Novel Enzymatic Reactions and Determination of Biodegradation Mechanisms and Pathways (Subtask 32.01S).

The overall objective of this project was to assist in the development of strategies for the bioremediation of dinitrotoluene contaminated soils. Recent work at Tyndall Air Force Base has demonstrated the treatment of 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT) contaminated soils in bench-scale bio-slurry reactors (Nishino et al., 1999). The present study was undertaken to test the treatability of DNT contaminated soils in a pilot-scale slurry reactor system, and to obtain the optimal operating parameters in a continuous fill-and-draw mode. Contaminated soils were obtained from two former ammunition plants, the Volunteer Army Ammunition Plant (VAAP, Chattanooga, TN) and the Badger Army Ammunition Plant (Baraboo, WI). The pilot-scale study was conducted in an Eimco bioreactor system to evaluate the rate and extent of dinitrotoluene biodegradation following augmentation with dinitrotoluene-mineralizing cultures available at Tyndall Air Force Base.

2 MATERIALS AND METHODS

2.1 Chemicals

2,4-Dinitrotoluene, 2,6-dinitrotoluene, and 2,4,6-trinitrotoluene were obtained from Aldrich. Other chemicals were HPLC grade acetonitrile (Fisher) and trifluoroacetic acid (Sigma) for HPLC mobile phase, and N-(1-naphthyl)-ethylene-diamine dihydochloride (Marshall's reagent) and sulfanilic acid (Baker Chemical Co., Phillisburgh, NJ) for nitrite analysis.

2.2 Bacterial culture

An induced culture containing the 2,4-DNT degrading strain *Burkholderia cepacia* JS872, and the 2,6-DNT degrading strains *B. cepacia* JS850 and *Hydrogenophaga palleronii* JS863 was grown in 18-L batches in nitrogen-free minimal medium (Bruhn et al., 1987) supplemented with 2,4-DNT (1 mM) and 2,6-DNT (100 - 500 μM) as sole source of carbon, nitrogen and energy. The culture was incubated in a Biostat C reactor (B. Braun) at 30°C, with stirring at 400 rpm and sparged with air at 15 L/min. Non-sterile additions of DNT were made daily. Cells were harvested by filtration on a 0.45 μ Pellicon cassette filter (Millipore), washed once with phosphate buffer (20 mM, pH 7.2) and suspended in 1 L of phosphate buffer before addition to the Eimco reactors.

2.3 Preparation and characterization of test soils

Contaminated soils were collected from two former army ammunition plants, the Volunteer Army Ammunition Plant (VAAP, Chattanooga, TN) and the Badger Army Ammunition Plant (BAAP, Baraboo, WI). At the VAAP site, soil was collected from 4 points along the drainage ditch outflow from the Acid Recovery House of TNT production Line 4. Soil containing up to 200 g/kg of 2,4-DNT was found within 5 m of the Acid Recovery House, and soil with lower concentrations of DNT (2-3 g/kg) but higher concentrations of TNT (1-2 g/kg) and 2,6-DNT (0.5-1 g/kg) were collected approximately 50 to 150 m down gradient. The soil was stored in 55-gallon drums at ambient temperature for 6 months before processing. At the BAAP site, soil core samples were collected in 1997 from Propellant Burning Ground Waste Pit-1, -2, and -3. Several drums of contaminated soils were shipped to AFRL/MLQR in February, 1999 (Table 1).

Table 1 Soil cores from Propellant Burning Ground (PBG).

Drum	Soil Boring[¶]	Boring Depth (feet)	DNT Concentration (mg/kg)
011	PBG9713	20-30	1910-8870
	PBG9714	0-30	0-14
	PBG9718	0-30	0-306
013	PBG9712	0-40	0-5
	PBG9713	0-20	0-8870
020	PBG9705	0-55	2180-30700
024	PBG9706	1-30	1770-49100
	PBG9707	1-40	0-4950
	PBG9708	1-30	2790-39300
	PBG9709	0-5	0

[¶] Data from Figures 4-12, Stone & Webster Environmental Technology & Services. Draft alternative feasibility study Propellant Burning Ground and Deterrent Burning Ground, waste pits, subsurface soil, Badger Army Ammunition Plant, Baraboo, Wisconsin: U.S. Army Corps of Engineers, Omaha District; June 1, 1998.

Soils were air-dried, gravel and large debris were removed, followed by repeated sieving and tumbling processes until all soils were passed through a 20-mesh sieve. Soils were then subjected to a homogenization procedure, including manual mixing followed by the use of a sample splitter (Model Sp-1, Gilson Screen Co., Malinta, OH) to obtain a homogeneous stock of soils for use in biodegradation studies. Table 2 presents results of soil characterization obtained following homogenization.

Table 2 Characteristics of test soils.

Soil	moisture (%)	Bulk density (g/mL)	pH	2,4-DNT (g/kg)	2,6-DNT (g/kg)	TNT (g/kg)
VAAP	3.07 ± 0.10	1.40 ± 0.02	4.60	18.54^{A}	1.38^{A}	0.28^{A}
				10.89 ± 1.75^{B}	0.87 ± 0.02^{B}	0.43 ± 0.01^{B}
BAAP	1.62 ± 0.02	1.69 ± 0.02	9.35	8.94 ± 0.95	0.48 ± 0.08	0.07 ± 0.01

A, B denote two batches of soil.

2.4 Slurry phase aerobic reactors

Two identical Eimco Biolift slurry reactors (Model B75LA, Tekno Associates, Salt Lake City, UT) were used. Each has a working volume of 75 L. The reactors were equipped with agitation, aeration, and temperature control. Temperature was maintained at 30°C, and pH was maintained in the range of 6.75 ~ 7.25. The pH was automatically controlled by a pH meter coupled with a peristaltic pump with a stock solution containing 12.5 N (50% w/v) NaOH. A small amount of acid was consumed at the start of each feeding cycle when the alkaline BAAP soil was used, 50% (1:1 v/v) hydrochloric acid was added manually to adjust the pH to ~ 7.0.

A schematic of the reactor is shown in Figure 1. The slurry reactor uses airlifts, bottom rakes and diffuser tubes to achieve mixing and aeration of soil slurry in order to sustain aerobic biodegradation processes. The diffuser tubes mounted on the rake arms supply the oxygen to the slurry, and provide suspension of the smaller particles as well. Once the heavier particles have settled to the bottom of the reactor, the rakes move settled material to the central airlift where they are lifted upward and released above the liquid.

2.5 Removal of large particulates by soil washing

The Eimco reactor has been successfully used for the treatment of oily sludges and contaminated fines from a soil washing operation (Brox and Hanify, 1991). When

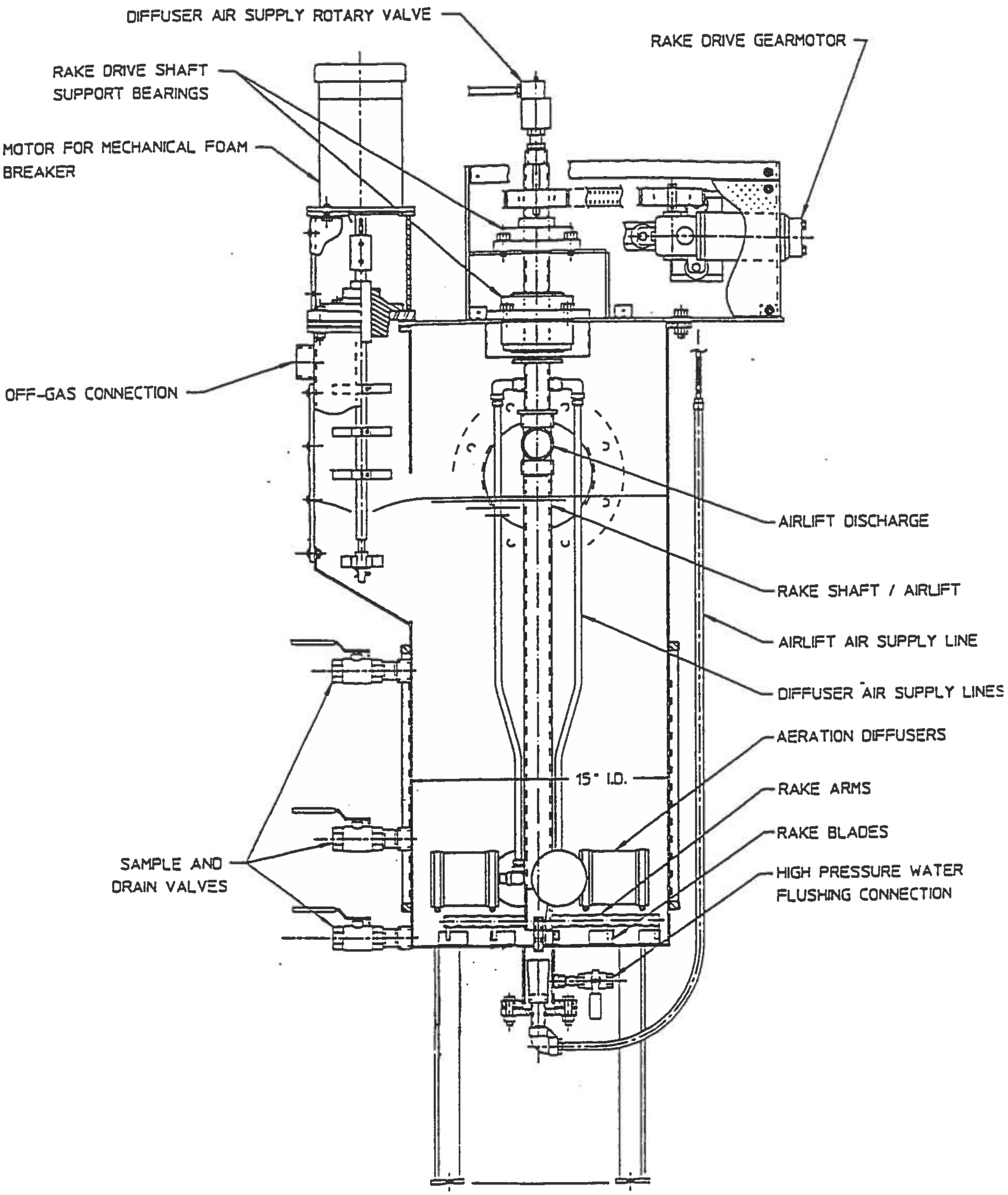

Figure 1 Schematic of Eimco Biolift Slurry Reactor

sandy BAAP soils were tested in the preliminary studies, the sand settled and interfered with the mechanical operation of the reactors. Therefore, all soils were washed with water to remove the sand prior to the introduction into the reactors. Repeated soil washings were performed in a 14-L cylinder with an upward jet flow of warm (60°C) tap water to separate the DNT and fines from the sand. A total of 60 L of water was generally used for each feeding of soil, however, the ratio of water to soil depended on the actual solids loading in a given feeding cycle. In this report, the term *soil wash* denotes the liquid slurry containing contaminated soil fine particulates after the repeated soil washing processes.

2.6 Solids loading rate

Solids loading rate is commonly referred to the mass of soil added per unit volume of reactor (w/v). In this report unless specified otherwise (i.e., Section 3.1 in which soil was added directly to the reactor without soil washing), soil was not added directly to the reactor. Instead, soil wash was added. Therefore in this report, solids loading rate is defined as the mass of soil used in the soil washing procedure per unit volume of reactor (w/v). The resulting solids concentration in the reactor depended on the percentage (weight basis) of large soil particulates removed during soil washing process. For clarity, the term "nominal solids loading rate" or "pre-soil washing solids loading rate" is used throughout this report to reflect this definition. In this pilot-scale study, VAAP soil was tested at nominal solids loading rates of 5, 20, and 30%, whereas BAAP soil was tested at nominal solids loading rates of 5, 10, 20, 30, and 40%. (Note that a nominal 10% solids loading is equivalent to 7 kg of soil used during soil washing which provided feed for each fill-and-draw operation, since the operating volume of the reactor was 70 L). A comparison of the nominal solids loading rates and actual solid concentrations is presented in Table 3.

Note that the actual solids concentration in the reactor was lower than the nominal solids loading rate. As shown in Table 3, the difference was more evident for sandy soils (e.g., BAAP soil) than clayed soil (VAAP soil) since a higher percentage of large soil particulates was associated with sandy soil. Note also that the *actual* solids concentration is a *calculated* concentration. It was calculated based on the *actual* mass of soil entered into the reactor. However, the calculation was based on the assumption that the reactor was a completely mixed system, which was not always observed.

Table 3 Nominal solids loading rates vs. actual solids concentrations in the reactor.

	Unit	Solids loading rates tested in this study				
VAAP soil						
Nominal solids loading rate	% (w/v)	5	20	30		
Actual solids loading rate[¶]	% (w/v)	4	16	25		
SS concentration[¶]	g/L	40	160	250		
BAAP soil						
Nominal solids loading rate	% (w/v)	5	10	20	30	40
Actual solids loading rate[¶]	% (w/v)	0.7	1.3	2.6	3.9	5.2
SS concentration[¶]	g/L	7	13	26	39	52

[¶] The removal of large soil particultes was assumed to be 18% for VAAP soil and 87% for BAAP soil, based on the averaged data from experiment. Reactor volume = 70 L.

2.7 Fill-and-draw procedure

Two reactors were operated in a sequential mode. The first reactor was charged with soil wash, and the effluent from the first reactor was then fed to the second reactor in series. Approximately 60 L of soil wash was pumped into the first reactor, followed by the addition of NaH_2PO_4 and Na_2HPO_4 as a buffer (20 or 1 mM) and phosphorous source. After the pH and temperature were equilibrated, the slurry was inoculated with the mixed bacterial culture during the start-up. The final volume of slurry in the reactor was brought to 70 L with tap water. No other nutrients were added, with the exception of a partial BLKN nitrogen-free minimal medium containing 1:1000 of 10.0 g/L $CaCl_2 \cdot 2H_2O$ (component B), 2.35 g/L sodium citrate and 2.16 g/L $FeCl_3$ (component C) and 20.0 g/L $MgSO_4 \cdot 7H_2O$ (component D). The additional nutrients were added only when high solids loading rates were tested (i.e., 20% and 30% for VAAP soil; 40% for BAAP soil).

During each fill and draw cycle, 10% (7 L) of the slurry was returned as the inoculum and the remaining 90% (63 L) was made up with soil wash and tap water. The effluent from the first reactor at the end of each feeding cycle was fed to the second reactor, which was operated primarily for the degradation of the less biodegradable isomer 2,6-dinitrotoluene, and secondarily for the extended aerobic degradation of any residual 2,4-dinitrotoluene from the first reactor. Additional inoculations with 2,6-DNT degrading bacteria were made occasionally in the second reactor when a lack of activity was observed in the reactor.

2.8 Summary of reactor operations

Two identical reactors were operated simultaneously and continuously for a period of 3 months. Initially, the first reactor (Reactor A) was fed VAAP soil wash at a 5% (w/v) nominal solids loading rate (mass of soil used in soil washing / 70 L reactor) and operated in a 10:90 (v/v) draw and fill mode, the effluent was fed to the second reactor (Reactor B). Both reactors were operated continuously for approximately 30 days (13 feeding cycles for Reactor A). The reactors were then switched to the BAAP soil wash. The first reactor in series (referred to as Reactor C when fed BAAP soil wash) was operated at a 5% nominal solids loading rate for 9 cycles, followed by step increase in nominal solids loading rates of 10%, 20%, 30% to 40% for 3, 3, 2, and 2 cycles, respectively. The second reactor in series (referred to as Reactor D when fed BAAP soil wash) was first operated in a continuous mode by automatically pumping soil wash directly to the reactor for approximately 180 hrs (8 days). It was then changed to an intermittent feeding mode by withdrawing effluent and replacing with the same volume of soil wash, i.e., 7.5 L for the first 4 cycles followed by 10 L for the next 3 cycles. The reactor was finally operated in a fill-and-draw mode with increasing volume of soil wash from 50:50 v/v (7 cycles), 35:65 v/v (2 cycle), to 10:90 v/v (1 cycle). Reactor C had a total operation time of 48 days, whereas Reactor D was operated for 55 days. After the completion of BAAP soil with Reactors C and D, both reactors were switched back to VAAP soil wash. The first reactor in series (referred to as Reactor E when fed VAAP soil wash) was tested at higher loading rates (20% and 30%) than previously tested in Reactor A (i.e., 5%) for a total of 11 days (5 cycles). The 2,6-DNT containing effluent was stored at room temperature with aeration, and tested for the degradation of 2,6-DNT (referred to as Reactor F) with accumulated bacterial biomass from Reactor D.

2.9 Sampling and analytical method

Three sampling ports (bottom, middle, and top) were located along the side-wall of the reactor at 2, 15, and 40 cm from the bottom of the reactors. An opening at the top of the reactor (central) permitted visual inspection of the mechanical action of the reactor and insertion of instrument probes, and allowed sampling from the central shaft of the air-lift. Routine samples were taken from the top sampling port of the reactor side-wall. Slurry samples taken from the reactor were stirred on a magnetic stirrer, and sub-samples were withdrawn through a large-bore pipette tip to prevent setting of solids.

Samples (200 μL) for HPLC analyses were filtered through a 0.22 μ centrifugal filter unit (Millipore, Co., Bedford, MA), and solids remained on the filter were extracted twice (200 μL, each for 10 minutes) with acetonitrile. The acetonitrile extracts were combined with the aqueous phase. Representative samples were also taken for the analysis of aqueous phase and solid phase concentrations, respectively. 2,4-DNT, 2,6-DNT and TNT were analyzed on a Hewlett Packard series 1050 HPLC equipped with an UV detector. Analytes were separated on a reverse-phase Hypercarb porous graphite column (5 μm, 100 x 3mm) (Hypersil, Runcorn, UK) with an isocratic mobile phase of 10:90 (v/v) of water in 0.1% trifluroacetic acid and acetonitrile in 0.05% trifluroacetic acid at a flowrate of 1 mL/min. Spectra were acquired between 190 and 400 nm and chromatograms extracted at 230 nm for quantitation.

Subsamples for nitrite analysis were centrifuged and the supernatant was immediately withdrawn and subjected to analysis using a modified colorimetric method (APHA, 1992). Absorbance readings were made at 560 nm on an EL340 Automated Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT).

Slurry samples taken from reactors were placed directly in a 300-mL BOD bottle for oxygen uptake rate analysis. After re-aeration at 30°C, dissolved oxygen concentrations were measured using a YSI Model 58 dissolved oxygen meter with a YSI 5905 BOD probe (Yellow Springs Instrument Co., Inc., Yellow Spring, OH). A typical oxygen consumption curve demonstrated zero-order kinetics, and the oxygen uptake rate was determined directly by linear regression.

The reactors were also routinely monitored by the measurement of temperature, pH, SS, and sodium hydroxide consumption. Treated soil wash (sludge) samples were also taken for the analyses of residual 2,4-DNT, 2,6-DNT and TNT. These samples were taken after the settling of the reactor effluent from representative runs and subsequent decanting of the supernatant. The wet sludge samples were oven-dried overnight at temperature below 55°C and subjected to homogenization prior to analysis.

3 RESULTS

3.1 Reactor Fed Soils without Inoculation

This section summarizes results from initial stage of this pilot study. Soils from BAAP and VAAP were added directly to slurry reactors at a solids concentration of 10% (w/v). Reactors were operated to evaluate solids distribution and concentration profile of target compounds under operational conditions. The main tasks were three folds: (1) To identify any potential problems associated with the operation of slurry reactors with the test soils, (2) To determine the concentration profiles in the slurry reactor and develop a sampling protocol for the routine monitoring of SS, 2,4-DNT, 2,4-DNT and TNT, and (3) To establish the baseline degradation (if any) by indigenous microorganisms (i.e., no inoculations were added).

3.1.1 SS profiles

Results of the suspended solid profiles at a 10% actual solids loading rate are presented in Figures 2 and 3. Clear differences existed between these two soils with respect to the ability of the reactors to maintain a homogeneous suspension. The high sand content of the BAAP soils did not allow for adequate mixing, and the majority of soils settled at the bottom of the reactor. By incrementally adding soils to the reactor to higher loading rates, operational problems were further identified, especially for the sandy BAAP soil. Solids concentrations in excess of 10% resulted in plugging of the

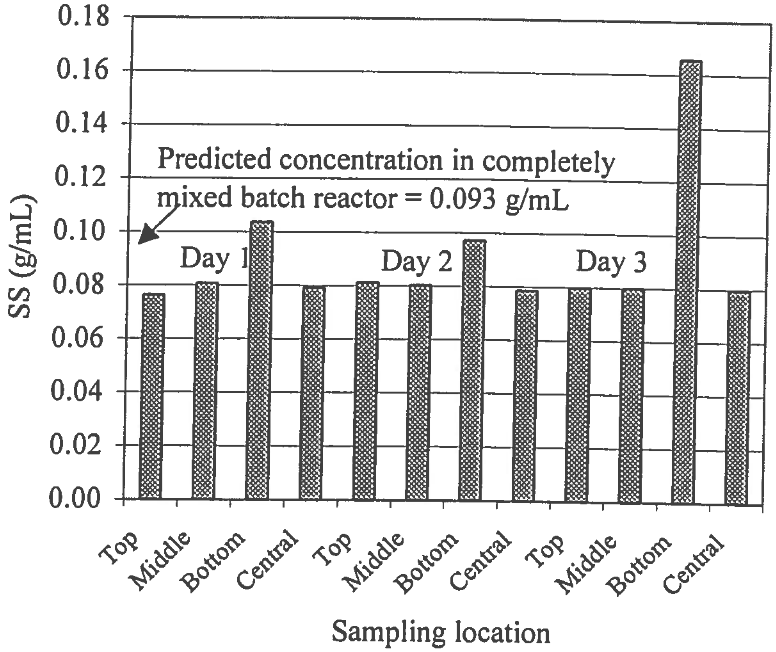

Figure 2 Suspended solid concentration profile: VAAP soil at 10% (w/v) solids loading rate

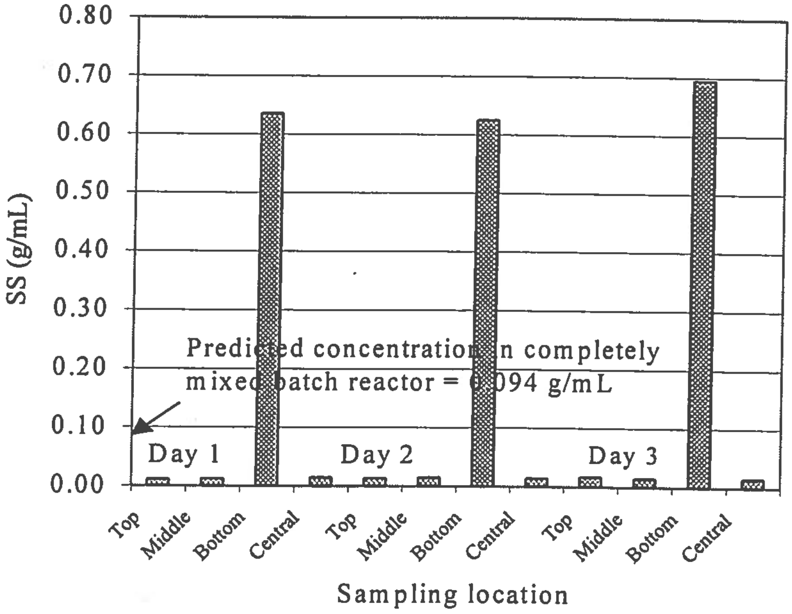

Figure 3 Suspended solid concentration profile: BAAP soil at 10% (w/v) solids loading rate

air-lift chamber. Furthermore, removal of BAAP sands from the reactor was very difficult and not amenable to routine fill-and-draw operation.

3.1.2 2,4-DNT, 2,6-DNT and TNT profiles

Nitroaromatic concentration profiles were obtained during 10% solids operation for both soils. Results for aqueous phase concentration profiles are presented in Figures 4 and 5 for VAAP and BAAP soils, respectively. It is obvious that aqueous concentrations were the same among samples taken from different locations of the reactor. However, larger variations in solid phase concentrations existed due to incomplete mixing (Figure 6 and 7). Concentrations of nitroaromatic compounds during a 3-day period remained constant, implying that the degradation by indigenous microbes did not occur.

3.2 Removal of Large Particulates by Soil Washing and Soil Washing Efficiency

In an attempt to overcome problems associated with the BAAP sands, soil washing studies were conducted to evaluate the potential to remove contaminants from the sand with a repeated resuspension in water – simulating a soil washing procedure. Preliminary results from bench studies (see **Section 3.7.1**) showed that soil washing could effectively remove three target nitroaromatic compounds from soil. Further studies were conducted to determine the ability to reduce the volume of water required, so that the procedure could be implemented for use as a pretreatment for BAAP soils under operational conditions.

3.2.1 Mass of large particulates removed in soil wash

Soil washing was shown to be very successful in removing larger particles and sands from soil (Figure 8). On a weight basis, 87% of larger particles in BAAP soil

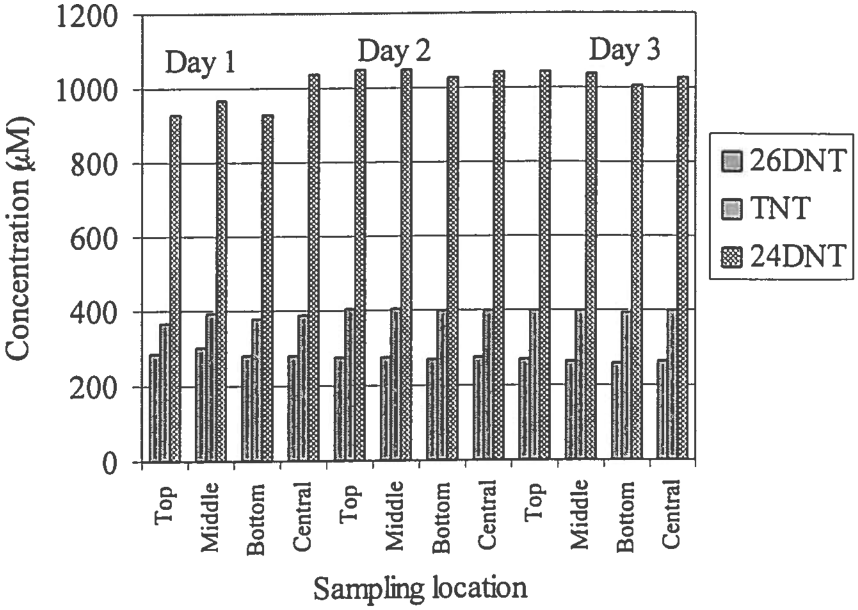

Figure 4 Aqueous phase contaminant concentration profile: VAAP soil at 10% solids loading rate

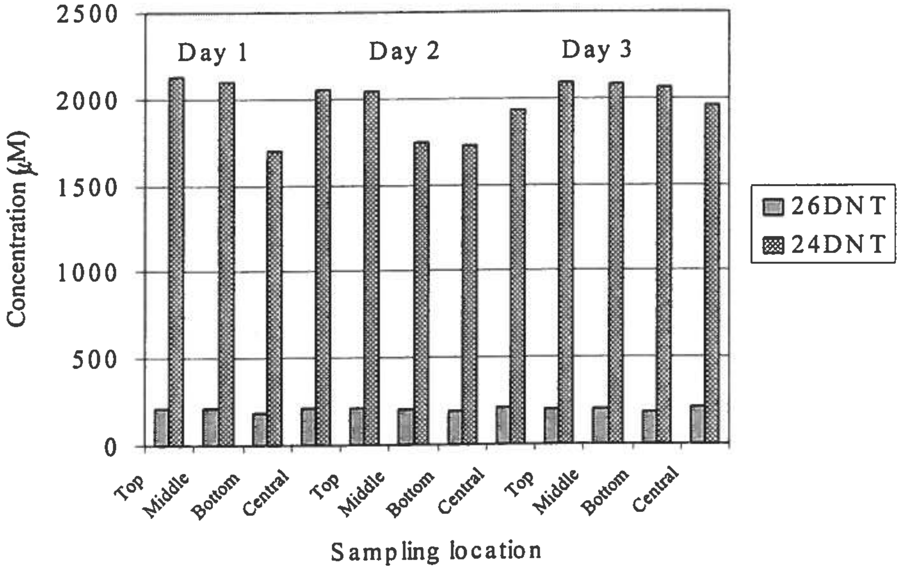

Figure 5 Aqueous phase contaminant concentration profile: BAAP soil at 10% solids loading rate

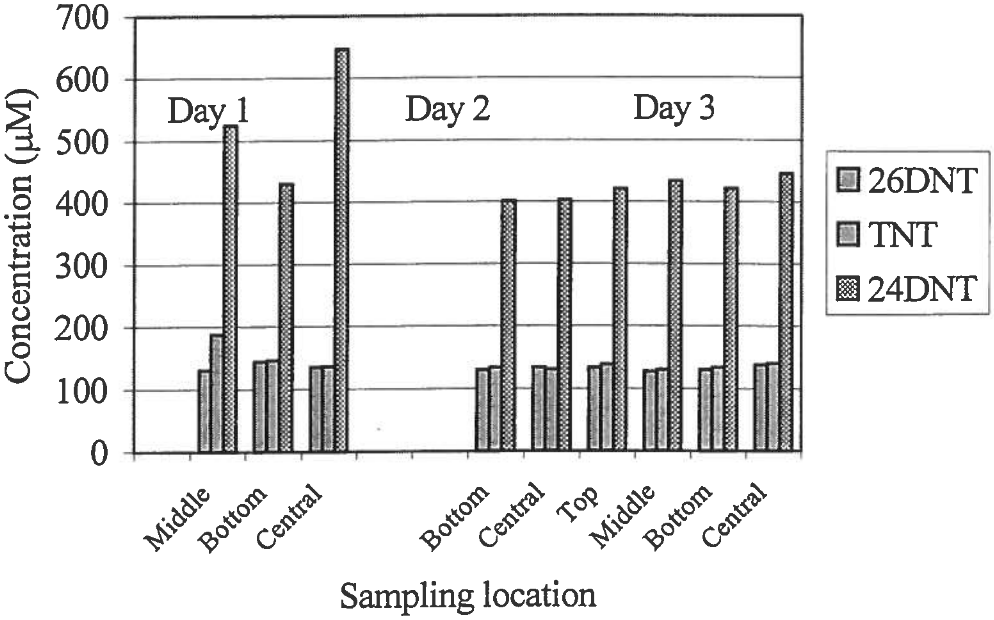

Figure 6 Solid phase contaminant concentration profile: VAAP soil at 10% solids loading rate

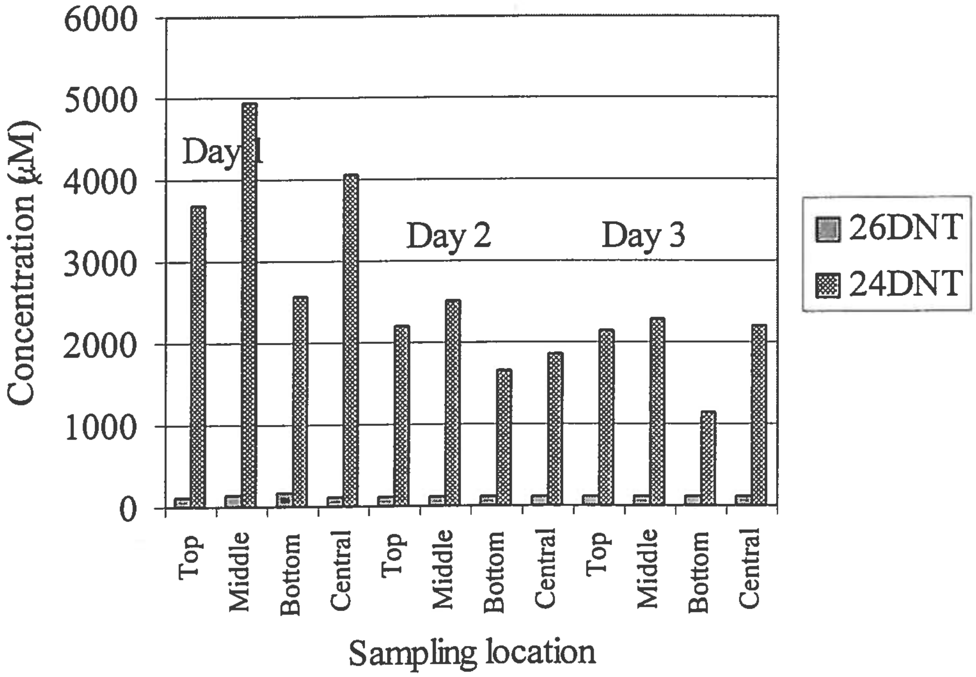

Figure 7 Solid phase contaminant concentration profile: BAAP soil at 10% solids loading rate

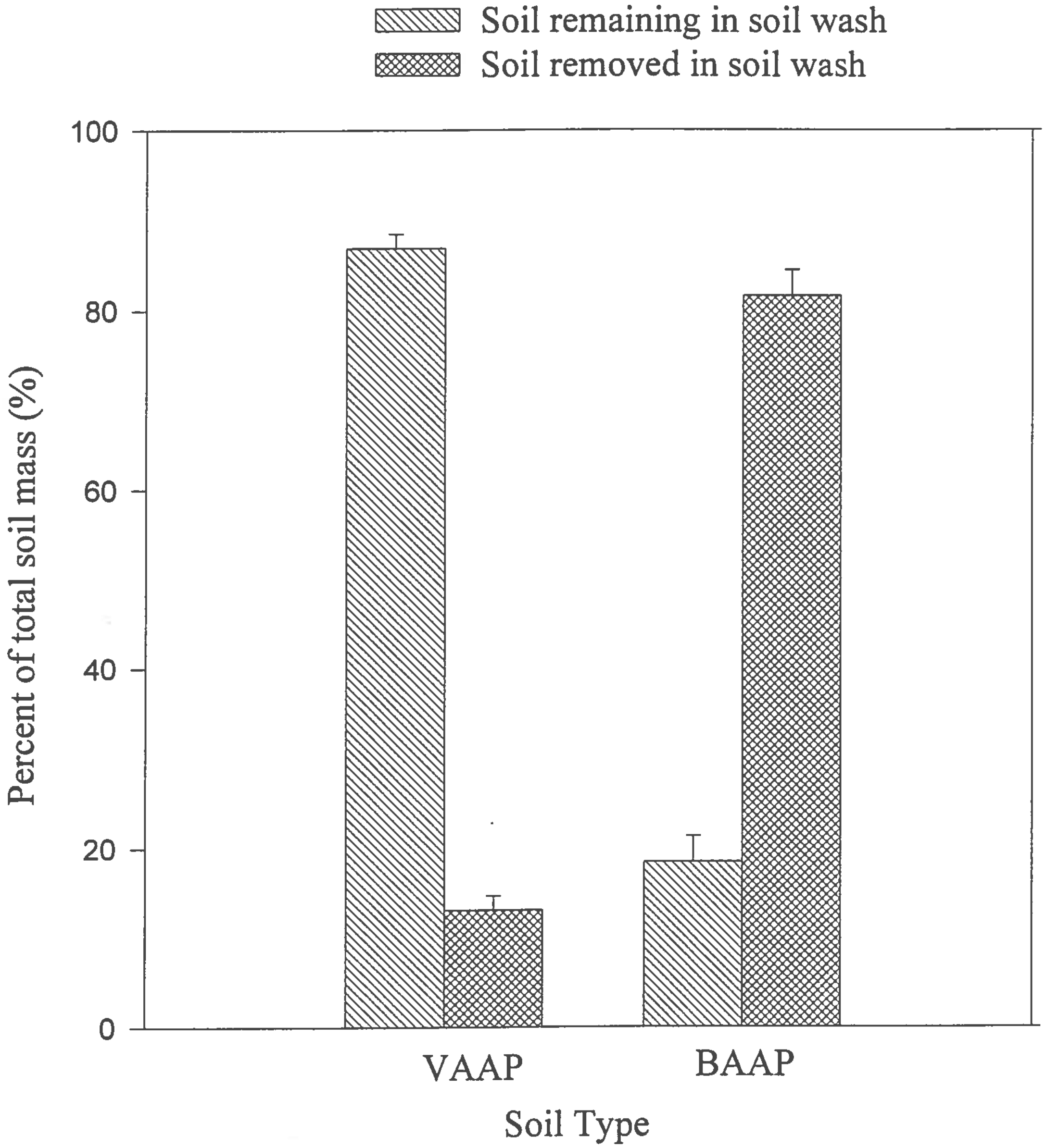

Figure 8 Soil mass distribution after soil washing

were removed after soil washing. The remaining 13% of soils were finer particles and remained in soil slurry. For VAAP soil, the distribution after soil washing was nearly reversed, as VAAP soil was comprised mostly of fine particles. Approximately 18% of VAAP soil (weight basis) was removed after soil washing, and 82% of the soil was fine particles that remained in the slurry phase.

3.2.2 Residual concentrations in soil removed in soil wash

The efficiency of soil washing at a 5% (w/v) nominal solids loading rate (i.e., mass of soil used in soil washing /70 L reactor) of BAAP soil was evaluated under two operational conditions (12 L x 5 washings vs. 6 L x 5 washings) with the only variable being the total volume of water used (60 vs. 30 L). Both tests produced satisfactory results. Figure 9 presents the residual concentrations of sands after 5 soil washings. These residual concentrations corresponded to a soil washing efficiency of greater than 99% (Figure 10 and 11). Note that soil washing efficiency in Figure 10 is based on the concentration reduction $(C_0 - C)/C_0$, while the efficiency in Figure 11 is calculated by the total mass of the contaminant, i.e., $(M_0 * C_0 - M * C) / M_0 * C_0$, where C_0 and M_0 are the concentration and total mass of soil before soil washing, respectively, and C and M are the residual concentration and total mass of sand removed after soil washing operation, respectively.

3.2.3 Effects of water/soil ratios on soil washing efficiency

The efficiency of soil washing for BAAP soil was also examined at different ratios of water/soil to determine the minimal water required under operational conditions. Results based on residual concentration, concentration reduction, and total mass of contaminants are presented in Figure 12, 13, and 14, respectively. These

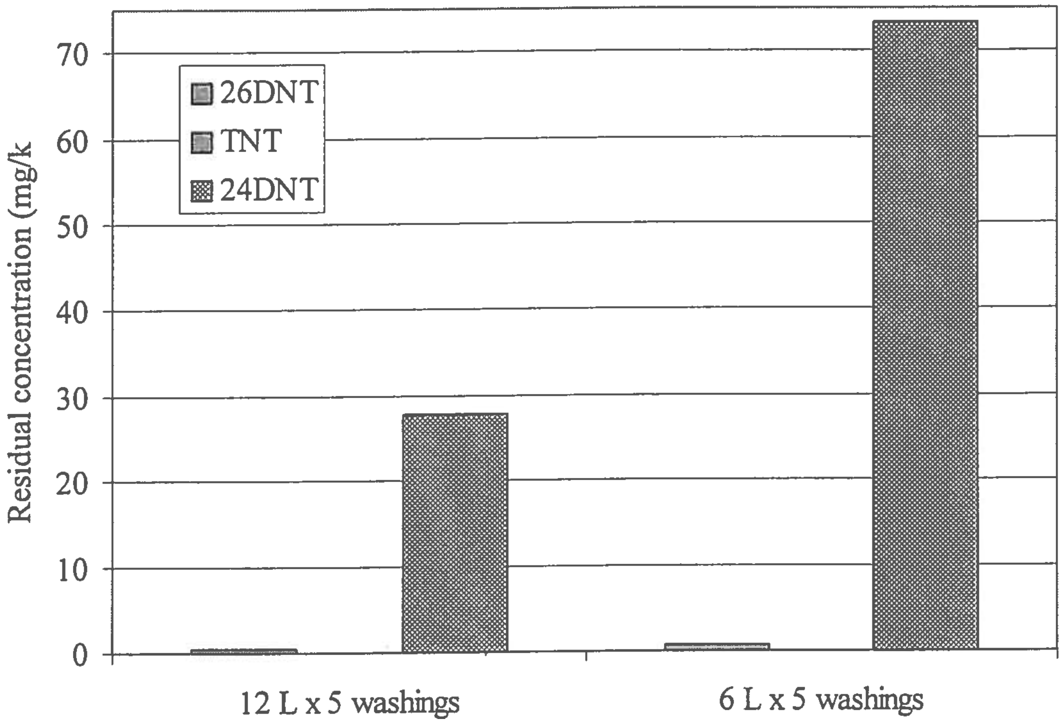

Figure 9 Soil washing efficiency based on residual concentration (BAAP soil): 12 L x 5 washings vs. 6 L x 5 washings

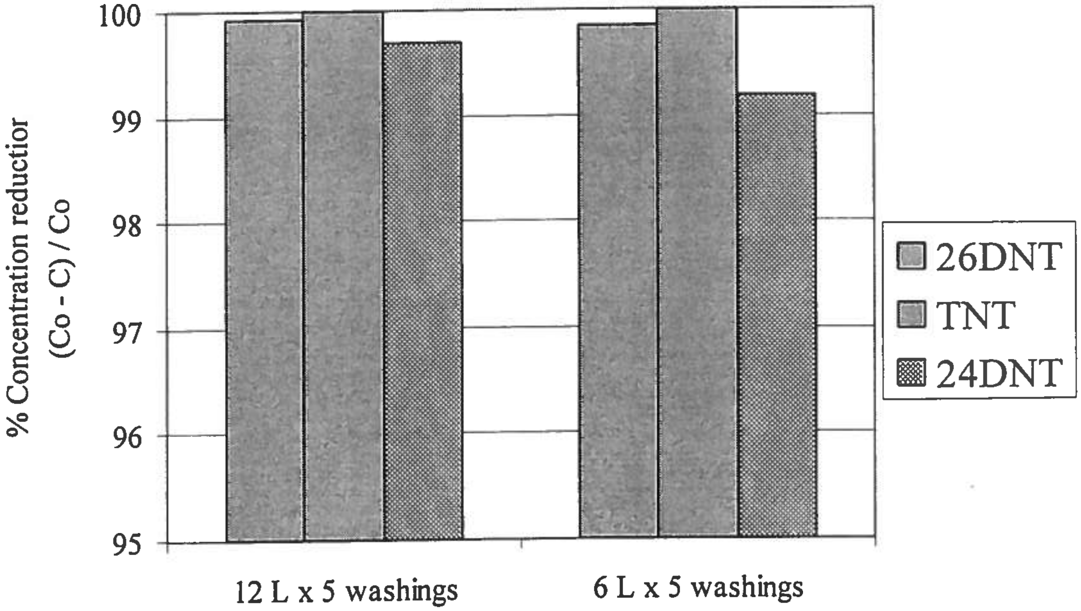

Figure 10 Soil washing efficiency based on concentration reduction (BAAP soil): 12 L x 5 washings vs. 6 L x 5 washings

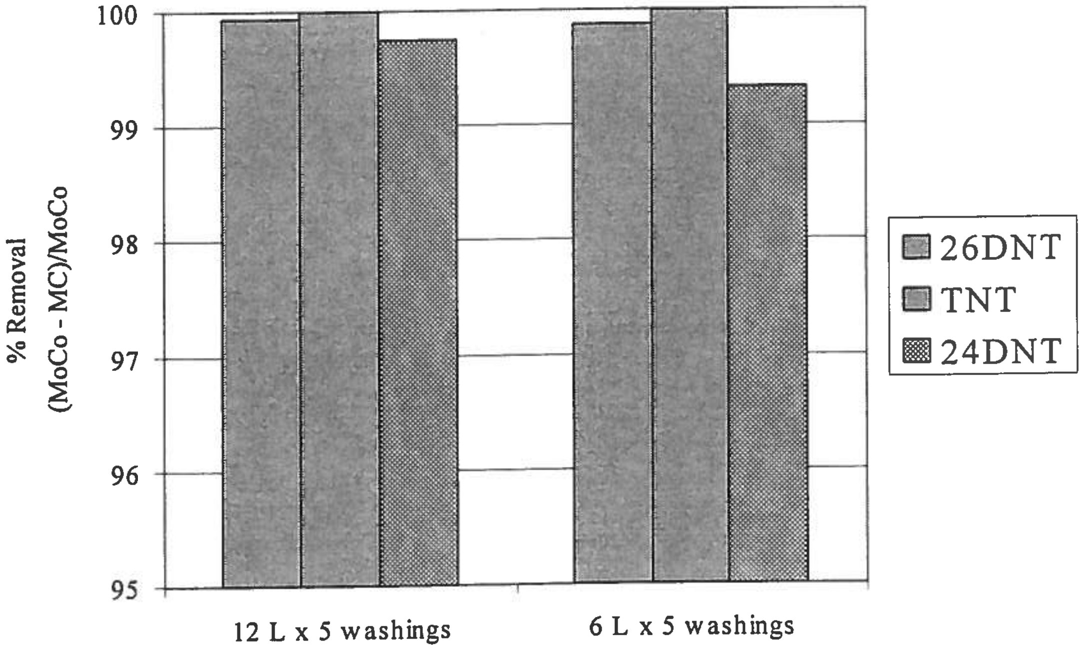

Figure 11 Soil washing efficiency based on total mass of contaminant (BAAP soil): 12 L x 5 washings vs. 6 L x 5 washings

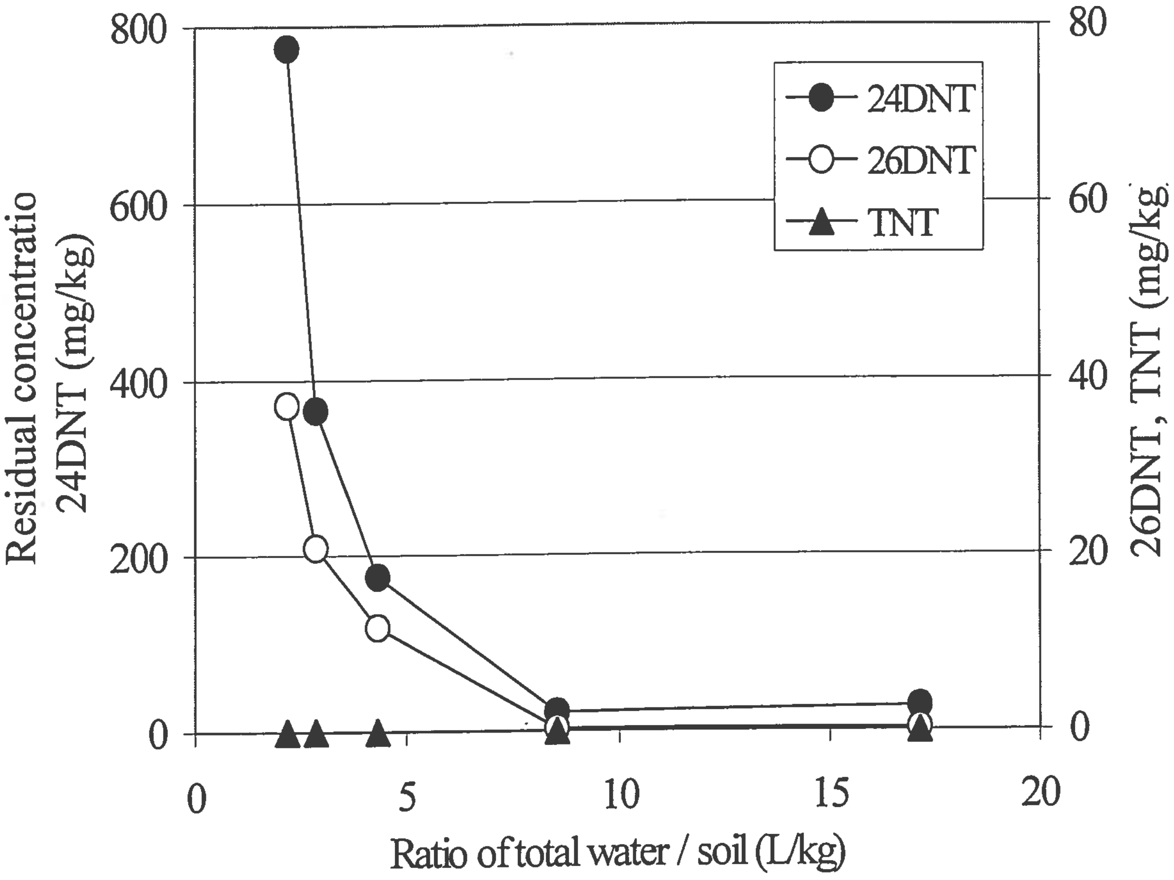

Figure 12 Effects of water/soil ratio on soil washing efficiency: Residual concentration in BAAP soil

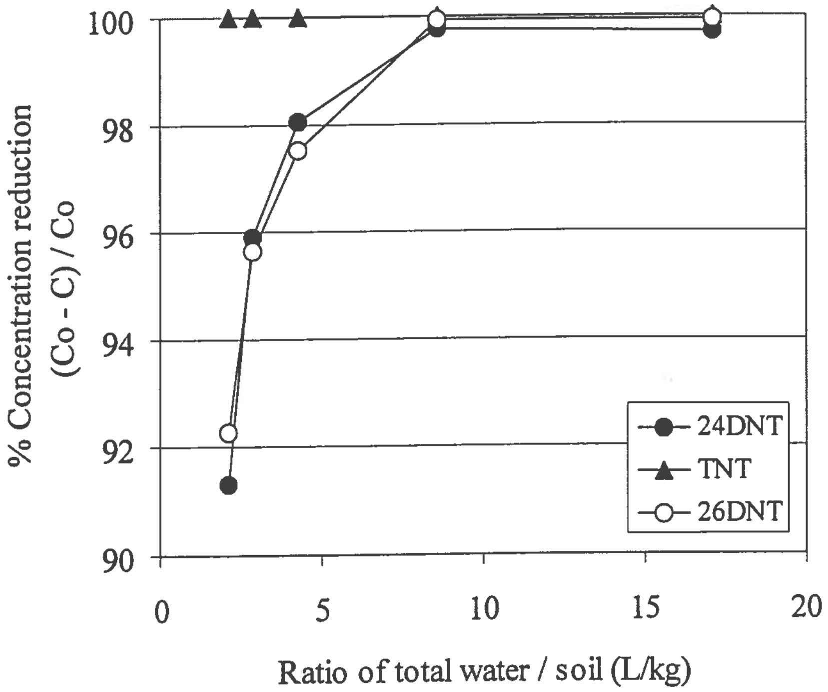

Figure 13 Effects of water/soil ratio on soil washing efficiency: Concentration reduction in BAAP soil

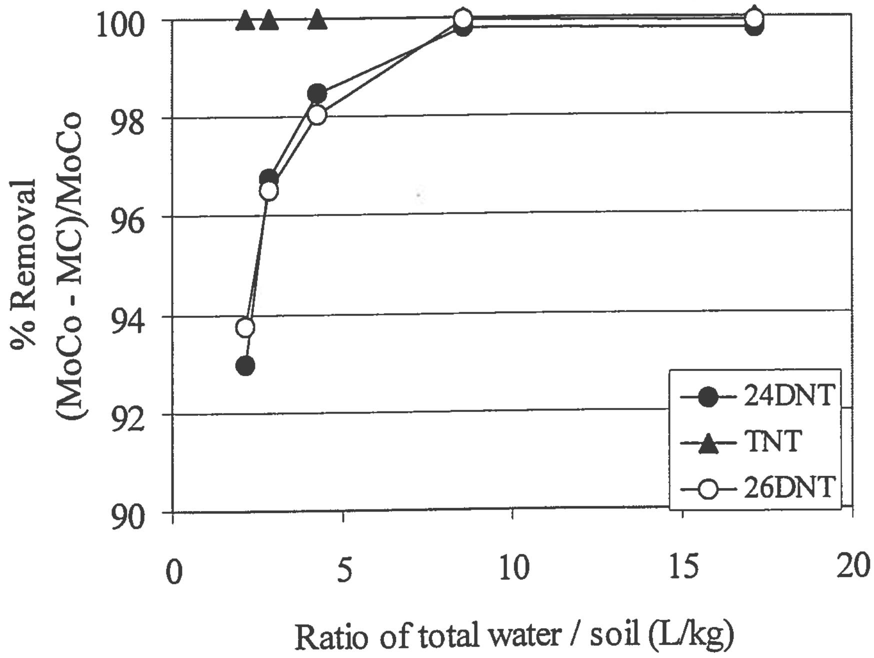

Figure 14 Effects of water/soil ratio on soil washing efficiency: Total mass of contaminant in BAAP soil

results suggest that a water/soil ratio of 10 L/kg or greater resulted in nearly complete removal of contaminants from soil. However, a ratio of 5 L/kg or less resulted in a significant amount of residuals, and dramatically reduced the efficiency (Figure 13 and 14).

For VAAP soil, a similar pattern was noticed regarding the effects of water/soil ratio on the efficiency of soil washing (Figure 15, 16, and 17). Increasing water/soil ratio apparently improved soil washing efficiency. However, VAAP soil appeared to have higher residual concentrations when compared to BAAP soil at an identical operational condition (Figure 15 vs. 9). A water/soil ratio of 17 L/kg was necessary to maintain a 98% reduction in concentration (Figure 16). It should also be noted that at all the operational conditions tested (3, 4, and 17 L/kg), an efficiency of 99.3% or greater was demonstrated if the efficiency is calculated based on the total mass of contaminant (Figure 17).

3.3 Reactor A and B Performance: VAAP Soil at Low Solids Loading Rates

3.3.1 Temperature, pH and SS

Variations of temperature, pH and SS in reactors A and B are shown in Figure 18 and 19, respectively. Reactor A had an average temperature of 30.0 ± 1.8°C (range: 24.5 ~ 33.0), and pH of 6.93 ± 0.09 (range: 6.73 ~ 7.10). Reactor B had an average temperature of 30.2 ± 1.0°C (range: 27.0 ~ 32.2), and pH of 7.00 ± 0.07 (range: 6.84 ~ 7.14). These results suggest that both reactors achieved satisfactory performance with regard to temperature and pH control.

Reactor A was operated at a nominal 5% (w/v) solids loading rate of VAAP soil wash throughout the study. The actual SS concentration in the reactor (sampled from the

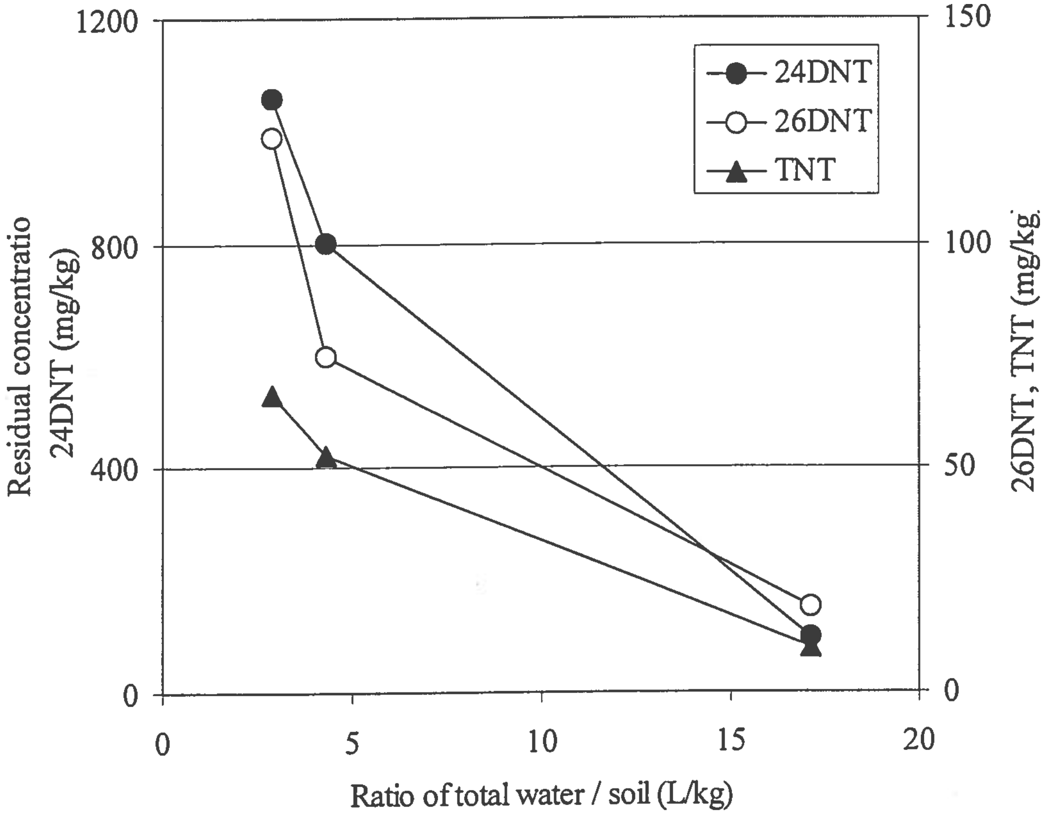

Figure 15 Effects of water/soil ratio on soil washing efficiency: Residual concentration in VAAP soil

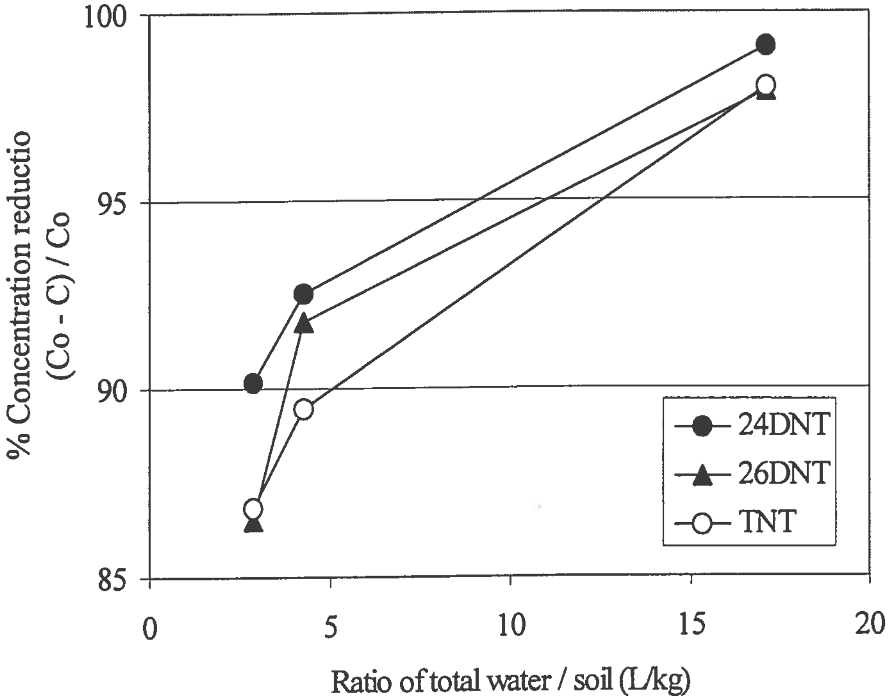

Figure 16 Effects of water/soil ratio on soil washing efficiency: Concentration reduction in VAAP soil

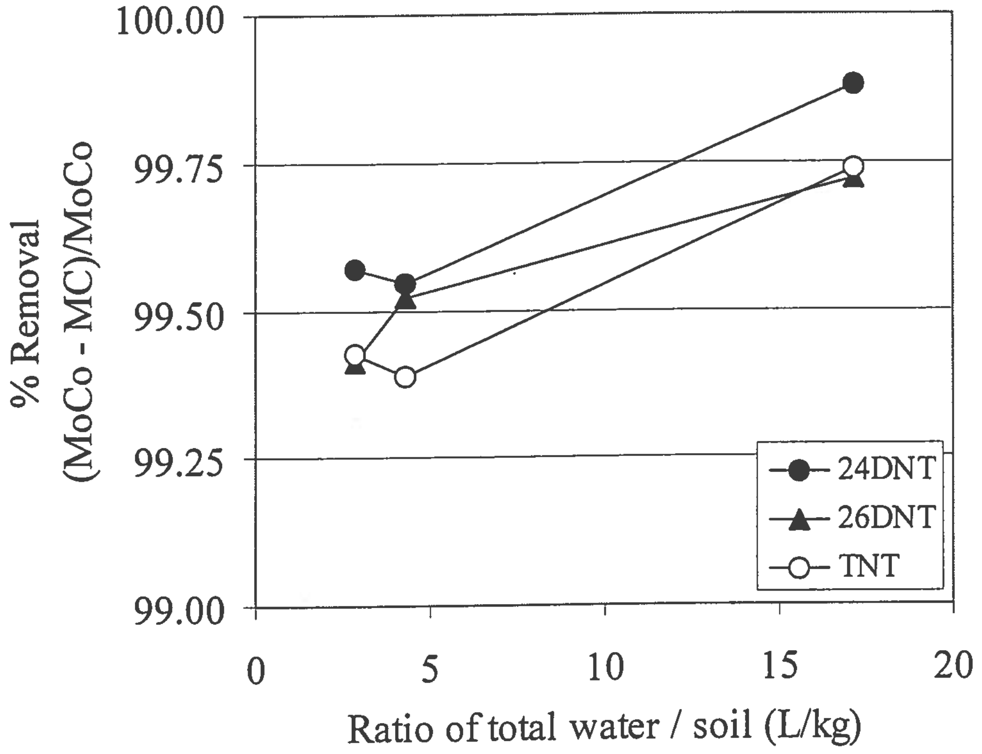

Figure 17 Effects of water/soil ratio on soil washing efficiency: Total mass of contaminant in VAAP soil

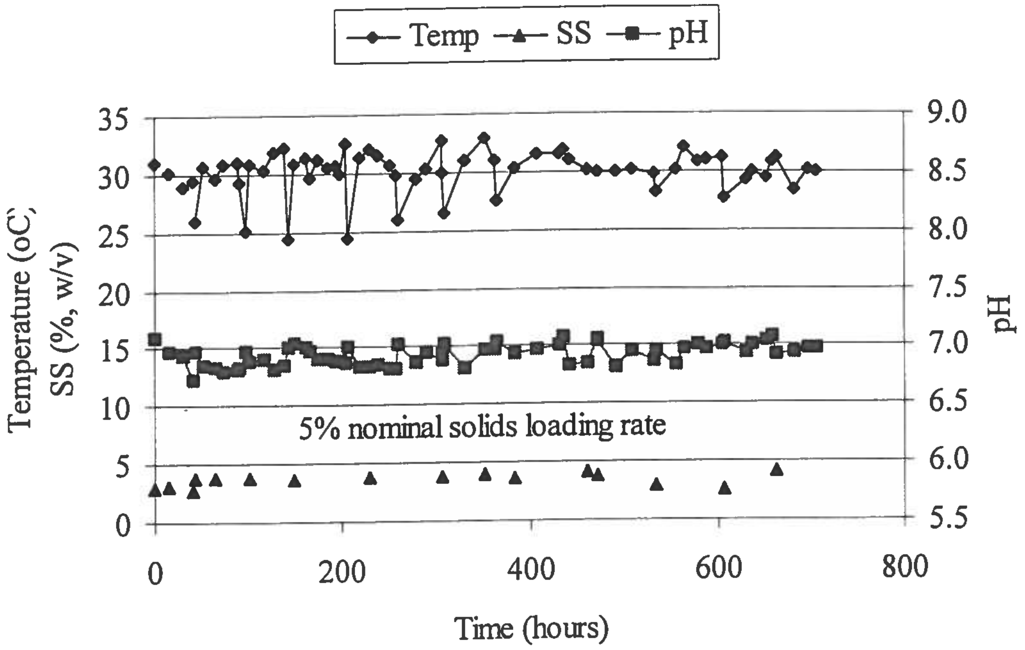

Figure 18 Variation of temperature, pH and SS in Reactor A

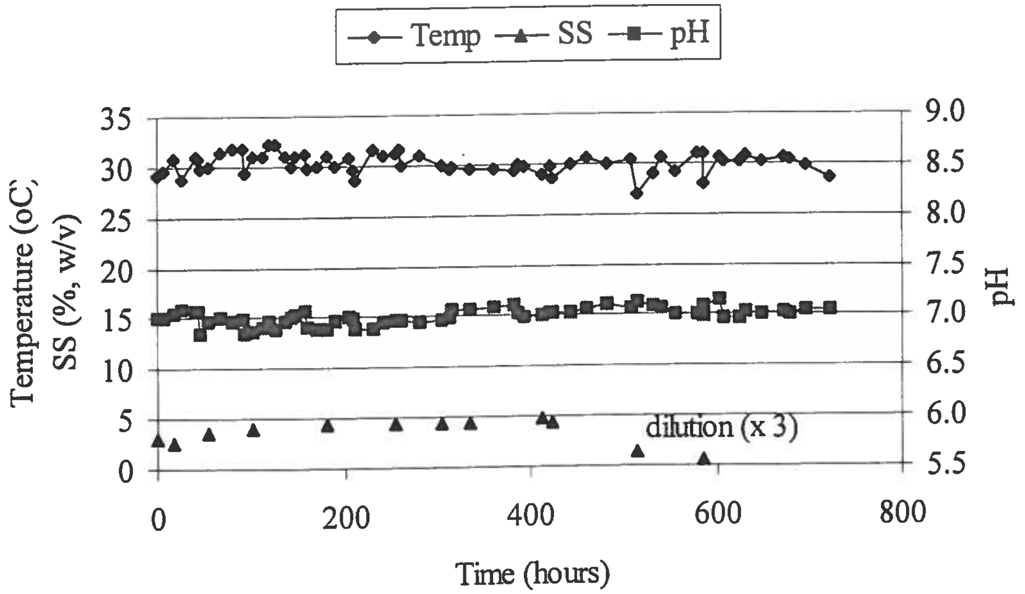

Figure 19 Variation of temperature, pH and SS in Reactor B

top) was 3.56 ± 0.50% (range: 2.65 ~ 4.22%). The discrepancy between the nominal loading rate and the actual SS concentration is expected because 18% of sand were removed during soil washing. In addition, the nonhomogeneous distribution of suspended solids in the reactor may attribute to this discrepancy. Reactor B was fed effluent from Reactor A during the first 9 cycles, and fed a 3-fold diluted Reactor A effluent during the last two cycles. The actual SS concentrations was 4.03 ± 0.69% (range: 2.63 ~ 4.75) for the first 9 cycles, and 1.35% and 0.53% for the last 2 cycles (Figure 19).

3.3.2 Degradation of 2,4-DNT, 2,6-DNT and TNT

Figure 20 presents the temporal concentrations of 2,4-DNT, 2,6-DNT and TNT throughout the 13 feeding cycles of continuous operation with VAAP soil wash. Although the reactor was tested at the same loading rate (5% VAAP soil), it is noted that there were marked differences in the initial concentrations due to two different batches of VAAP soil used during the course of the test. Almost complete degradation of 2,4-DNT was observed during a 2-day period of each feeding cycle, despite the presence of high concentration of 2,4-DNT in the slurry reactor (~ 4000 μM). The rate of 2,6-DNT degradation was much slower, as is evident from Figure 20. Only 40% of 2,6-DNT were degraded in one feeding cycle even though the initial concentration of 2,6-DNT was about 10-times lower than 2,4-DNT. Similar patterns of TNT degradation can also be seen from Figure 19. It should be pointed out that the slurry was initially supplemented with 20 mM phosphate buffer to maintain the buffering capacity and supply the phosphorous nutrient. Phosphate concentrations were reduced to 1 mM after 435 hours of operation. No changes in the rate and extent of degradation were noted, indicating that

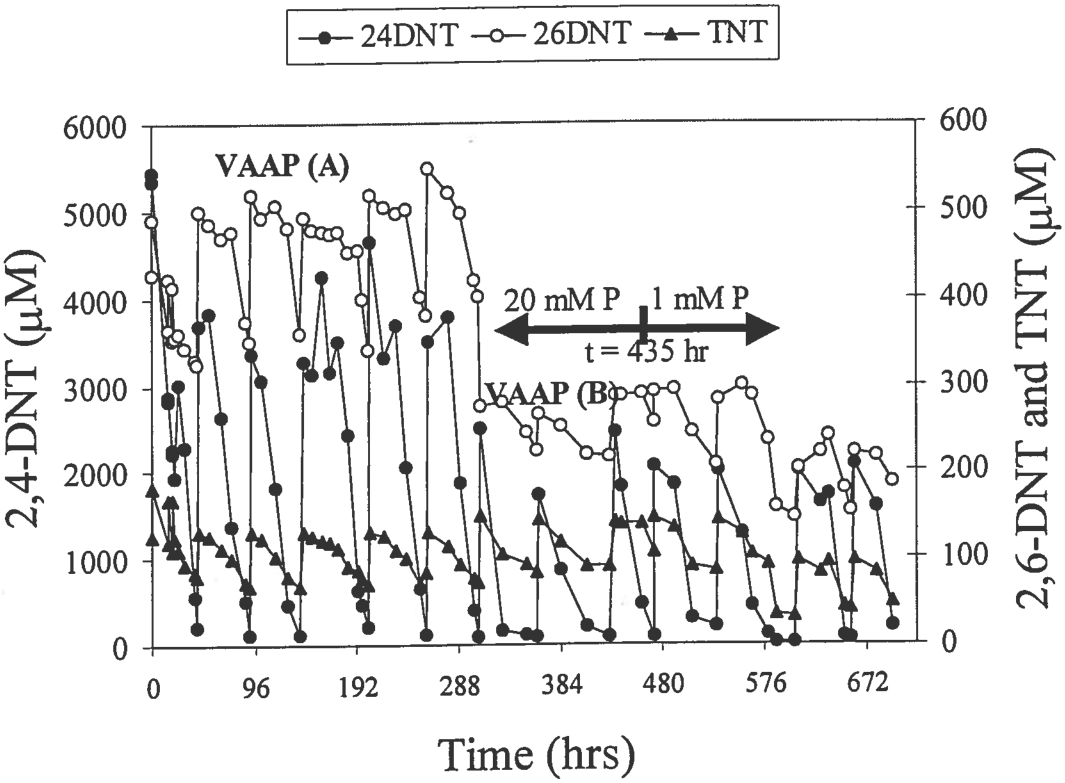

Figure 20 Degradation of 2,4-DNT, 2,6-DNT and TNT in Reactor A

1 mM of phosphate was sufficient at the test loading rates. Therefore, 1 mM of phosphate was used throughout the remaining studies.

Reactor B was operated in a sequential mode with Reactor A, i.e., fed undiluted effluent from Reactor A. It was monitored for the degradation of 2,6-DNT and TNT that was not degraded in Reactor A, and for any additional degradation of 2,4-DNT. The concentration profiles of 2,4-DNT, 2,6-DNT and TNT are shown in Figure 21. The reactor was inoculated with a mixed bacterial culture during the start-up, and 10% (7 L) of the slurry was carried over for microbial seeds during the subsequent runs. Phosphorous nutrient was carried over from Reactor A, no additional nutrients were supplied. Although slight degradation of 2,6-DNT was observed in occasional feeding cycles, complete degradation was not sustained and performance was inconsistent. Since the conditions of temperature, pH (Figure 19) and oxygen supply were adequate throughout the test period, these results were attributed to the loss of 2,6-degrading bacteria activity. To test if the limited activity was due to high 2,6-DNT concentrations or the nitrite produced during 2,4-DNT degradation, two successive 3-fold dilution of the slurry and re-inoculations (t = 516 hr, 587 hr) were conducted. Again, the results demonstrated little 2,6-DNT degradation activity.

3.3.3 Nitrite release

Figure 22 shows the production of nitrite in 13 feeding cycles of VAAP soil wash in Reactor A. The pattern of the nitrite production corresponded very well to the disappearance of dinitrotoluenes shown in Figure 20. In addition, by comparing the amount of nitrite released with the total amount of dinitrotoluenes degraded in each

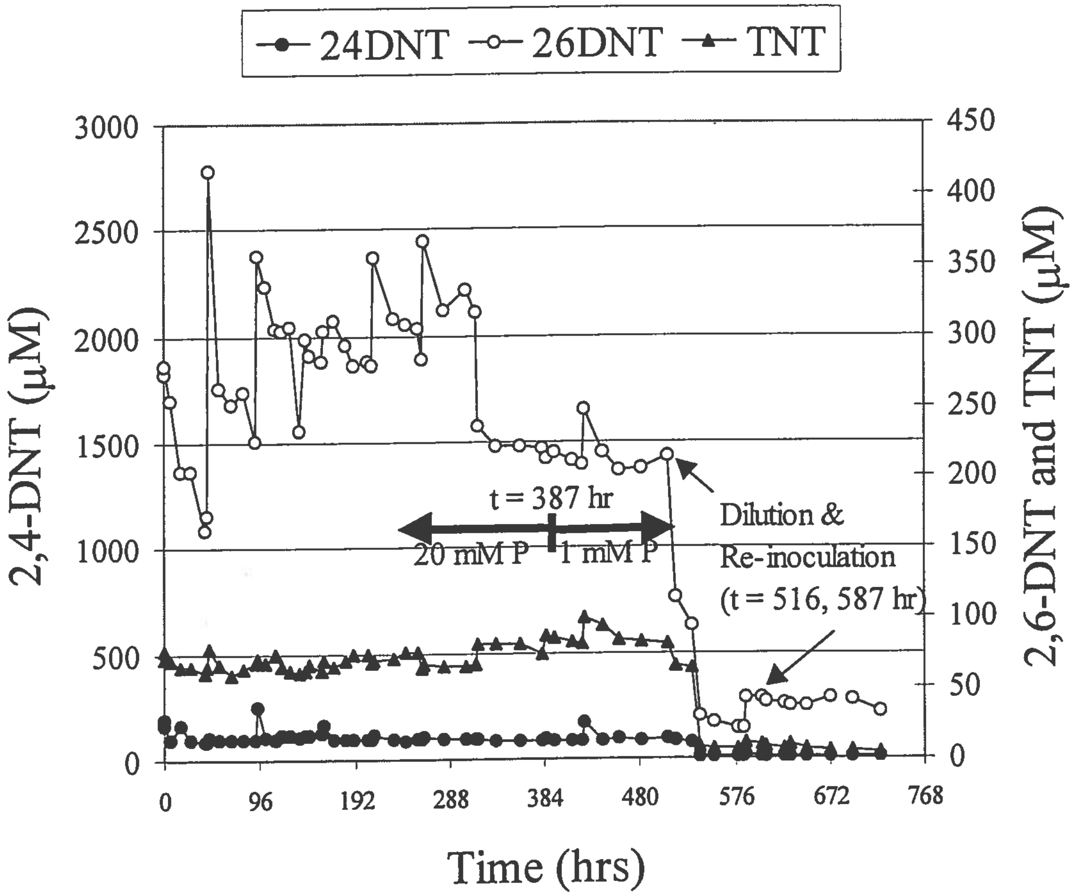

Figure 21 Degradation of 2,4-DNT, 2,6-DNT and TNT in Reactor B

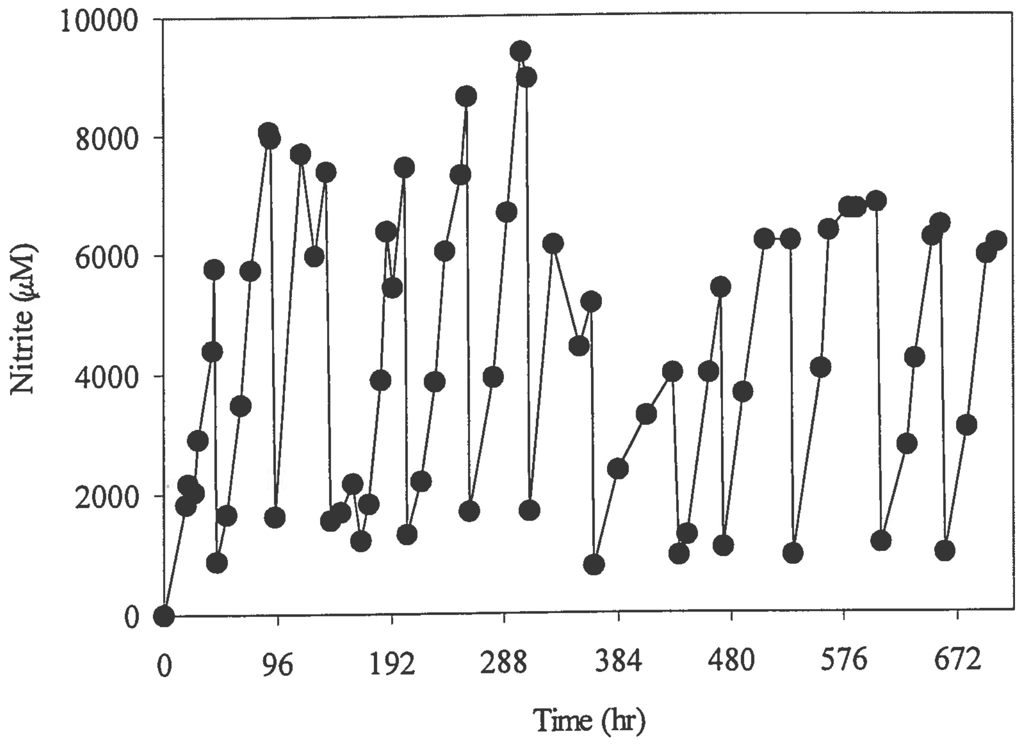

Figure 22 Nitrite concentrations in Reactor A

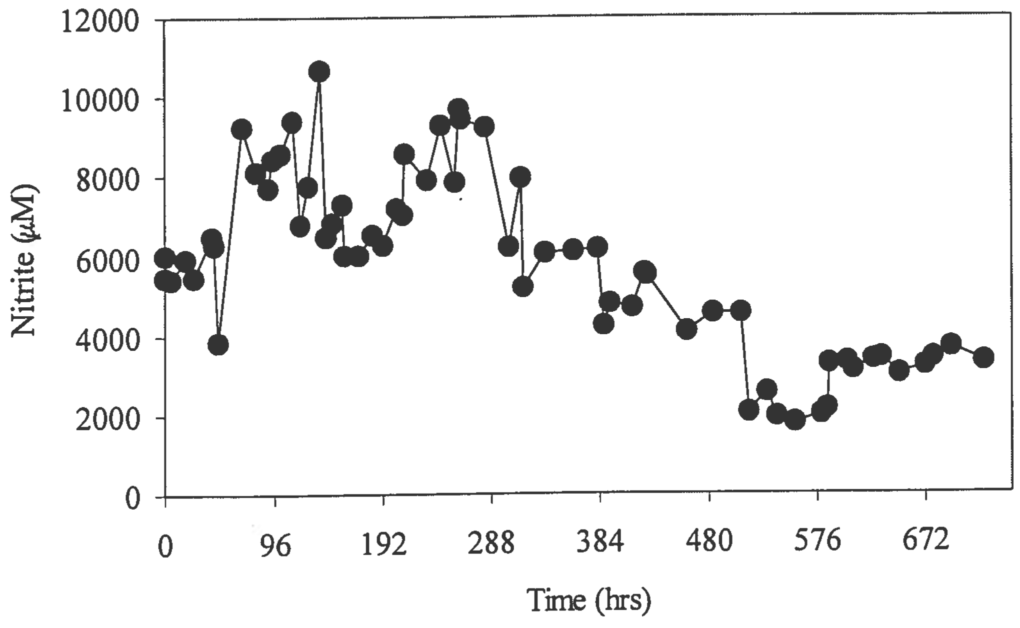

Figure 23 Nitrite concentrations in Reactor B

feeding cycle, a stoichiometric release of 2 moles of nitrite per mole of dinitrotoluene was observed.

The nitrite concentration profiles in Reactor B are given in Figure 23. Since this reactor was fed the effluent from Reactor A, the concentration fluctuations reflect the nitrite from Reactor A at the end of each feeding cycle plus the additional nitrite produced in Reactor B. In Reactor A, nitrite was released primarily from the degradation of 2,4-DNT. As the concentration of 2,6-DNT was about ten times lower than 2,4-DNT, little nitrite release was observed in Reactor B, even if 2,6-DNT degradation was occurring.

3.3.4 Oxygen uptake

In Reactors A and B, oxygen uptake rates were not routinely measured. Hence, only scatter plots of the oxygen uptake rates during the course of the study are given (Figure 24 and 25). It is clear that oxygen uptake rates corresponded to the microbial activities in both reactors. The maximum oxygen uptake rate in Reactor A was 1.4 mg/L·min, which was approximately 20 times the observed maximum oxygen uptake rate in Reactor B (0.07 mg/ L·min). For a typical feeding cycle in Reactor A, oxygen was initially consumed slowly (right after fill and draw), then increased reaching a maximum uptake rate, followed by a decrease in the rate towards the end of each feeding cycle. Oxygen consumption in Reactor B was much lower, and dilution of the slurry at the end of test run resulted in even lower oxygen uptake rates (Figure 25).

3.3.5 NaOH consumption

The consumption of sodium hydroxide (12.5 N, 50% w/v) is given on a per cycle basis, and the results are shown in Figure 26 and 27 for Reactors A and B, respectively.

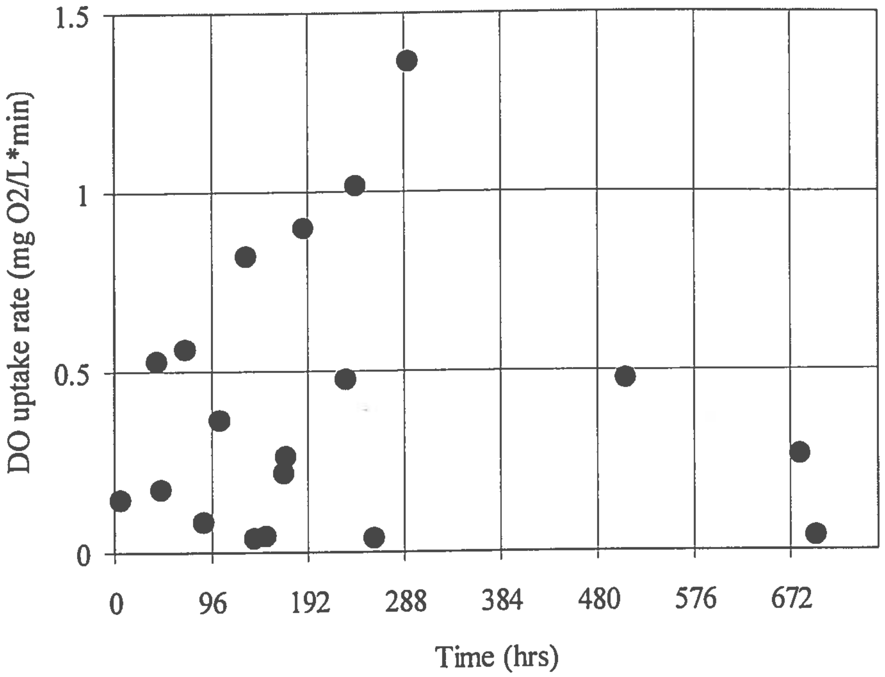

Figure 24 Oxygen uptake rate in Reactor A

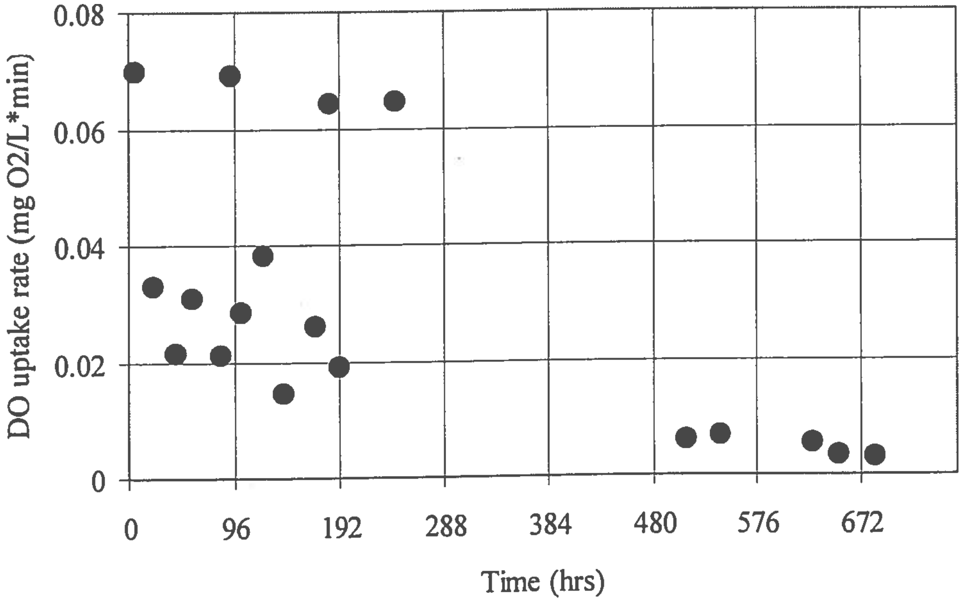

Figure 25 Oxygen uptake rate in Reactor B

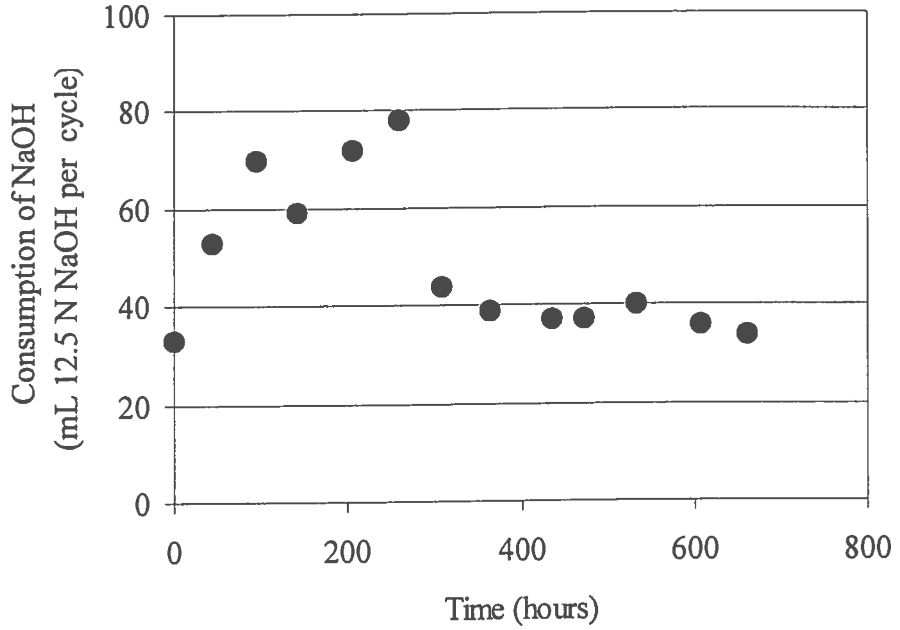

Figure 26 NaOH consumption in Reactor A

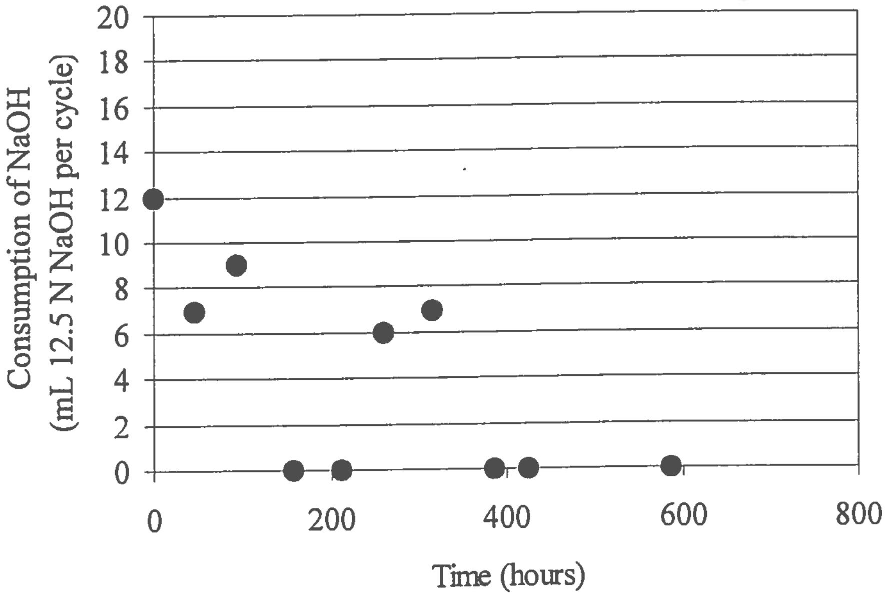

Figure 27 NaOH consumption in Reactor B

The results of NaOH consumption were generally consistent with the degradation pattern of dinitrotoluenes, the nitrite release, and the oxygen uptake rate presented in Figure 20-25. In Reactor A, a distinct difference can be seen between two batches of VAAP soil. In Reactor B, only a small amount of NaOH was consumed during the first several cycles, no NaOH was consumed when the bacterial activity in the slurry reactor was low.

3.4 Reactor C and D Performance: BAAP Soil at Various Solids Loading Rates

3.4.1 Temperature, pH and SS

Variations of temperature, pH and SS in reactors C and D are shown in Figure 28 and 29, respectively. Reactor C had an average temperature of 29.6 ± 1.1°C (range: 26.0 ~ 33.7), and pH of 7.03 ± 0.10 (range: 6.77 ~ 7.24). Reactor D had an average temperature of 28.5 ± 1.2°C (range: 24.0 ~ 31.3), and pH of 7.10 ± 0.09 (range: 6.75 ~ 7.25). These results suggest that both reactors achieved satisfactory performance with regard to temperature and pH control.

Reactor C was operated at solids concentrations ranging from 0.7% (7 g/L) to 5.2% (52 g/L), corresponding to pre-soil washing loading rates of 5% to 40% solids (mass of soil used in soil washing/70 L reactor) per feeding cycle. The actual SS concentration was much lower than the nominal loading rate, as discussed previously in Section 2.6. The SS concentrations after pretreatment with soil washing should be distinguished from the results presented earlier (Figure 3) in which BAAP soils were added directly without the removal of large particultaes. Note that when soils were directly added to the reactor without washing, clogging and mixing problems were observed even at an actual solids loading rate of 10%. Reactor D was fed continuous and

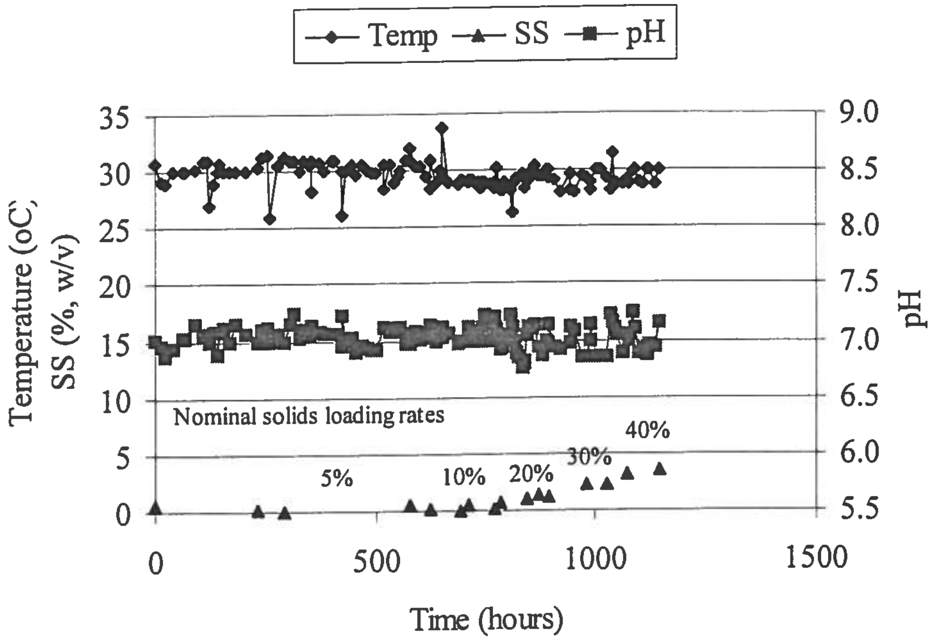

Figure 28 Variation of temparature, pH and SS in Reactor C

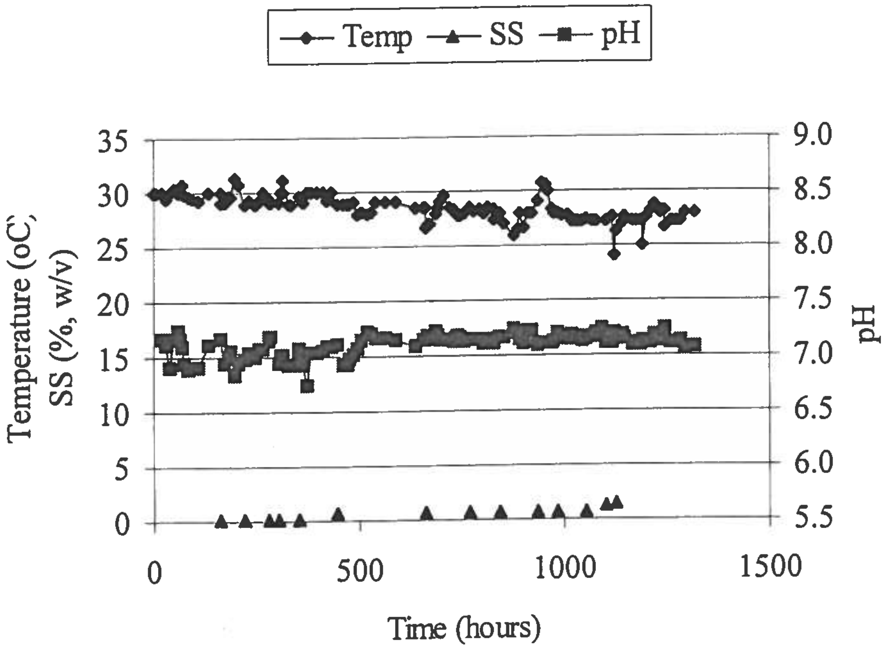

Figure 29 Variation of temperature, pH and SS in Reactor D

intermittent soil wash followed by various amount of effluent from Reactor C, the SS concentration had a range of 0.65 ~ 1.52%.

3.4.2 Degradation of 2,4-DNT, 2,6-DNT and TNT

System performance for the BAAP soil was examined in Reactor C at five nominal solids loading rates, 5%, 10%, 20%, 30%, and 40% (Figure 30), corresponding to actual solids concentrations of 0.7%, 1.3%, 2.6%, 3.9% and 5.2% in the reactor (Table 3 in Section 2.6). The performance of the reactor was very stable over a 7-week period with regard to 2,4-DNT degradation. Several observations can be made from the data shown in Figure 30. First, complete degradation of 2,4-DNT was demonstrated in the slurry reactor with BAAP soil at a nominal solids loading rate of up to 40%. This loading rate corresponded to an initial 2,4-DNT concentration of 11,230 μM (2,045 mg/L). The rate of degradation was 71.6 g 2,4-DNT/day for each reactor, assuming an observed average residence time of ~ 2 days. These results indicate that the bacteria were capable of degrading 2,4-DNT at high concentrations without noticeable inhibition by 2,4-DNT. Second, the degradation of 2,6-DNT was negligible, especially at higher solids loading rates of BAAP soil, believed to be a result of the inhibition of 2,6-DNT degradation by 2,4-DNT. This assumption is further supported by a shake flask experiment presented in Section 3.7.2. Third, the rate of 2,4-DNT degradation depended largely on the microbial activity regardless of the loading rates. For instance, a lag and/or a lower degradation rate were observed when the feeding of soil wash was delayed. This was apparent when comparing the performance between the first 6 cycles and the subsequent 3 cycles, when the reactor was fed at a constant loading rate of 5%. Finally, the effects of nutrient additions and re-inoculation on Reactor C performance were minimal. As shown in

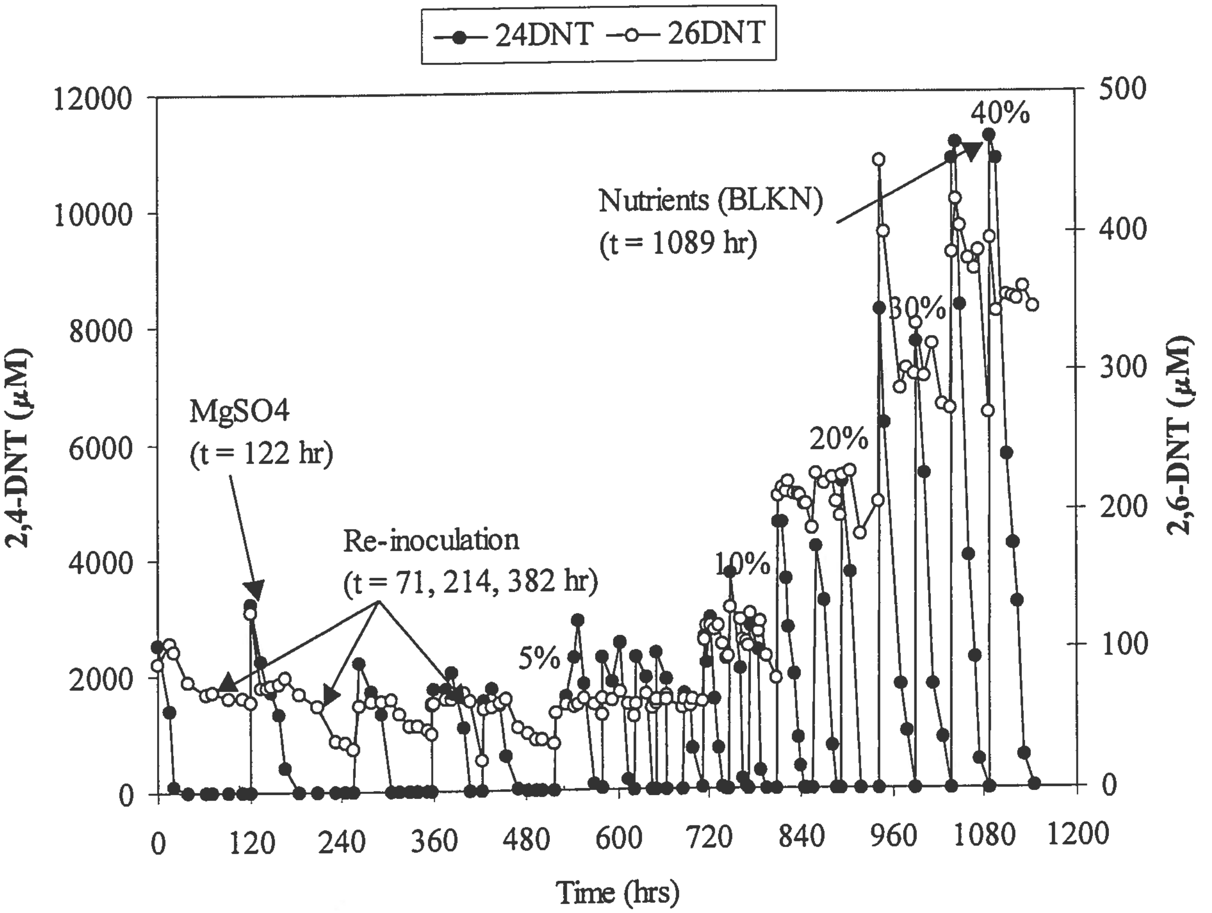

Figure 30 Degradation of 2,4-DNT and 2,6-DNT in Reactor C

Figure 30, additional nutrients were supplemented at t = 122 hr ($MgSO_4$) and t = 1089 hr (BLKN medium). The reactor was also re-inoculated several times (t = 71 hr, 214 hr and 382 hr).

Studies performed in Reactor D emphasized the degradation of 2,6-DNT. The reactor was first operated in a continuous feeding mode (i.e., continuously pumping soil wash to the reactor), followed by an intermittent mode with feedings of soil wash instead of effluent from Reactor C (Figure 31). After approximately 600 hours, the reactor was fed effluent from Reactor C in a fill-and-draw mode with increasing volume of effluent from 50:50, 35:65, to 10:90 (v/v). The results are given in Figure 32. Significant degradation of 2,6-DNT in Reactor D as shown in Figure 31 and 32 present a marked difference with the performance in Reactor B for VAAP soil. Although Reactor D lost activity of 2,6-DNT degradation in certain cycles, the activity recovered after re-inoculation. Note that the initial concentrations of 2,6-DNT in continuous mode, intermittent mode, and the first 6 cycles of fill and drawn mode were all below 50 μM in Reactor D.

As mentioned above, several inoculations of 2,6-DNT degrading bacteria were made in Reactor D when a lack of activity was observed. The effects of re-inoculation were significant as compared to the response in Reactor C. Following the re-inoculation at t = 401 hr and 645 hr, 2,6-DNT degradation occurred immediately. Reactor D was also supplemented with additional BLKN nutrients after a prolonged lag phase at t = 956 hr, the activity was recovered again. These results demonstrated the role of DNT-degrading bacteria from the bacterial culture and the significant impact of additional nutrients.

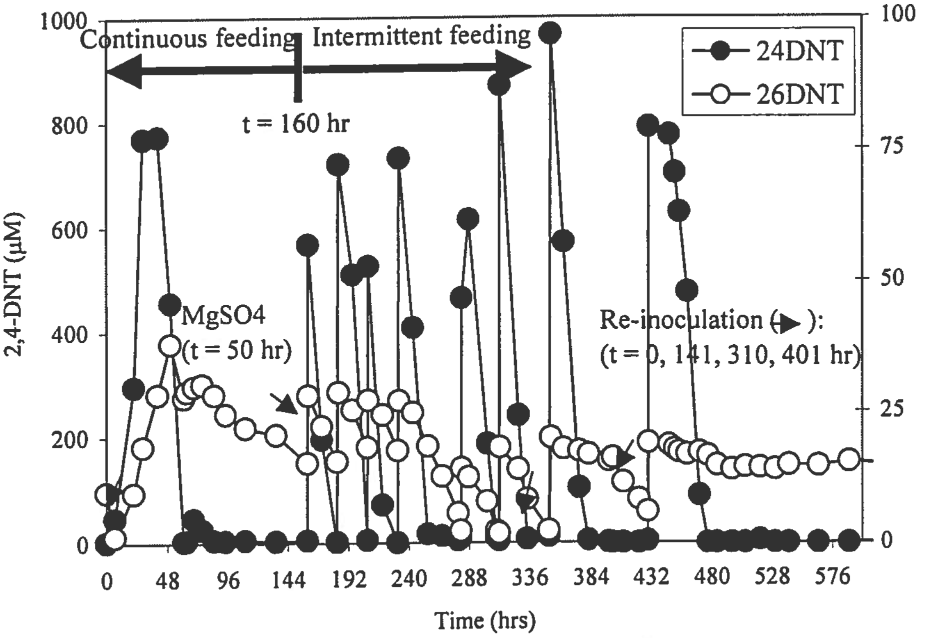

Figure 31 Degradation of 2,4-DNT and 2,6-DNT in Reactor D: Continuous and intermittent feeding modes with BAAP soil wash

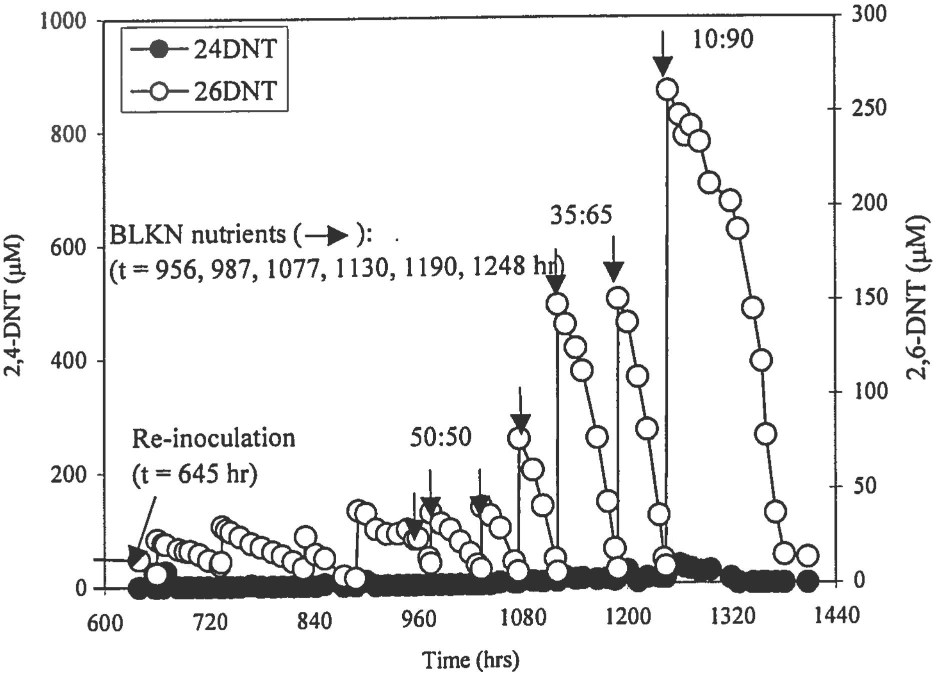

Figure 32 Degradation of 2,4-DNT and 2,6-DNT in Reactor D: Fill-and-draw mode with effluent from Reactor C

However, the physiological reason for the loss of activity in degrading 2,6-DNT is unknown.

3.4.3 Nitrite release

Figure 33 shows the production of nitrite in 20 feeding cycles of BAAP soil wash in Reactor C. Similar to the VAAP soil in Reactor A, the pattern of the nitrite production corresponded to the disappearance of dinitrotoluenes shown in Figure 30. In addition, the concentrations of nitrite increased proportionally with the increase in solids loading rates. Nitrite release calculated on the basis of dinitrotoluene degraded averaged of 1.61 mole NO_2^-/mole DNT. This is close to the stoichiometric release of 2 moles NO_2^- per mole of dinitrotoluene.

The nitrite concentration profile in Reactor D is given in Figure 34. During the initial period of continuous feeding, nitrite concentration increased gradually. The concentration remained constant between 5,000 and 10,000 μM during the intermittent feeding. Following the increase in the effluent ratio (therefore the concentration of 2,6-DNT) in the fill-and-draw mode, nitrite concentrations increased significantly from about 5,000 to 35,000 μM. Similar concentrations (~ 40,000 μM) were observed at the conclusion of Reactor C with a 40% of solids loading rate (Figure 33). It is noteworthy that degradation of 2,6-DNT still occurred at these high nitrite concentrations.

3.4.4 Oxygen uptake

In Reactor C, oxygen uptake rates were routinely measured and O_2 uptake rates corresponded to activity in each feeding cycle (data not available for the first cycle) of soil wash was clearly defined (Figure 35). It is clear that oxygen uptake rates correlated with the microbial activity of the reactor. The peak oxygen uptake rates increased with

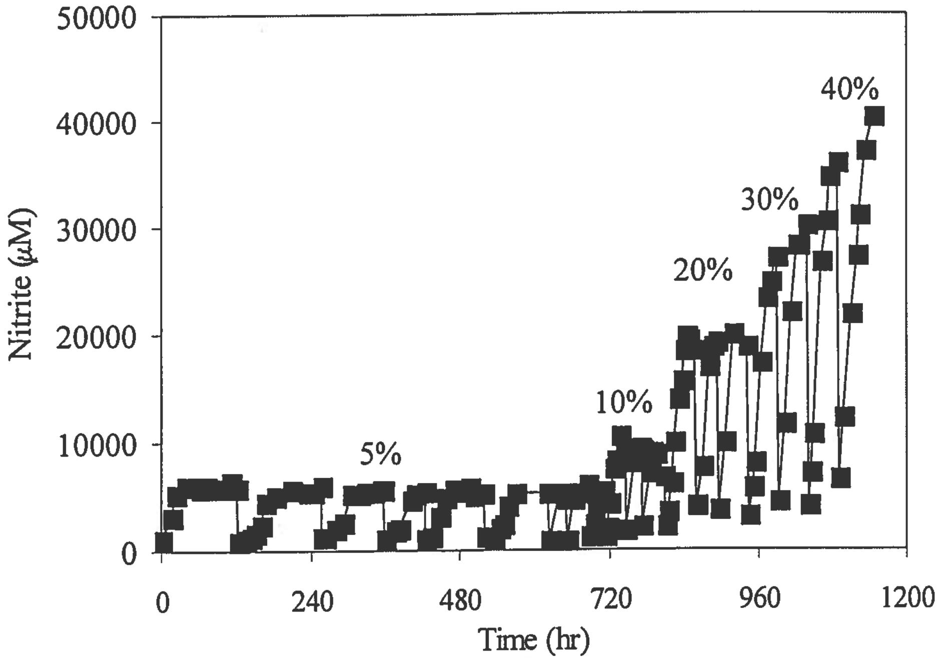

Figure 33 Nitrite concentration in Reactor C

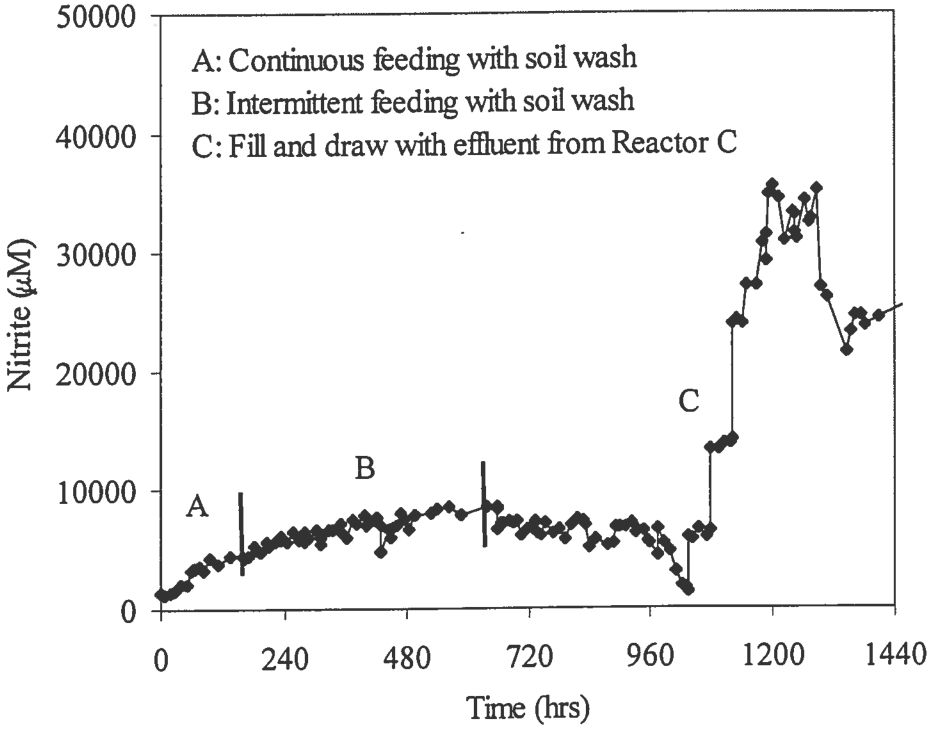

Figure 34 Nitrite concentration in Reactor D

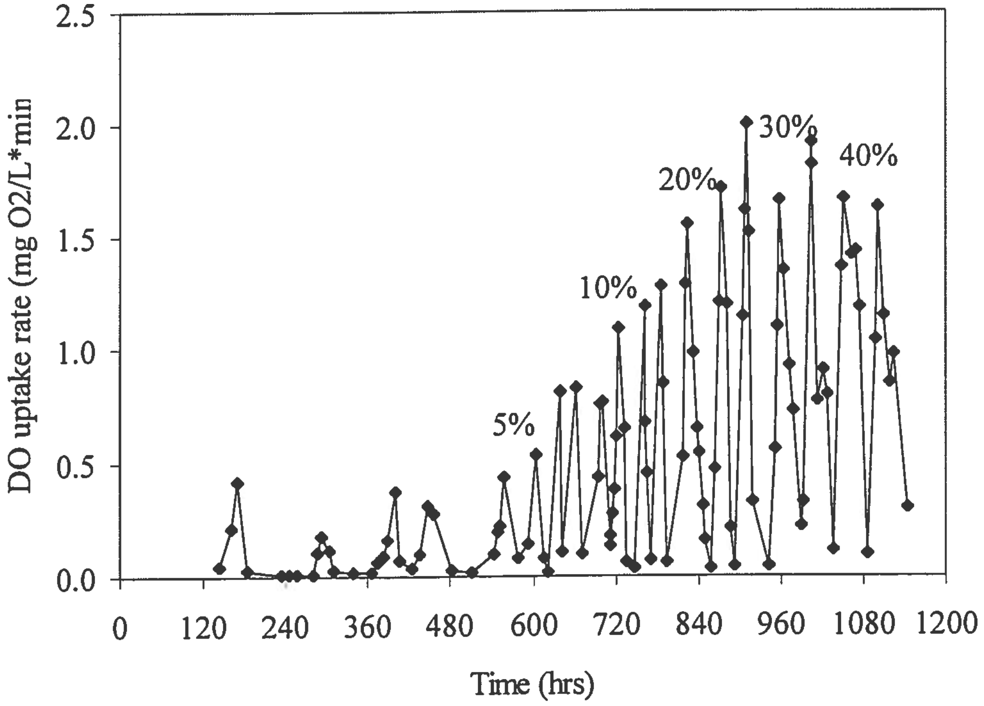

Figure 35 Oxygen uptake rate in Reactor C

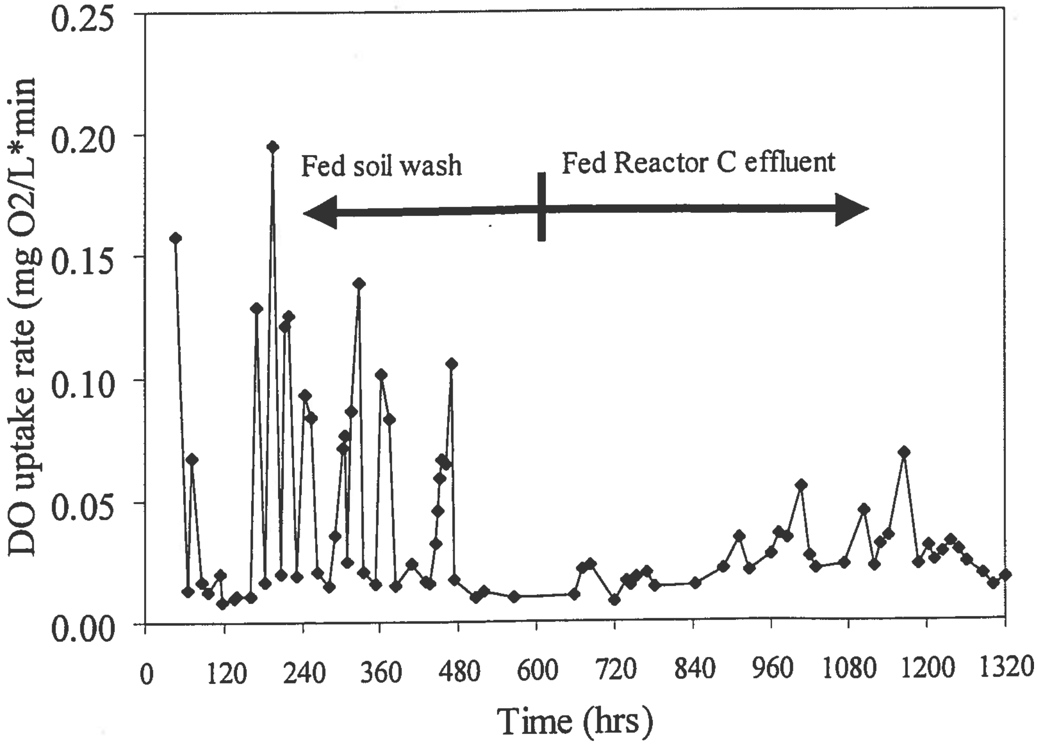

Figure 36 Oxygen uptake rate in Reactor D

increasing solids loading rates, but reached a maximum when the nominal solids loading rate was 20%. The maximum oxygen uptake rate in Reactor C was 2.0 mg/L·min. Oxygen consumption in Reactor D was much lower, especially when the reactor was fed Reactor C effluent containing 2,6-DNT. High oxygen demand was observed only during the continuous and intermittent feeding of soil wash when 2,4-DNT was fed to the reactor (Figure 36).

3.4.5 NaOH consumption

The consumption of sodium hydroxide (12.5 N, 50% w/v) is given on a per cycle basis, and the results are shown in Figure 37 and 38 for Reactor C and D, respectively. The results of NaOH consumption were generally consistent with the degradation pattern of dinitrotoluenes, the nitrite release, and the oxygen uptake rate presented in Figure 30-36. In Reactor C, more NaOH was consumed as solids loading rates were increased. A linear relationship was found between the two ($R^2 = 0.998$), with a correlation of 4.6 mL of 12.5 N NaOH per 1% (w/v) of BAAP soil. Based on dinitrotoluene degraded, this is equivalent to 1.7 mole of NaOH per mole of DNT. In reactor D, however, NaOH consumption was minimal, except when it was fed soil wash containing 2,4-DNT. The theoretical consumption (based on 2 mole NaOH/mole DNT) of 12.5 N NaOH is very small (2.8 mL) for 250 μM of 2,6-DNT. This explains why NaOH consumption was not observed while it was fed Reactor C effluent.

3.4.6 Residual 2,4-DNT, 2,6-DNT and TNT in treated BAAP soils

Residual concentrations of 2,4-DNT, 2,6-DNT and TNT in treated BAAP soils are presented in Table 4. Results for Reactor C are based on 9 samples taken from the reactor effluent that were previously loaded with 10 to 40% of BAAP soil. A total of 7

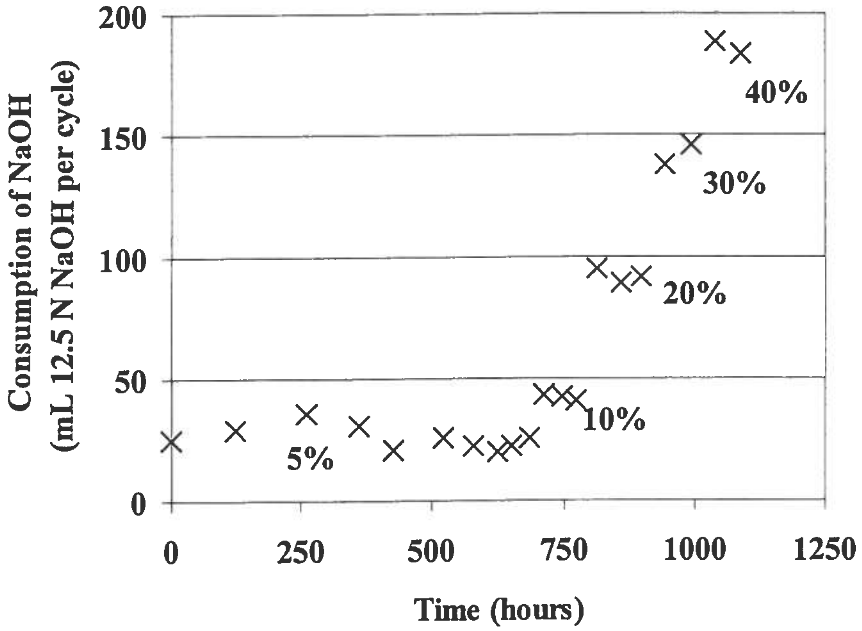

Figure 37 NaOH consumption in Reactor C

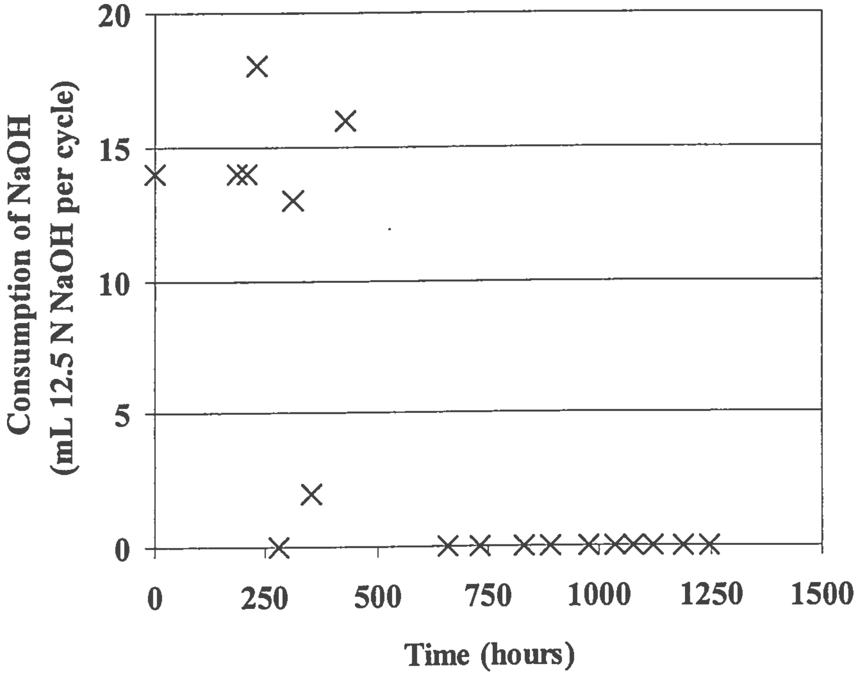

Figure 38 NaOH consumption in Reactor D

samples were taken from Reactor D, 2 of these samples were spiked with additional 2,6-DNT (for the purpose of maintaining 2,6-DNT degradation activity) and therefore were excluded for the calculation. It is clear that 2,6-DNT concentration was significantly reduced after the sequential treatment of 2,6-DNT in Reactor D (99.1%) as compared to the concentration in Reactor C (95.6%). Further concentration reduction (99.0% in Reactor C vs. 99.2% in Reactor D) was also observed for 2,4-DNT. For both reactors, TNT concentrations were below the detection limit. An unknown compound with a retention time of approximately 4.1 minutes was observed for most of the treated VAAP soil samples. Note that the concentration reductions in soil were the combined effects of biodegradation and desorption from soil phase. This explained why residual concentration of 2,6-DNT in soil was low in Reactor C even though no significant biodegradation was occurred.

Table 4 Residual concentrations of 2,4-DNT, 2,6-DNT and TNT in treated BAAP soil.

Reactor	Reactor C (n = 9)		Reactor D (n = 5)	
Contaminant	2,4-DNT	2,6-DNT	2,4-DNT	2,6-DNT
Original soil (mg/kg)	8,940	480	8,940	480
Residual soil (mg/kg)				
Mean (x ± s)	91.5 ± 33.5	21.0 ± 15.7	70.9 ± 31.0	4.1 ± 0.6
Minimum	46.8	N.D.	28.9	3.6
Maximum	161.8	44.0	102.2	4.7
% Reduction	99.0	95.6	99.2	99.1

¶ TNT concentration in original soil was 70 mg/kg, and residual TNT was not detectable in treated soil. N.D. — not detected.

3.5 Reactor E and F Performance: VAAP Soil at High Solids Loading Rates

3.5.1 Temperature, pH and SS

Variations of temperature, pH and SS in Reactor E are shown in Figure 39. Reactor E had an average temperature of 29.8 ± 0.8°C (range: 28.0 ~ 31.0), and pH of 6.93 ± 0.06 (range: 6.75 ~ 7.05). These results demonstrate that the reactor achieved

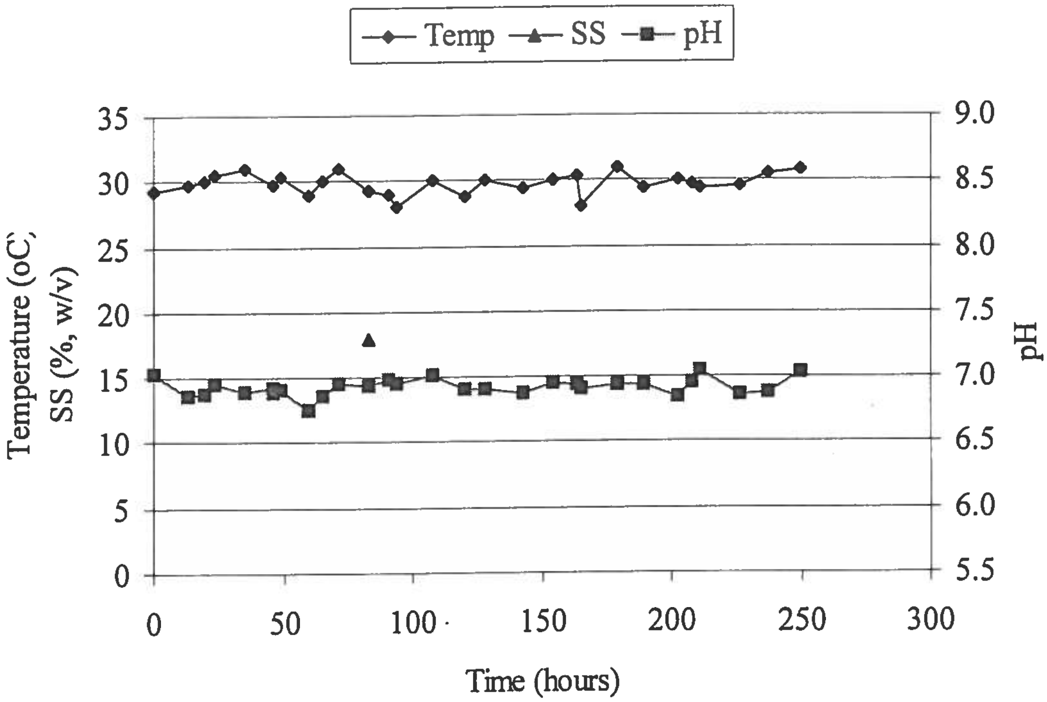

Figure 39 Variation of temperature, pH and SS in Reactor E

satisfactory performance with regard to temperature and pH control. Reactor F was fed effluent from Reactor E that had been stored at room temperature till use. During storage, the effluent was aerated with an air sparger to maintain aerobic conditions. The stored effluent was added to the emptied and rinsed reactor that had previously been Reactor E. Reactor F had an average pH of 6.88 ± 0.14 (range: 6.56 ~ 7.14), and SS of 10.62%. Routine temperature measurements were not performed.

3.5.2 Degradation of 2,4-DNT, 2,6-DNT and TNT

For VAAP soil, nearly complete degradation of 2,4-DNT was demonstrated in the slurry reactor at a nominal solids loading rates of 20% (Figure 40). When the reactor was fed VAAP soil at a 30% nominal solids loading rate (17,000 μM 2,4-DNT, 1,500 μM 2,6-DNT, and 700 μM TNT), the degradation of 2,4-DNT was not complete, as is shown in Figure 40. It was not known whether this was the result of limiting nutrients or the inhibition by nitrite, or both. However, after adding a new batch of soil wash at a reduced loading rate (e.g., 20%), the system performed as previously observed.

As presented in Section 3.3.2, stable performance of 2,6-DNT degradation from VAAP soil was not achieved in Reactor B. Therefore, studies in Reactor F was initiated to focus on developing the degradation of 2,6-DNT. This was conducted using Reactor E effluent containing residual 2,6-DNT, and the finished effluent from Reactor D as the active bacterial culture. Reactor F started with an initial 2,6-DNT concentration of approximately 100 μM. The initial 2,6-DNT concentrations were increased continuously over the 8 fill-and-draw cycles to ascertain whether the bacteria were able to degrade 2,6-DNT and to define the maximum tolerable concentration to the bacteria. As shown in Figure 41, degradation of 2,6-DNT was successfully demonstrated at a concentration

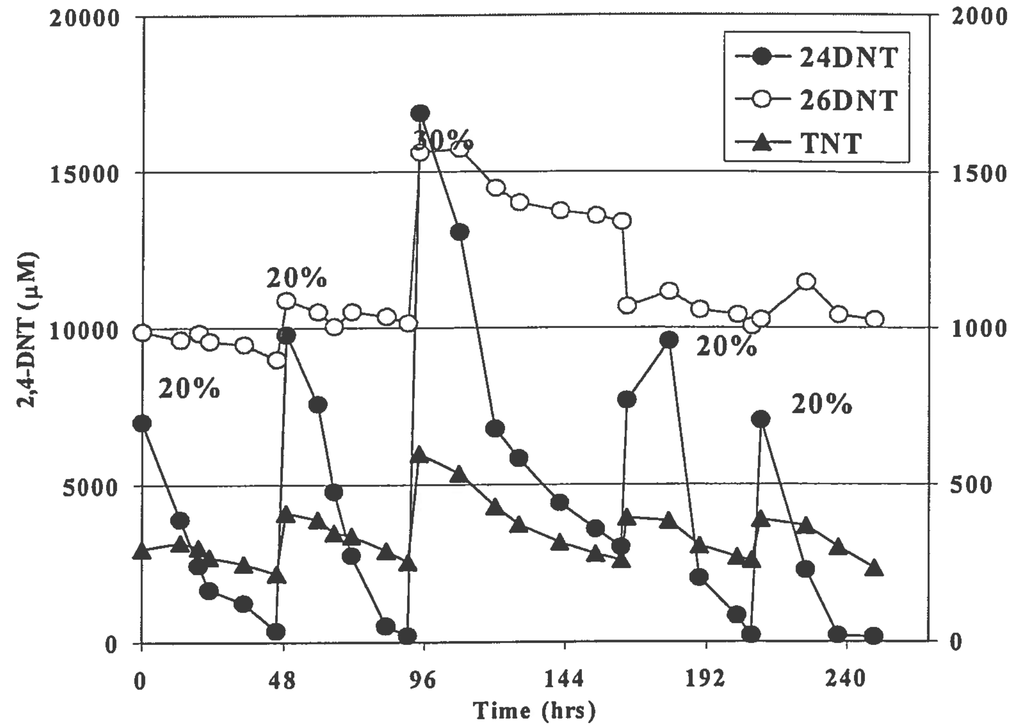

Figure 40 Degradation of 2,4-DNT, 2,6-DNT and TNT in Reactor E

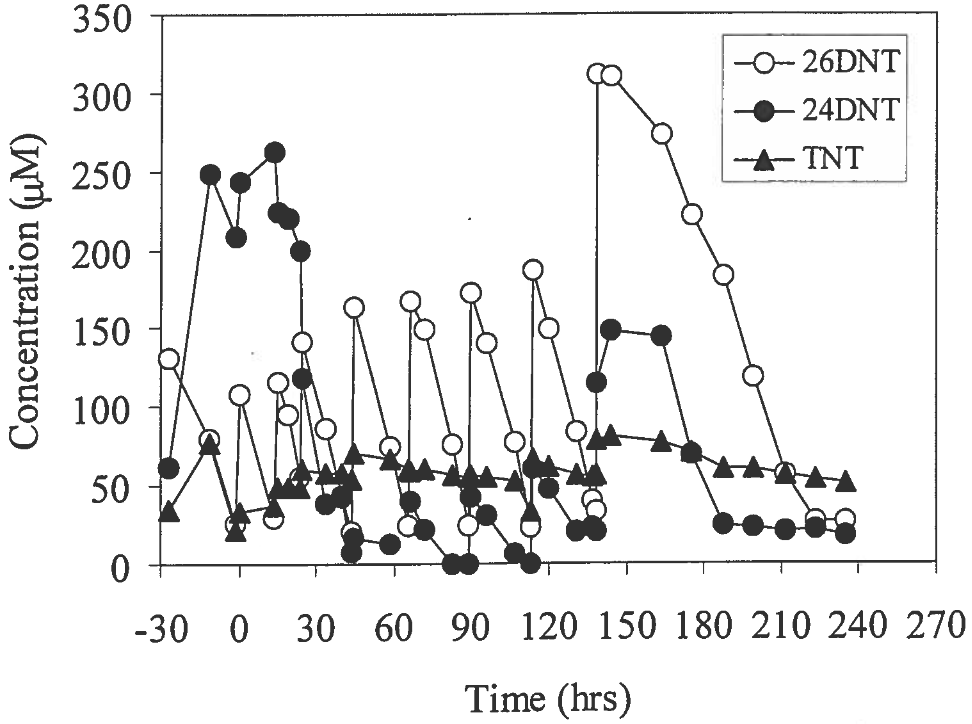

Figure 41 Degradation of 2,4-DNT, 2,6-DNT and TNT in Reactor F

range of 100 ~ 300 μM. 2,6-DNT at concentrations up to 300 μM showed only a brief lag phase, but the rate of 2,6-DNT degradation appeared to be similar to previous feeding cycles. It was also noted that TNT degradation in Reactor F was minimal. An initial increase in 2,4-DNT concentration in the reactor was observed, this may have been the result of the desorption from residues within the reactor.

3.5.3 Nitrite release

The nitrite concentration profiles in Reactor E are given in Figure 42. The maximum concentrations were about 35,000 μM at a 20% nominal solids loading rate. Again, the pattern of the nitrite production corresponded to the disappearance of dinitrotoluenes shown in Figure 40. Note that the maximum concentration at a 30% loading rate remained about the same as the concentrations at a 20% loading rate. This is because a fill-and-draw was initiated prior to the complete degradation of 2,4-DNT.

Similar to the concentration profile in the other two reactors in series (Reactors B and D), the nitrite concentrations in Reactor F fluctuated and did not correspond to the fill-and-draw patterns of 2,6-DNT degradation in the reactor (Figure 43). Again, this is presumably due to the high background concentrations carried over from the first reactor, and the small amount of nitrite released from the relatively low concentrations of 2,6-DNT.

3.5.4 Oxygen uptake

Oxygen uptake rates in Reactor E were routinely measured, no oxygen uptake data are available in Reactor F. The shape of the curve corresponding to each feeding cycle of soil wash in Reactor E was evident (Figure 44). It is clear that oxygen uptake rates responded to the microbial activities in the reactor. The peak oxygen uptake rates

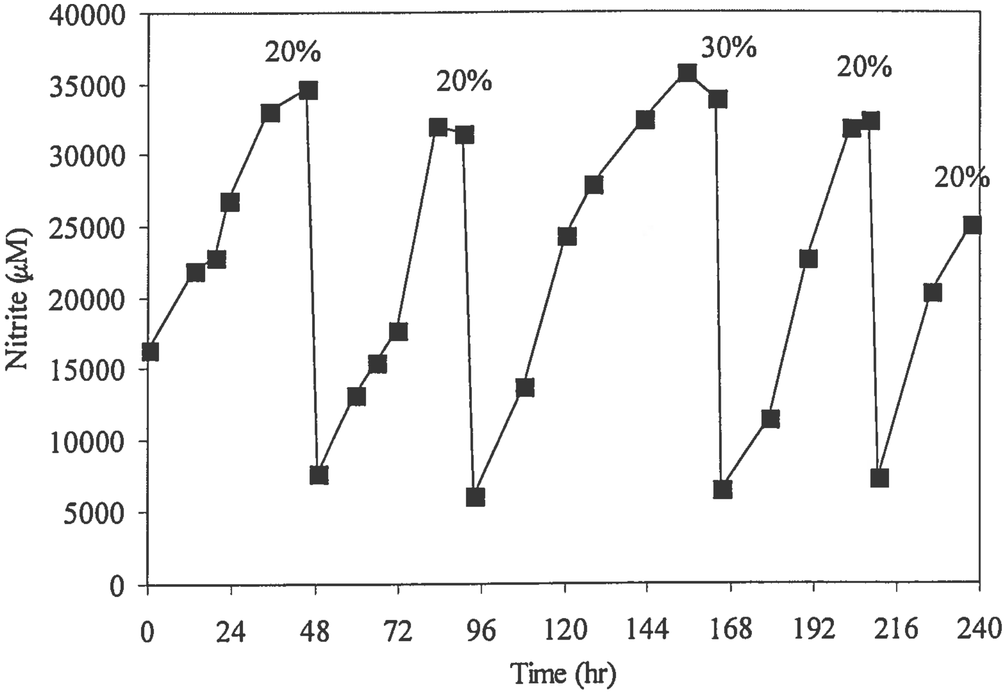

Figure 42 Nitrite concentration in Reactor E

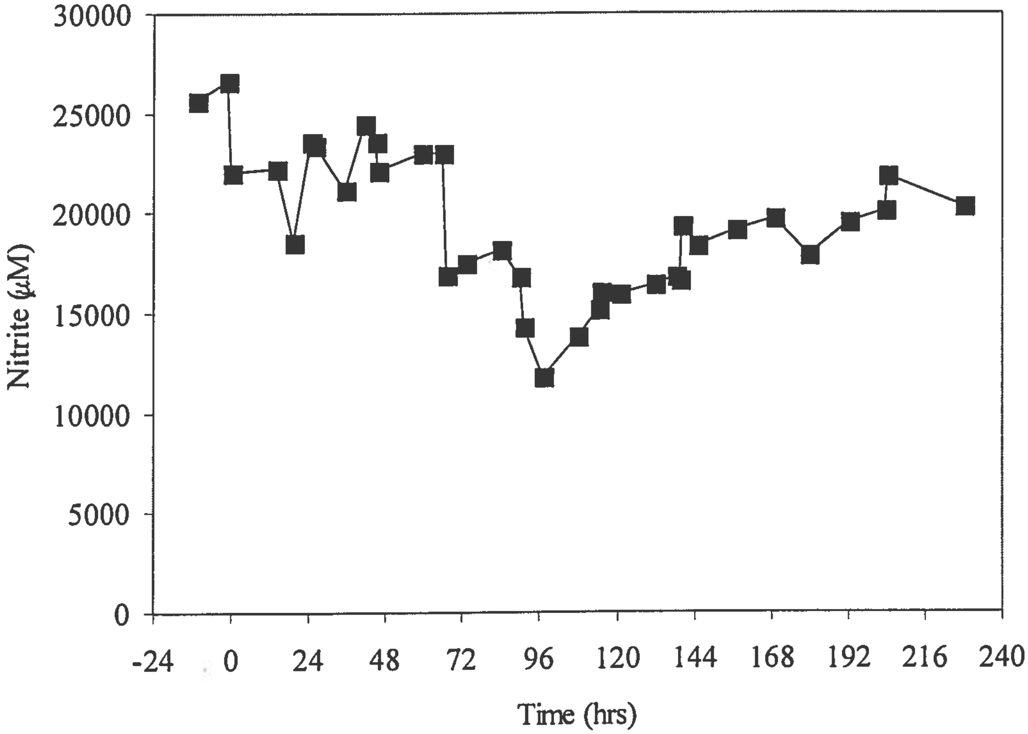

Figure 43 Nitrite concentration in Reactor F

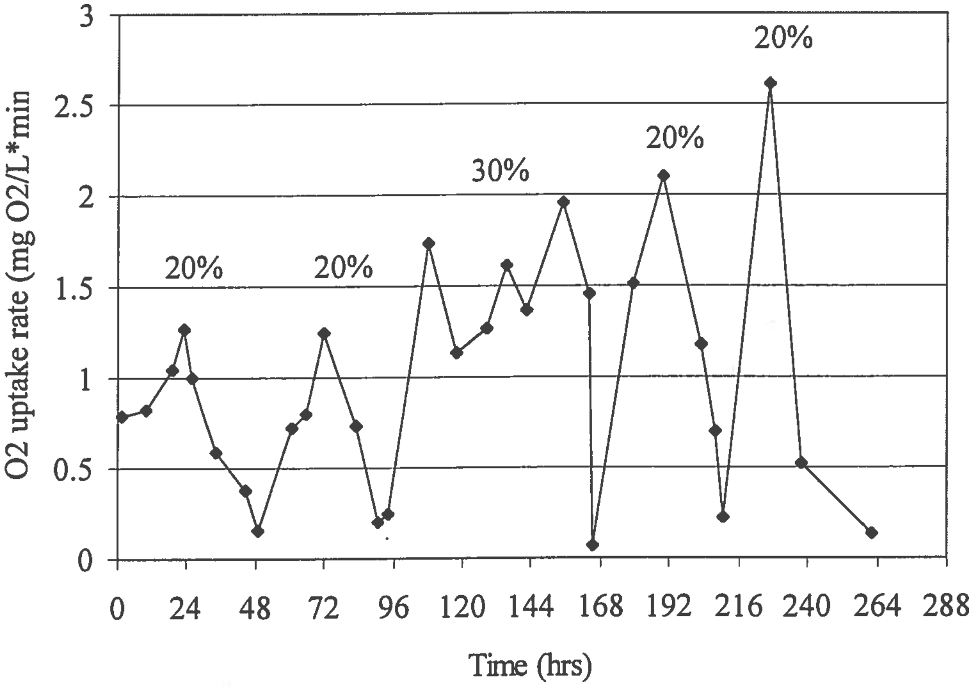

Figure 44 Oxygen uptake rate in Reactor E

remained high between 1.3 and 2.6 mg/L·min, as compared to the observed maximum oxygen uptake rate in Reactor C of 2.0 mg/L·min.

3.5.5 NaOH consumption

The consumption of sodium hydroxide (12.5 N, 50% w/v) in Reactor E is given on a per cycle basis, and the results are shown in Figure 45. In Reactor F, pH remained neutral, no consumption of NaOH was observed.

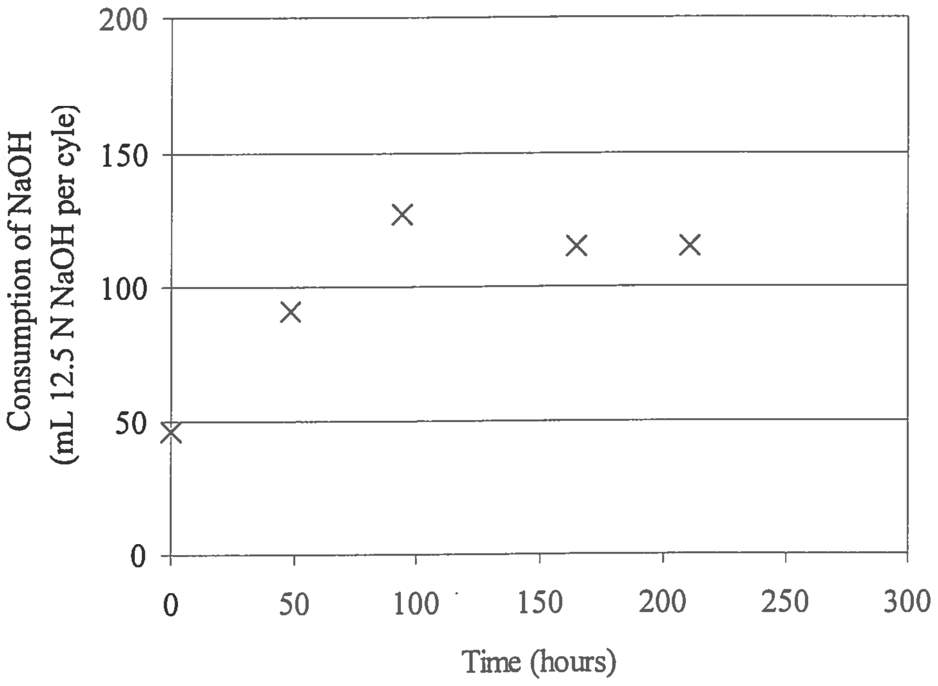

Figure 45 NaOH consumption in Reactor E

3.5.6 Residual 2,4-DNT, 2,6-DNT and TNT in treated VAAP soil

Residual concentrations of 2,4-DNT, 2,6-DNT and TNT in treated VAAP soils are presented in Table 5. No samples were taken from the sequential Reactor F. Results for Reactor E are based on 4 samples taken from the reactor effluent that were previously loaded with 20 to 30% of VAAP soil. The residual concentrations for three target compounds in treated VAAP soils were all higher than in treated BAAP soils (Table 5). The residual concentrations in treated VAAP soils were equivalent to a removal of 97.9, 90.4 and 94.3% for 2,4-DNT, 2,6-DNT and TNT, respectively.

Table 5 Residual concentrations of 2,4-DNT, 2,6-DNT and TNT in treated VAAP soil.

Reactor	Reactor E (n = 4)		
Contaminant	2,4-DNT	2,6-DNT	TNT
Original soil (mg/kg)	10,890	870	430
Residual soil (mg/kg)			
Mean (x ± s)	224.3 ± 241.0	83.9 ± 31.9	24.6 ± 10.4
Minimum	38.0	46.1	N.D.[¶]
Maximum	567.5	123.3	N.D.
% Reduction	97.9	90.4	94.3

[¶] N.D. — Not detected.

3.6 Abiotic Processes of 2,4-DNT, 2,6-DNT and TNT in Slurry Reactors

3.6.1 Suspended solid (SS) concentration profiles: Effect of mixing

Suspended solid (SS) profiles in the reactor were examined with the sandy BAAP soil in an attempt to better understand the mixing by mechanical apparatus and its effects on the phase behaviors of 2,4-DNT, 2,6-DNT and TNT in the test soil. Results also were used for the development of sampling protocols from the slurry reactor. This study was performed in Reactor C when it was loaded with a nominal solids loading rate of 10% BAAP soil. Note that soils were added after the pretreatment with soil washing rather than the direct addition of soils as presented in Figure 3 (Section 3.1).

SS concentration profiles at three different sampling locations are given in Figure 46. The calculated concentration was obtained by the actual amount of soil added into the reactor after the removal of large particulates, assuming the reactor was a completely mixed system. From Figure 46, SS concentrations at the top, middle and bottom ports were 0.72%, 0.83% and 2.44%, respectively, as compared to a calculated concentration of 1.87%. In contrast to pretreated soil, SS concentration without the removal of large particulates at top, middle and bottom were 1.4%, 1.46% and 65.2%, respectively, as compared to a calculated concentration of 9.4% (Figure 3). It is very obvious that larger amount of soils were deposited at the bottom of the reactor in the case of untreated soil.

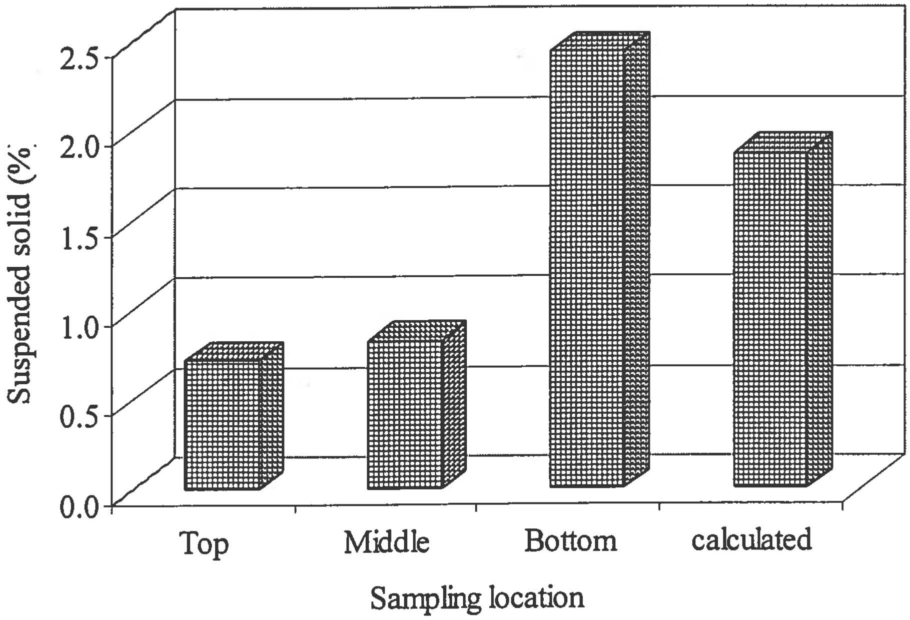

Figure 46 Suspended solid profile in Eimco reactor: BAAP soil at 10% nominal solids loading rate

It is also important to note that the reactor had a nonhomogeneous distribution of SS in both cases (i.e., soil wash vs. direct soil addition). Attempts were also made to improve the homogeneity by increasing the mixing intensity of the reactor by doubling the flowrate of the air-lift (20 to 40 SCFH) and the flowrate of the air diffuser (30 to 60

SCHF). Results given in Figure 47 indicate that increasing the mixing intensity resulted in only a minimal improvement.

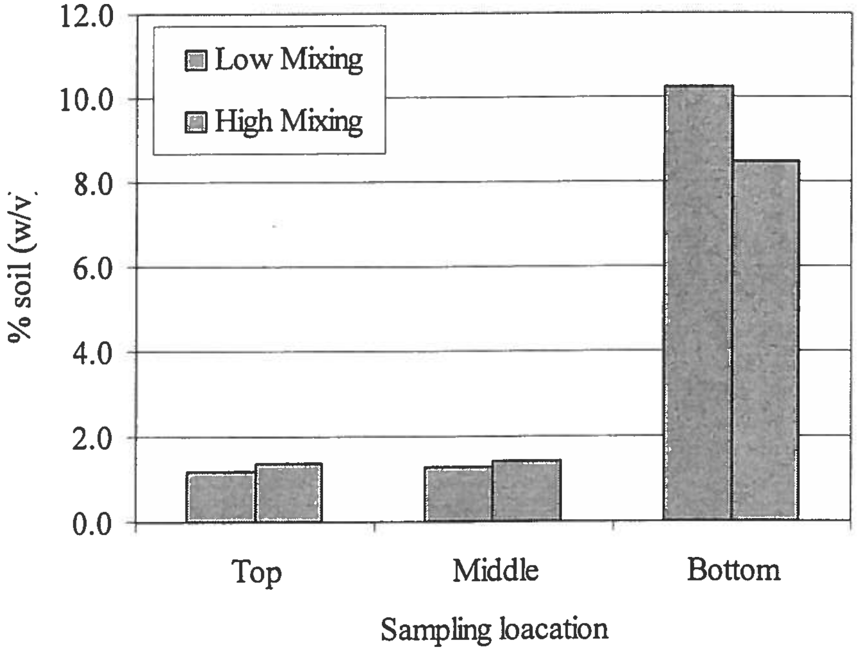

Figure 47 Suspended solid profile in Eimco reactor: Effects of mixing

3.6.2 Nitroaromatic compound concentration profiles: Effect of mixing

Similar to the study on the SS profile in the reactor, samples were also taken from Reactor C for the analysis of 2,4-DNT and 2,6-DNT in both aqueous and solid phases. Concentration profiles at three sampling locations and the effects of mixing are given in Figure 48 for 2,4-DNT and Figure 49 for 2,6-DNT.

Results in Figure 48 and 49 indicate that aqueous phase concentrations of both 2,4-DNT and 2,6-DNT were the same among three vertical locations in the reactor. While the variations of solid phase 2,6-DNT concentration were small, significant differences were observed for 2,4-DNT. With low mixing, solid phase concentration of the bottom

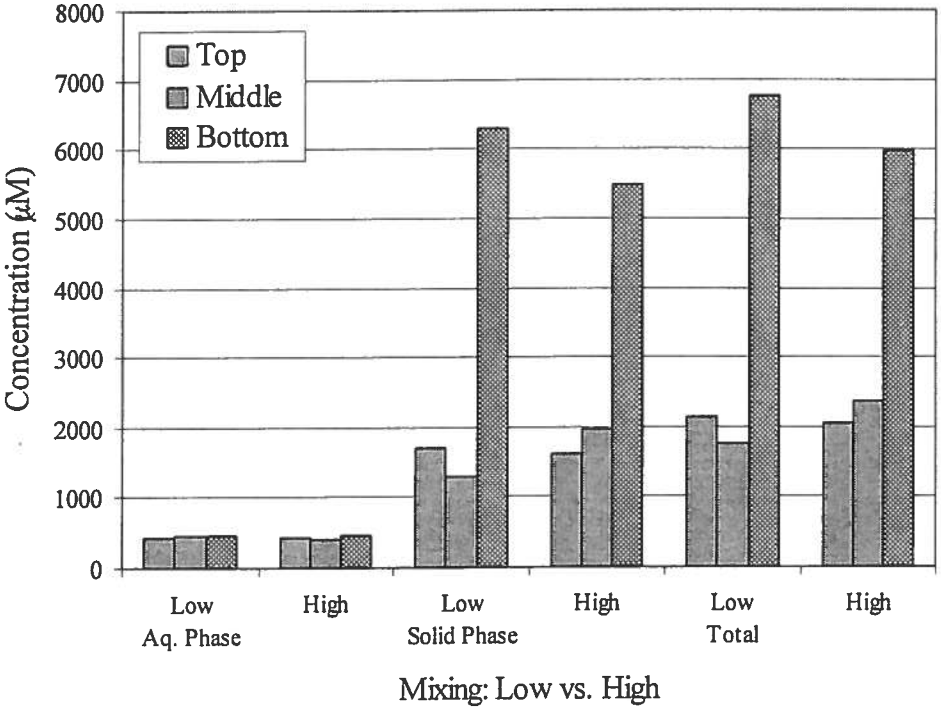

Figure 48 2,4-DNT profile in slurry reactor: BAAP soil at 20% nominal solids loading rate

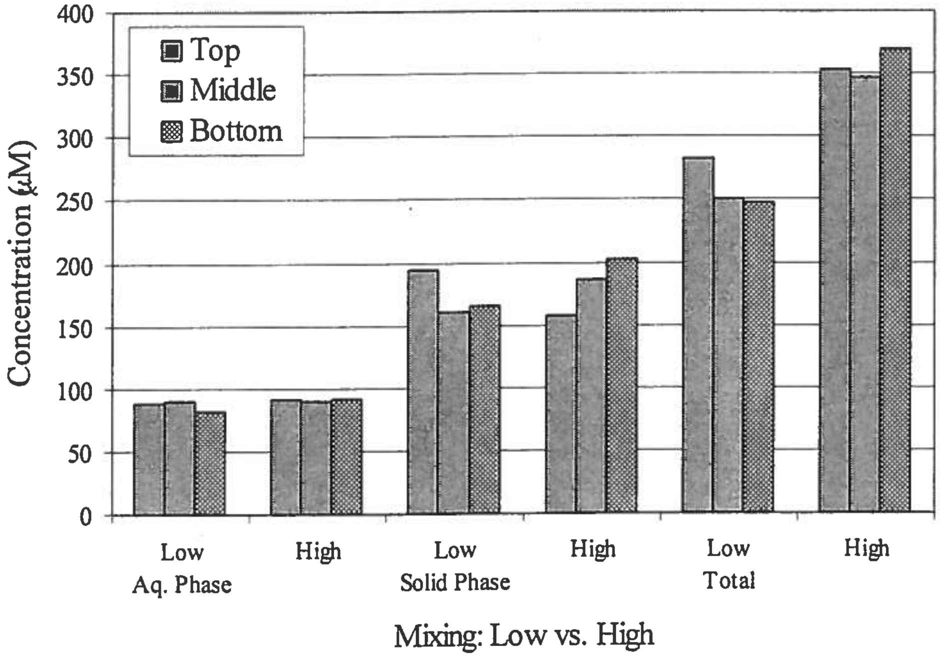

Figure 49 2,6-DNT profile in slurry reactor: BAAP soil at 20% nominal solids loading rate samples were about 3.7 times higher than the concentrations at the top. Increased mixing appeared to have a little improvement, the concentration ratio of the bottom to top was decreased to 3.4.

3.6.3 Partitioning of 2,4-DNT and 2,6-DNT in BAAP soil

The partitioning of 2,4-DNT and 2,6-DNT between aqueous and solid phases was investigated in Reactor C at three nominal solids loading rates (5%, 10% and 20%) of the BAAP soil. Intensive samplings were conducted for a selected feeding cycle at each loading rate. Data are provided in two formats, with concentrations in Figure 50 to Figure 55 and with percent distribution in Figure 56 to Figure 61.

Several observations can be made from these figures. 1) Right after the addition of soil wash, the percentage of aqueous phase 2,4-DNT decreased with the increase in solids loading rate. For instance, aqueous phase 2,4-DNT was about 60% of the total at 5% solids loading rate. In comparison, only about 20% of 2,4-DNT were in aqueous phase at 20% of loading rate. The reduced partitioning in aqueous phase at higher solids loading rates may be the result of a dissolution-limited process, since the concentration of 2,4-DNT was far above the solubility limit (~ 1500 μM). 2) During the course of 2,4-DNT degradation in a given cycle, the concentrations in both aqueous and solid phases decreased. However, the percentage of 2,4-DNT in solid phase increased steadily as 2,4-DNT degradation progressed. 3) At the end of each feeding cycle, the majority of 2,4-DNT remained in solid phase. 4) 2,6-DNT had approximately equal distribution between aqueous and solid phases. The partition remained constant throughout the feeding cycle, this is because no significant degradation of 2,6-DNT occurred in Reactor C at all three loading rates of BAAP soil.

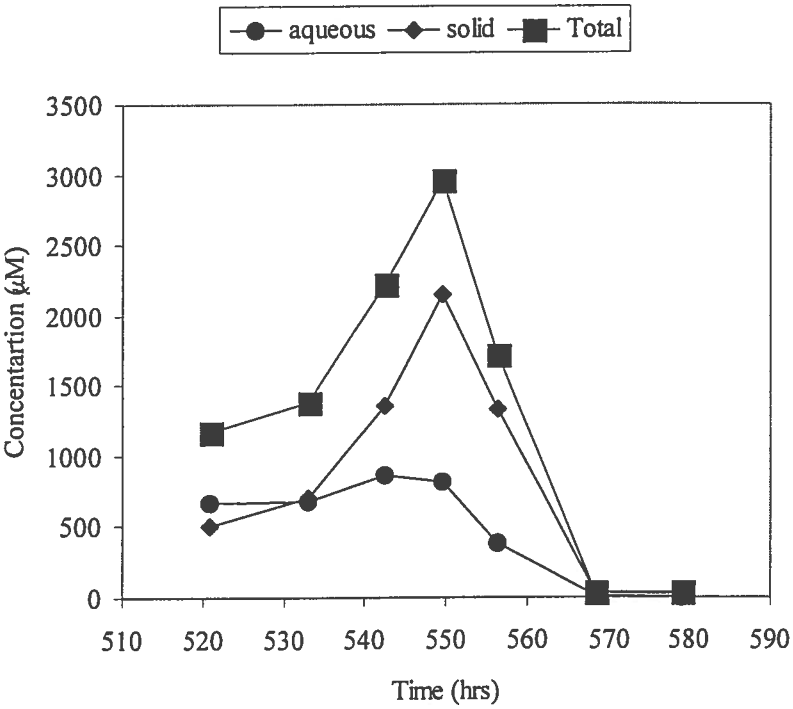

Figure 50 Partitioning in Reactor C at 5% solids loading rate of BAAP soil: Aqueous vs. solid phase concentration of 2,4-DNT

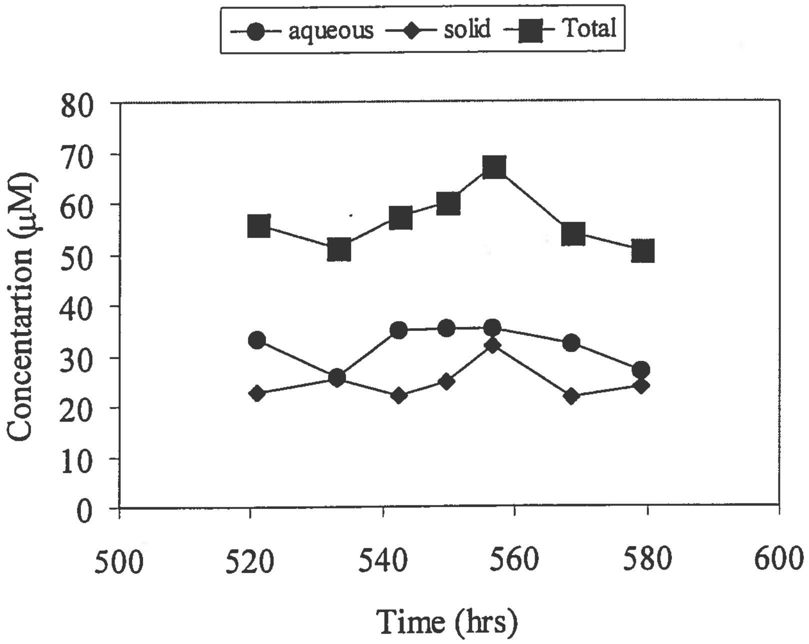

Figure 51 Partitioning in Reactor C at 5% solids loading rate of BAAP soil: Aqueous vs. solid phase concentration of 2,6-DNT

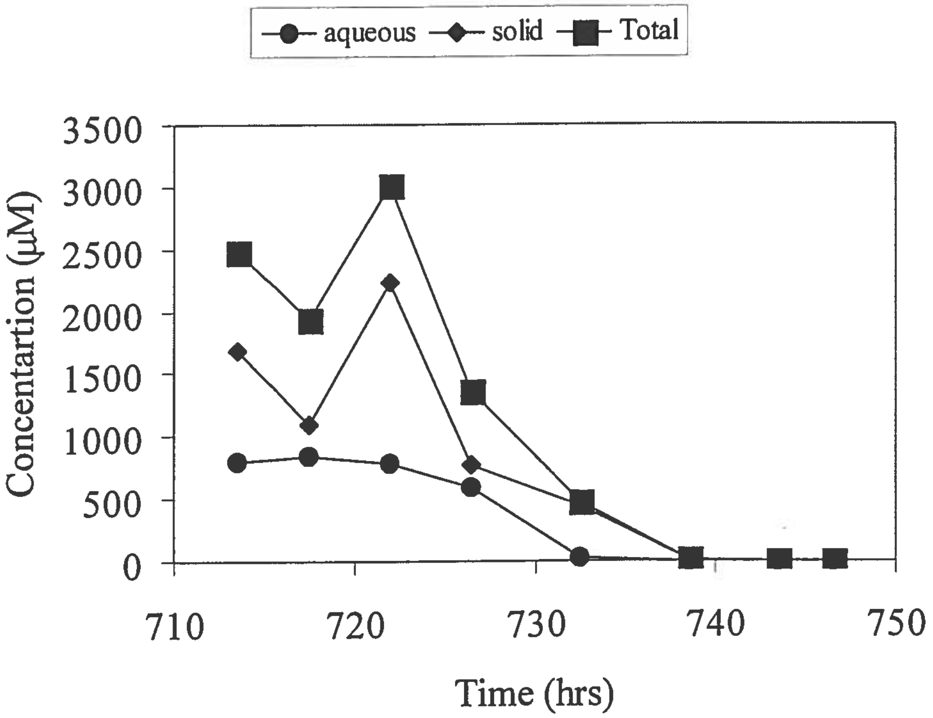

Figure 52 Partitioning in Reactor C at 10% solids loading rate of BAAP soil: Aqueous vs. solid phase concentration of 2,4-DNT

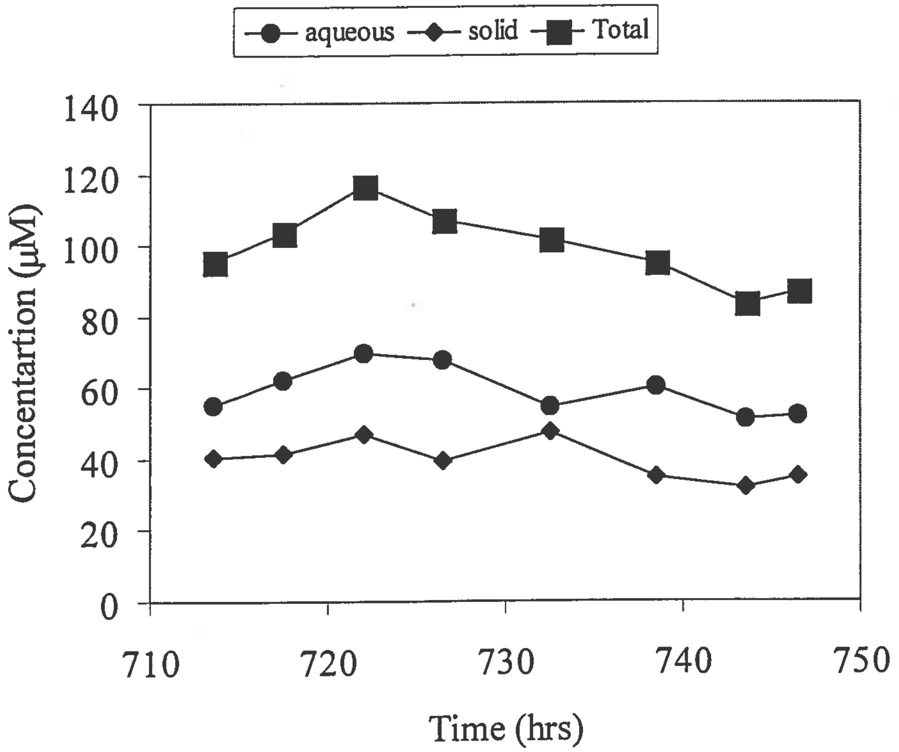

Figure 53 Partitioning in Reactor C at 10% solids loading rate of BAAP soil: Aqueous vs. solid phase concentration of 2,6-DNT

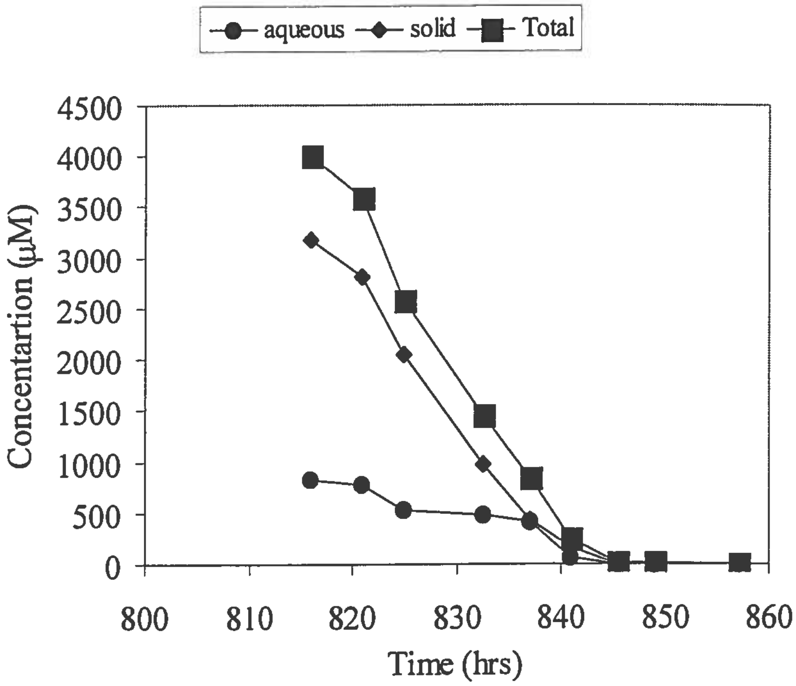

Figure 54 Partitioning in Reactor C at 20% solids loading rate of BAAP soil: Aqueous vs. solid phase concentration of 2,4-DNT

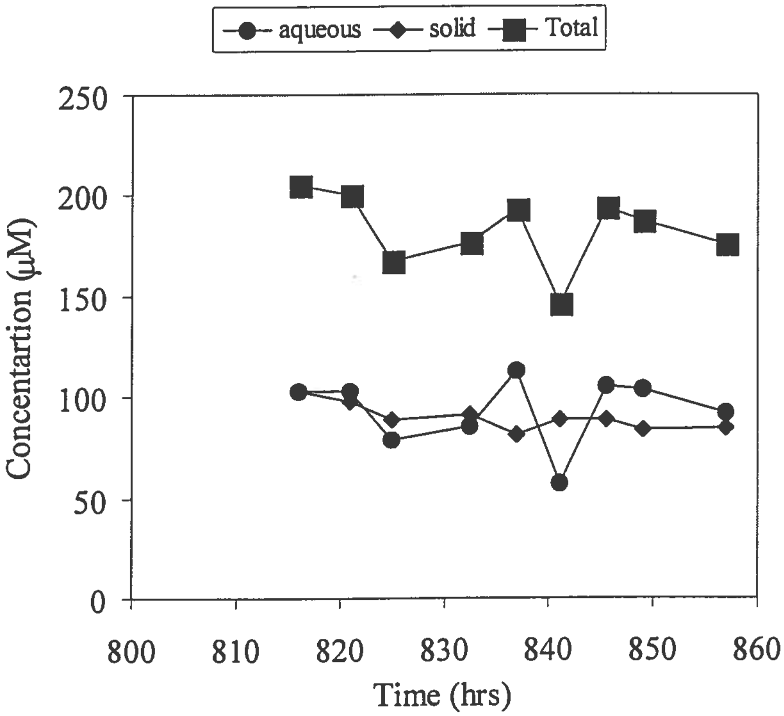

Figure 55 Partitioning in Reactor C at 20% solids loading rate of BAAP soil: Aqueous vs. solid phase concentration of 2,6-DNT

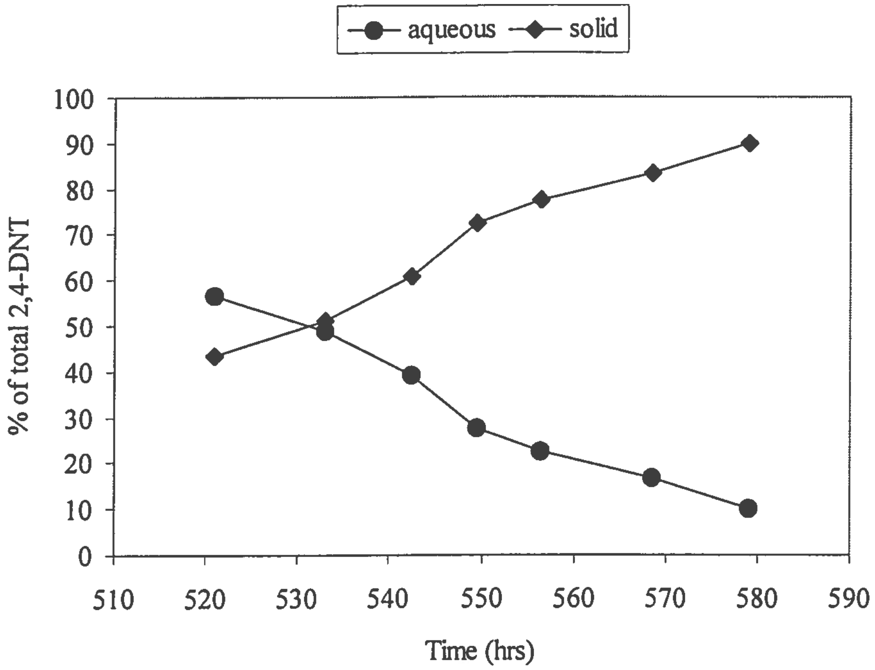

Figure 56 Partitioning in Reactor C at 5% solids loading rate of BAAP soil: Percentage of aqueous phase vs. solid phase 2,4-DNT

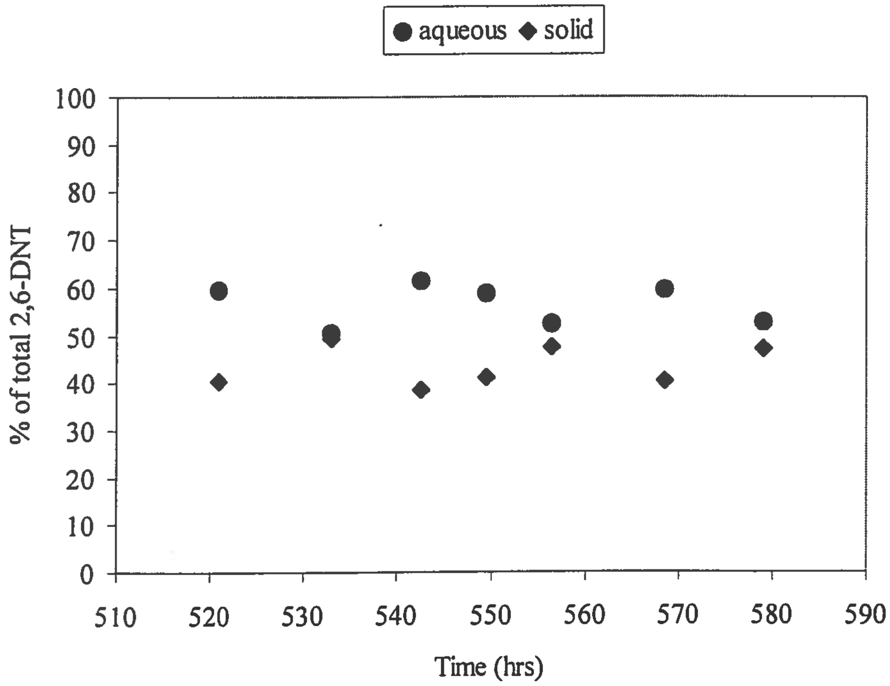

Figure 57 Partitioning in Reactor C at 5% solids loading rate of BAAP soil: Percentage of aqueous phase vs. solid phase 2,6-DNT

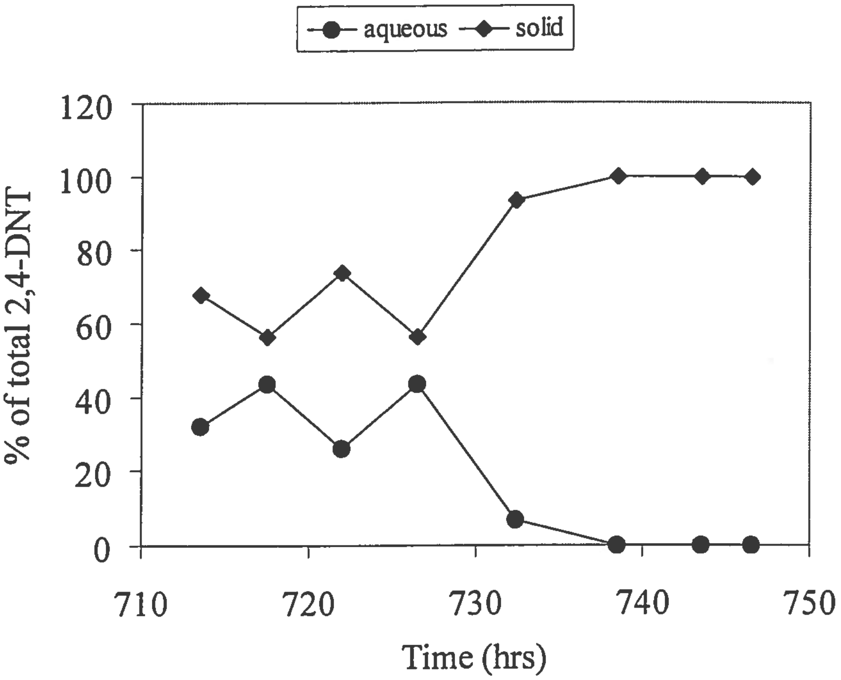

Figure 58 Partitioning in Reactor C at 10% solids loading rate of BAAP soil: Percentage of aqueous phase vs. solid phase 2,4-DNT

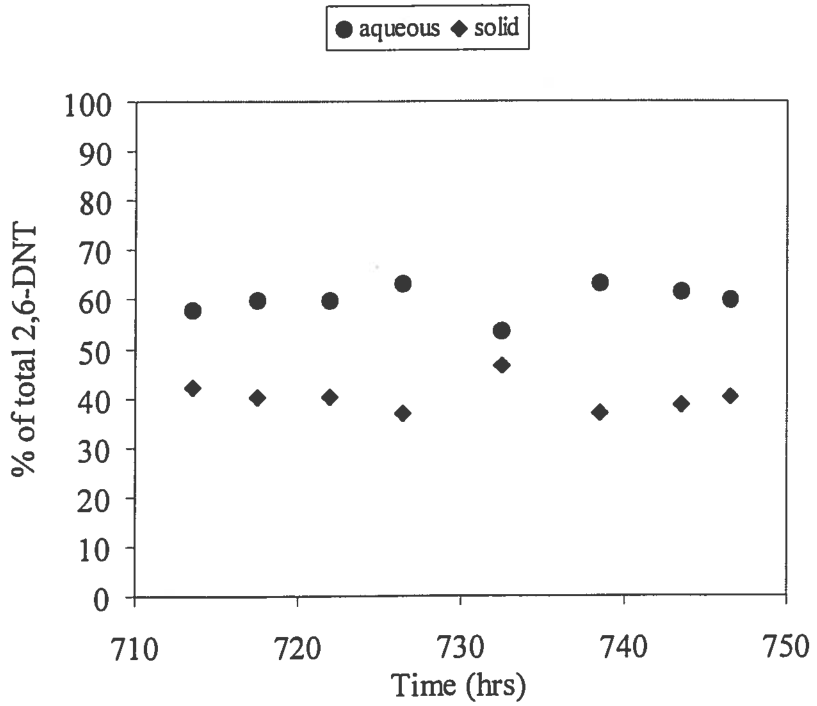

Figure 59 Partitioning in Reactor C at 10% solids loading rate of BAAP soil: Percentage of aqueous phase vs. solid phase 2,6-DNT

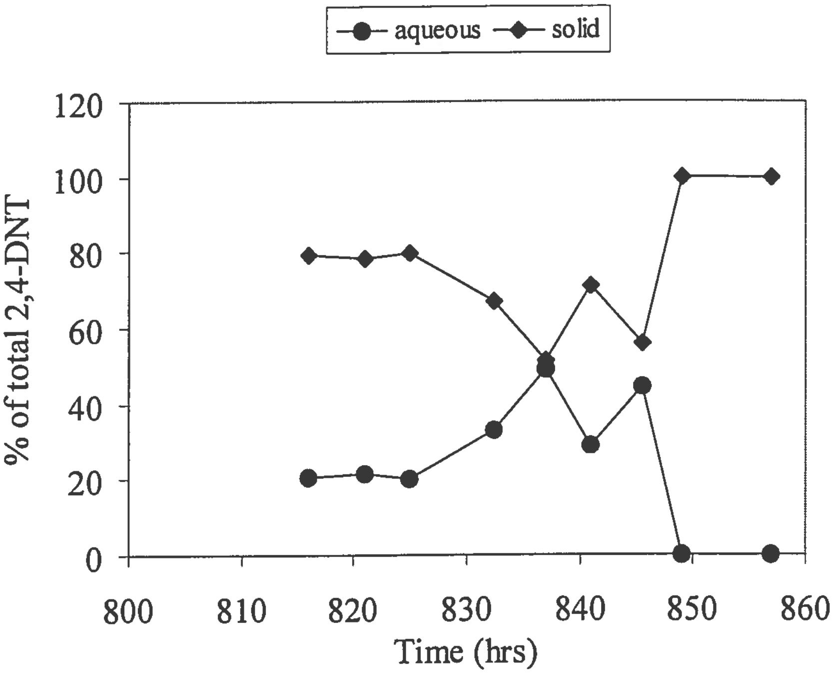

Figure 60 Partitioning in Reactor C at 20% solids loading rate of BAAP soil: Percentage of aqueous phase vs. solid phase 2,4-DNT

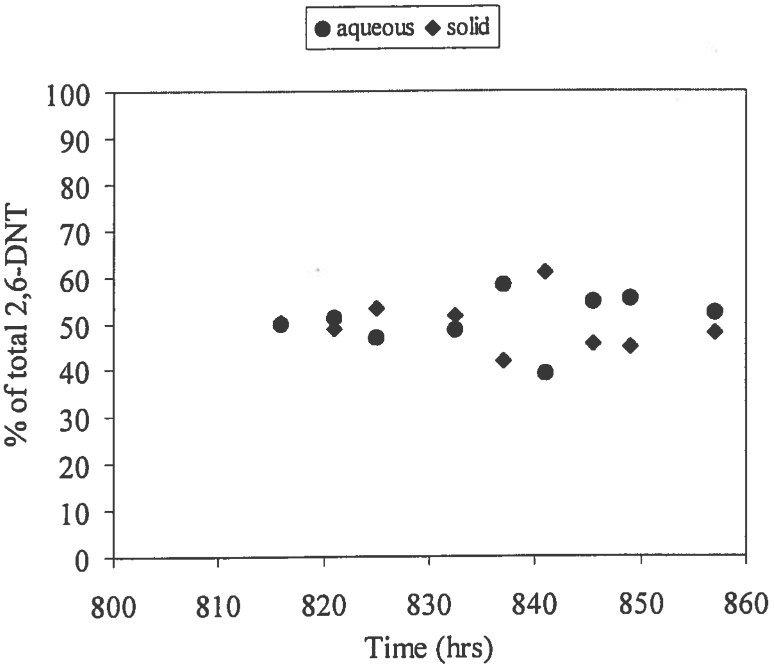

Figure 61 Partitioning in Reactor C at 20% solids loading rate of BAAP soil: Percentage of aqueous phase vs. solid phase 2,6-DNT

3.6.4 Partitioning of 2,4-DNT, 2,6-DNT and TNT in VAAP soil

The partitioning of 2,4-DNT, 2,6-DNT and TNT between aqueous and solid phases was investigated in Reactor E (2nd cycle) at a 20% nominal solids loading rate of the VAAP soil. Data are provided in the same formats as described for BAAP soil, with concentrations of 2,4-DNT, 2,6-DNT and TNT in Figure 62, 63 and 64 and with percent distribution of 2,4-DNT, 2,6-DNT and TNT in Figure 65, 66 and 67, respectively.

The partition behaviors of test compounds appeared to differ significantly between these two soils. Unlike the BAAP soil in Reactor C, 2,4-DNT existed mostly in the solid phase right after fresh soil wash was added. In addition, the percentage in the aqueous phase increased over time during the course of biodegradation. At the end of the feeding cycle, residual 2,4-DNT was distributed about 40% in aqueous phase and 60% in the solid phase. Similar increases in the percentage of aqueous phase concentration can also be noted for 2,6-DNT and TNT. However, since degradation was minor for both 2,6-DNT and TNT, the concentrations in both aqueous and solid phases were decreased only slightly over time.

3.7 Supporting Data from Shake-Flask Experiments

3.7.1 Soil washing efficiency: Effects of number of soil washings

This study was initiated to investigate the feasibility of soil washing processes for the removal of large soil particulates and the recovery of nitroaromatic compounds from contaminated soils. Ten grams of BAAP soil sample with 200 mL of DI water were placed in duplicate 500-mL Erlenmeyer flasks. The soil slurry was vigorously mixed on a magnetic stirrer at room temperature for 10 minutes to mimic soil washing processes with an equivalent actual solids loading rate of 5% (w/v). Immediately after settling of large

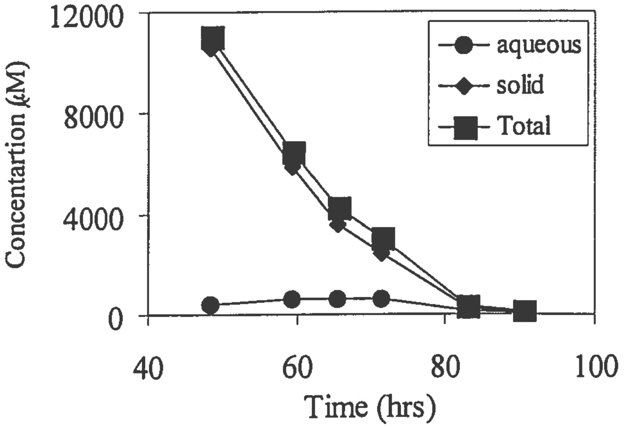

Figure 62 Partitioning in Reactor E at 20% solids loading rate of VAAP soil: Aqueous vs. solid phase concentration of 2,4-DNT

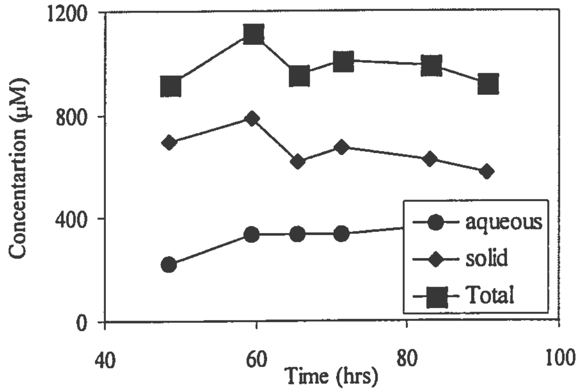

Figure 63 Partitioning in Reactor E at 20% solids loading rate of VAAP soil: Aqueous vs. solid phase concentration of 2,6-DNT

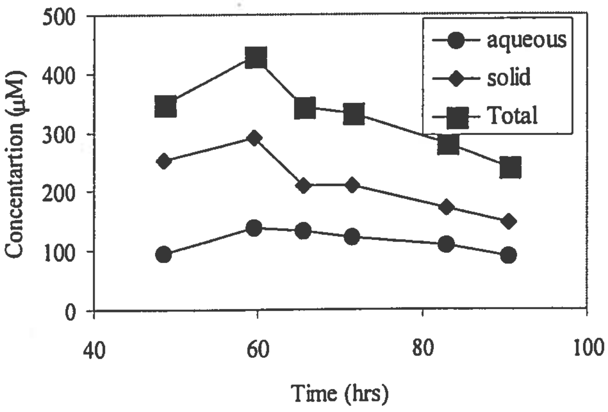

Figure 64 Partitioning in Reactor E at 20% solids loading rate of VAAP soil: Aqueous vs. solid phase concentration of TNT

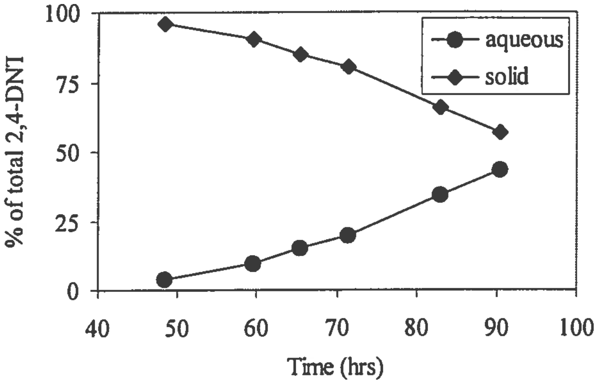

Figure 65 Partitioning in Reactor E at 20% solids loading rate of VAAP soil: Percentage of aqueous phase vs. solid phase 2,4-DNT

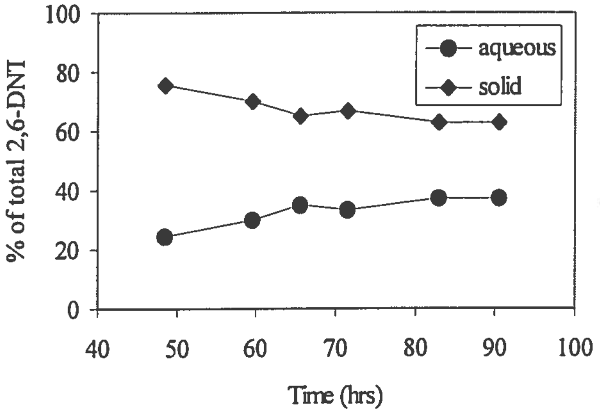

Figure 66 Partitioning in Reactor E at 20% solids loading rate of VAAP soil: Percentage of aqueous phase vs. solid phase 2,6-DNT

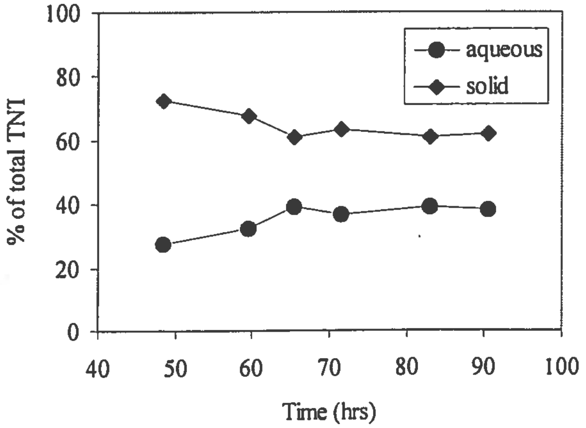

Figure 67 Partitioning in Reactor E at 20% solids loading rate of VAAP soil: Percentage of aqueous phase vs. solid phase TNT

particulates (several seconds), supernatant samples containing fine particles were taken for the analysis of 2,4-DNT and 2,6-DNT. The flasks were refilled with another 200 mL of DI water. This process was repeated till a total of 5 washings were completed. Figure 68 is a plot of the total amount of 2,4-DNT or 2,6-DNT recovered versus the number of soil washings. The first wash removed only 83.6% of total 2,6-DNT and 49.2% of 2,4-DNT, and the first 3 washings removed 97.1% of 2,6-DNT and 90.5% of 2,4-DNT. Results also showed that a 99.7% washing efficiency was achieved for 2,4-DNT after 5 washings.

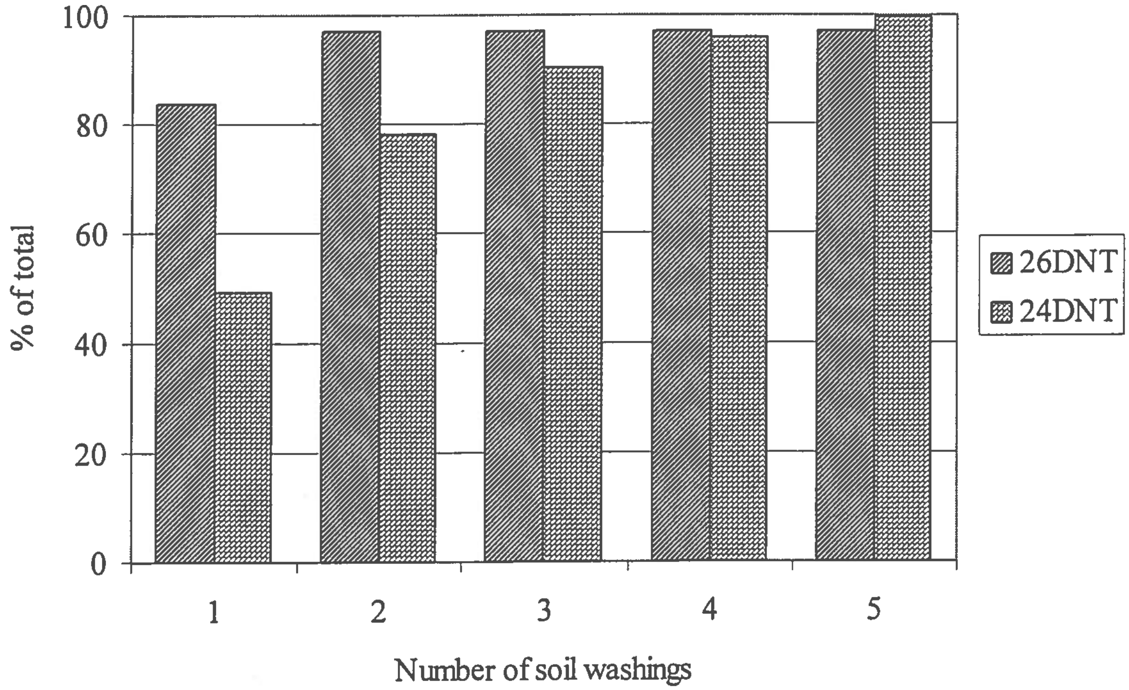

Figure 68 Soil washing efficiency: Shake-flask study with 5% (w/v) BAAP soil

3.7.2 Inhibition of 2,4-DNT on 2,6-DNT degradation

The purpose of this shake-flask study was to investigate whether 2,4-DNT inhibited the degradation of 2,6-DNT and to quantify the inhibitory levels of 2,4-DNT. The concentrations of 2,4-DNT tested were 0, 50, 500, 1000, 2500, and 5000 µM, which were equivalent to the concentrations tested in the slurry reactor. Note that the solubility limit of 2,4-DNT is ~1500 µM at 25°C. Prior to the experiment, 2,4-DNT was dissolved in a small amount of acetone. By swirling the flask and flushing with N_2 gas, a thin and uniform layer of 2,4-DNT was coated on the flask after acetone was evaporated. A total of 500 mL of soil slurry was then added to each of the 5 flasks with the supplementation of BLKN mineral nutrients (components B, C and D). The flasks were then placed on a rotary shaker (New Brunswick Scientific Co. Inc., Edison, NJ) at a constant temperature of 30°C. The pH of the slurry was adjusted manually at least twice a day, and samples were taken for the analysis of both 2,4-DNT and 2,6-DNT. Results are shown in Figure 69 and 70 for 2,6-DNT and 2,4-DNT, respectively.

Note that the slurry withdrawn from Reactor D had an initial concentration of approximately 40 µM of 2,6-DNT. Results presented in Figure 69 clearly demonstrate the effects of 2,4-DNT on the degradation of 2,6-DNT. 2,4-DNT at a concentration of 50 µM had no effects on the degradation of 2,6-DNT. When the concentrations were between 500 and 1000 µM, the inhibition was observed and was reversible after the disappearance of 2,4-DNT. However, the inhibition of 2,4-DNT at a concentration above 2500 µM appeared to be irreversible, since degradation of 2,6-DNT did not occur (Figure 69) even though 2,4-DNT was completely degraded (Figure 70).

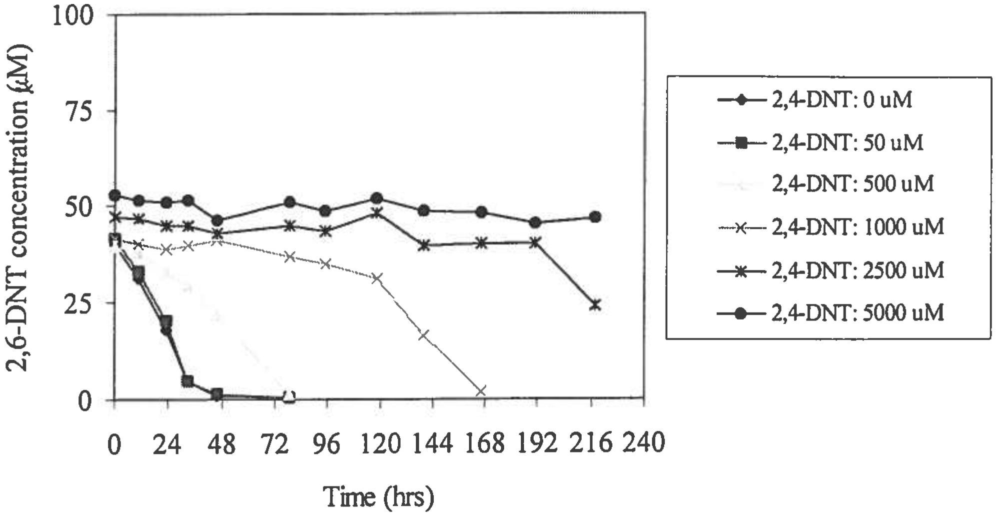

Figure 69 Inhibition of 2,4-DNT on 2,6-DNT degradation: Degradation of 2,6-DNT

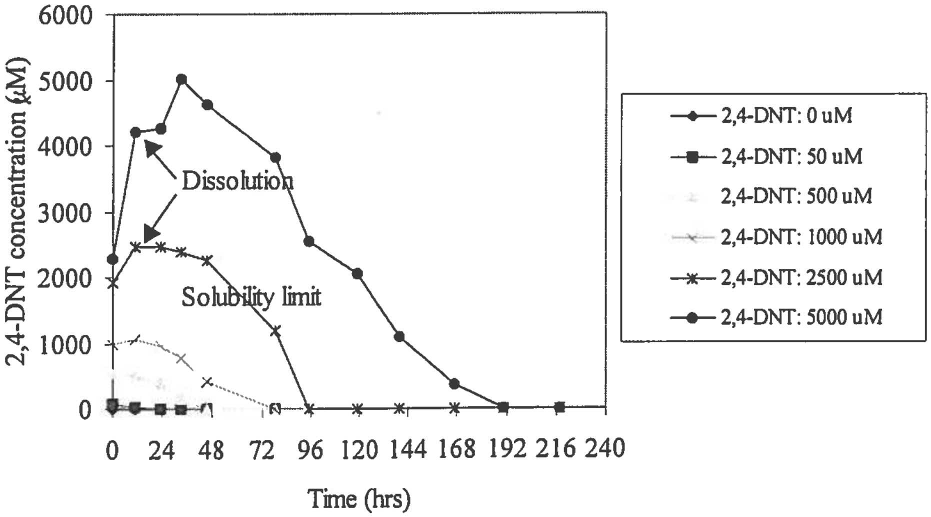

Figure 70 Inhibition of 2,4-DNT on 2,6-DNT degradation: Degradation of spiked 2,4-DNT

3.7.3 Effects of 2,6-DNT concentrations

The purpose of this shake-flask study was to investigate whether high concentrations of 2,6-DNT inhibited the bacterial activity of 2,6-DNT degradation. Similar tests as described in Section 3.7.2 were performed in 4 Erlenmeyer flasks containing 500 mL of slurry from Reactor D and different concentrations of 2,6-DNT (0, 100, 200, 500 μM). Results in Figure 71 clearly show that bacteria sustained their activity at a concentration up to 500 μM of 2,6-DNT. Results also demonstrated a complete degradation of 2,6-DNT at a concentration range of 40 ~ 500 μM.

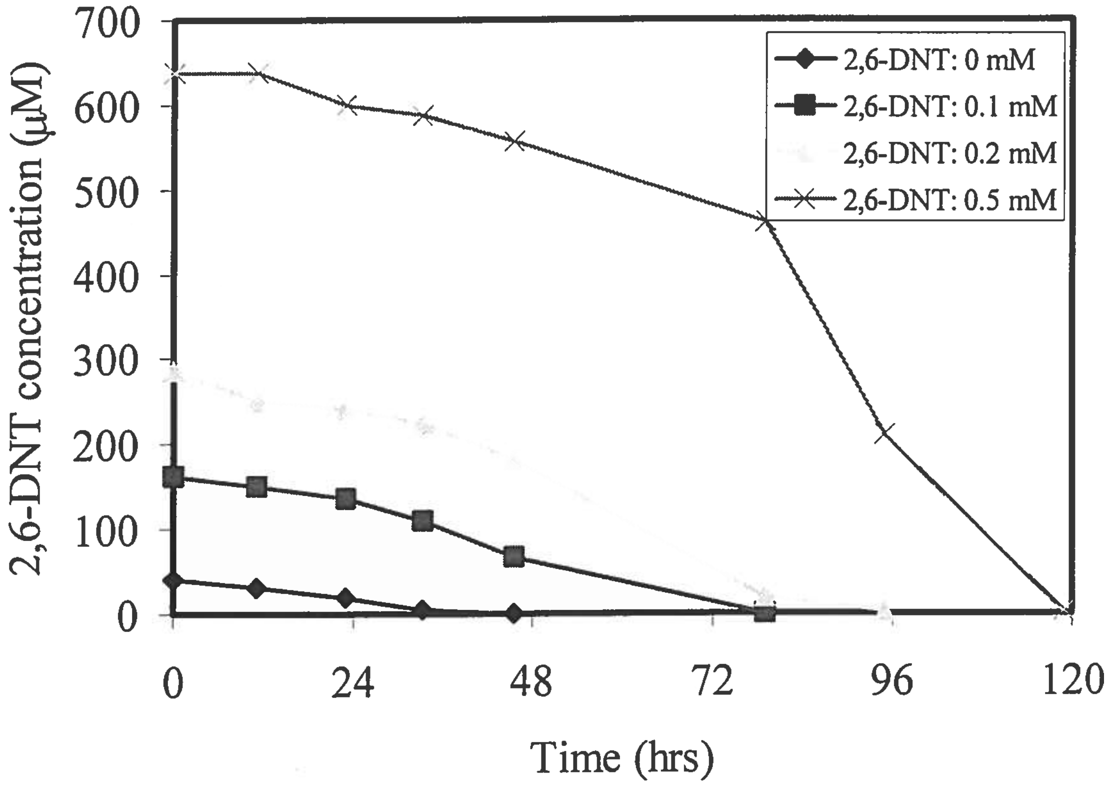

Figure 71 Degradation of 2,6-DNT at various initial concentrations

3.7.4 Extended aeration of reactor effluent with BAAP soil

Using effluent at the end of representative feeding cycles, extended aeration studies were performed to investigate the extent of degradation of residual 2,4-DNT, 2,6-DNT and TNT. For BAAP soil, effluent from both reactors (C and D) was tested. The effluent was placed in 2 L Erlenmeyer flasks, and was shaken for 10-12 days at 30°C. Extended aeration for Reactor C effluent was tested under two highest nominal solids loading rates, i.e., 30% and 40%. Similar results were obtained, Figure 72 presents the residual concentrations of 2,4-DNT, 2,6-DNT and TNT with a 30% of BAAP soil loading. It can be seen that the concentrations remained constant during a 12-day period.

Reactor D effluent was taken at the end of a feeding cycle (~ 1034 hr) that was previously run at 50:50 (v/v) fill-and-draw mode. The effects of extended aeration are given in Figure 73. In contrast to Reactor C, further degradation of 2,6-DNT was achieved. Note that the residual concentrations of 2,4-DNT and 2,6-DNT were one order of magnitude lower than the concentration in Reactor C. Residual concentrations of 2,4-DNT and 2,6-DNT were close to the detection limit.

3.7.5 Extended aeration of reactor effluent with VAAP soil

For the extended aeration of VAAP effluent, only effluent from Reactor E was studied. This effluent was taken at the end of the second feeding cycle of 20% VAAP solids loading. No studies were conducted for the effluent from the second reactor in series (Reactor F). As can be seen in Figure 74, the effluent had much higher residual concentrations of 2,6-DNT (919 μM) and TNT (233 μM) than in Reactor C. This is expected because higher concentrations of 2,6-DNT and TNT were present in VAAP soil. It is noted that both 2,4-DNT and TNT underwent further degradation during the

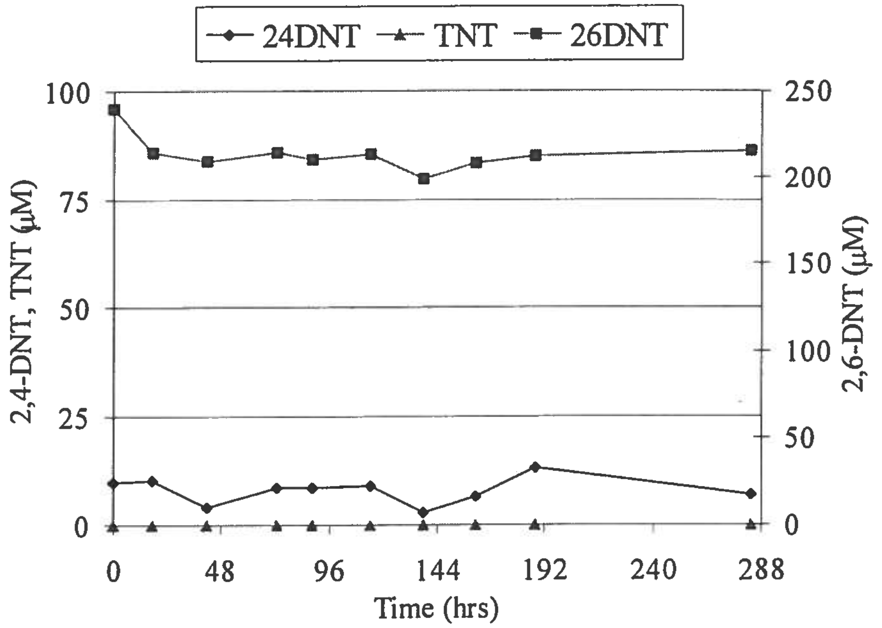

Figure 72 Extended aeration of Reactor C effluent

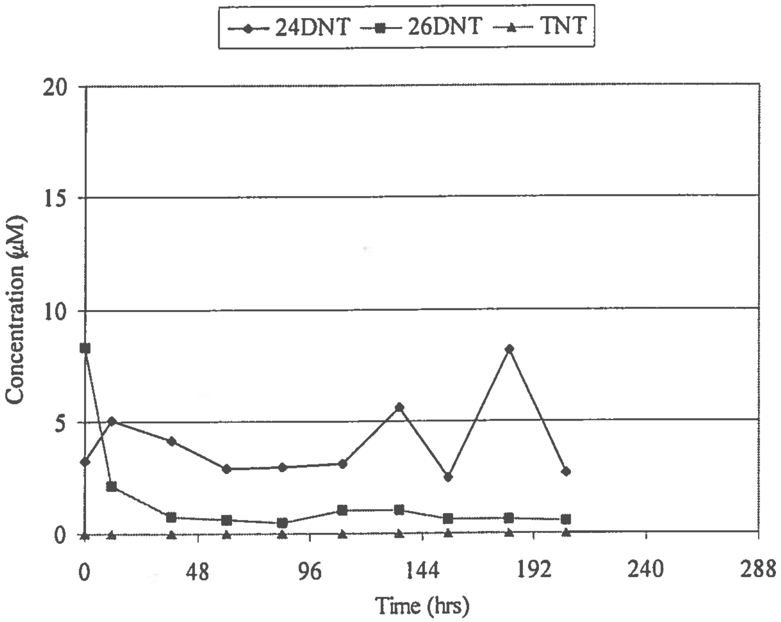

Figure 73 Extended aeration of Reactor D effluent

extended aeration. However, the concentration of 2,6-DNT remained constant, implying the lack of activity of 2,6-DNT bacteria in the first reactor. This is consistent with the results shown in Figure 72 for BAAP soil.

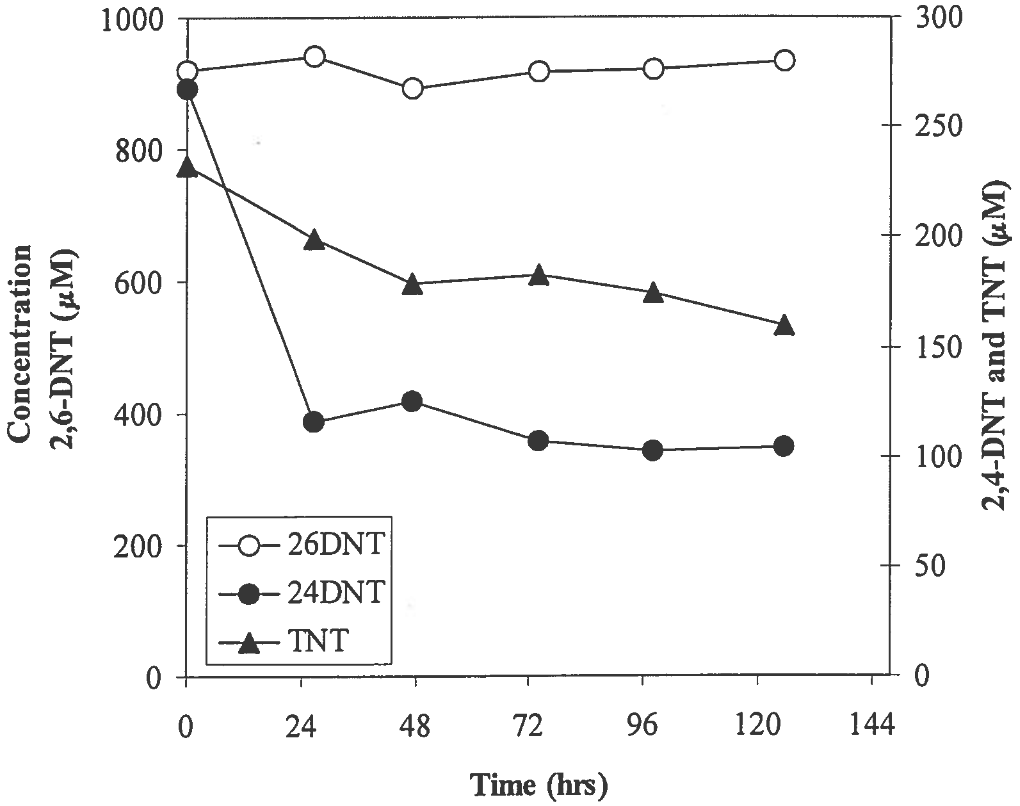

Figure 74 Extended aeration of Reactor E effluent

At the end of extended aeration studies, slurry samples were also taken for the analysis of both aqueous and solid phase concentrations of 2,4-DNT, 2,6-DNT and TNT. Results are summarized in Table 6 for the comparison between 2 reactors in series with BAAP soil (Reactor C vs. D) and the comparison between two soils (BAAP vs. BAAP). Between two reactors in series, it is expected that both aqueous and solid phase concentrations were much lower in Reactor D than in Reactor C after the extended aeration. It is also interesting to note the difference in the partitioning (aqueous vs. solid phases) between two soils. For the sandy BAAP soil, the residual concentrations were about equally distributed between two phases. For the VAAP soil, however, more residuals were associated with solid phase than with aqueous phase (60:40).

Table 6 Residual concentration and partitioning in aqueous and solid phase after extended aeration.

		Concentration (μM)			% of total (aqueous + solid)		
		24DNT	26DNT	TNT	24DNT	26DNT	TNT
Reactor C (BAAP soil)	Aqueous	6.0	149	N.D.[¶]	38.3	50.6	-
	Solid	9.6	145	N.D.	61.7	49.4	-
Reactor D (BAAP soil)	Aqueous	1.3	0.29	N.D.	48.1	53.3	-
	Solid	1.4	0.26	N.D.	51.9	46.7	-
Reactor E (VAAP soil)	Aqueous	40.6	342.5	60.3	39.0	36.7	37.9
	Solid	63.4	591.0	99.0	61.0	63.3	62.1

[¶] Not detected.

3.8 Design and cost consideration

3.8.1 Design basis for a hypothetical full-scale bioslurry treatment system

The treatment cost of a slurry reactor system depends mainly on three process parameters: (1) solids loading rate, (2) residence time, and (3) soil removed during slurry preparation (Dupont, 1997). Due to the differences in soil characteristics and the allowable loading rates, the treatment costs will differ significantly between two soils. A schematic of a hypothetical full-scale bioslurry reactor system is shown in Figure 75. The design for the treatment of VAAP soil is based on the pilot-scale study using the following assumptions:

- 8,100 m^3 (10,000 yd^3) or 215,000 metric ton of contaminated soil are treated. The VAAP soil has average concentrations of 11.0 and 0.9 g/kg for 2,4-DNT and 2,6-DNT, respectively.

- 13% of large particulates are removed in soil washing processes.
- A heating device is used, and the reactors are operated at 30°C. At this operating temperature, the residence times for 2,4-DNT and 2,6-DNT are approximately 2 and 4 days, respectively.
- Each reactor has an operating volume of 720 m^3 (180,000 gal).
- The reactors are operated in a sequential mode. The first two reactors in parallel are fed soil wash directly at a 20% nominal solids loading rate, followed by the amendment of 1 mM phosphate buffer. These two reactors have a 10:90 (v/v) draw-and-fill operation every two days. The effluent streams are further treated in four sequential reactors. The reactors in series are operated in 4 days per feeding cycle, with fill-and-draw every two days for two of the sequential reactors. A total of six reactors are needed to sustain such operation (Figure 75).
- The processed slurry is dewatered and the recovered water is recycled to the slurry preparation/soil washing process. Note that the feasibility for the reuse of recovered water was not tested in the pilot-scale study.
- The treatment system is operated 7 days a week for the duration of the project.

Similar to VAAP soil, a hypothetical full-scale treatment system for BAAP soil can be designed based on the same assumptions except:

- The BAAP soil has average concentrations of 9.0 g/kg and 0.5 g/kg for 2,4-DNT and 2,6-DNT, respectively.
- 87% of large particulates are removed in soil washing processes.
- The reactors are operated at a 40% nominal solids loading rate.

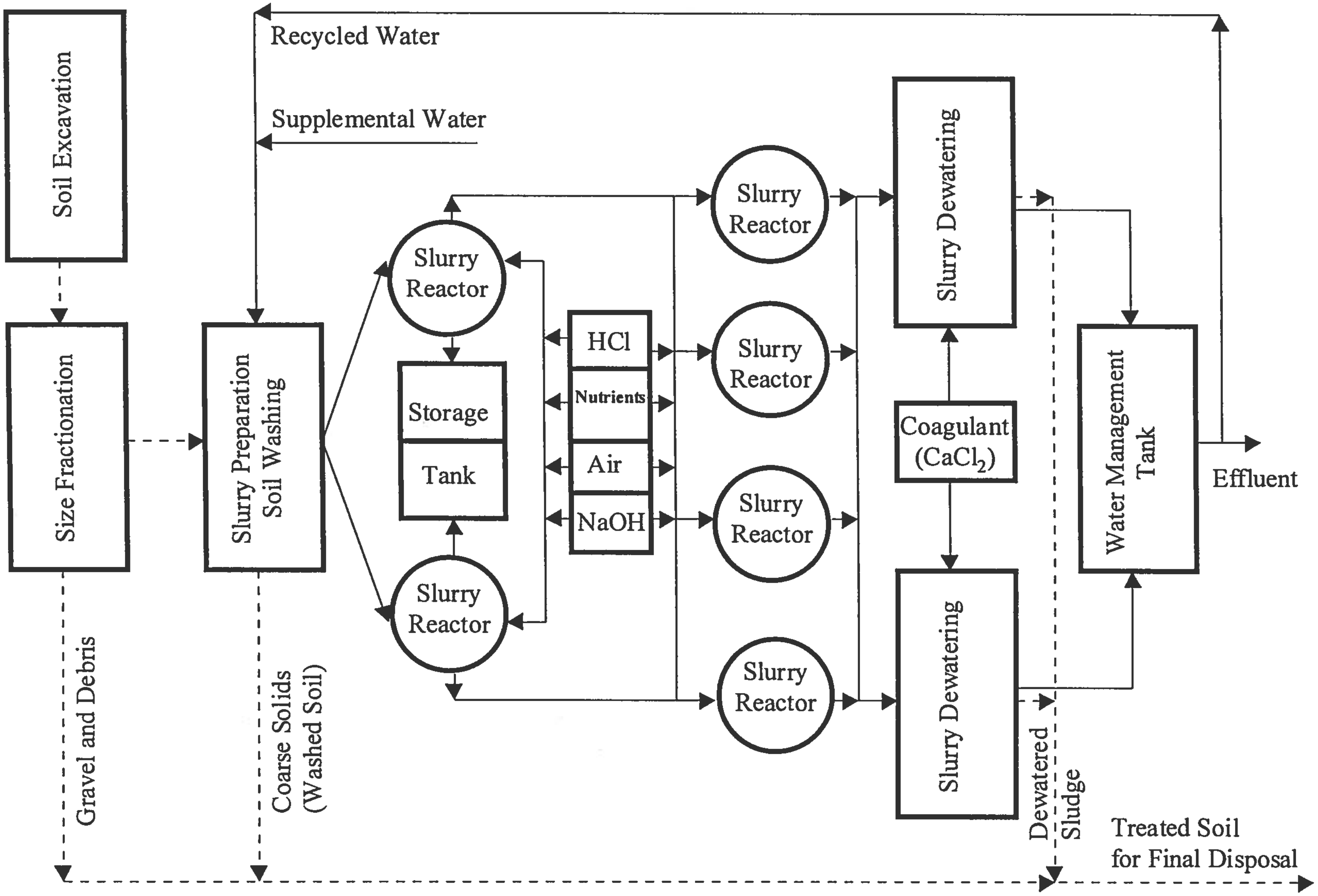

Figure 75 Conceptual flowchart of a hypothetical full-scale slurry reactor system

Table 7 is a summary of the comparison between two soils in a full-scale hypothetical bioslurry system. As shown in the table, it needs 2.5 months to treat 8,100 m^3 contaminated BAAP soil. The duration for the treatment of the same amount of VAAP soil is doubled since the system is allowed to operate at one-half of the solids loading rate (i.e., 20% for VAAP vs. 40% for BAAP, based on pilot-scale study), indicating a significant increase in operating and maintenance cost. Table 7 also indicates that a significantly larger fraction of VAAP soil is associated with sludge in the slurry effluent, implying an added remediation cost due to the requirement of sludge dewatering. The amount of chemicals required per cubic meter of soils are similar between two soils, but differs significantly on a per day basis due to different project duration (Table 7).

Table 7 Chemicals and oxygen requirement, solid material generation and project duration for a hypothetical full-scale slurry reactor system.

Soil[1]	VAAP soil	BAAP soil
Na_2HPO_4 (buffer and P-nutrient)	1.88 kg/m^3 (110 kg/day)	0.94 kg/m^3 (97 kg/day)
NaOH (pH control)	13.7 kg/m^3 (724 kg/day)	10.9 kg/m^3 (1126 kg/day)
Oxygen[2]	3.74 kg/m^3 (slurry) /day	2.88 kg/m^3 (slurry) /day
Soil removed in soil wash[3]	25 ton/day	238 ton/day
Soil remaining in slurry effluent[4]	115 ton/day	36 ton/day
Project duration	5 months	2.5 months

1 — Reported as per m^3 of soil for Na_2HPO_4 and NaOH, and per m^3 of liquid slurry for oxygen. 2 — Based on the peak oxygen uptake rate observed in pilot-scale slurry reactors. 3 — Dry weight; 4 — Dry weight, the biomass and possible precipitates are neglected.

3.8.2 Cost elements associated with bioslurry reactor systems

The total project costs of a slurry reactor system include the treatment costs and many other elements as well. The procedures for the cost estimates of slurry reactors are not standardized, so the following discussion gives a general cost analysis. A summary of cost factors for a slurry reactor treatment system is presented in Table 8. Note that the exact cost estimates are not given, since many of these elements are site specific or process specific.

The capital investment can be grouped into 3 categories: pretreatment for slurry preparation, treatment with bioslurry reactors, and post-treatment for the handling and disposal of sludge. As shown in the table, major components of the capital costs include the equipment for soil excavation and soil pretreatment (screening, milling, soil washing), reactors and accessories (e.g., pump, storage tanks, etc.), and sludge thickening/dewatering facilities such as clarifiers, pressure filters, vacuum filters, and sand drying beds. Note that excavations of contaminated soils are site-specific and can be very expensive. Nonhomogeneous soils and clayed soils may also add extra remediation costs due to potential handling problems.

Table 8 Summary of cost-contributing tasks and activities.

Preliminary	Pre-treatment	Treatment	Post-treatment
Site assessment Bench-scale testing Pilot-scale testing Design Permitting	Site preparation Excavation & transport Screening Milling Soil washing	Bioslurry reactor & accessories Monitoring & analysis Compliance	Thickening/ dewatering Sidestream treatment Final disposal Site closure

Source: Christodoulatos and Koutsoapyros (1998).

Major components for operating and maintenance costs for a slurry reactor system include the costs for chemicals, oxygen supply, power, and labor, etc. The chemical and oxygen requirements are illustrated previously in Table 7. According to the vendor, the energy consumption with Emico reactor is typically less that one-half of that required when turbine mixtures or surface aerators are employed. Typical installed energy for a mobile 20,000 – 25,000 gallon reactor ranges from 2 – 3 hp per 1,000 gallons (EPA, 1990). When sediments are treated, much lower power input (0.1 – 1 hp per 1,000 gallons) is expected since sediments are generally less dense than soils and can be more easily maintained in suspension (Dupont, 1997). Labor costs could be a major component of the O&M costs, and may reach ~50% of the total remediation cost for a full-scale implication.

3.8.3 Comparison with other competing technologies

Due to a cost-prohibitive issue and environmental concern with the traditional methods (e.g., incineration), innovative technologies for the treatment of explosives compounds are emerging. A list of these technologies tested in several U.S. army ammunition plants is given in Table 9 for an illustrative purpose. The lists are not exhaustive and the cost data are not available in all cases, but it appears that bioslurry and windrow composting offer two competitive methods for the remediation of explosives contaminated soils. Phytoremediation is generally economically favorable and environmentally acceptable relative to other more intrusive remediation technologies, but commercialization has been limited to date.

A general comparison of the overall cost for the remediation of contaminated soils using different technologies is further presented in Table 10. Caution should be excised when using these data, since overall cost depends on the contaminants of interest, scales, and other site or process specific parameters. Slurry reactor system cost compare favorably over traditional methods, but appears to be generally more expensive than land farming and composting. It offers a preferred alternative over land treatment when dealing with highly contaminated soil, and a distinct advantage over composting since slurry reactor provides a better control of environmental conditions (pH, temperature, aeration, nutrients, etc.) and therefore more rapid treatment of explosive compounds. More importantly, the slurry reactor augmented with DNT-degrading bacteria achieved mineralization of DNT without the production of aminonitrotoluenes.

Table 9 Innovative technologies for the clean-up of explosives contamination at selected army ammunition plants in the U.S.

Contaminated Sites¶	Technologies	Process Performance	Cost Data
UMDA	Windrow composting	> 99.5% removal of TNT, HMX and 96.8% for RDX in soil	< $210/ton soil for larger site greater than 20,000 tons of soil
LAAP	Aerobic static pile composting	Removal of 99.6-99.9% (TNT), 94.8-99.1% (RDX) and 86.9-95.6% (HMX) in lagoon sediments	N.A.
BAAP	Aerated static pile composting	Nitrocellulose reduction > 99.5%	N.A.
JAAP	Aerobic bioslurry	99+% removal of TNT, RDX and HMX in soil	Best suited for small sites where incineration is cost-prohibitive
MAAP	Gravel-bed constructed wetlands	> 95% removal of TNT, RDX, HMX, TNB, 2DNT and 4DNT in groundwater	$1.8/1,000 gallons compared to $4.0/1,000 gallons for GAC
VAAP	Flow-through systems with aquatic and wetland plants	> 90% removal of TNT, 2,4-DNT, 2,6-DNT in groundwater	N.A.

¶ UMDA: Umatilla Army Depot Activity, Hermiston, OR; LAAP: Louisiana Army Ammunition Plant, Shreveport, LA; BAAP: Badger Army Ammunition Plant, Baraboo, WI; JAAP: Joliet Army Ammunition Plant, IL; MAAP: Milan Army Ammunition Plant, Milan, TN; VAAP: Volunteer Army Ammunition Plant, Chattanooga, TN.

Table 10 General cost comparison between slurry reactor and other competing technologies.

Technologies	Overall cost ($ per metric ton)
Land farming	25 – 40
Composting	30 – 50
Slurry reactor	50 – 100
Solvent extraction	100 – 500
Stabilization / solidification	120 – 520
Incineration	400 – 1000

Source: Christodoulatos and Koutsospyros (1998)

4 DISCUSSIONS

A number of pilot- or field-scale demonstrations have been reported for the bioremediation of explosives compounds, these studies were limited primarily for the biodegradation of TNT, RDX and HMX in aerated composting systems (EPA, 1996). Anaerobic slurry reactors were tested at the Joliet Army Ammunition Plant, IL, with a reported removal of 99+% for TNT, RDX and HMX in soil. The present study provides important data on the treatability of soils contaminated with both isomers of DNT in a pilot-scale sequential bioslurry reactor system using bacteria obtained at Tyndall AFB (Nishino et al., 1999). Results from this unique study are expected to provide a general basis for system design, optimum operating parameters, routine monitoring, and process control for a full-scale slurry reactor system. Results from this study indicated the different kinetic rates between two isomers of DNT and the inhibitory effects on 2,6-DNT degradation in the presence of high concentrations of 2,4-DNT. The inhibition and differential rates of degradation were mitigated by the sequential mode of reactor operation. Results also demonstrated stoichiometric release of nitrite and NaOH consumption from the degradation of dinitrotoluene, and the accurate assessments of microbial activity by the measurement of oxygen uptake rates. These results suggested that cost-effective monitoring could be achieved through the use of these inexpensive measures for routine process control.

Slurry reactors provide optimum control of operating conditions (e.g., pH, temperature, aeration, nutrients and mixing), and therefore the rapid treatment of explosives compounds. The slurry system, however, produce large volumes of effluent. High concentrations of suspended solids in the effluent must be settled, and sludge so generated needs to be dewatered for ultimate disposal. It was found that the effluent containing soil fine particles settled very slowly, and only the addition of $CaCl_2$ at a concentration of approximately 3 mM effectively clarified the effluent. This was presumably due to the precipitation of residual phosphate and hence the destabilization of the colloidal system. However, it is not known why settling was not achieved with the use of other chemical coagulants such as $FeSO_4$ and alum. Another issue should be addressed in scale-up application is the feasibility of recovered water for reuse in slurry preparation. On the basis of process economics, it is desirable to reuse the effluent for soil washing during slurry preparation. A potential problem associated with the reuse of recovered water is the high concentrations of nitrite accumulated in the effluent. No inhibitory effects were observed in this study when nitrite concentration was as high as 4 mM (56 mg NO_2^--N/L). However, nitrite at higher concentrations was shown to be inhibitory to both 2,4-DNT and 2,6-DNT degradation in a batch-scale experiment.

An important characteristic of slurry reactor is its ability to treat highly contaminated soils. As shown in Table 11, successful demonstrations have been achieved for both soils contaminated heavily with 2,4-DNT and 2,6-DNT. The initial concentrations of 2,4-DNT and 2,6-DNT in the first reactors in series were as high as 2,011 and 71 mg/L, respectively, for BAAP soil at a 40% nominal solids loading rate, or 1,434 and 189 mg/L, respectively for VAAP soil at a 20% nominal solid loading rate. After the treatment with sequential slurry reactors, 2,4-DNT and 2,6-DNT concentrations in the effluent were, respectively, below the detection limit and 2.7 mg/L for BAAP soil, or 3.5 mg/L and 4.9 mg/L for VAAP soil. Further concentration reductions were achieved if the effluent was subjected to extended

Table 11 Summary of reactor performance.

		VAAP Soil at 20% loading rate[1]			BAAP Soil at 40% loading rate[2]		
		2,4-DNT	2,6-DNT	TNT	2,4-DNT	2,6-DNT	TNT
Contaminated soil (mg/kg, dry basis)		1089	870	430	8940	480	70
First Reactor	Initial conc. in µM (mg/L)[3]	7875 (1434)	1040 (189)	376 (86)	11039	390 (71)	N.D.[7]
	Effluent in µM (mg/L)	213 (39)	985 (179)	243 (55)	13 (2)	308 (56)	N.D.
	Residence time (days)	1.8			2.1		
Second Reactor	Initial conc. in µM (mg/L)[4]	115 (21)	311 (57)	79 (19)	32 (6)	261 (48)	N.D.
	Effluent in µM (mg/L)	19 (3.5)	27 (4.9)	52 (12)	N.D.	15 (2.7)	N.D.
	Residence time (days)	4.0			12.6[8]		
Soil removed in soil wash	Water/soil = 17 L//kg	97	19	10	28	0.4	N.D.
	Water/soil = 2 ~ 4 L/kg[5]	907	95	58	775	37	N.D.
Soil remaining in reactor	First reactor (mg/kg, dry basis)	224	84	25	92	21	N.D.
	Second reactor (mg/kg, dry basis)	N.A.[6]	N.A.	N.A.	71	4	N.D.

1 — Average of 4 feeding cycles at a 20% loading rate. 2 — Average of 2 feeding cycles at a 40% loading rate.
3 — Observed concentrations might be lower than calculated concentrations due to solubility limits and the nonhomogeneous distribution of contaminants in the reactor.
4 — The second reactor was fed a mixture of effluent from the first reactor. The effluent was stored at room temperature with aeration, followed by dilution prior to uses.
5 — VAAP soil: water/soil = 4 L/kg; BAAP soil: water/soil = 2 L/kg. 6 — Data not available. 7 — Not detectable.
8 — The residence time depended upon the initial concentrations of 2,6-DNT.

aeration. For instance, residual concentrations of both 2,4-DNT and 2,6-DNT were reduced to 0.49 and 0.10 mg/L, respectively, after several days of extended aeration.

A key criteria with regard to reactor performance is the residual concentrations of DNT and TNT in processed effluent and solid materials (i.e., soil removed in soil wash and sludge produced in reactor) as compared to the regulatory safety limits. The U.S. EPA lists both 2,4-DNT and 2,6-DNT as priority pollutants, but numerical effluent standards are not available for both compounds in the current NPDES regulations. Residual dinitrotoluenes present in effluent at mg/L level may present a concern, as it is substantially higher than the safety drinking water limits. The U.S. EPA Health Advisory limits for 2,4-DNT, 2,6-DNT, and TNT in drinking water are 50, 40, and 2.0 μg/L, respectively. The residual concentrations of 2,4-DNT in the effluent compare favorably with the RCRA standard established in the Code of Regulations (Title 40, Section 261.24). The characteristic hazardous levels in the TCLP leachate is 0.13 mg/L for 2,4-DNT, but no standard is available for 2,6-DNT and TNT.

It should be pointed out that the observed residual concentrations of 2,6-DNT in the effluent or solid materials might be overestimated due to the analytical method employed. An impurity co-eluted with 2,6-DNT with a UV/VIS spectrum that resembled 3,4-dinitrotoluene. Efforts were made in order to quantify these two isomers in samples, but separation was not successful. The observed residual concentration, therefore, is the sum of 2,6-DNT and 3,4-DNT. It is known that technical grade of DNT has 76% of 2,4-DNT, 19% of 2,6-DNT, and 5% of other isomers (e.g., 2,3-DNT, 3,4-DNT). However, the initial composition of these isomers present in the soil is unknown and the percentage composition may change during the course of biodegradation, these factors attributed to the difficulty in determining the final concentration of 2,6-DNT and 3,4-DNT in soil. Moreover, the presence of residual 3,4-DNT may present a greater concern in residual management, since an aquatic toxicity test showed that DNT isomers with *ortho* (2,3 and 3,4) and *para* (2,5) nitro groups were about 10-20 times more toxic than those with *meta* (2,4, 2,6 and 3,5) nitro groups (Rosenblatt et al, 1991). Information is needed regarding the toxicity, biodegradability and regulatory limit for the risk assessment of residual 3,4-DNT in treated soil.

5 CONCLUSIONS AND RECOMMENDATIONS

The following major conclusions can be drawn from this study:

- Soil washing was an effective pretreatment for slurry reactors by removing large particlulates and recovering 2,4-DNT, 2,6-DNT and TNT from contaminated soils.
- Augmentation of slurry reactor with dinitrotoluene-mineralizing bacteria resulted in the oxidative biodegradation of 2,4-DNT and 2,6-DNT with both VAAP and BAAP soils.
- A sequential mode of operation optimized the rate and extent of 2,4-DNT and 2,6-DNT degradation because high concentrations of 2,4-DNT inhibited 2,6-DNT degradation.
- Optimal operating parameters have been defined for both VAAP and BAAP soils, including the ratio of water/soil, solid loading rates, phosphorous nutrient requirement, resident time, etc.

- Stoichiometry of nitrite release and sodium hydroxide consumption during the course of dinitrotoluene biodegradation has been established in pilot-scale study.

The following recommendations can be made based on findings and experience gained from this study:

- Fill-and-draw operations of bio-slurry reactors require extensive and expensive monitoring of DNT and TNT concentrations in the reactor. Inexpensive measures such as nitrite release, oxygen uptake rate and NaOH consumption are recommended for routine monitoring and process control.
- 2,6-DNT bacteria are more restricted in sustaining degradation activities as compared to 2,4-DNT degrading bacteria. Cost-effective measures for process control are critical in sustaining bacterial activity (especially 2,6-DNT degrading bacteria) without the prolonged period of insufficient DNT supply.

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