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13. ABSTRACT ***(Maximum 200 Words)***

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In this report are the results of a three-year program designed to test and demonstrate the GAC-DBR process for a number of problem wastewaters facing the U.S. Armed Forces. Both laboratory-pilot and field demonstrations using small commercial scale GAC-FBR systems were conducted.

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TREATMENT OF INDUSTRIAL PROCESS EFFLUENTS AND CONTAMINATED GROUNDWATER USING THE BIOLOGICAL GRANULAR ACTIVATED CARBON-FLUIDIZED BED REACTOR (GAC-FBR) PROCESS

Improving Mission Readiness Through Environmental Research

TREATMENT OF INDUSTRIAL PROCESS EFFLUENTS AND CONTAMINATED GROUNDWATER USING THE BIOLOGICAL GRANULAR ACTIVATED CARBON-FLUIDIZED BED REACTOR (GAC-FBR) PROCESS

FINAL REPORT

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EXECUTIVE SUMMARY

In 1992, Congress allocated funds for development of expertise in applied environmental bioremediation restoration technology [Congressional Record, Senate, 22 Sept 92 S14633] including work on process integration, scale-up and demonstration of the Granular Activated Carbon-Fluidized Bed Reactor (GAC-FBR) process. Specific targets included the treatment of chlorinated solvents, nitrated compounds and aromatic hydrocarbons. The goal of this SERDP funded project was to conduct experimental work at the bench-scale through field demonstration using the GAC-FBR as the platform for degradation of these compounds of concern.

The GAC-FBR process is a high-rate platform for performing biologically catalyzed reactions. The process can be operated as an aerobic, anoxic (denitrifying) or anaerobic system. The use of an adsorptive biomass carrier in the form of granular activated carbon (GAC) affords the process robustness during adverse environmental conditions beyond that possible with other biological fixed film systems. This makes the process an ideal candidate for ensuring compliance with effluent discharge limits for wastewaters produced during munitions manufacturing and remediation operations.

In this report are the results of a three-year program designed to test and demonstrate the GAC-FBR process for a number of problem wastewaters facing the U.S. Armed Forces. Both laboratory-pilot and field demonstrations using small commercial scale GAC-FBR systems were conducted.

Based on needs identified at a kickoff meeting held with representatives of the U.S. Army, Navy and Air Force, four laboratory-pilot tests were conducted. The intent was to determine the potential of the GAC-FBR to meet the treatment needs for different waste streams of concern for DoD overall and develop a sufficient information base upon which field demonstrations could be subsequently conducted for two of these applications. The four laboratory-pilot projects included:

1. Treatment of propylene glycol dinitrate (PGDN) in Biazzi nitration effluents.
2. Removal of trinitrobenzene (TNB) from the effluent of an advanced oxidation process (AOP) treating TNT in water.
3. Treatment of ketones in groundwaters, with emphasis on biological viability at low ketone concentrations.
4. Cometabolic transformation of trichloroethylene (TCE) at sites where both TCE and hydrocarbon contamination are found, such as at many Air Force bases.

A total of three field demonstrations, using custom-designed, small commercial-scale GAC-FBRs were conducted as part of this work. These included application of the GAC-FBR under anaerobic, anoxic and aerobic conditions. The demonstrations conducted included:

1. Demonstration of anaerobic reduction of DNT in high to moderate strength munitions wastewater at the Radford Amy Ammunition Plant, Radford, VA.
2. Aerobic treatment of ketones in groundwater.
3. Treatment of PGDN in Biazzi nitration effluent under denitrifying (anoxic) conditions at the Indian Head Naval Surface Warfare Center, Indian Head, MD.

In addition, the design, procurement and start-up and technical assistance for anaerobic treatment of a wastewater containing chlorinated phenols at a chemical plant in Hungary was also completed.

Laboratory-Pilot Tests

Presented below is a summary of results from the four laboratory-pilots and field demonstrations conducted during this work effort.

Treatment of Propylene Glycol Dinitrate (PGDN) in a Nitration Plant Effluent

During munitions manufacturing, wastewater streams containing nitrated compounds of regulatory concern are generated. At the Indian Head Division, Naval Surface Warfare Center, wastewater containing propylene glycol dinitrate (PGDN) and high nitrate concentrations is generated from nitrating propylene glycol (PG) to produce PGDN. The purpose of this project was to evaluate the effectiveness of biological treatment to reduce the concentration of PGDN from hundreds of mg/L to below 1 mg/L in the Biazzi plant process effluent water.

Tests were performed using a 7-liter pilot scale reactor with both real and synthetic nitration process wastewater. The effect of applied organic loading rate, PGDN loading rate and the ratio of primary substrate COD to PGDN were examined. Both ethanol (EtOH) and PG were examined as primary growth substrates.

Conclusions drawn from the pilot scale tests include:

- PGDN could be removed under denitrifying conditions with either EtOH or PG as the primary substrate.
- Effluent PGDN concentrations below the 1 mg/L regulatory limit could be achieved at PGDN loading rates of up to 0.22 Kg COD/m^3-d and overall OLR of 5 Kg COD/m^3-d. PGDN removal rates of greater than 98% were achieved at PGDN loading rates up to 0.89 Kg COD/m^3-d at primary COD/PGDN ratios of 4:1.
- Increasing the PGDN loading rate at a fixed total COD loading led to an increase in effluent PGDN concentration. Decreasing the primary COD/PGDN ratio at a fixed PGDN loading rate resulted in an increase in effluent PGDN concentration.
- The GAC-FBR proved robust to suspensions in feeding of both primary substrate and PGDN. The microbial population in the system was able to degrade PGDN

after a three-month interval of exposure to only the primary substrate. The reactor biomass remained viable after a three-week period of starvation (no substrate feed).

- No pH control was necessary for stable reactor performance. The reactor pH self-regulated between 8.0 and 8.6.

Treatment of Trinitrobenzene (TNB)

Bench-scale feasibility tests for biodegradation of trinitrobenzene (TNB) under aerobic and microaerophilic conditions were conducted. TNB is produced as a result of treatment of TNT containing waters via advanced oxidation systems such as peroxone (ozone and peroxide). The driving force for this test was to determine if the TNB produced could be readily degraded biologically. Results obtained indicated TNB could be readily treated.

- Complete biological removal of TNB was achieved under aerobic and microaerophilic conditions using ethanol as a primary substrate to support growth.
- Under microaerophilic conditions, TNB was transformed to dinitroaminobenzene (DNAB), then to nitrodiaminobenzene (NDAB) prior to being removed. Degradation of NDAB required the addition of a supplemental nitrogen source (NH_4NO_3 or NH_4Cl).
- Under aerobic conditions, DNAB was the only observed transformation product of TNB. This occurred in the absence of an additional nitrogen source.
- No intermediates were observed when an additional nitrogen source was supplied to the chemostat under aerobic conditions.
- Under aerobic conditions, approximately 10-15 mg ethanol/mg TNB removed was achieved in a batch-fed reactor. Complete removal of TNB over a 41-day period was achieved in a continuously fed reactor, with the ethanol/TNB consumption ratio at 50 mg/mg.
- Biological treatment significantly reduced toxicity caused by TNB. Microtoxicity assays indicated that no toxicity was observed in the aerobic and microaerophilic reactor effluents.

Treatment of Ketones

Ketones such as acetone, MEK and MIBK are organic solvents that are used for nitrocellulose and for removal of paraffins and long-chain hydrocarbons. These compounds are present in groundwaters at a number of sites and in some process effluent streams. The treatment of ketones in groundwater was initially tested using a 7-liter laboratory-pilot GAC-FBR. Results obtained indicated that at OLRs ranging from 0.025 to 2.3 Kg COD/m^3-d, acetone, MEK and MIBK were consistently removed to below analytical detection limits (and well below any regulatory limits).

There was little decrease in the reactor bed height and, therefore, little loss of biomass when the OLR was decreased from 2.3 to ca. 0.025 Kg COD/m^3-d, a 100-fold reduction. A

viable biomass population was retained in the reactor even at OLRs as low as 0.025 Kg COD/m^3-d.

The results of this work clearly illustrate that the GAC-FBR is effective at degrading ketones most often encountered at contaminated sites. This was true at low feed concentrations and high volumetric throughput (7 min HRT).

Cometabolic Oxidation of TCE

The final laboratory-pilot work was conducted to determine if cometabolic removal of TCE could be achieved by commingling groundwater plumes containing TCE and BTEX. A GAC-FBR system, fed synthetic groundwater containing BTEX and TCE, was started-up under ambient temperature conditions (21-22°C). The synthetic groundwater contained 190 mg TCE/L and 6000 mg BTEX/L. The hydraulic retention time (HRT) was 5.9 minutes. Key observations include:

- A stable biofilm could be formed on the GAC carrier within 10 days; BTEX removal efficiency was greater than 99%.
- TCE degradation was examined at four different steady-state conditions under ambient temperature conditions with one-pass feed at a 5.6 minute HRT. Under all test conditions, with BTEX loading rates ranging from 1.9 to 4.6 Kg COD/m^3-d, BTEX removal efficiencies were greater than 99.9%. The effluent BTEX concentrations were, in general, below detection limits.
- Under the first steady-state condition, the reactor was fed a moderate TCE concentration (380 µg/L) with a BTEX/TCE loading ratio of 17/1 (mg/mg); average TCE removal efficiency was 32.7% with a BTEX/TCE consumption ratio of 44.9 mg/mg.
- At an increased BTEX/TCE loading ratio (37/1) and reduced TCE concentration (160 mg/L), the average TCE removal efficiency was 30.8%; the BTEX/TCE consumption ratio was 110.9 mg/mg.
- At a reduced BTEX/TCE loading ratio (17/1) and constant TCE concentration (180 mg/L), the average TCE removal efficiency was 36.3% at a BTEX/TCE consumption ratio of 41.8 mg/mg.
- The results obtained from batch assays and reactor profile analyses indicated that cometabolic TCE degradation rate was inhibited in the presence of BTEX. The batch assays indicated that the biomass taken from upper portion of the fluidized bed had sufficient TCE degradation capability when the BTEX/TCE and toluene/TCE consumption ratios were as low as 42 and 21 mg/mg, respectively. Cometabolic TCE transformation could be described using first order kinetics for the range of TCE concentrations tested.
- The biomass from the GAC-FBR were capable of degrading TCE, cis-1,2-DCE, trans-1,2-DCE, and vinyl chloride. The degradation rates for cis-1,2-DCE and VC, two major anaerobic metabolic intermediates of TCE, were almost three-fold of that for TCE with much less inhibition observed.

- Based on the results obtained from reactor operation and batch assays, TCE removal performance by the GAC-FBR with different HRT and influent TCE concentrations was estimated using kinetic analysis and modeling. High TCE removal efficiency was predicted at increased HRTs.
- In fact, 70% TCE removal efficiency was achieved at an HRT of 26.9 minutes (effluent TCE concentration of 48.3 mg/L) when recirculation of reactor effluent was used. This confirmed modeling results.

Field Demonstrations

Based on results obtained during laboratory-pilot work from this effort and parallel work conducted at the University of Cincinnati under the direction of Dr. Makram Suidan, three demonstrations were planned. These included 1) anaerobic reduction of DNT in a munitions effluent stream, 2) treatment of ketones in groundwater and 3) treatment of PGDN in Biazzi nitrate effluent at the Indian Head Naval Surface Warfare Center. In addition, a project on treatment of chlorinated phenols in a chemical plant effluent was initiated.

Anaerobic Treatment of DNT in a Munitions Wastewater

A field demonstration was conducted to examine the effectiveness of a commercial scale Granular Activated Carbon-Fluidized Bed Reactor (GAC-FBR) for removal of dinitrotoluene (DNT) from water-dry wastewater generated during munitions manufacturing. The location for this demonstration was the Radford Army Ammunition Plant (RAAP). The system is intended for use as pretreatment to remove DNT present in water-dry wastewater at a low flow, high concentration point source prior to entering the RAAP wastewater treatment system. The demonstration included monitoring during eight operational periods each of which differs from the others in one or more key operational characteristics. The demonstration included installation and start-up, acclimation to water-dry wastewater, technical evaluation of reactor performance, decommissioning of the GAC-FBR, and design and economic analysis of a full scale GAC-FBR for the site. Throughout the entire project DNT removal efficiencies were greater than required to meet the plant effluent discharge limitations on RAAP's NPDES permit.

System performance assessment was based on analysis of the influent and effluent streams. The influent and effluent wastewater streams were analyzed for DNT, DAT, ethanol, ether, short chain fatty acids, and COD. Added to the influent wastewater was the measured flow of a supplemental ethanol/sucrose stream used to adjust the COD loading rate to the reactor independent of the DNT loading rate. The effluent gas stream was directly measured using a wet test gas meter and analyzed using an in-line infra-red gas analyzer. The methane concentration in the effluent wastewater was determined from the gas phase methane concentration using Henry's Law. The concept of "usable COD" (i.e. COD that could be readily used by the anaerobic microbial consortia in the GAC-FBR system) was used as a measure of the primary substrate and thus of available reducing power.

Throughout the demonstration, when sufficient primary substrate was present, the anaerobic GAC-FBR system removed DNT to within the facility discharge limits for RAAP. The net average effluent DNT level throughout the course of the demonstration was 0.03 mg/L vs. a facility discharge average limit of 0.113 mg/L. Even when there was insufficient primary substrate present during one period, the effluent DNT only reached 0.6 mg/L (>99% removal), a level which would not cause the facility discharge limit to be exceeded when this source was blended with other wastewater sent to the existing treatment facility.

During the course of this work, the limited availability of water-dry wastewater prevented increasing the DNT loading rate beyond the capability of the GAC-FBR system. The maximum applied DNT loading rate, and thus the minimum equipment size required and operating costs could not, therefore, be identified.

During the eight operational periods, the applied DNT loading rate was varied from 370 to 1390 g DNT/m^3-d. The usable COD:DNT ratio was varied from a high of 18.1 during the first period (initial operation) to a low of 1.2 during the forth period. DNT removal efficiencies were above 99.95% and DNT effluent levels were below the permitted plant discharge levels for all COD:DNT ratios of 2.1 or higher. A ratio of 3 was conservatively selected for the economic analysis.

In six of the eight operational periods, the mass balance on usable COD generally gave an excellent accounting of recovery of influent usable COD between DNT reduction, methane generation, biomass production, and effluent usable COD. Results from the first and last periods gave a slight under-accounting. These periods both had a high driving force for biomass accumulation that could account for the observed results.

GAC from the reactor was sampled several times during the course of the study and analyzed for adsorbed DNT and DAT. No DNT was recovered from any of the samples. This confirmed that the complete transformation of DNT to DAT and other reduced species occurred. DAT was observed in all GAC samples taken from 4/17/95 on. The measured DAT on the GAC samples was directionally less than the amount calculated from measured throughput and influent and effluent analyses. This is consistent with a combination of irreversible adsorption onto the GAC, polymerization of a portion of the adsorbed DNT, and less than full recovery of DAT from the trapping resin in the thermal desorption - GC/FID analysis method used herein.

The design criteria for economic analysis was based on a production level of 4.0 million pounds of DNT containing propellant per year, their estimated production level for the next five years. The design treatment rate of 4 gpm is the maximum generation rate that would result from simultaneous use of all eight currently active water-dry buildings on an 11 day cycle. At this rate, the annual production would be completed in less than half of the year. The design influent concentrations for DNT and usable COD were based on the analysis of results during the longest period of sustained operation during this demonstration.

The GAC-FBR system recommended for installation at RAAP had a working bed capacity of 5.2 m^3. Based on the maximum DNT loading rate successfully demonstrated during this project, this off-the-shelf model has the capacity to treat 7.2 Kg DNT/d, 85% over the design criteria. In continuous use, this system has the capability to successfully treat RAAP's DNT containing water-dry wastewater even if production requirements quadrupled.

The capital cost for the anaerobic GAC-FBR was estimated at $225,000. The total operating and maintenance (O&M) cost, less labor, was estimated at $2,940 per year, over half of which is for electrical power. The liquid phase GAC adsorption system currently in use at RAAP had an initial setup cost of $5,360, an annual rental cost of $22,500, and a change-out service charge of $5,710 per event. At the design loading rate, there would be 16.5 changeout services required at an annual cost of $94,215. The total O&M cost for the GAC adsorption system is thus $122,075. The high O&M costs for GAC adsorption offset the GAC-FBR's higher initial cost in less than two years.

Results of this project are incorporated into AEC Report SFIM-AEC-ETD-CR-95048 "Treatment of Propellant Production Wastewaters Containing 2,4-Dinitrotoluene" prepared by IT Corporation. The AEC reports provides information on alternate technology tested at RAAP for treating DNT including advanced oxidation and adsorption using GAC.

Site support and site preparation costs for installation of the anaerobic GAC-FBR were paid for, in part, through the AEC funded project.

Treatment of Ketones in Groundwater

A four-month demonstration, using a small commercial-scale biological Granular Activated Carbon-Fluidized Bed Reactor (GAC-FBR) system was conducted to determine the potential of biologically treating ketones in groundwater. Due to logistical problems, the demonstration was moved from the U.S. Army Waterways Experiment Station in Vicksburg, MS to the pilot plant at MBI International. The system was operated at range of flow rates and applied organic loading rates during these four months. The key results obtained were:

- Acetone, MEK and MIBK could be consistently reduced in concentration by >99%. Effluent concentrations were well below regulatory limitations and, in general, below the method detection limits.
- The rates of degradation observed from highest to lowest were MIBK > MEK > acetone.
- An 86-fold reduction in applied mass loading of ketones per unit volume per day from typical Organic Loading Rates (OLRs) of 3.2 Kg COD/m^3-d to 0.037 Kg COD/m^3-d, resulted in a 20-fold decrease in net kinetic capacity for degradation of acetone, MEK and MIBK in the aerobic GAC-FBR.
- The remaining biomass population still possessed the capacity to degrade 4.4 (acetone) to 20 (MIBK) times more mass of ketones than was fed at this OLR rate, indicating considerable excess capacity.

- The cost of treatment using the GAC-FBR appears quite reasonable, ranging from $0.27 to $1.33 ($/1000 gallons) for 30 to 350 gpm, and a total COD of 43 mg/L and a groundwater temperature of 10°C.

Treatment of PGDN at Indian Head

During munitions manufacturing, wastewater streams containing nitrated compounds of regulatory concern are generated. A wastewater containing propylene glycol dinitrate (PGDN) and high nitrate concentrations is generated from nitrating propylene glycol (PG) to produce PGDN. The purpose of this project was to evaluate the effectiveness of biological treatment under denitrifying conditions to reduce the concentration of PGDN to below 1 mg/L in the Biazzi plant process effluent water at the Indian Head Naval Surface Warfare Center in Maryland.

The commercial scale GAC-FBR was transported from Radford AAP to the Indian Head Division, Naval Surface Warfare Center, erected and hydraulically tested. A feed storage and effluent handling system was set up and connected to the reactor. Several modifications to the FBR system were installed to improve reliability including: a new wastewater feed pump, PG feed pump and holding tank, effluent discharge pump, foam control pump, and in-line water heater. Extensive weather protection and heat tracing were added to the reactor skid and to the influent and effluent systems to permit winter operation.

Due to mechanical problems with the Biazzi nitration plant, PGDN was not manufactured and wastewater, therefore, was not available for conducting the demonstration during this work effort.

Several conclusions can, however, be drawn from the start-up and initial operation of the biological GAC-FBR using a synthetic feed containing the primary substrate of choice for this work, propylene glycol (PG).

- The GAC-FBR unit integrated readily into the established production and treatment train at the Indian Head facility. Safety features provided on the unit were generally adequate to pass the site safety and hazards review.
- A mature biofilm developed rapidly using PG with the 6% TDS synthetic wastewater containing sodium nitrate and other salts.
- The GAC-FBR proved robust to cessation in feeding of the primary substrate tested, PG. The microbial population in the system was able to quickly degrade PG after intervals of starvation (no substrate feed).
- Because of the high nitrate feed concentration and relatively low COD feed rate ($COD:NO_3\text{-}N < 4.5:1$), some of the nitrate was only partially reduced resulting in some accumulation to nitrite and presumably other reduced intermediates, such as N_2O.
- No pH control was necessary for stable reactor performance. The reactor pH self-regulated between 8.0 and 8.6.

It is anticipated this demonstration will be conducted at Indian Head during the final quarter of 1996 using Navy personnel and internal funding.

Treatment of an Industrial Effluent Containing Chlorophenols

During 1993, the Environmental Protection Agency developed a program entitled the Environmental Technologies Initiative, which contained a section for demonstration of U.S. technologies in the former Soviet bloc countries. With Mr. Richard Brenner of the EPA's Risk Reduction Engineering Laboratory in Cincinnati as the lead and partners EFX Systems, Inc., USACERL and the University of Cincinnati, a proposal for demonstration of the GAC-FBR was developed, using funds from this SERDP project for acquisition of the GAC-FBR. The proposal was accepted by the ETI program, and site selection, followed by installation/operation, commenced.

Eight industrial sites were visited in Poland, Hungary and the Czech Republic, from which three were selected for further consideration. Each of the three were asked to submit a full proposal for operation of the demonstration. Based on the proposals received, Nitrokémia, a chemical plant located near Lake Balaton and about 90 miles southwest of Budapest, Hungary was selected. The plant manufacturers pesticides and herbicides, and has chlorinated phenols as a waste by-product.

EFX participated in site selection along with EPA, USACERL and the University of Cincinnati. EFX was then responsible for the acquisition of the GAC-FBR, and installation/start-up at the site in Hungary. The system was installed in April 1996 and will be operated in the host country until February 1977. EFX also provided consultation to the operators in Hungary after the system was in operation. The results of the demonstration are outside the scope of this project. Further information about this demonstration should be available from the University of Cincinnati, USACERL and EPA in mid-1977.

SUMMARY

The GAC-FBR was tested under aerobic, denitrifying (anoxic) and anaerobic conditions. In all cases, effective removal of the contaminants of concern in process waters and groundwater was achieved. The GAC-FBR appears to have a somewhat higher initial capital cost than physical-chemical removal processes such as adsorption onto GAC. The O&M costs are, however, considerably lower for the GAC-FBR. In many cases, payback of the capital cost will be realized within several years due to this difference in operating costs.

Both nitroaromatics tested, DNT and TNB, were readily treated in GAC-FBR systems. The GAC-FBR appears to be well-suited both technically and economically for treatment of munitions wastewaters and groundwater containing nitroaromatics.

For the treatment compounds such as ketones which are not readily treated by air stripping or adsorption, the GAC-FBR would appear to be an extremely economically attractive alternative.

For treatment of chlorinated solvents, such as TCE, the GAC-FBR appears to hold promise. More work will be required to bring the GAC-FBR to commercialization for this application.

SECTION 1 - INTRODUCTION AND OVERVIEW OF WORK

The GAC-FBR process is a high-rate platform for performing biologically catalyzed reactions. The process can be operated as an aerobic, anoxic (denitrifying) or anaerobic system. The use of an adsorptive biomass carrier in the form of granular activated carbon (GAC) affords the process robustness during adverse environmental conditions beyond that possible with other biological fixed film systems. This makes the process an ideal candidate for ensuring compliance for wastewaters produced during munitions manufacturing and remediation operations.

In this report are the results of a three-year program designed to test and demonstrate the GAC-FBR process for a number of problem wastewaters facing the U.S. Armed Forces.

The work can be divided into two separate segments:

- Laboratory-piloting testing of candidate wastewaters
- Field demonstrations using a small commercial-scale GAC-FBR

An overview of these different segments is provided below.

Laboratory-Pilot Work

Four separate studies were conducted as a part of this portion of the overall work effort. The selection of the respective wastewater treatment projects was based on needs identified at a kickoff meeting held with representatives of the U.S. Army, Navy and Air Force. The intent of these projects was to determine the potential of the GAC-FBR to meet the treatment needs for different waste streams of concern for DoD overall. A second objective was to develop a sufficient information base upon which field demonstrations could be subsequently conducted. The four laboratory-pilot projects included:

- Treatment of propylene glycol dinitrate (PGDN) in Biazzi nitration effluents
- Removal of trinitrobenzene (TNB) from the effluent of an advanced oxidation process (AOP) treating TNT in water
- Treatment of ketones in groundwaters, with emphasis on biological viability at low ketone concentrations
- Cometabolic transformation of trichloroethylene (TCE) at sites where both TCE and hydrocarbon contamination are found, such as at many Air Force bases

The reports prepared for each of these projects are presented as Sections 2-5, respectively, of this final report.

Field Demonstrations

A total of three field demonstrations, using custom-designed, small commercial-scale GAC-FBRs were conducted as part of this work. These included application of the GAC-FBR under anaerobic, anoxic and aerobic conditions. The demonstrations conducted included:

- Demonstration of anaerobic reduction of DNT in high to moderate strength munitions wastewater at the Radford Amy Ammunition Plant, Radford, VA
- Aerobic treatment of ketones in groundwater
- Treatment of PGDN in Biazzi nitration effluent under denitrifying (anoxic) conditions at the Indian Head Naval Surface Warfare Center, Indian Head, MD

In addition, the design, procurement and start-up and technical assistance for anaerobic treatment of a wastewater containing chlorinated phenols at a chemical plant in Hungary was also completed. Results of the three demonstrations are presented as Sections 6 through 8, respectively, of this final report.

The work at RAAP was a continuation of CERL support laboratory testing conducted by the University of Cincinnati under the direction of Dr. Makram Suidan. The objective was to demonstrate that DNT, in a point source within the facility (water dry wastewater from munitions production), could be transformed (reduced) to DAT, which would be readily mineralized at the plant treatment facility thereby ensuring compliance with regulatory effluent discharge requirements.

The project for aerobic treatment of ketones (acetone, MEK and MIBK) in groundwater was originally scheduled to be conducted at the U.S. Army Waterways Experiment Station, Vicksburg, MS. Due to logistical problems, the effort was eventually conducted in the high bay pilot plant at MBI International using groundwater at flows up to 18 gpm spiked with a mixture of ketones. This work was performed using a commercial-scale GAC-FBR loaned by Ms. Alison Thomas from Tyndall AFB.

Following completion of the demonstration at RAAP, the GAC-FBR system used there was decommissioned and shipped to the Indian Head Naval Surface Warfare Center, MD. The system was installed during early fall of 1995. The system was biologically started to obtain a denitrifying population with using nitrate and propylene glycol in anticipation of wastewater production. Due to design flaws in the refurbished Biazzi nitration system at Indian Head, production was stalled. By the conclusion of this project, no production of PGDN containing wastewater had occurred.

As a part of the overall work effort an anaerobic GAC-FBR was designed for operation in Central Europe, procured, shipped and started up at a chemical plant in Hungary. This was in support of an EPA funded Environmental Technology Initiative (EIT) involving CERL and the University of Cincinnati. During 1993, the Environmental Protection Agency

developed a program entitled the Environmental Technologies Initiative, which contained a section for demonstration of U.S. technologies in the former Soviet bloc countries. With Mr. Richard Brenner of the EPA's Risk Reduction Engineering Laboratory in Cincinnati as the lead and partners EFX Systems, Inc., USACERL and the University of Cincinnati, a proposal for demonstration of the GAC-FBR was developed, using funds from this SERDP project for acquisition of the GAC-FBR. The proposal was accepted by the ETI program, and site selection, followed by installation/operation, commenced.

Eight industrial sites were visited in Poland, Hungary and the Czech Republic, from which three were selected for further consideration. Each of the three were asked to submit a full proposal for operation of the demonstration. Based on the proposals received, Nitrokémia, a chemical plant located near Lake Balaton and about 90 miles southwest of Budapest, Hungary was selected. The plant manufacturers pesticides and herbicides, and has chlorinated phenols as a waste by-product.

EFX participated in site selection along with EPA, USACERL and the University of Cincinnati. EFX was then responsible for the acquisition of the GAC-FBR, and installation/start-up at the site in Hungary. The system was installed in April 1996 and will be operated in the host country until February 1977. EFX also provided consultation to the operators in Hungary after the system was in operation. The results of the demonstration are outside the scope of this project. Further information about this demonstration should be available from the University of Cincinnati, USACERL and EPA in mid-1977.

Final reports have been prepared on each of these four laboratory-pilot efforts and the three field demonstrations. These reports are presented herein as a compendium of the overall work effort. Sections 2 through 5 are the final reports for the laboratory-pilot work and Sections 6 through 8 are the final reports for the field demonstrations.

SECTION 1 - INTRODUCTION AND OVERVIEW OF WORK

The GAC-FBR process is a high-rate platform for performing biologically catalyzed reactions. The process can be operated as an aerobic, anoxic (denitrifying) or anaerobic system. The use of an adsorptive biomass carrier in the form of granular activated carbon (GAC) affords the process robustness during adverse environmental conditions beyond that possible with other biological fixed film systems. This makes the process an ideal candidate for ensuring compliance for wastewaters produced during munitions manufacturing and remediation operations.

In this report are the results of a three-year program designed to test and demonstrate the GAC-FBR process for a number of problem wastewaters facing the U.S. Armed Forces.

The work can be divided into two separate segments:

- Laboratory-piloting testing of candidate wastewaters
- Field demonstrations using a small commercial-scale GAC-FBR

An overview of these different segments is provided below.

Laboratory-Pilot Work

Four separate studies were conducted as a part of this portion of the overall work effort. The selection of the respective wastewater treatment projects was based on needs identified at a kickoff meeting held with representatives of the U.S. Army, Navy and Air Force. The intent of these projects was to determine the potential of the GAC-FBR to meet the treatment needs for different waste streams of concern for DoD overall. A second objective was to develop a sufficient information base upon which field demonstrations could be subsequently conducted. The four laboratory-pilot projects included:

- Treatment of propylene glycol dinitrate (PGDN) in Biazzi nitration effluents
- Removal of trinitrobenzene (TNB) from the effluent of an advanced oxidation process (AOP) treating TNT in water
- Treatment of ketones in groundwaters, with emphasis on biological viability at low ketone concentrations
- Cometabolic transformation of trichloroethylene (TCE) at sites where both TCE and hydrocarbon contamination are found, such as at many Air Force bases

The reports prepared for each of these projects are presented as Sections 2-5, respectively, of this final report.

Field Demonstrations

A total of three field demonstrations, using custom-designed, small commercial-scale GAC-FBRs were conducted as part of this work. These included application of the GAC-FBR under anaerobic, anoxic and aerobic conditions. The demonstrations conducted included:

- Demonstration of anaerobic reduction of DNT in high to moderate strength munitions wastewater at the Radford Amy Ammunition Plant, Radford, VA
- Aerobic treatment of ketones in groundwater
- Treatment of PGDN in Biazzi nitration effluent under denitrifying (anoxic) conditions at the Indian Head Naval Surface Warfare Center, Indian Head, MD

In addition, the design, procurement and start-up and technical assistance for anaerobic treatment of a wastewater containing chlorinated phenols at a chemical plant in Hungary was also completed. Results of the three demonstrations are presented as Sections 6 through 8, respectively, of this final report.

The work at RAAP was a continuation of CERL support laboratory testing conducted by the University of Cincinnati under the direction of Dr. Makram Suidan. The objective was to demonstrate that DNT, in a point source within the facility (water dry wastewater from munitions production), could be transformed (reduced) to DAT, which would be readily mineralized at the plant treatment facility thereby ensuring compliance with regulatory effluent discharge requirements.

The project for aerobic treatment of ketones (acetone, MEK and MIBK) in groundwater was originally scheduled to be conducted at the U.S. Army Waterways Experiment Station, Vicksburg, MS. Due to logistical problems, the effort was eventually conducted in the high bay pilot plant at MBI International using groundwater at flows up to 18 gpm spiked with a mixture of ketones. This work was performed using a commercial-scale GAC-FBR loaned by Ms. Alison Thomas from Tyndall AFB.

Following completion of the demonstration at RAAP, the GAC-FBR system used there was decommissioned and shipped to the Indian Head Naval Surface Warfare Center, MD. The system was installed during early fall of 1995. The system was biologically started to obtain a denitrifying population with using nitrate and propylene glycol in anticipation of wastewater production. Due to design flaws in the refurbished Biazzi nitration system at Indian Head, production was stalled. By the conclusion of this project, no production of PGDN containing wastewater had occurred.

As a part of the overall work effort an anaerobic GAC-FBR was designed for operation in Central Europe, procured, shipped and started up at a chemical plant in Hungary. This was in support of an EPA funded Environmental Technology Initiative (EIT) involving

CERL and the University of Cincinnati. The actual operation and results of this demonstration are outside the scope of this work.

Final reports have been prepared on each of these four laboratory-pilot efforts and the three field demonstrations. These reports are presented herein as a compendium of the overall work effort. Sections 2 through 5 are the final reports for the laboratory-pilot work and Sections 6 through 8 are the final reports for the field demonstrations.

SECTION 2 - PILOT SCALE EVALUATION OF THE GRANULAR ACTIVATED CARBON-FLUIDIZED BED REACTOR (GAC-FBR) FOR THE TREATMENT OF PROPYLENE GLYCOL DINITRATE (PGDN) IN MUNITIONS WASTEWATER

Pilot Scale Evaluation of the Granular Activated Carbon-Fluidized Bed Reactor (GAC-FBR) for the Treatment of Propylene Glycol Dinitrate (PGDN) in Munitions Wastewater

Final Report

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EXECUTIVE SUMMARY

During munitions manufacturing, wastewater streams containing nitrated compounds of regulatory concern are generated. At the Indian Head Division, Naval Surface Warfare Center, wastewater containing propylene glycol dinitrate (PGDN) and high nitrate concentrations is generated from nitrating propylene glycol (PG) to produce PGDN. The purpose of this project was to evaluate the effectiveness of biological treatment to reduce the concentration of PGDN to below 1 mg/L in the Biazzi plant process effluent water.

The biological fluidized bed reactor (FBR) using granular activated carbon (GAC) as the biomass carrier was chosen to demonstrate PGDN degradation under denitrifying conditions. The fluidized bed bioreactor is a high rate, biological fixed-film treatment process in which the water to be treated is passed upwards through a bed of fluidized fine-grained media on which microorganisms grow. Biofilms attached to the media remove the organic pollutants from the water.

Tests were carried out in a pilot scale reactor with both real and synthetic nitration process wastewater. The effect of applied organic loading rate, PGDN loading rate and the ratio of primary substrate COD to PGDN were examined. Both ethanol (EtOH) and PG were tested as primary growth substrates. In addition, performance was evaluated after a period of feeding primary substrate only (no PGDN) and after a period of starvation (suspension of primary substrate feed).

Several conclusions can be drawn from the pilot scale tests presented in this report.

- The biological GAC-FBR removed PGDN under denitrifying conditions in munitions wastewater with either EtOH or PG as the primary substrate.

- Effluent PGDN concentrations below the 1 mg/L regulatory limit could be achieved with the system at PGDN loading rates of up to 0.22 Kg COD/m^3-d and overall OLR of 5 Kg COD/m^3-d. PGDN removal rates of greater than 98% were achieved at PGDN loading rates up to 0.89 Kg COD/m^3-d at primary COD/PGDN ratios of 4:1.
- Increasing the PGDN loading rate at a fixed total COD loading led to an increase in effluent PGDN concentration. Decreasing the primary COD/PGDN ratio at a fixed PGDN loading rate resulted in an increase in effluent PGDN concentration.
- The GAC-FBR proved robust to suspensions in feeding of both primary substrate and PGDN. The microbial population in the system was able to degrade PGDN after a three-month interval of exposure to only the primary substrate. The reactor biomass remained viable after a three-week period of starvation (no substrate feed).
- No pH control was necessary for stable reactor performance. The reactor pH self-regulated between 8.0 and 8.6.

1. INTRODUCTION AND BACKGROUND

1.1 Background

During munitions manufacturing, wastewater streams containing nitrated compounds of regulatory concern are generated. At the Indian Head Division, Naval Surface Warfare Center, wastewater containing propylene glycol dinitrate (PGDN) and high nitrate concentrations is generated from production of PGDN in a Biazzi nitration process. The purpose of this project was to evaluate the effectiveness of biological treatment to reduce the concentration of PGDN to below 1 mg/L in the Biazzi plant process effluent water.

The wastewater is produced during final stages of purification after the nitration of propylene glycol with a mixed acid containing concentrated nitric and sulfuric acids. The PGDN is first separated from the spent acid, then purified by a series of carbonate and water washes. The spent carbonate solution and wastewater from these washes are combined into one wastewater stream that is passed through a series of settling tanks to remove any suspended PGDN. The resulting waste stream contains 220 ppm of PGDN on average, 1200 ppm maximum. The concentration of salts may be as high as 6% by weight. The approximate composition of these salts on a dry weight basis is: 70-75% $NaNO_3$, 2-3% Na_2SO_4, 20% Na_2CO_3, 2-3% $NaHCO_3$. The maximum flow rate of the stream is 20 gpm with 7000 gallons a day average total flow.

This wastewater stream has been treated at Indian Head by GAC adsorption of the PGDN. The high nitrate concentration does, however, make this stream a candidate for a biological process, anoxic denitrification. Anoxic denitrification is a process where facultative bacteria in the absence of dissolved oxygen and in the presence of nitrate use nitrate as the electron acceptor in place of oxygen. The

nitrate is converted (reduced) to molecular nitrogen while organic carbon is oxidized to carbon dioxide. In this process, the PGDN is co-metabolically degraded.

For this project, the biological fluidized bed reactor was chosen to demonstrate PGDN degradation under denitrifying conditions. The biological FBR process has proved robust under aerobic, anaerobic and anoxic (denitrifying) conditions for industrial wastewater and contaminated groundwater. A denitrifying FBR has been installed to treat wastewater from a commercial nitration operation for adipic acid production (Radian 1993).

1.2 Process Description

The fluidized bed bioreactor is a high rate, biological fixed-film treatment process in which the water to be treated is passed upwards through a bed of fluidized, fine-grained media, such as sand, granular activated carbon or ion exchange resins, on which contiguous films of microorganisms grow. As the water to be treated is passed upward through the bed of media, biofilms attached to the media remove the organic pollutants from the water. Water is passed through the bed at a velocity sufficient to impart motion or fluidize the media. This occurs when the drag forces caused by the liquid moving past the individual media particles are equal to the net downward force exerted by gravity (buoyant weight of the media).

Fluidization of fine grained media allows the entire surface of each individual particle to be colonized by bacteria in the form of a biofilm. Surface areas on the order of 300 m^2/m^3 of bed are common in fluidized bed reactor systems. This results in accumulation of biomass concentrations of up to 50,000 mg VSS/L of fluidized bed, which is an order of magnitude greater than the cell mass concentrations obtained in most suspended growth biological processes. Fluidization is key to the ability of this process to concentrate active bacterial mass

to high levels on small diameter media (<2 mm) without the clogging encountered with downflow or upflow filter systems. This superior ability to concentrate active bacterial mass in the reactor has considerable theoretical and kinetic advantages to the performance of the reactor. By manipulating the volume of media added to a system and the fluidization velocity, a great deal of control can be exerted on the average biofilm thickness and mean cell retention time to optimize overall process performance. The advantages of biological fluidized bed reactor systems over conventional biological processes include:

- Large surface area for biomass attachment;
- High biomass concentrations;
- Ability to control and optimize biofilm thickness;
- Minimal plugging, channeling or gas hold-up; and
- High mass transfer properties, maximum contact between biomass and substrate.

In the mid-1980s, engineers recognized that this technology might have the potential of substantially reducing the cost of treating groundwater contaminated with industrial wastes. Currently, at thousands of contaminated sites in the U.S., interdiction wells are used to contain VOC pollutants in the subsurface. Water that is pumped from these wells is usually treated with conventional air stripping processes and the effluent air is passed through a granular activated carbon (GAC) module to control VOC emissions. This conventional system of treating interdicted water with 10 ppm or less of VOCs can cost $1-3/1000 gallons, due mainly to the expense for GAC replacement/regeneration.

This indicated that the opportunity for cost reduction lies in the use of biological treatment to destroy most of the pollutant mass instead of loading it on GAC. Yet it was also recognized that a bioprocess that was designed to replace

this type of conventional treatment would have to achieve stringent removal capabilities. These included: 1) the ability to remove xenobiotic pollutants (chemicals foreign to biological organisms) at high efficiencies, 2) mobility, 3) the ability to handle a wide range of concentrations and loadings, and 4) resistance to process upsets due to sudden changes in influent concentration and composition. This pointed to the need for implementing the concept of integrating GAC into the biological fluidized bed reactor as the biomass carrier.

1.3 The GAC-FBR Process

The granular activated carbon fluidized bed reactor (GAC-FBR) process is a fluidized bed which employs GAC as the solid support for biofilm growth. The use of an adsorbent carrier offers three advantages. First, essentially complete contaminant removal occurs as soon as the system is commissioned due to adsorption. After rapid development of a mature biofilm, removal is due to biological degradation. Second, the effects of shock loads of pollutants and other perturbations may be buffered by the adsorptive capacity of the GAC resulting in a more stable, robust overall performance. Third, the process provides general removal of a broad range of pollutants whether they are biodegradable or not.

Over the past six years, personnel of EFX Systems, Inc. a spin-off company formed by MBI International of Lansing, MI and Ecolotrol, Inc. of Westbury, NY, have pursued the application of the GAC-FBR for the cleanup of groundwater contaminated with gasoline, complex wastewaters and a number of industrial process effluents. Laboratory and field-pilot data indicated that the GAC-FBR has the capability of removing >99% of the total VOCs from groundwater, with high removals of semi-volatile compounds as well. Full-scale systems are now operation at field sites with groundwater flow rates as high as 4,000 gpm (5.8 million gallons/day).

2. MATERIALS AND METHODS

2.1 Laboratory-Pilot Scale GAC-FBR System

A single fluidized bed reactor (FBR) was set up and operated under denitrifying conditions for all the tests discussed in this report. The two-inch diameter, 7-liter working bed volume, all glass reactor was equipped with pH and biomass control systems, which were operated as needed during the tests (Figures 2-1 and 2-2). Peristaltic tubing pumps were used to fluidize the Granular Activated Carbon (GAC) biomass support carrier in the reactor and to deliver salt solution, nutrients, buffer, and wastewater. Syringe pumps were used to meter propylene glycol (PG), ethanol (EtOH), and mixtures of EtOH and propylene glycol dinitrate (PGDN) to the system. All tests were conducted at room temperature (18° to 22°C).

An initial charge of 2.7 liters of GAC was added as the biomass support. This resulted in an initial settled bed height of 147 cm. The GAC used was Calgon Type MRX-P, 10x30 mesh, the same material used in commercial-scale GAC-FBR systems supplied by EFX Systems/Envirex. After carbon addition, the system was flushed for 4 days to remove carbon fines and to rinse the GAC. No carbon was added or removed from the reactor during the operational periods. The fluidization pump was set for 1 liter/min to yield a flux rate of 12 gpm/ft^2 and an initial fluidized bed height of 199 cm.

For most of the operational periods, the reactor was fed a 1:1 mixture of two salt solutions, prepared to mimic Biazzi process wastewater from PGDN production. One solution contained sodium nitrate, sodium sulfate and sodium bicarbonate, the primary salts in the Biazzi wastewater. The second solution contained phosphate and ammonia salts and trace minerals (Appendix C). The total dissolved solids

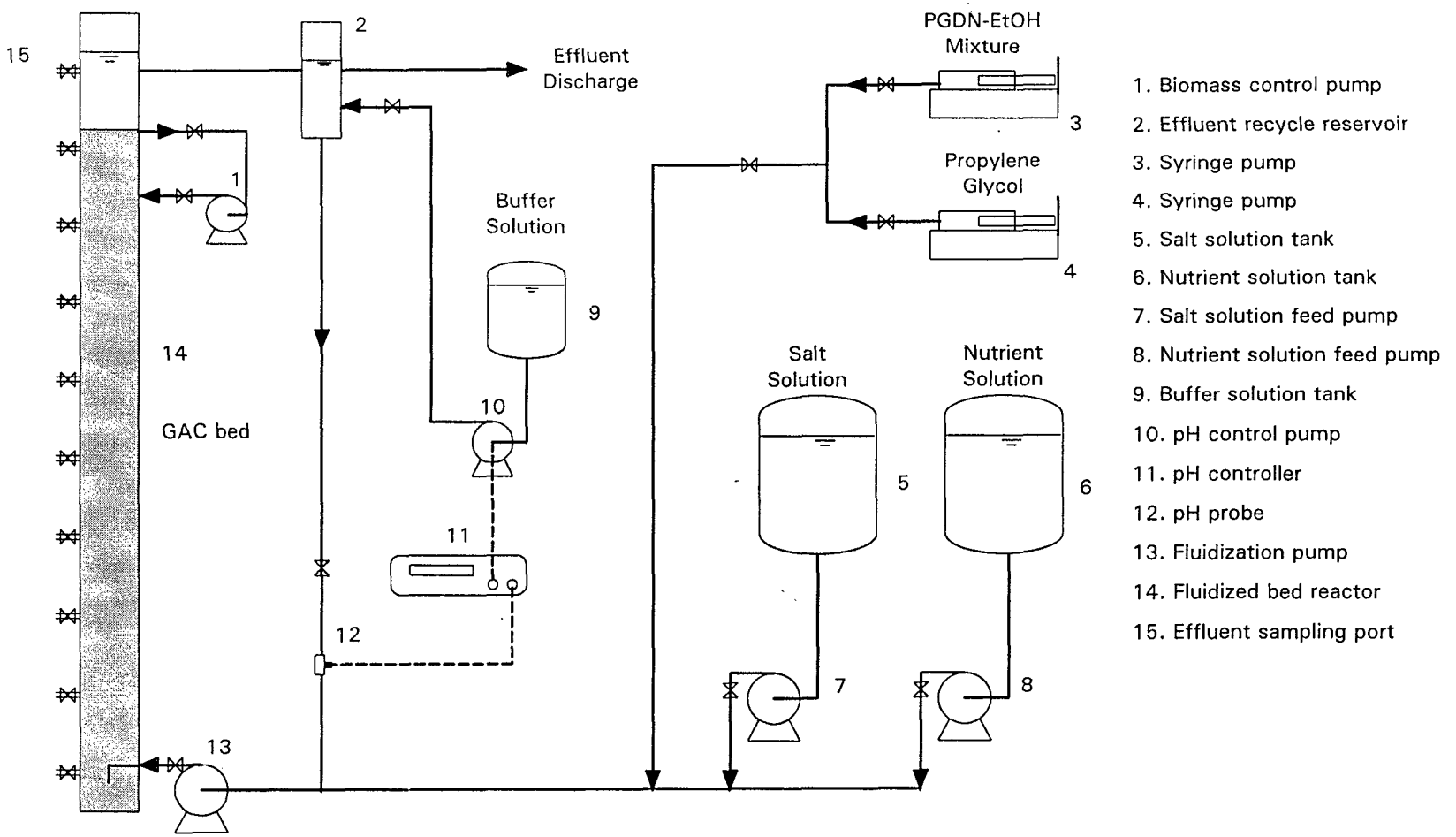


Figure 2-1. Schematic Diagram of a GAC-FBR System for Treating Synthetic PGDN Wastewater.

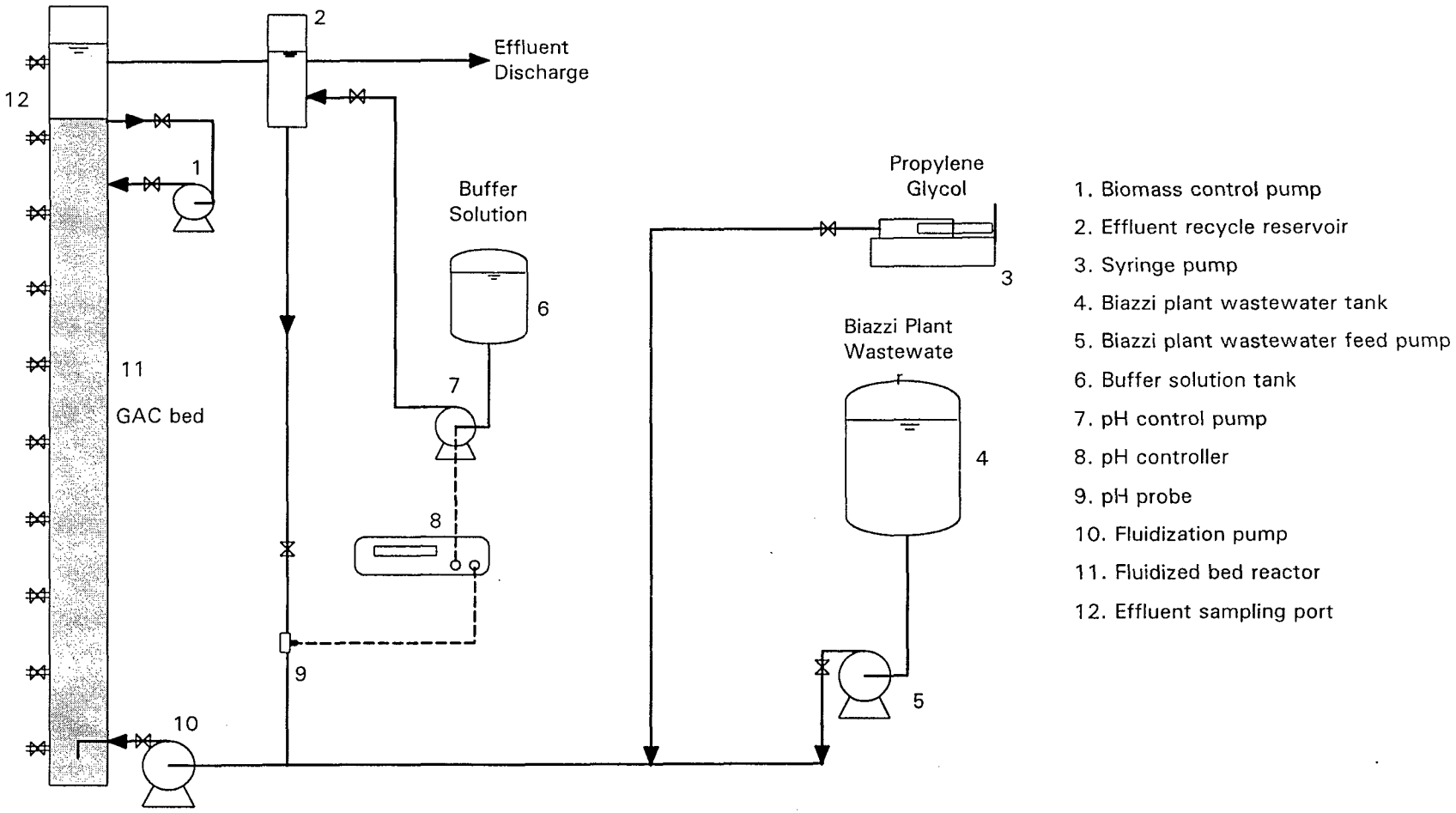


Figure 2-2. Schematic Diagram of a GAC-FBR System for Treating Biazzi Plant Wastewater.

(TDS) concentration in the combined feed to the reactor was about 4.6%. Biazzi wastewater from the Indian Head Division, Naval Surface Warfare Center (Indian Head, MD) was treated during two operational periods. This water was collected during nitroglycerin (NG) production and then passed through GAC to remove residual NG. The water, which contained about 2.1% TDS, was spiked with PGDN to achieve a concentration of about 240 to 250 mg/L.

The feed rate of the synthetic wastewater was set at either 12 or 15 ml/min for all operational periods. This resulted in hydraulic residence times (HRTs) of between 8 and 10 hours, based on the nominal empty bed volume of 7 liters. During the periods when Biazzi wastewater was treated, the system HRT was varied between 5 and 17 hours. The Biazzi wastewater was spiked with phosphate, ammonium, and trace minerals immediately before it was fed to the reactor. No attempt was made to exclude or remove dissolved oxygen from the wastewater feeds.

PGDN and growth substrates (PG and EtOH) were metered into the feed stream using syringe pumps. A PGDN solution (5% in EtOH) was provided by Indian Head Division, Naval Surface Warfare Center (Indian Head, MD). The ratio of EtOH to PGDN was adjusted by rotary evaporation of the EtOH to give final PGDN concentrations of 10, 20, and 40%. To achieve the desired primary growth substrate loading rates, syringes were filled with EtOH, PG, 50% PG and water solution, or 50% PG and EtOH solutions.

The pH control system was used intermittently during operations. The denitrification process produces alkalinity. With the high nitrate concentrations used, the pH self-regulated at about 8.6. During certain periods, the pH control system was used to maintain pH at 7.75, 8.0 or 8.4.

The growth control system was also used intermittently during operations. Nitrogen gas produced in the reactor tended to shear off enough biomass to control the bed height at about 310 cm or 7.4 liters of bed. When in operation, the peristaltic growth control pump was used to withdraw biomass coated carbon from near the top of the bed (293 cm), to shear the excess biomass and then return the GAC to the reactor. The GAC returns to the fluidized bed while the sheared excess biomass exits the system with the effluent flow.

2.2 Monitoring, Sampling, and Analysis

The reactor was monitored daily during most operational periods. Feed flow rates were measured via bucket and stopwatch, and pH and bed height were recorded. During campaign sampling periods, effluent samples were withdrawn once or twice per day, filtered immediately and extracted for analysis of PGDN (see Appendix D). Feed PGDN concentrations were calculated for all the synthetic wastewater experimental periods based on the feed rates of the synthetic wastewater salt solution and PGDN solution. The PGDN concentration of the Biazzi wastewater was measured using the protocols used for effluent samples. Applied loading rates of PG, EtOH, and NO_3-N were all calculated from the known solution concentrations and flow rates. During campaign sampling periods, occasional feed and effluent samples were collected and analyzed for NO_3-N, NH_4-N, and COD.

In this report, volumetric applied organic loading rates and volumetric removal rates are based on the nominal bed volume available in the reactor (7 liters). The concentrations and applied OLR of the two primary substrates are presented as COD. Conversion to COD was based on a COD/PG ratio of 1.68 g/g; COD/EtOH of 2.10 g/g. PGDN concentrations are given in mg of compound per liter while volumetric loading rates and volumetric removal rates are presented on a COD basis. The COD/PGDN ratio is 0.77 g COD/g PGDN.

The analytical methods used throughout this effort are listed in Table 2-1. Sampling, analysis, and quality assurance/quality control protocols are detailed in Appendices D and E.

Table 2-1. Sample Analysis Methods.

Component	Method*
PGDN and PGMN	Solid phase extraction, HPLC with UV detection
PG	Ion exchange to eliminate Na interference, direct aqueous injection GC analysis with FID detection
NO_3-N	Isocratic ion chromatography with conductivity detection
*Detailed methods are presented in Appendix D	

3. RESULTS AND DISCUSSION

3.1 Preliminary Batch Tests

In order to determine under what conditions PGDN would be most readily degraded, some preliminary batch tests (Appendix A) were conducted under both aerobic and anoxic (denitrifying conditions). Results indicated that PGDN did not degrade well under aerobic conditions, especially at the concentrations expected in the Biazzi Plant wastewater (200-250 mg/L). PGDN did degrade under denitrifying conditions. Accordingly, all further testing was conducted under denitrifying conditions.

3.2 Start-up and Acclimation (Start-up Periods No. 1 and 2)

After carbon fines had been purged from the system, the reactor was filled with synthetic wastewater and the forward feed rate was established at about 12 mL/min. The GAC-FBR system was inoculated using waste activated sludge obtained from an industrial treatment system acclimated to a high salt, high nitrate wastewater. Three aliquots of sludge (100 mL, 60 mL, and 60 mL) were applied at two day intervals. The fluidized bed was operated in complete recycle for 30 to 90 minutes after each inoculation to encourage microorganism attachment.

Ethanol was selected as the sole substrate to grow biofilm on the GAC. The reactor was operated for 42 days at an organic loading rate (OLR) of 4 Kg COD/m^3-d with ethanol as the primary substrate (Table 3-1). A low COD:NO_3-N ratio (0.2:1) was used to ensure excess electron acceptor was available during this period. For systems designed to completely remove nitrate, a COD:NO_3-N ratio of 4:1 is typically used. The objective here was to co-metabolically remove the PGDN and

not necessarily remove all the nitrate. Gas production in the fluidized bed was visible 10 days following the initial inoculation.

Table 3-1. Periods and Conditions for Laboratory Pilot-Scale GAC-FBR Treatment Trials.

Designation in Figure 3-1	Period Type	Primary Substrate(s)	Contaminant	Applied OLR ($KgCOD/m^3$-d)	Days
ST #1	Start-up	100% EtOH	None	4.0	1 to 42
ST #2	Start-up	100% EtOH	PGDN	10.0	43 to 66
EP # 1	Evaluation	100% EtOH	PGDN	10.0	67 to 93
EP # 2	Evaluation	100% EtOH	PGDN	4.8	101 to 112
EP # 3	Evaluation	100% EtOH	PGDN	4.5	115 to 127
EP # 4	Evaluation	100% EtOH	PGDN	4.4	135 to 144
MTP	Maintenance	100% EtOH	PGDN	10.0	145 to 163
MTP	Maintenance	65% EtOH & 35% PG	PGDN	5.2	164 to 174
MTP	Testing	55% EtOH & 45% PG	PGDN	10.1	183 to 186
MTP	Testing	100% EtOH	PGDN	9.9	188 to 191
MTP	Transition	100% EtOH	None	10.2	192 to 211
MTP	Transition	75% EtOH & 25% PG	None	10.3	212 to 231
MTP	Transition	75-25% EtOH & 25-75% PG	None	10.3	232 to 248
MTP	Transition	100% PG	None	10.8	249 to 280
EP # 5	Evaluation	23% EtOH & 87% PG	PGDN	13.2	281 to 285
EP # 6	Evaluation	100% PG in Biazzi Wastewater	PGDN	11.7	286 to 294
EP # 7	Evaluation	100% PG in Biazzi Wastewater	PGDN	11.1	295 to 317
FSP	Feed	100% PG	None	10.8	318 to 337
FSP	Starvation	None	None	None	338 to 345
FSP	Feed	100% PG	None	10.8	346 to 352
FSP	Starvation	None	None	None	353 to 372
FSP	Feed	100% PG	None	10.8	373 to 380

ST - start-up period
EP - experimental period
MTP - maintenance transition period
FSP - feed/starvation experimental period

After 40 days at these conditions a mature biofilm became established causing the bed to expand to about 233 cm. Increases in biofilm thickness result in a decrease in the specific gravity of the GAC particles. This results in greater bed expansion at the same fluidization flow rate. Bed height, therefore, is a good gauge of the accumulation of biomass in the GAC-FBR. In general, the biofilms grown with EtOH feed were thin and tended to shear easily from the GAC carrier particles.

On Day 43, 100% EtOH was replaced with a 5% PGDN in EtOH solution (wt/wt) as the feed. The applied OLRs for Start-up Period No. 2 were 9.8 Kg COD/m^3-d of EtOH and 0.19 Kg COD/m^3-d of PGDN (Table 3-2). The addition of PGDN caused no visible change in the biofilm or in nitrogen gas production rate. This acclimation period with PGDN was continued for 24 days. The increase in the applied OLR from ST1 resulted in an increase in the thickness of biofilm on the GAC. Bed height at the end of the period was about 248 cm, a 13 cm increase.

Table 3-2. Conditions During Start-up for a Laboratory Pilot-Scale GAC-FBR.

Period Designation	SP1	SP2
Period Type	Start-up	Start-up
Primary Substrate	100% EtOH	100% EtOH
Contaminant	None	PGDN
Organic Loading Rate (KgCOD/m^3-d)	4.0	10.0
Effluent pH	ca. 8	7.7 to 8.5
Hydraulic Residence Time (hours)	9.7	9.7
Bed Volume (liters)	4.3	5.0
Day No.	1 to 42	43 to 66

3.3 Overall Results for PGDN Removal under Anoxic Conditions

A total of seven tests were conducted to evaluate what factors could influence PGDN removal efficiency in the GAC-FBR (Tables 3-1 and 3-3). Two of these evaluations were performed using wastewater from the Biazzi Plant at Indian Head, while the remaining tests were conducted using a synthetic mixture designed to simulate the actual wastewater. Initial tests (EP1-EP4) were conducted using

ethanol as the primary substrate (electron donor). Because propylene glycol (PG) is readily available at the Indian Head site, subsequent tests were conducted using PG as the primary substrate. Preliminary batch tests showed that biomass from the GAC-FBR system that was consistently degrading PGDN could readily utilize PG as a substrate (Appendix B).

Table 3-3. Summary of Laboratory Pilot-Scale GAC-FBR Treatment of PGDN in Biazzi Plant Wastewater and Synthetic Wastewater.

Evaluation Period	EP1	EP2	EP3	EP4	EP5	EP6	EP7
Primary Substrate	100% EtOH	100% EtOH	100% EtOH	100% EtOH	23% EtOH, 87% PG	100% PG	100% PG
Wastewater Type	Synthetic	Synthetic	Synthetic	Synthetic	Synthetic	Biazzi	Biazzi
PGDN Concentration (mg/L)							
Feed	96	93	186	373	360	241	251
Effluent	0.5	0.9	1.8	4.3	7	3.8	1.8
PGDN Removal (%)	99.4	99.0	99.0	98.8	98.1	98.5	99.3
PGDN Removal Rates ($KgCOD/m^3$-d)	0.19	0.22	0.44	0.88	0.84	0.87	0.27
Primary COD/PGDN Ratio	52	21	9	4	14	12	40
Organic Loading Rate ($KgCOD/m^3$-d)	10.0	4.9	4.6	4.4	13.2	11.7	11.1
Effluent pH	7.7-8.1	8.0-8.2	8.0-8.3	8.0-8.3	7.5-7.6	7.5-8.0	7.9-8.7
Hydraulic Residence Time (hours)	9.7	7.8	7.8	7.8	7.8	5.1	17.4
Bed Volume (liters)	6.2	4.8	4.6	4.7	6.4	6.6	6.6

During several periods when no PGDN was available, the GAC-FBR was placed in maintenance operation. The system continued to receive primary substrate without PGDN addition during these periods. The effect of starvation (no primary substrate) on system viability was tested at the end of the project. The entire matrix of experimental tests and other operational periods is presented in Table 3-1 and Figure 3-1. These results are described in detail by experimental period below.

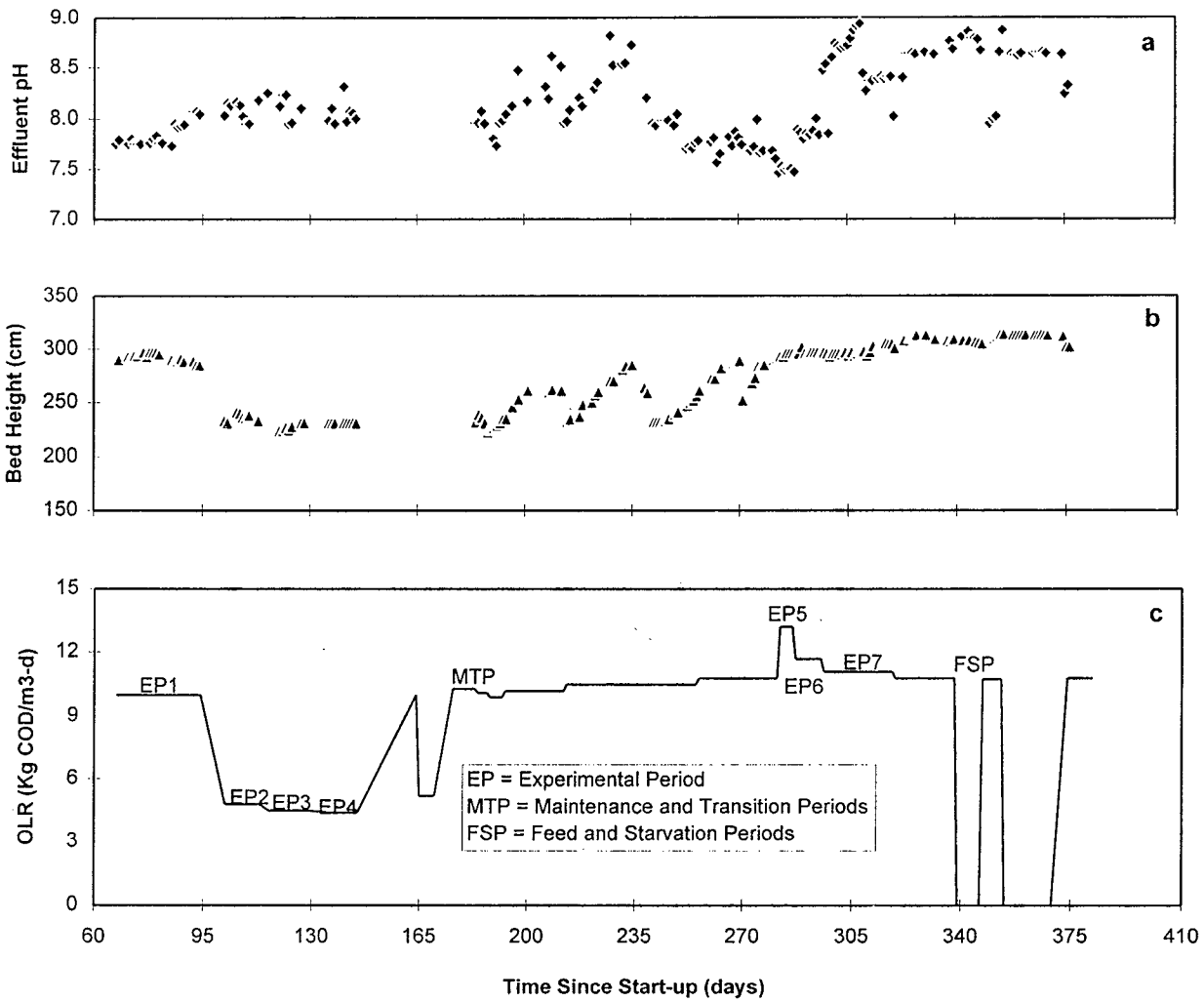


Figure 3-1. Summary of a) Effluent pH, b) Fluidized Bed Height, and c) Applied OLR During the Entire Experiment Program.

3.4 PGDN Degradation with EtOH as the Primary Substrate (Experimental Periods 1 through 4)

During Experimental Periods 1 through 4, PGDN degradation was tested with EtOH as the primary COD source. During these four experimental periods (EP), the effect of applied OLR, PGDN loading rate and the ratio of primary substrate COD to PGDN were examined. During EP1 through EP3, quasi steady-state removal efficiency and effluent concentration of PGDN were achieved. PGDN removal efficiency did not stabilize during EP4.

Start-up Period No. 2 (days 43 to 66) served as an acclimation period for PGDN removal. Applied OLR, and ethanol and PGDN concentrations were maintained constant for ST2 and EP1. Short acclimation periods (3 to 7 days) were allowed after each change in conditions. For EP1 - EP4, nitrate (electron acceptor) was provided in excess. The pH was controlled between 7.7 and 8.3. The bed height control pump was operated but bed heights were generally below the 293 cm growth control height.

Overall, high removal efficiency of PGDN was observed under denitrification conditions using ethanol as the primary electron donor (Table 3-3 and Figure 3-2). Typical removal efficiency for PGDN was ≥99%. The effluent quality target of 1 mg/L was achieved at the PGDN loading rates used during EP1 and EP2 (ca. 0.19-0.22 Kg COD/m^3-d).

During EP1, at an overall OLR of 10 Kg COD/m^3-d and influent PGDN concentration of 96 mg/L, an effluent PGDN concentration of 0.5 mg/L was achieved (99.4% removal). The ratio of primary COD (ethanol) to PGDN was 52/1 during this period.

For EP2, the concentration of PGDN was held constant while the overall COD applied OLR was reduced to 4.9 Kg COD/m^3-d. This resulted in an increase in the effluent PGDN concentration to 0.9 mg/L, still less than the 1 mg/L regulatory limit.

During EP3, the applied loading rate of PGDN was doubled, while the ethanol feed concentration was held constant. This reduction in the overall OLR and the ratio of primary substrate to PGDN (9:1) resulted in the effluent PGDN concentration increasing above the regulatory limit. The average effluent PGDN concentration during this period was 1.8 mg/L (99.0% removal efficiency).

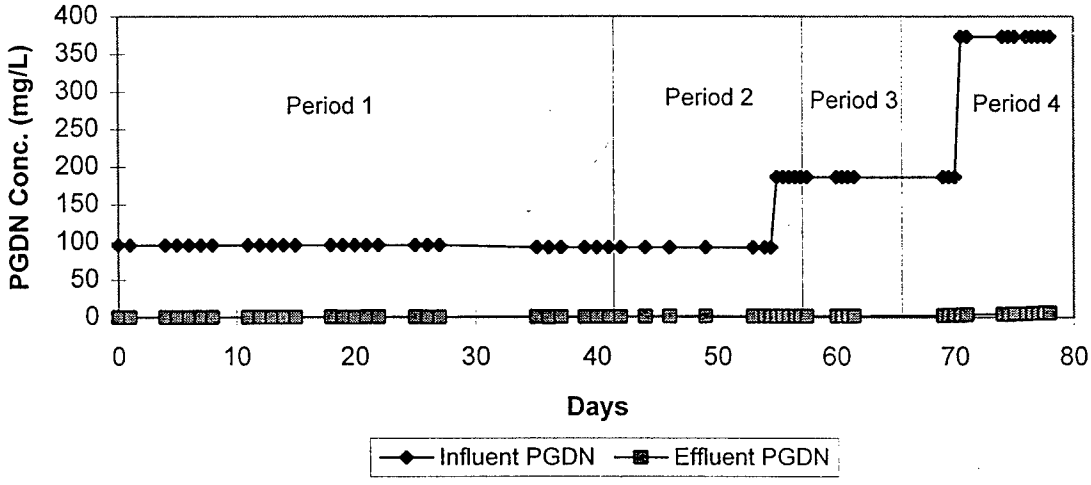


Figure 3-2. Influent and Effluent PGDN Concentration During Periods 1 to 4.

For EP4, the ratio of primary substrate to PGDN was decreased to 4:1. This was accomplished by increasing the PGDN concentration to 373 (essentially doubling the influent concentration) accompanied by a slight reduction in the ethanol feed rate. PGDN removal performance did not stabilize during EP4, rising from ca. 3 to 6 mg/L over the 10 days of data collection. It is likely that the steady-state PGDN effluent concentration at the 4:1 primary COD/PGDN ratio applied would be higher than the average of 4.3 mg/L reported. EP4 was concluded when the supply of available PGDN was exhausted.

Definitive separation of the effects of applied OLR, PGDN loading rate and primary substrate to PGDN ratio cannot be obtained from the experimental matrix in EP1 through EP4. However, certain conclusions can be drawn. Decreasing the COD/PGDN ratio at a nearly constant PGDN loading rate resulted in an increase in effluent PGDN concentration. In addition, increasing the applied PGDN loading rate at approximately the same primary COD loading rate led to increased PGDN effluent concentrations. PGDN loading rates of ca. 0.2 Kg COD/m^3-d and overall applied OLRs of 5 to 10 Kg COD/m^3-d produced an effluent PGDN concentration below the 1.0 mg/L regulatory limit.

As a consequence in the reduction of OLR between EP1 and EP2, a slow decrease in biomass concentrations and a concurrent decrease in fluidized bed height was observed (Figure 3-3). Nitrogen gas evolution also decreased (as anticipated) from an average of 11.7 L/day during EP1 to 6.7 L/day as a result in the change in OLR. The bed level eventually stabilized at 231 cm. The ratio of primary substrate COD to PGDN (21:1) was adequate, however, to reduce PGDN concentration below the 1 mg/L effluent criteria.

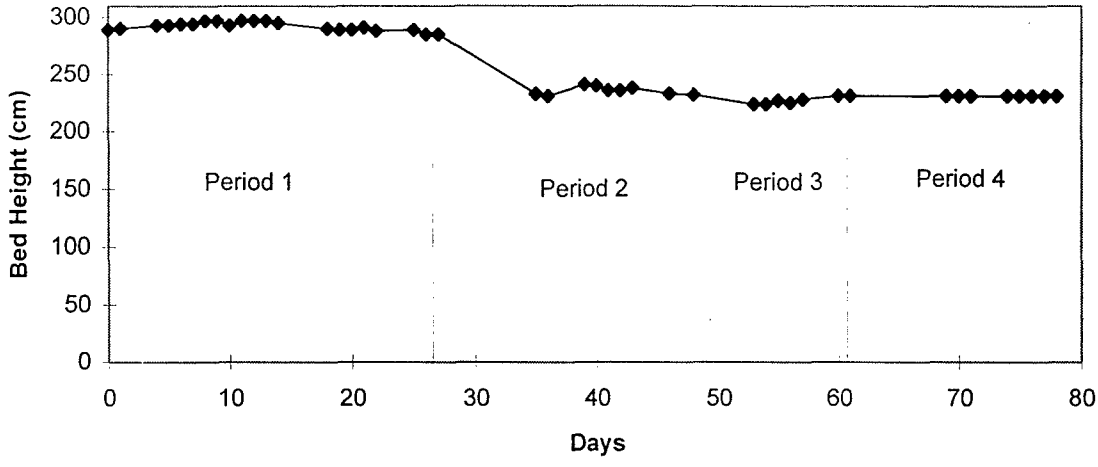


Figure 3-3. Bed Height During Periods 1 to 4.

3.5 Reactor Maintenance and Transition from Ethanol to Propylene Glycol as the Primary Substrate

Limited availability of PGDN stock solution, made it impossible to continue operation of the GAC-FBR at the high PGDN loading conditions of EP4. Therefore, the same EtOH and PGDN loading rates (4.7 and 0.2 Kg COD/m^3-d, respectively) used for Start-up (SP2) were established and maintained for the next 19 days (Tables 3-2 and 3-4). Bed height remained at about 231 cm during this period.

On Day 98, attempts were made to start acclimating the system biomass to propylene glycol (PG) as the primary growth substrate. PG is the feed stock used in PGDN manufacture and, therefore, readily available at the Indian Head, Naval Surface Warfare Center. The applied OLR was set 5 Kg COD/m^3-d with 65% of the COD from EtOH and 35% from PG (Table 3-4). The PGDN loading rate was ca. 0.1 Kg COD/m^3-d. Bed height remained at about 231 cm during the next three weeks.

Table 3-4. Conditions During Maintenance (A) and Transition Periods (B) for a Laboratory Pilot-Scale GAC-FBR.

Table 3-4(A)				
Period Designation	**MTP**	**MTP**	**MTP**	**MTP**
Period Type	**Maintenance**	**Maintenance**	**Testing**	**Testing**
Primary Substrate	**100% EtOH**	**65% EtOH, 35% PG**	**55% EtOH, 45% PG**	**100% EtOH**
Contaminant	**PGDN**	**PGDN**	**PGDN**	**PGDN**
Organic Loading Rate ($KgCOD/m^3$-d)	10.0	5.2 to 10.3	10.1	9.9
Effluent pH	8.0 to 8.5	8.0 to 8.5	8.0 to 8.1	7.5 to 7.8
Hydraulic Residence Time (hours)	7.8	7.8	7.8	7.8
Bed Volume (liters)	4.7	4.7	4.7	4.6
Days	145 to 163	164 to 182	183 to 186	188 to 191

Table 3-4(B)				
Period Designation	**MTP**	**MTP**	**MTP**	**MTP**
Period Type	**Transition**	**Transition**	**Transition**	**Transition**
Primary Substrate	**100% EtOH**	**75% EtOH, 25% PG**	**75-25% EtOH, 25-75% PG**	**100% PG**
Contaminant	**None**	**None**	**None**	**None**
Organic Loading Rate ($KgCOD/m^3$-d)	10.2	10.3	10.3	10.1
Effluent pH	8.0 to 8.6	8.0 to 8.8	7.7 to 8.7	7.6 to 7.9
Hydraulic Residence Time (hours)	7.8	7.8	7.8	7.8
Bed Volume (liters)	5.1	5.1	5.0	5.6
Days	192 to 211	212 to 231	232 to 248	249 to 280

During this period, biofilm coverage on GAC particles decreased visibly, although this was not reflected by a concurrent reduction in bed height. It appears that the microorganisms present did not acclimate readily to the added PG. The concentration of PG in the reactor, if not degraded by the microorganisms, would have accumulated to a high concentration. This may have resulted in some inhibition of the system biomass. Unfortunately, quantitation of the effluent PG

concentration was not performed during this period. A more gradual introduction of the PG may have ameliorated this problem.

During this several week period, some testing on PGDN removal efficiency was conducted (Table 3-4, Appendix G). With an inlet PGDN concentration of 162 mg/L, 97.3% removal was achieved (effluent concentration of 4.4 mg/L). This was considerably lower than the prior results using ethanol only as the primary COD source. Because of the loss of biomass from the system and resultant reduction in PGDN removal efficiency, the system feed was switched back to 100% ethanol. A slower acclimation or transition for replacing the ethanol with propylene glycol as the primary substrate was then initiated.

3.5.1 Gradual Transition Period

This transition phase of substituting PG for ethanol took place over a six-week period during which PG concentration was increased in four steps. From an initial condition of 100% EtOH, the carbon source feed was switched in steps to 75% EtOH/25% PG, 50% EtOH/50% PG, 25% EtOH/75% PG, and finally 100% PG. The applied OLR was maintained at about 10 Kg COD/m^3-d with an HRT of 7.8 hours throughout this period. The same salt and nutrient concentrations used in the initial PGDN treatment tests were used during this period.

During the transition period, biofilm development on the GAC particles generally improved and the fluidized bed height increased (Figure 3-1). In general, biofilm that grew was somewhat denser and provided more uniform coverage of the GAC surface than previously observed when feeding ethanol only as the primary growth substrate (electron donor).

The pilot reactor was operated on 100% PG at about 11 Kg COD/m^3-d with a 7.8 hr HRT for four weeks after ethanol was eliminated completely. During operation with 100% PG, the height of the fluidized bed was controlled at ca. 295 cm. GAC particles in the upper two-thirds of the bed typically were coated with a contiguous biofilm.

3.6 PGDN Degradation with PG as the Primary Substrate

Three additional experimental runs were conducted using PG as the added electron donor. An initial experimental run was conducted with a synthetic wastewater. This was followed by two experimental runs during which the GAC-FBR was fed actual Biazzi Plant wastewater spiked with PGDN to achieve an influent concentration of ca. 250 mg/L (Tables 3-1 and 3-3).

3.6.1 Treatment of Synthetic Biazzi Plant Wastewater (EP 5)

After transition to PG as the primary substrate was complete, the degradation of PGDN was evaluated in synthetic wastewater with PG as the primary substrate. Since the PGDN was dissolved in EtOH, a portion of the substrate COD (ca. 23%) was necessarily contributed by EtOH. The test was conducted at a PGDN loading rate of 0.86 Kg COD/m^3-d and a total organic loading rate of 13.2 Kg COD/m^3-d. Unlike the other evaluation periods, sampling began immediately following PGDN addition. This was due to the limited amounts of PGDN available. The biomass had not been exposed to PGDN for 90 days prior to this test.

The primary substrate combination of 77% PG and 23% EtOH supported PGDN degradation. A PGDN removal efficiency of 98% with an effluent concentration of 7.0 mg/L (influent concentration of 300 mg/L) was observed. The capacity to degrade PGDN appeared to have been retained by system biomass

even after a prolonged absence of exposure (3 months) to the compound. This implies that a GAC-FBR could be kept in standby mode by maintaining some biomass fed with PG. The system could be subsequently placed on-line when batches of wastewater were generated.

3.6.2 Treatment of Biazzi Plant Wastewater (EP6 and EP7)

The wastewater for these treatment tests was provided by John Stacy from the Indian Head Division, Naval Surface Warfare Center. The water was collected during a nitroglycerin (NG) production run and treated with activated carbon to remove residual NG. The water was spiked with PGDN to give an initial concentration of ca. 250 mg/l. The total dissolved solids (TDS) of the NG wastewater was 2.1%, lower than the concentration of the synthetic wastewater used (4.5%) and of the concentration expected for PGDN wastewater (up to 6% TDS).

Two barrels of wastewater totaling 500 liters (about 70 reactor volumes - empty bed) were treated during these tests. Nutrients (phosphorus and ammonia) were added to each barrel at a ratio of COD:N:P of 100:5:1.

Two different conditions were tested. These were designated EP6 and EP7 (see Tables 3-1 and 3-3). A PGDN loading rate of 0.9 Kg COD/m^3-d was applied during EP6. This was decreased to 0.3 Kg COD/m^3-d for EP7. This was accomplished by reducing the flow rate of wastewater to approximately 30% of the flow (20.6 ml/min) used during EP6. Thus, HRT was lower during EP6 (5.1 hrs) than during EP7 (17.4 hrs). For both periods, the primary carbon source (PG) loading rate was maintained at 10.8 Kg COD/m^3-d.

Removal efficiencies for PGDN were high, about 99% for both experimental runs. Effluent quality was better during EP7. The PGDN effluent concentration was at 1.8 mg/l compared to 3.8 mg/l during EP6. The lower PGDN loading rate and higher primary COD (PG) PGDN ratio are potential reasons for the higher removal efficiency.

During EP5 through EP7, removal efficiency for PG was nearly 100% (Appendix G). Influent PG concentrations of 2000 to 8000 mg/L were reduced to between 8 and 18 mg/L. Nitrate was present in excess at all times.

The Biazzi plant wastewater also contained low concentrations of Propylene Glycol Mononitrate (PGMN). This compound apparently was degraded in the GAC-FBR (Appendix G). The measurements taken indicate that PGDN is either not converted to PGMN as a major pathway or that PGMN is degraded more rapidly than it is formed from PGDN.

The treatment of PGDN using PG as the primary carbon source resulted in effluent quality similar to that observed when EtOH was used as the primary carbon source at similar PGDN loading rates. The loading rates of PGDN during EP3 (0.4 Kg COD/m^3-d) and EP4 (0.9 Kg COD/m^3-d) resulted in effluent PGDN concentrations of 1.8 and 4.3 mg/l, respectively. This is closely matched by results for EP7 (1.8 mg/L; 0.3 Kg COD/m^3-d) and EP6 (3.8 mg/L; 0.9 Kg COD/m^3-d). Results based on the synthetic wastewater appear to be essentially the same as for the actual wastewater.

A trace study was performed on the reactor to determine if nitrogen gas production caused high by-pass flow in the reactor. By-pass flow can carry untreated feed water into the reactor effluent and thus produce elevated effluent PGDN concentrations.

Results of a lithium tracer study performed at a loading of 10 Kg COD/m^3-d show low dispersion, essentially plug flow in the reactor (Appendix F). Therefore, by-pass flow did not seem significant in this system at the OLRL tested in these experiments.

3.7 Control of Biofilm and Bed Height During EP6 and EP7

Biomass growth during these two periods was vigorous. The biomass control pump tended to remove the biofilm coating from each GAC particle in clam-shell shaped pieces. These suspended biomass particles tended to form a layer at the top of the GAC bed. Some of the biomass also was recirculated through the bed with the recycled water. This combination of suspended and fixed biomass confounded accurate assessment of performance of the GAC-FBR with attached biomass only, which is typical of full-scale system operation.

Therefore, the biomass pump was turned off during the seventh day of EP6. The suspended biomass washed out of the system after several days and the fluidized bed slowly expanded from about 6.4 to 6.6 liters. The biofilm tended to become more uniform in coverage and thickness from the top to the bottom of the bed. Turbulence and shearing from nitrogen gas production was sufficient to control the bed height at between the 296 to 298 cm mark (from ca. 292), without any need for active bed height control.

3.8 Effect of Starvation on System Viability

The production of PGDN based propellants at the Indian Head facility is a batch process. There may be extended periods between production runs. Therefore, a qualitative test was conducted to determine the influence of periods of starvation (i.e., no substrate for prolonged periods) on system viability. Two

starvation periods of 8 days and 20 days were alternated with three feed periods during which PG was used as the sole organic carbon source (Table 3-5). The GAC carrier particles were maintained fluidized during the starvation periods using 100% recycle. The water in the reactor at the beginning of the starvation periods contained an excess of nitrate for the COD present (ca. 15- 20:1 of COD: NO_3-N). Nitrogen gas production and biomass coverage were used as a measure by which to judge the effect of starvation and recovery when feed was reestablished.

Table 3-5. Conditions During Feed-Starvation Periods for a Laboratory Pilot-Scale GAC-FBR.

Period Designation	FSP	FSP	FSP	FSP	FSP
Period Type	Feed	Starvation	Feed	Starvation	Feed
Primary Substrate	100% PG	None	100% PG	None	100% PG
Contaminant	None	None	None	None	None
Organic Loading Rate ($KgCOD/m^3$-d)	10.8	0	10.8	0	10.8
Effluent pH	8.0 to 8.6	8.7 to 8.9	8.0 to 8.8	8.6 to 8.9	7.8 to 8.6
Hydraulic Residence Time (hours)	7.8	Reactor in 100% recycle	7.8	Reactor in 100% recycle	7.8
Bed Volume (liters)	7.4	7.3	7.3	7.6	7.2
Days	318 to 337	338 to 345	346 to 352	353 to 372	373 to 380

Biofilm coverage on the GAC media decreased during the two starvation periods (Table 3-6). These losses did not result in a significant decrease in bed height. Bed height remained constant throughout the feed and starvation periods. The increase in net particle density (due to loss of much of the biofilm) was apparently compensated for by the increase in apparent viscosity of the water due to the recirculation of the sloughed cells (biomass). Some of the biomass sloughed from the GAC media formed a sludge blanket of about 10 cm at the top of the bed. Nitrogen gas production ceased within one hour of stopping the feed of PG. Water in the reactor took on a light amber color during the starvation periods (due to sloughed biomass).

Table 3-6. Biofilm Coverage of GAC Particles During Periods of Substrate Withdrawal.

Segment of Bed	Beginning of First Starvation Period	End of First Starvation Period	Beginning of Second Starvation Period	End of Second Starvation Period	End of Final Feed Period
0 to 100 cm	ca. 95 %	ca. 90 %	80 to 90 %	50 to 70 %	80 to 90 %
100 to 200 cm	80 to 90 %	60 to 80 %	60 to 70 %	40 to 50 %	40 to 70 %
200 to 300 cm	50 to 70 %	40 to 50 %	45 to 60 %	20 to 30 %	20 to 40 %
Length of Periods: First starvation - 8 days, Intermediate feed - 7 days, Second starvation - 20 days, Final feed - 8 days					

Denitrification began almost immediately after PG and nitrate feeds were reestablished. Nitrogen gas bubbles were evident less than two hours after restarting the feeds. Gas production visibly reached prestarvation rates within 24 hours. Biofilm regrowth was rapid. For example, after 20 days of starvation, biomass coverage recovered to near initial levels in the bottom 100 cm of the bed within 8 days. The suspended solids present during the starvation periods washed out of the system in about 3 days. The amber color of the water also dissipated concurrently after feeding was restarted.

For the first several hours following restart of forward flow to the reactor, the effluent had a faint ammonia odor. It is possible that part of the nitrate was being reduced to ammonia during this time.

The pH control system was also turned off during this period. Alkalinity produced during the denitrification reaction regulated the pH between 8.0 and 8.8. No operational difficulties were associated with allowing the biological reactions to regulate pH.

4. CONCLUSIONS

Several conclusions can be drawn from the pilot scale tests presented in this report.

1. The biological GAC-FBR removed PGDN under denitrifying conditions in munitions wastewater with either EtOH or PG as the primary substrate.
2. Effluent PGDN concentrations below the 1 mg/L regulatory limit could be achieved with the system at PGDN loading rates of up to 0.22 Kg COD/m^3-d and overall OLR of 5 Kg COD/m^3-d.
3. Increasing the PGDN loading rate at a fixed total COD loading led to an increase in effluent PGDN concentration.
4. Decreasing the primary COD/PGDN ratio at a fixed PGDN loading rate resulted in an increase in effluent PGDN concentration.
5. PGDN removal rates of greater than 98% were achieved at PGDN loading rates up to 0.89 Kg COD/m^3-d at primary COD/PGDN ratios of 4:1.
6. The GAC-FBR proved robust to suspensions in feeding of both primary substrate and PGDN. The microbial population in the system was able to degrade PGDN after a three-month interval of exposure to only the primary substrate. The reactor biomass remained viable after a three-week period of starvation (no substrate feed).
7. No pH control was necessary for stable reactor performance. The reactor pH self-regulated between 8.0 and 8.6.

5. REFERENCES

Radian Corporation. 1993. Draft Report #2 - Bench-scale Treatability, E.I. DuPont de Nemours and Company, Singapore Project. Radian Project No. 228-139. Prepared for Envirex Ltd., Waukesha, WI 53186.

APPENDIX A

BIODEGRADATION OF PGDN

Table of Contents

List of Tables and Figures

1. Objectives

The primary objectives of this test were to 1) examine the biodegradation of propylene glycol dinitrate (PGDN) under aerobic and anoxic conditions in the presence of high nitrate concentrations and 2) determine if PGDN could be metabolized at a significant rate or if there was the need for addition of a primary growth substrate.

2. Materials and Methods

2.1 Examination of Abiotic Losses of PGDN

As a first step, abiotic losses of PGDN in the experimental system were examined. Two different tests were conducted. In the first, loss of PGDN due to periods of gas purging (O_2 or N_2), which was an initial procedure in the PGDN degradation test, was examined. The second test was designed to study potential abiotic losses of PGDN.

2.1.1 Test One: PGDN loss due to gas purging

A dilute PGDN solution (20 mL) and 5 mL of biomass were transferred to 40 mL glass vials sealed with Teflon coated septa and screw caps. A 0.3 mL aliquot of concentrated H_3PO_4 (85%) was added to adjust the pH to 1.9. Samples were then purged with oxygen (for aerobic condition) or nitrogen (for anoxic condition) for 30 seconds. A control sample was not purged. Vials were closed and shaken at 20°C for 24 hours. After 24 hours, a 10 mL sample was filtered (0.45 mm) and concentrated with a solid phase extraction procedure (C-18) for PGDN measurement by HPLC (see Appendix D).

2.1.2 Test Two: potential abiotic losses of PGDN

The tests were conducted in 40 mL glass vials using the test conditions described in Table A-1. An acidified PGDN solution (25 mL, pH 1.8) was added in each vial. The

PGDN concentration was determined before the experiment. Samples were taken after 3 to 6 days of the testing.

Table A- 1. Conditions Tested to Examine Abiotic Losses of PGDN.

Testing Vials	Screw Cap	Aluminum Foil Covered	Mixing
Control without mixing	Yes	Yes	No
Control with mixing	Yes	Yes	Yes
Continuous air bubbled through headspace	No	Yes	Yes
Photolytic Loss	Yes	No	Yes

2.2 PGDN Degradation Test without Added Substrate

A concentrated PGDN solution (5% wt/wt) was transferred to 40 mL glass vials sealed with Teflon coated septa and screw caps along with salt and nutrient solutions (see Table A-2) used in the laboratory-pilot GAC-FBR. The final volume was 20 mL. Two different concentrations of PGDN (200 mg/L and 10 mg/L) were tested. Biofilm coated GAC (200 mL) was collected from a fluidized bed reactor which was fed with PG under anoxic conditions. The biomass was removed from the GAC into the salt solution. Then, 5 mL of the biomass solution was transferred to each of the vials. Each vial was purged with oxygen (for aerobic conditions) or nitrogen (for anoxic conditions) for 30 seconds. Vials were closed and shaken at 20°C for one day. Control vials were prepared by adding 0.3 mL concentrated H_3PO_4 (85%) before sealing the vials to reduce pH below 2.0 and, therefore, arrest biological activity. After incubation, 10 mL samples were removed, filtered (0.45 mm) and concentrated with a solid phase extraction procedure (C-18) for PGDN measurement by HPLC.

2.3 PGDN Degradation under Aerobic Conditions with PG Addition

Biomass was collected from an aerobic batch reactor which was fed with propylene glycol (PG) as the sole carbon source for three weeks. A PGDN stock solution (5% wt/wt), biomass solution, salt and nutrient solution were transferred to the 250 mL glass bottles sealed with Teflon mini-inert valves. The final volume of the mixture was 150 mL. The

initial PGDN concentration was 23 mg/L. The initial biomass concentration was 1257 ± 12 mg VSS/L. To test the effect of PG addition on degradation of PGDN, two different initial concentrations of PG were used (Table A-3). A control sample (biomass only), in which PG and PGDN were not added, was also prepared to monitor the oxygen consumption by the endogenous decay of the biomass. An inactivated biomass control with PGDN and PG present was prepared by adding 1 mL concentrated H_3PO_4 (85%) to reduce the pH to 1.2. Pure oxygen was added to each vial before sealing the caps. The bottles were mixed on a shaker (Controlled Environment Incubator Shaker, New Brunswick Scientific Co. Inc. Edison, NY) at 200 rpm and 22°C. Oxygen concentration in the gas phase was monitored using GC-TCD. PG and PGDN concentrations were also measured. After the PG and PGDN samples were taken, the headspace was recharged with pure oxygen.

Table A- 2. Salt and Nutrient Solutions.

Compound	Concentration
$NaNO_3$	42.7 g/L
Na_2SO_4	1.8 g/L
$NaHCO_3$	1.8 g/L
KH_2PO_4	40 mg/L
NH_4Cl	530 mg/L
HCl	110 mg/L
H_3BO_3	0.010 µg/L
$CaCl_2 \cdot 2H_2O$	0.099 µg/L
$CoCl_2 \cdot 6H_2O$	0.169 µg/L
$CuCl_2 \cdot 2H_2O$	0.020 µg/L
$FeSO_4 \cdot 7H_2O$	0.099 µg/L
$MnCl_2 \cdot 4H_2O$	0.099 µg/L
$Na_2MoO_4 \cdot 2H_2O$	0.010 µg/L
$NiCl_2 \cdot 6H_2O$	0.026 µg/L
NaCl	0.993 µg/L
Na_2SeO_3	0.017 µg/L
$ZnCl_2$	0.099 µg/L

Table A- 3. Experimental Conditions and Sampling Schedules.

	Aerobic Condition		Anoxic Condition	
Test Identification	0-200	0-10	N-200	N-10
Initial PGDN Conc. (mg/L)	200	10	200	10
Initial Biomass Conc. (mg VSS/L)	6299	4434	6299	4434
Sampling Schedule				
1 day	Yes/C	Yes/C	Yes/C	Yes/C
12 days		Yes/C		
14 days	Yes		Yes	
26 days	Yes/C			
C - included in sampling control.				

At day 14, 40 mL samples from PG-0, PG-100 and PG-400 were transferred to 250 mL glass bottles sealed with Teflon mini-inert valves, pure nitrogen was added to the headspace to create the anoxic conditions.

3. Results and Discussion

3.1 Abiotic Losses of PGDN

As part of the PGDN degradation test, the solution needed to be aerated for the aerobic samples or purged with nitrogen to remove oxygen for the anoxic samples. It is important to determine whether this purging procedure would result in losses of PGDN. Three groups of vials (each in triplicate, one set controls) were prepared and tested for losses due to gas purging. The pH of all the solutions were adjusted to less than 2 to inhibit biological degradation. There were no significant differences (95% confidence) among the different treatments (Figure A-1). The 30 second purging caused no significant loss of PGDN.

To further evaluate potential abiotic losses of PGDN, two potential loss mechanisms (volatilization and photolytic) were examined (Table A-4). There was significant loss of PGDN due to the volatilization, the result of constant aeration, while photolytic losses were

not significant. Simple mixing with a magnetically driven stir bar did not result in loss of PGDN (Figure A-2).

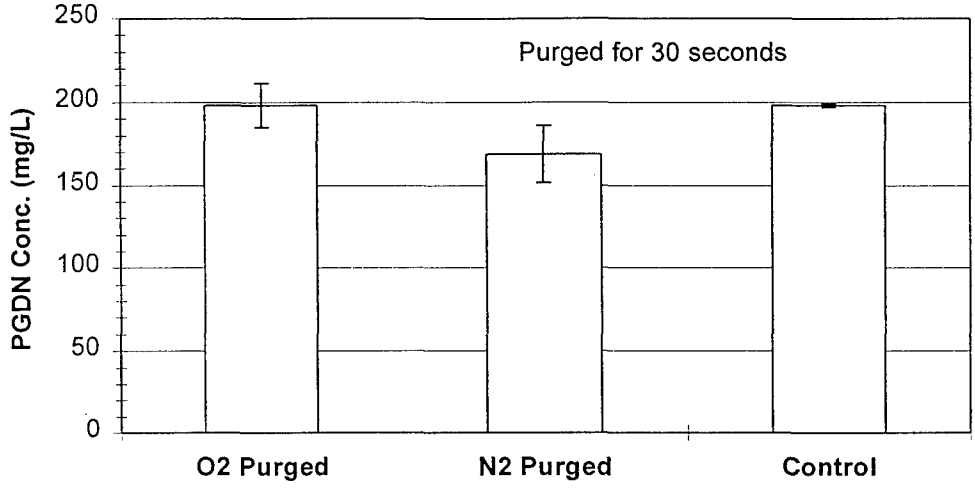


Figure A- 1. PGDN Loss During a Short Period Gas Purging.

Table A- 4. Experimental-Conditions for the PGDN Degradation Tests with PG Addition.

Testing Vial Identification	PGDN Conc.	PG Conc.	pH	Headspace Gas
Biomass	0 mg/L	0 mg/L	7.5	Oxygen
Control	25 mg/L	200 mg/L	1.2	Oxygen
PG-0	25 mg/L	0 mg/L	7.5	Oxygen
PG-100	25 mg/L	50 mg/L	7.5	Oxygen
PG-400	25 mg/L	250 mg/L	7.5	Oxygen
N2-0	At day 14, transfer 40 mL from PG-0		7.08	Nitrogen
N2-100	At day 14, transfer 40 mL from PG-100		7.09	Nitrogen
N2-400	At day 14, transfer 40 mL from PG-400		7.05	Nitrogen

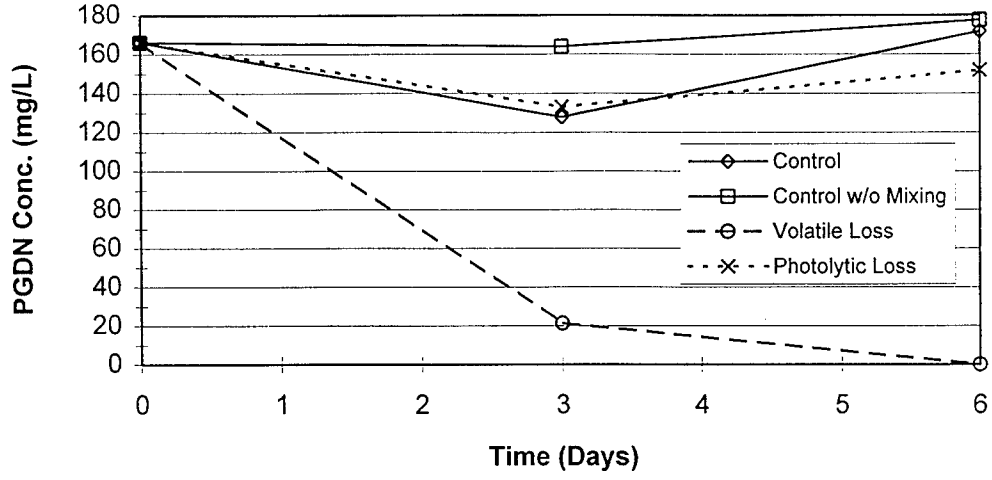


Figure A- 2. Test for PGDN Loss Mechanism - Comparison of Volatile Loss and Photolytic Loss (bioactivity was inhibited by low pH).

3.2 PGDN Degradation without PG Addition

Two initial PGDN concentrations were selected (200 mg/L and 10 mg/L) with two initial biomass concentrations (Table A-3). The concentrations of VSS were 6299 mg/L and 4434 mg/L for the high PGDN concentration samples and low PGDN concentration samples, respectively. Because of the high carbonate concentration in the solution (Table A-2), the value of VSS included both the biomass and the amount of carbonate that decomposed at 550°C.

These initial experiments were planned to be for a one-day incubation period. Because the PGDN concentration did not change significantly after one day under aerobic conditions, additional samples were taken at days 14 and 26. The shaker, however, was stopped after a one day incubation.

There was no significant disappearance of PGDN in samples after 24 hours under the aerobic conditions (Figures A-3 and A-4). The dissolved oxygen (DO) was 18 mg/L after 24 hours. The samples at 14 days and 12 days from the high and low PGDN initial concentration vials did show some degradation of PGDN. However, due to a technical

difficulty, the DO could not be measured after 14 days incubation. Because the samples were not continually mixed, it is possible that local anoxic conditions formed in the vials and that degradation occurred as a result of these anoxic conditions.

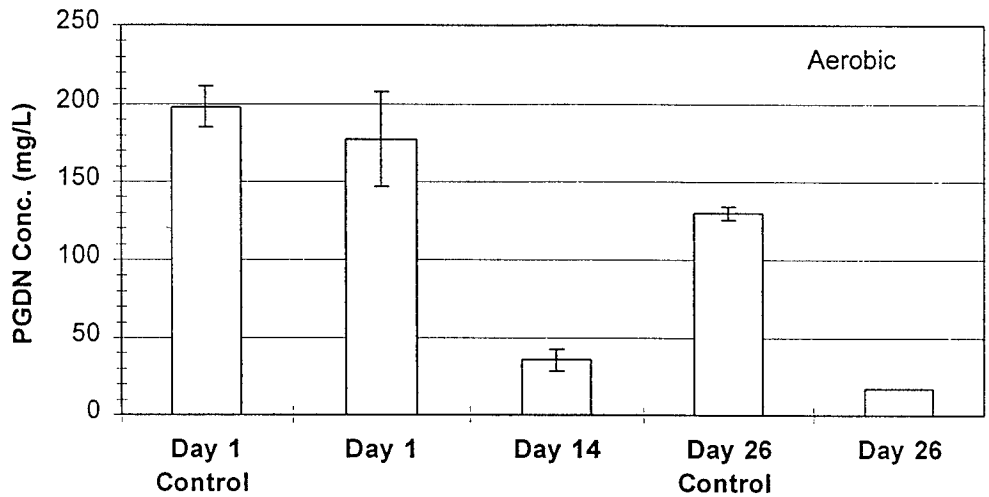


Figure A- 3. PGDN Degradation Test under Aerobic Conditions without PG Addition (agitation stopped after day 1).

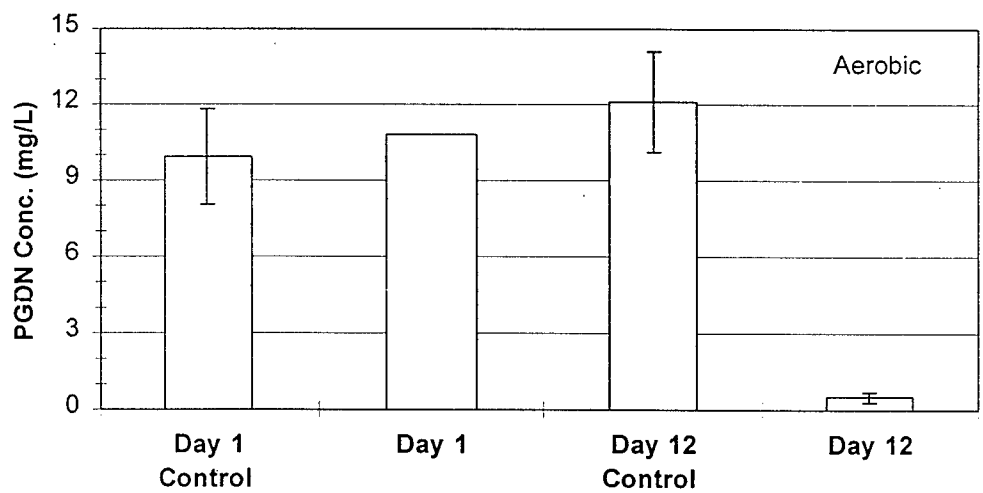


Figure A- 4. PGDN Degradation Test under Aerobic Conditions (low initial PGDN concentration; without PG addition; agitation stopped after day 1).

Under anoxic conditions, the degradation of PGDN occurred during the initial 24 hours of incubation (Figure A-5). After 14 days, the concentration of PGDN decreased

further. The degradation rate was 0.0097 mg PGDN/mg VSS/day based on a zero order reaction rate.

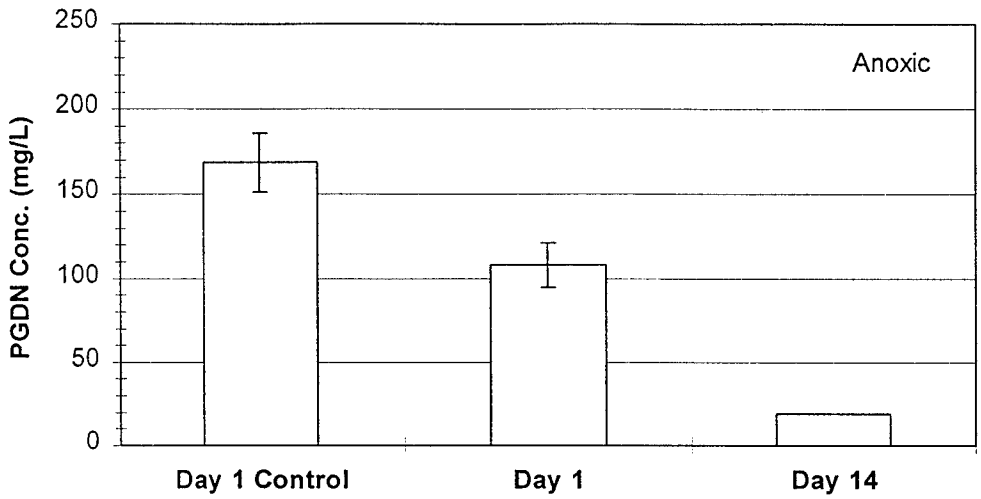


Figure A- 5. PGDN Degradation Test under Anoxic Conditions (without PG addition; agitation stopped after day 1).

3.3 PGDN Degradation with PG Addition

The degradation of PGDN under aerobic conditions with PG addition was also evaluated. Two concentrations of PG (200 and 50 mg/L) were used. A sample without PG addition was prepared as a reference to monitor loss of PGDN. Biomass was grown under aerobic conditions using PG as the sole carbon source. The seed came from the initial degradation test which showed possible PGDN degradation under aerobic conditions. Because of the limited available supply of PGDN, no selection/acclimation procedure for the biomass was possible. The initial biomass concentration was 1257 ± 12 mg VSS/L. The oxygen in the headspace of the closed reaction bottles was monitored to verify the aerobic conditions (Figure A-6). The gas phase oxygen concentration was higher than 85% (v/v) which was sufficient to maintain the DO higher than 18 mg/L. The oxygen consumption for the biomass decay was continuous after two days incubation. The degradation of PG began quickly and PG was consumed completely after three days (Figure A-7).

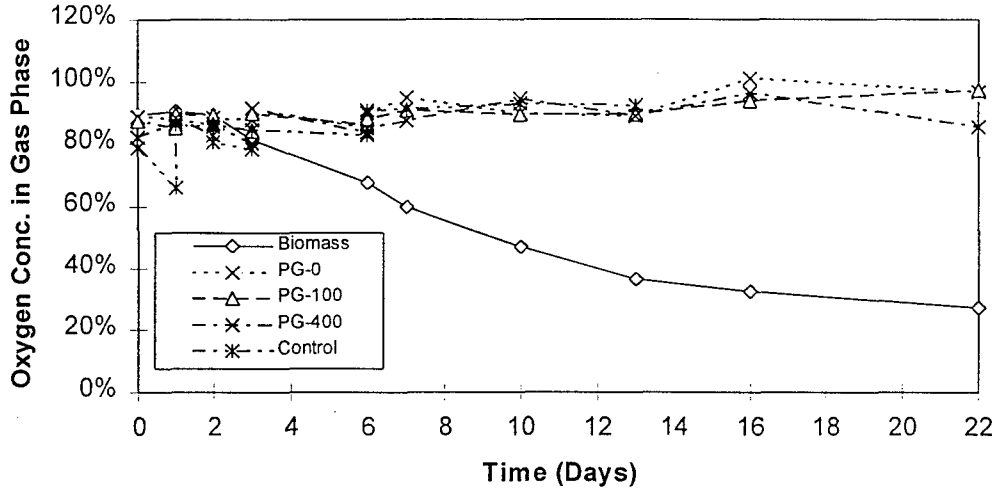


Figure A- 6. Oxygen Concentration in the Gas Phase of PGDN Degradation Test with PG Addition.

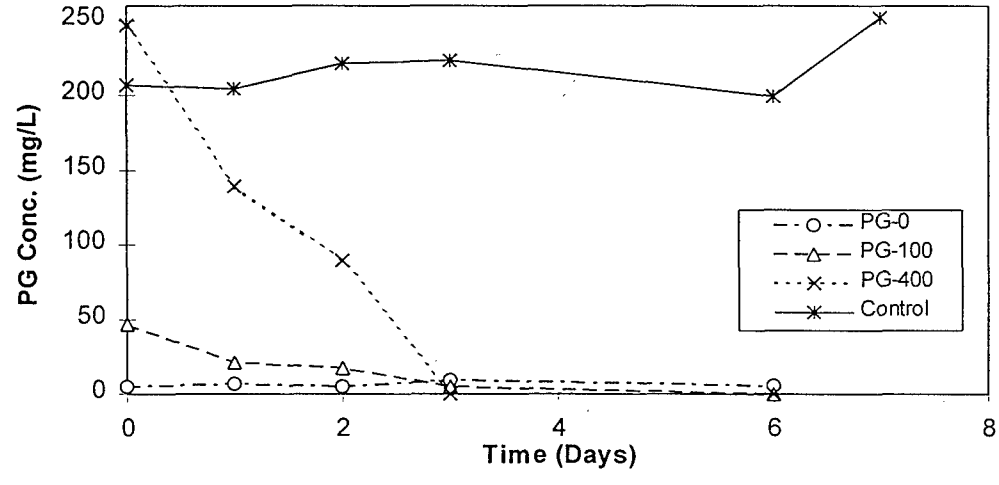


Figure A- 7. PG Concentration in the Liquid Phase of PGDN Degradation Test with PG Addition.

Degradation of PGDN was not, however, observed under the aerobic conditions (Figure A-8a). A sampling problem during the first three days resulted in artificially low PGDN concentrations. This is an artifact of low recovery of PDGN. The sampling procedure was modified after that and any loss of PGDN was eliminated.

Because the degradation of PGDN did not occur after 13 days incubation under aerobic conditions, on day 14, a portion of liquid from each vial was transferred to a new bottle and purged with nitrogen to provide anoxic conditions. No additional substrate (PG) was supplied. After 9 days incubation, degradation of PGDN was not observed (Figure A-8b).

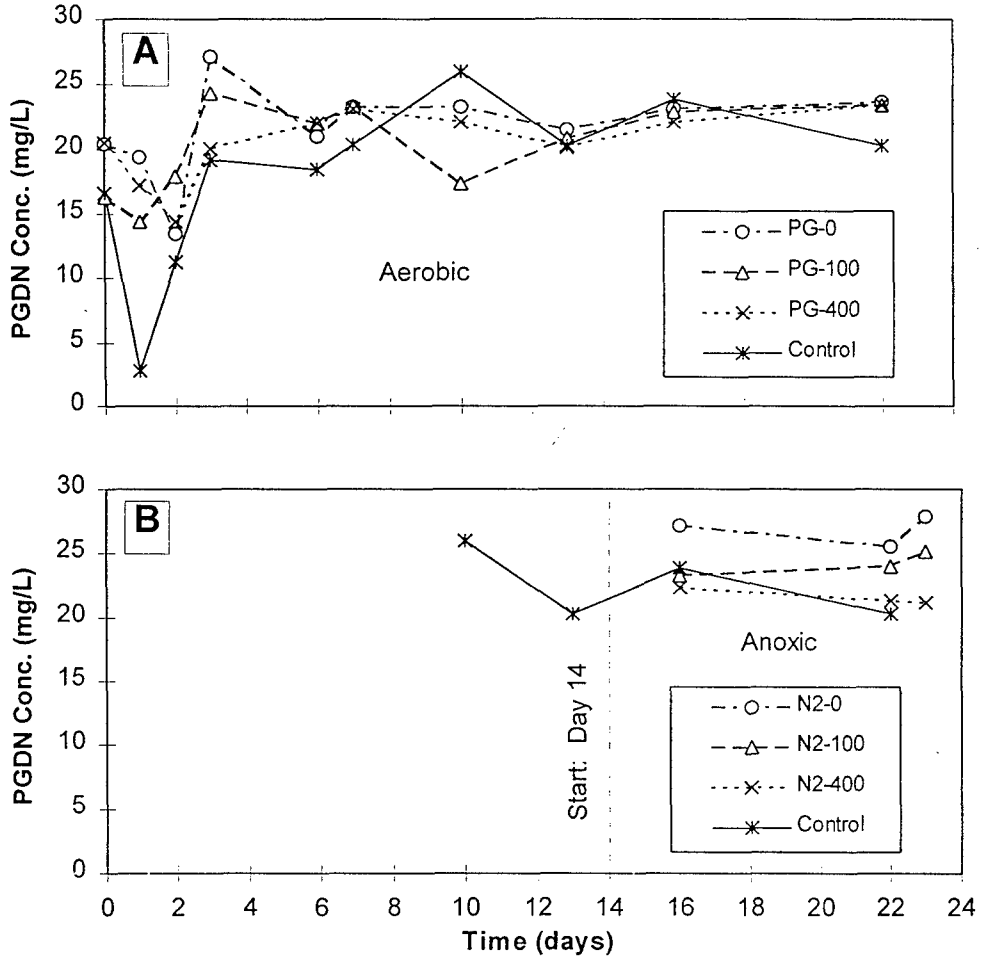


Figure A- 8. PG Concentration in the Liquid Phase of PGDN Degradation Test with PG Addition: A - aerobic condition; B - anoxic condition (anoxic test was started at day 14 by transferring 40 mL liquid from aerobic testing bottles).

4. Conclusions

The degradation of PGDN was investigated under aerobic and anoxic conditions. The biodegradation of PGDN occurred only under anoxic conditions. The degradation of PGDN under aerobic conditions did not occur under the conditions tested, with or without addition of a primary substrate.

APPENDIX B
PROPYLENE GLYCOL BATCH DEGRADATION TESTS

Propylene Glycol Batch Degradation Tests

Introduction

Propylene Glycol (PG) is used as the carbon skeleton in a nitration process to yield the propellant Propylene Glycol Dinitrate (PGDN). Since PG is already used in the manufacturing process, it is the preferred supplemental carbon and energy source for PGDN degradation. Batch serum bottle tests were conducted to determine whether the denitrifying organisms could use PG as a carbon and electron source.

Experimental Method

A 40 mL aliquot of a 50/50 mixture of the salt and trace mineral solutions used to feed the laboratory-pilot GAC-FBR system was added to each of nine 158 mL serum bottles. A 10 mL aliquot of biomass sheared from the GAC carrier in the FBR was added to each vial. Each bottle was then spiked with 12 μL neat PG. The biomass from the GAC-FBR had been grown in the reactor using EtOH as the primary growth substrate. The serum bottles were vacuum flushed and purged with N_2 prior to adding the PG to ensure anoxic conditions existed during the experiment. The bottles were shaken in a water bath (room temperature) until sacrificed for organic carbon analysis. One bottle was sacrificed immediately following preparation. One sample bottle was equipped with a valve connected to a water lubricated syringe, which was inserted through the bung using a 23 gauge needle to monitor gas production during the experimental period.

Organic Carbon Sampling

At discrete time intervals, the serum bottles were opened and a 15 mL sample withdrawn with a 25 mL glass syringe. The sample was filtered through a Whatman GF/F filter into a 40 mL vial containing 2 drops of concentrated HCl. Dissolved, non-purgable organic carbon (DNPOC) was measured with a Rosemount Analytical Dorhmann DC-190 organic carbon analyzer.

Sampling Schedule

One bottle was sacrificed immediately after PG addition and analyzed for DNPOC content. One of each of the remaining bottles were sacrificed and analyzed every hour for the next five hours. Three additional bottles were sacrificed about two days later.

Results

Denitrifying microorganisms were able to degrade PG (Figures B-1 through B-3). Nitrogen gas was produced and DNPOC values decreased over the two days of the test. From the DNPOC measures, the concentration of PG was reduced from 270 mg/L to about 50 mg/L.

These results indicated that the PG can serve as a growth substrate and that PG could be substituted for ethanol as the carbon and energy source for PGDN treatment.

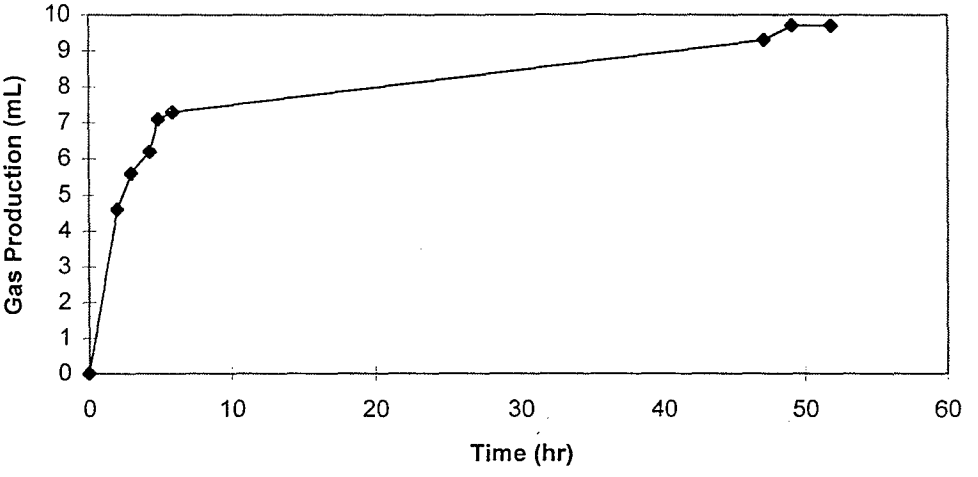


Figure B- 1. Gas Production During Propylene Glycol Degradation.

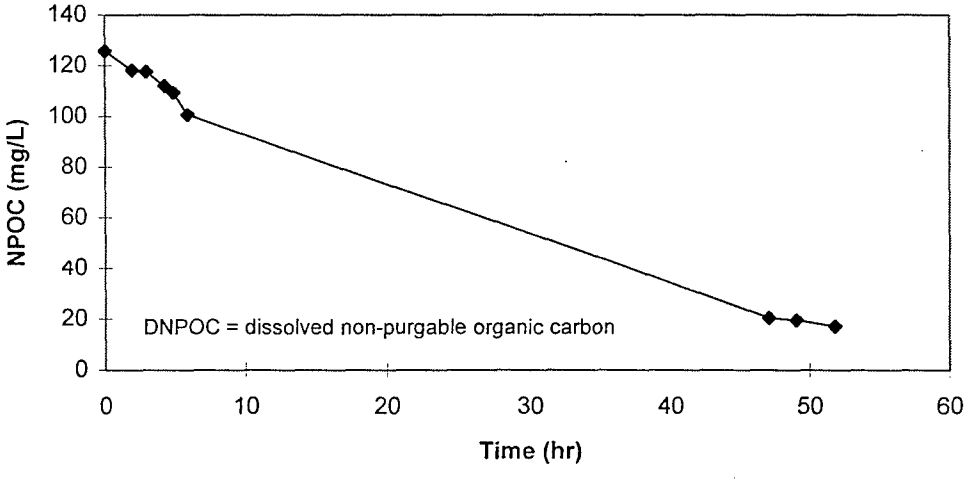


Figure B- 2. Propylene Glycol Degradation as Measured by Reduction in DNPOC.

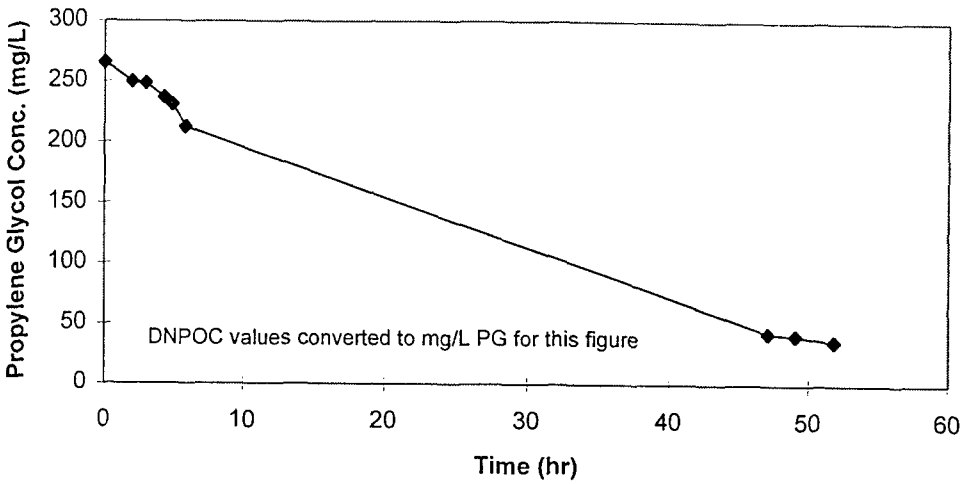


Figure B- 3. Propylene Glycol Degradation.

APPENDIX C
PGDN REACTOR NUTRIENTS, BUFFER SOLUTION

PGDN Reactor Nutrients, Buffer Solution

Primary Salts

Carboy I	Amount	Volume	Concentration
$NaNO_3$	4.8 kg	56.7 L (15 gal)	84.7 g/L
Na_2SO_4	204 g		3.6 g/L
$NaHCO_3$	204 g		3.6 g/L

Nutrients

Carboy II	Amount	Volume	Concentration
Trace solution	80 mL	56.7 L (15 gal)	see table below
Phosphorus stock	227 mL		0.04 gp/L (see below)
HCl	30 mL		
NH_4Cl	30 g		0.14 gN/L

Trace Metal Solution Composition

Metal	Compound	Compound Conc. (g/L)	Metal Conc. (mg/L)
B	H_3BO_3	0.014	0.002
Ca	$CaCl_2$ $2H_2O$	0.141	0.038
Co	$CoCl_2$ $6H_2O$	0.239	0.059
Cu	$CuCl_2$ $2H_2O$	0.028	0.010
Fe	$FeSO_4$ $7H_2O$	0.141	0.028
Mn	$MnCl_2$ $4H_2O$	0.141	0.039
Mo	Na_2MoO_4 $2H_2O$	0.014	0.006
Ni	$NiCl_2$ $6H_2O$	0.037	0.009
Na	NaCl	1.408	0.555
Se	Na_2SeO_3	0.024	0.011
Zn	$ZnCl_2$	0.141	0.068

Phosphorus Stock Solution

Element	Compound	Compound Conc. (g/L)	P Conc. (g/L)
P	K H_2PO_4	8.5	1.94
P	K_2 HPO_4	21.75	3.88
P	Na_2HPO_4 $7H_2O$	33.4	3.86
P	Total P	--	9.68

pH Control Buffer	Volume	Concentration
1 kg $NaHCO_3$	20 L	50 g/L
30 g NaOH		1.5 g/L

APPENDIX D
ANALYTICAL METHODS

PROPYLENE GLYCOL DINITRATE AND PROPYLENE GLYCOL MONONITRATE BY HPLC/UV ANALYSIS

1. SCOPE AND APPLICATION

This method has been used to quantitatively analyze for Propylene Glycol Dinitrate (PGDN) and Propylene Glycol Mononitrate (PGMN) in aqueous samples.

2. SUMMARY OF METHOD

This method provides subsample preparation by solid phase extraction to Acetonitrile and HPLC conditions for the detection of PGDN and PGMN. A PAH Hypersil column and a UV Detector are used in this method.

3. INTERFERENCES

No interferences have been observed in our samples.

4. APPARATUS AND MATERIALS

High Performance Liquid Chromatography: A Spectra-Physics HPLC system is used for this analysis. The HPLC hardware includes:

Gradient pump: Spectra System P4000.

Autosampler: Spectra System AS 3000. The autosampler is equipped with a 120 position sample carousel. The injection volume range runs from 0.1 - 200 µL.

Columns: PAH Hypersil 150 x 4.6 mm Keystone Scientific.

Detector: Spectra System UV 2000.

Extraction Columns and Vacuum Manifold: A 200 mg octadecyl solid phase extraction columns and manifold (Burdick and Jackson) are used for subsamples preparation.

Filters: 0.45 µm syringe filter (Nalgene).

Syringes: 20 mL all glass syringe with 3 inch, 15 gauge needle are used for sampling.

Microsyringes: 10, 50 and 100 µL syringes are used for standard preparation (Hamilton and Unimetrics).

Glassware: 10 mL volumetric flask, 2 mL screw cup vials for analysis, disposable glass pipettes, 15 mL centrifuge tubes.

Data Acquisition and Analysis: A Windows based chromatographic data acquisition system, PE-Nelson Turbochrom 3.3, is used. The system is interfaced to the HPLC via a 900-Series A/D link box, to provide for continuous storage of raw chromatograms in a PC, for subsequent off-line batch analysis.

5. REAGENTS

Standards: PGDN and PGMN solution in Acetonitrile from Indian Head Division, Naval Surface Warfare Center.

Calibration Standards: These are prepared from Standards by serial dilution in Acetonitrile.

Reagent Water: Deionized water (> 18 MOhm) is prepared on demand with a Milli-Q plus water system (Millipore). System cartridges include mixed ion exchange resins, activated carbon and 0.45 μm filter.

Solvents: Acetonitrile - HPLC analysis grade (Fisher Scientific) - is used as a mobile phase and as a solvent for standards and subsamples.

Methanol - HPLC analysis grade (Fisher Scientific) is used in shutdown program.

6. SAMPLE COLLECTION AND HANDLING.

Samples are drawn from the influent and effluent ports using an all glass, 20 mL syringe with needle. The 15 mL samples are drawn, one at a time, over a period of 1 minute time to provide an integrated sample. The syringes are rinsed with deionized water before using and pre-rinsed with sample prior to withdrawal of 15 mL sample volume. The samples are filtered to remove suspended solids using syringe filters. The filtered sample (10 mL) is collected in a centrifuge tube.

For each influent and effluent sample, a solid phase extraction column is placed on a vacuum manifold drawing 15 in. Hg vacuum. The columns are pre-rinsed with 7.5 mL of acetonitrile. Between each elution step, the columns are allowed to air dry (via vacuum) for about 2 min to prevent carryover of residual liquids. The entire 10 mL sample is eluted through the pre-washed columns. The column is washed with 10 mL water to remove media salts and other reactor contaminants. The column is then washed with 7 mL acetonitrile which is collected in a volumetric flask.

The 10 mL volumetric flask is removed from manifold and brought up to volume using acetonitrile. Using a disposable glass pipette, a 2 mL subsample is transferred from the

volumetric flask to a 2 mL HPLC vial for analysis. All waste is collected in 50 mL waste flasks within the vacuum manifold and discarded following sample preparation.

7. PROCEDURE

Summary

PGDN subsamples are analyzed using HPLC with a UV detector. The shutdown program is run after every sequence to make sure that the column is clean.

Recommended HPLC Conditions:

Gradient Pump - program for analysis:

	Time	Acetonitrile	Water	Flow
Solvent Program:	(min)	(%)	(%)	(mL/min)
	0	45	55	1.25
	15	45	55	1.25
Maximum pressure	4000 psi			
Minimum pressure	0 psi			
Equilibration time	0 min			
Gradient curve	linear			
Solvents	acetonitrile, water			

Gradient Pump - shutdown program:

	Time	Acetonitrile	Water	Methanol	Flow
Solvent Program:	(min)	(%)	(%)	(%)	(mL/min)
	0	45	55	0	1.25
	15	100	0	0	1.25
	60	0	0	100	1.25
	120	0	55	45	1.25
	316	45	55	0	0
Maximum pressure	6000 psi				
Minimum pressure	0 psi				
Time from ready	60				

Autosampler:

Injection:	Injection volume	20 µL
	Injection/sample	1
	Cycle time	15 min
More:	Equilibration time	0 min
	Gradient delay	0 min
	Sample viscosity	normal
	Flush volume	500 µL
	Injection type	push hi
	Injection range	1-200 µL
	Needle height	2.0

UV Detector:

Wavelength Program:

Program Time	**Single Wavelength Wavelength**
0	210
15	210
Options:	
Rinse time	0.1
Autozero time	0.0
Range 1	0.05
Range 2	0.05

Fluorescence Detector:

Lamp Status OFF

Calibration: Six-level calibration curves were generated for individual components, in concentrations ranging from 0.5 mg/L to 21 mg/L. Linear regression with forced origin was used to calculate the calibration factors. Sample calibration curve is attached.

HPLC Analysis: The following are representative retention times for individual components:

Compound	RT (minutes)
Propylene Glycol 2-mononitrate	1.7
Propylene Glycol Dinitrate	4.2

8. QUALITY ASSURANCE/ QUALITY CONTROL

Two injection of a high level standard are run at the beginning of each run, to ensure the integrity of the HPLC system and the retention time stabilization. QC acceptance criteria (typically ±15%) are established for the response of check standards. If acceptance criteria are not met, the problem is identified, fixed and a new calibration curve is generated.

9. METHOD PERFORMANCE

Accuracy and Precision: Single operator precision, overall precision and method accuracy are directly related to analyte concentrations and not affected by the sample matrices that have been analyzed using this method.

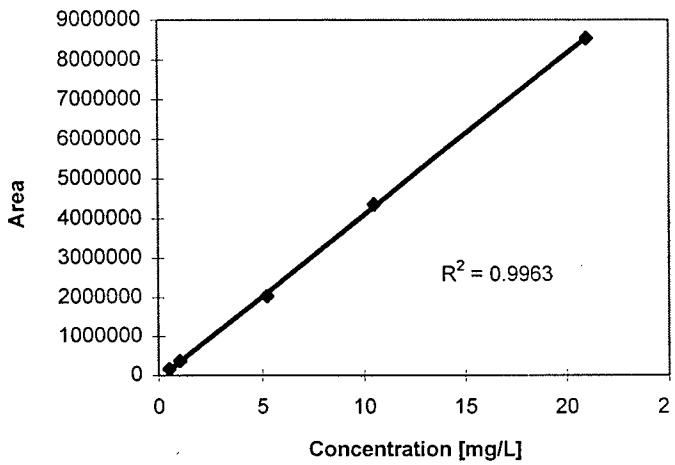
Method Detection Limits: Detection limits are established periodically, by analyzing the standard deviation of response to 7 replicates of a very low level standard.

10. REFERENCES

Spectra System Reference Manual, Spectra-Physics

Sample PGDN Calibration Curve

PGDN Concentration [mg/L]	Area
0.525	159525
1.05	365520
5.25	2029450
10.5	4359121
21	8537967



PROPYLENE GLYCOL BY GC/FID ANALYSIS

1. SCOPE AND APPLICATION

This method has been used to quantitatively analyze for Propylene Glycol (PG) in aqueous samples. The method is primarily designed for measuring PG in a high salt concentration water. This method with slight modification can be used also for glycol derivatives such as triethylene glycol and others.

2. SUMMARY OF METHOD

This method provides subsample preparation by solid phase extraction in 10% Methanol and GC conditions for the detection of PG. A Capillary column and a FID detector are used. The method is suitable for high salt concentration wastewater samples.

3. INTERFERENCES

No interferences have been observed in our samples.

4. APPARATUS AND MATERIALS

Gas Chromatography: A Varian 3400 is used for this analysis.

The GC hardware includes:

Injector: a septum-equipped programmable on-column injector (SPI).
Columns: a 15m Nucol capillary column (Supelco), 0.53 mm I.D., 0.5 μm film.
Detector: a capillary FID, with a ceramic tip.
Autosampler: a Varian 8100 Autosampler with 48-sample carrousel.
Extraction Columns: A 600 mg AccuCat (10 LRC) solid phase extraction Varian columns are used for subsamples preparation to decrease the concentration of salts.
Filters: 0.45 μm cellulose acetate syringe filter (Nalgene).
Syringes: 10 mL plastic syringes are used for drawing a sample through an AccuCat.
Microsyringes: Hamilton and Unimetrics 10, 50 and 100 μL syringes are used for standard preparation.
Glassware: 2 mL screw cup vials for analysis, pipettes and beakers for a sample preparation.

Data Acquisition and Analysis: A Windows based chromatographic data acquisition system, PE-Nelson Turbochrom 3.3, is used. The system is interfaced to the GC via a 900-Series A/D link box, to provide for continuous storage of raw chromatograms in a PC, for subsequent off-line batch analysis.

5. REAGENTS

Calibration Standards: These are prepared from propylene glycol (P-1009, Sigma Chemical) by a serial dilution in 10% Methanol.

Reagent Water: Deionized water (>18 MOhm) is prepared on demand with a Milli-Q plus water system (Millipore). System cartridges include mixed ion exchange resins, activated carbon and 0.45 μm filter.

Solvents: Fisher HPLC analysis grade Methanol.

6. SAMPLE COLLECTION AND HANDLING

Samples (5 mL) are drawn from the influent and feed ports using an all glass, 20 mL syringe (one dedicated to each sampling location) and a 3 inch, 15 gauge needle. The samples are drawn, one at a time, over a period of 1 minute to provide an integrated sample. The syringes are rinsed with deionized water before using and pre-rinsed with sample prior to withdrawal of 5 mL sample volume. The samples are filtered to remove suspended solids using a syringe filters. The filtered sample is collected in 15 mL centrifuge tubes.

For each influent and feed sample, a 600 (10 LRC) mg AccuCat solid phase extraction cartridge (Varian) is used to decrease concentration of salts (nitrate).These salts cause problems during sample analysis by forming a salt deposit in the GC column.

Feed samples are first diluted 10x and effluent samples 2-4 x with methanol and water to get 10% methanol solution in a subsample. The presence of methanol helps improve peak shape. A dilution is necessary because of the AccuCat columns limited capacity. After dilution, subsamples are drawn through the AccuCat. During this process concentration of Sodium Nitrate is decreased to about 5 to 10 mg /L.

The high salt concentrations present in the raw wastewater form salt deposits in the capillary GC column. No operational problems have been experienced with 0.5-10 mg/L salt.

Extraction: The AccuCat cartridge is pre-rinsed with 2.0 mL of 10% methanol in water at approximately 2 mL/min. Each subsample (2.0 mL) is manually drawn slowly (1-2 min.) through the wet cartridge with the 10 mL plastic syringe attached to the top of the cartridge. The first part of the subsample is thrown away to keep the sample from being diluted by the

pre-rinse. The major part of subsample (1 - 1.5 mL) is collected to a 2 mL GC vial for analysis. The recovery of PG after being drawn through the AccuCat column is approximately 85%. Reported concentrations are adjusted to account for the 85% recovery factor.

7. PROCEDURE

Summary

PG solution with 10% methanol is introduced into the GC using an autosampler. Detection is by FID. This method is appropriate for feed and effluent samples.

Recommended GC Conditions:

Injector Temperature: 220°C
Column Head Pressure: 7 psi for 90°C
Initial Column Temperature: 90°C
Initial Column Hold Time: 0 min.
Column Rate: 8°C/min
Program 1 Final Column Temperature: 160°C
Program 1 Column Hold Time: 2.5 min
Detector Temperature: 250°C
FID Attenuation: 8
FID Range: 10^{12}
FID AutoZero: OFF
Time Program FID: NO
Injection volume: 2µL
Fast Injection Rate: 5µL/s
Run Time: 9.0 min.

Calibration: Six-level calibration curves were generated for individual components, in concentrations ranging from 10 mg/L to 400 µg/L. Linear regression with forced origin was used to calculate the calibration factors. Sample calibration curve is attached.

GC Analysis: The following is a representative retention time for PG.

Compound	RT (minutes)
Propylene Glycol	2.6

8. QUALITY ASSURANCE/ QUALITY CONTROL

Two injections of a high level standard are run at the beginning of each run, to ensure the integrity of the GC system and the retention time stabilization. QC acceptance criteria

(typically ±15%) are established for the response of check standards. If acceptance criteria are not met, the problem is identified, fixed and a new calibration curve is generated.

9. METHOD PERFORMANCE

Accuracy and Precision. Single operator precision, overall precision and method accuracy are directly related to analyte concentrations and not affected by the sample matrices that have been analyzed using this method.

Method Detection Limits. Detection limits are established periodically, by analyzing the standard deviation of response to 7 replicates of a very low level standard.

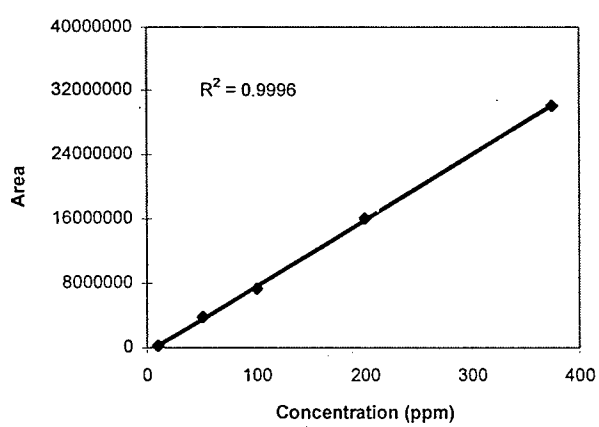
Estimated Quantitation Limits. Quantitation limits are established for new sample matrices by the analysis of variance in replicates spiked with low level standards.

10. REFERENCES

- 3300/3400 Operator's Manual, Varian
- 8100 Autosampler Operator's Manual, Varian
- Chromatography Products, Supelco, 1996
- Mike D'Amico, Glycol Analysis, Separation Times, Vol. 9, No. 3.

Sample Propylene Glycol Calibration Curve

PG Concentration (ppm)	Area
10	232547
50	3776401
100	7303820
200	16045628
375	30056268



ANALYSIS OF LITHIUM AND SODIUM IONS BY FLAME EMISSION SPECTROSCOPY

1. SCOPE AND APPLICATION

This method has been used to quantitatively analyze for Lithium and Sodium ions in aqueous samples, mainly for tracer studies of fluidized bed reactors.

This method can be also used to analyze for potassium ions.

2. SUMMARY OF METHOD

This method provides Flame Emission Spectroscopy settings for the detection of lithium and sodium ions in aqueous samples. An atomic absorption spectrophotometer with air-acetylene flame for the atomization and photomultiplier detection is used in this method.

3. INTERFERENCES

To compensate for background absorption , a blank is run at the beginning of each sequence.

4. APPARATUS AND MATERIALS

Spectrometer: SpectraAA 20 Plus Atomic Absorption Spectrometer (Varian).
Printer: ThinkJet (Hewlett Packard).
Sample Introduction: manual aspiration.
Data Acquisition and Analysis: SpectraAA system is equipped with the utilities disk. The disk can be used for both flame and furnace analysis for the following purposes:

- storage of methods for different elements
- storage of analytical data
- editing of results

The system is interfaced to the printer.
Filters: 0.45 μm cellulose acetate syringe filter (Nalgene).
Glassware: 1000 mL and 100 mL volumetric flasks for standard preparation, pipettes, 10 mL all plastic disposable syringes for a sample preparation, 10 mL all plastic vials for samples.

5. REAGENTS

Stock Standards. Standards are prepared in-house from LiCl or NaCl. Salts are dried at 100°C and dissolved in reagent water. 100 mg Li/L and 1000 mg Na/L solutions were used.
Aqueous Calibration Standards. These are prepared in reagent water from stock standards. They are prepared fresh every two weeks.
Reagent Water. Deionized water (> 18 MOhm) is prepared on demand with a Milli-Q plus water system (Millipore). System cartridges include mixed ion exchange resins, activated carbon and 0.45 μm filter.

6. SAMPLE COLLECTION AND HANDLING

10 mL samples are collected in plastic vials and stored at 4°C. Before analysis they are filtered by syringe filter and returned to the original vials rinsed with a small fraction of a filtered sample.

7. PROCEDURE

Summary

Samples are aspirated into the SpectraAA system manually. Mean Emissions (relative numbers) are measured and concentrations are automatically inserted into the table as they are obtained.

Recommended SpectraAA System Conditions for Lithium Ions:

Optimization:

Photomultiplier [volts] ca. 350
The calibration standard with the highest concentration is used for the optimization of signal. It determines the maximum measurable concentration in the run.

Flame emission	
Sample introduction	Manual
Delay time [sec]	2
Measurement time [sec]	1
Replicates	3
Flame	air-acetylene
Wavelength [nm]	671.4
Slit width [nm]	1
Oxidant [flow units]	4.3
Acetylene [flow units]	1.5

Recommended SpectraAA System Conditions for Sodium Ions:

Optimization:

Photomultiplier [volts] ca. 350

The calibration standard with the highest concentration is used for the optimization of signal. It determines a maximum measurable concentration in the run.

Flame emission	
Sample introduction	Manual
Delay time [sec]	2
Measurement time [sec]	1
Replicates	3
Flame	air-acetylene
Wavelength [nm]	589.6
Slit width [nm]	0.2
Oxidant [flow units]	4.3
Acetylene [flow units]	1.5

Calibration. Reagent water is used to set an instrumental zero. Five-level calibration curves are generated for individual components, in concentrations ranging from 10 mg/L to 200 mg/L for lithium ion and 5 mg/L to 30 mg/L for sodium ion in the beginning of each run. Obtained mean emission is a relative quantity and depends on concentration of standard which has been used for the signal optimization. Sample calibration curve is attached.

8. QUALITY ASSURANCE/ QUALITY CONTROL

The same concentration of standard picked for the signal optimization is used for quality control checks. QC acceptance criteria (typically ±15%) are established for the response of standards. If acceptance criteria are not met, new calibration standard are prepared.

Samples are allowed to reach room temperature before analysis to insure samples and standards have similar viscosity and aspiration rate.

The plastic tubing for sample introduction is cleaned with the wire after each 20-30 samples.

9. METHOD PERFORMANCE

Accuracy and Precision. Single operator precision, overall precision and method accuracy are directly related to analyte concentrations and not affected by the sample matrices that have been analyzed using this method.

Method Detection Limits. Detection limits are established periodically, by analyzing the standard deviation of response to 7 replicates of a very low level standard.

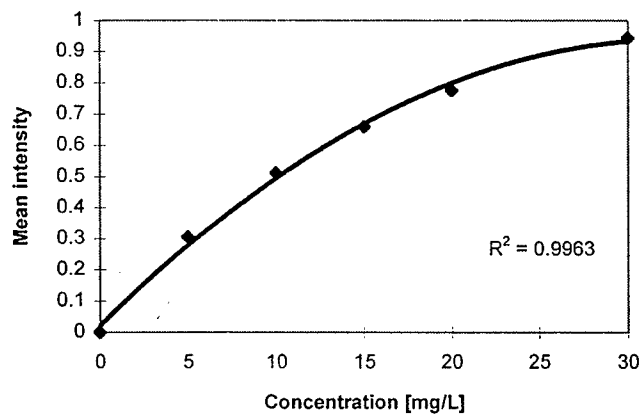
Estimated Quantitation Limits. Quantitation limits are established for new sample matrices by the analysis of variance in replicates spiked with low level standards.

10. REFERENCES

- SpectraAA - 10/20 Operation Manual, Varian, 1991
- Sodium by Spectra AA, Varian

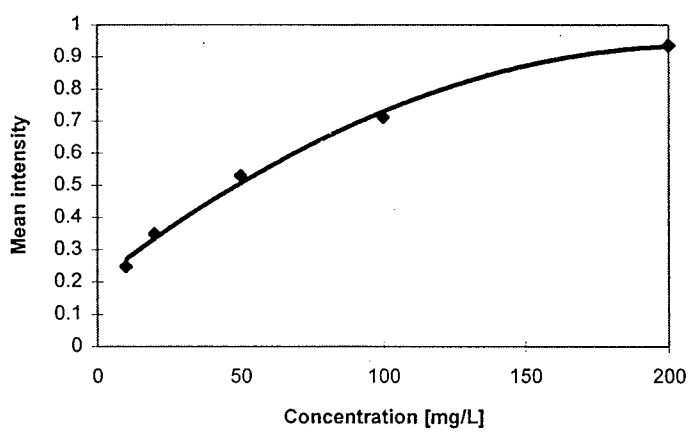
Sample Lithium Ion Calibration Curve

Li Concentration [mg/L]	Mean Intensity
0	0
5	0.306
10	0.512
15	0.659
20	0.776
30	0.943



Sample Lithium Ion Calibration Curve

Na Concentration [mg/L]	Mean Intensity
10	0.247
20	0.349
50	0.53
100	0.712
200	0.937



INORGANIC ANIONS BY ION CHROMATOGRAPHY ANALYSIS

1. SCOPE AND APPLICATION

This method has been used to quantitatively analyze for the following compounds in aqueous samples:

- Chloride
- Nitrite
- Nitrate
- Sulfate
- Phosphate

This method could be also used for the analysis of the other inorganic and organic anions.

2. SUMMARY OF METHOD

This method provides ion chromatographic conditions and sample preparation for analysis of inorganic anions important in the biological water treatment process. A Dionex chromatograph with a conductivity detector is used in this method.

3. INTERFERENCES

No interferences have been observed in our samples, however a dilution for samples must be chosen carefully because of similar chloride and nitrite retention time. A big chloride peak can mask a nitrite peak in samples with high concentration of chloride.

4. APPARATUS AND MATERIALS

Ion Chromatography: An IC (Dionex) is used for this analysis. The IC hardware includes:

Eluent Degas Module
Gradient Pump
BioLC equipped with:
Columns:
InoPack ASII analytical column 4 mm
InoPack AGII guard column
IonPack trap column (ATC -1)
Anion Self-regenerating Suppressor
ASRS-1, 4 mm

Detector: A conductivity detector

Automated Sampler: equipped with two types of carousels - for 0.5 mL and 5 mL plastic vials. Vial lids have integrated filters. Injection loop is 50 µL.

Filters: 0.45 µm cellulose acetate syringe filter (Nalgene)

Syringes: 20 mL all glass syringe with 3 inch, 15 gauge needle are used for sampling.

Glassware: 1 mL plastic vials are used

Microsyringes: 10, 50 and 100 µL microliter syringes (Unimetric) are used.

Analytical Balance: 0.0001 g accuracy.

Data Acquisition and Analysis: A Windows based chromatographic data acquisition system, PE-Nelson Turbochrom 3.0, is used. The system is interfaced to the GC via a 900-Series A/D link box, to provide for continuous storage of raw chromatograms in a PC, for subsequent off-line batch analysis.

5. REAGENTS

Stock Standards: Aqueous stock standards in concentration 1000 mg/L (as a component) were prepared in our laboratory for each anion.

Aqueous Calibration Mix Standards: Those are prepared in concentration range 5 - 200 mg/L (as a component) in reagent water.

Eluents:

E1 -reagent water
E2 - 5.0 mM NaOH
E3 - 100 mM NaOH

Reagent Water: Deionized water (> 18 MOhm) is prepared on demand with a Milli-Q plus water system (Millipore). System cartridges include mixed ion exchange resins, activated carbon and 0.45 µm filter.

6. SAMPLE COLLECTION, PRESERVATION AND HANDLING

Samples are collected and preserved with concentrated phosphoric acid (if concentration of phosphate is not measured). Before analysis, are samples filtered and diluted (usually 5-500 fold, depending on salt concentration) with reagent water.

7. PROCEDURE

Summary

Anions are introduced into the IC using an autosampler. This method is used directly on waste water samples and aqueous process effluents. Data are acquired by Turbochrom and concentration is presented as chloride , nitrite - N, nitrate - N, sulfate - S, Phosphate - P.

IC Conditions:

Flow: 2.0 mL/min
Sample Loop Volume: 0.5 mL
Expected System Operating Back Pressure: 1100 psi
Pressure Limit: 1600 psi
Gradient Pump Program:

Time (min)	%E1	%E2	%E3	Comments
Equilibration				
0.1	90	10	0	0.5 mM NaOH for 7 minutes
7.0	90	10	0	
Analysis				
0	90	10	0	0.5 mM NaOH, inject (valve 5:ON)
1.0	90	10	0	Inject valve to load position (valve 5:OFF)
2.5	90	10	0	0.5-5.0 mM NaOH in 3.5 minutes
6.0	0	100	0	5.0-38.25 mM NaOH in 12 minutes
18	0	65	35	

Calibration: Five to seven-level calibration curves were generated for individual components, in concentrations ranging from 5 mg/L to 120 mg/L. Linear regression with forced origin was used to calculate the calibration factors.

IC Analysis: The following are representative retention times for individual components:

Compound	*RT (minutes)*
Chloride	5.4
Nitrite	6.0
Nitrate	7.5
Sulfate	9.7
Phosphate	12.7

8. QUALITY ASSURANCE/ QUALITY CONTROL

Laboratory blanks are analyzed at the beginning of each run, followed by two levels of standards, to ensure the integrity of the IC system. Vials with 0.5 mL reagent water are used for blank analysis. QC acceptance criteria (typically ±15%) are established for the response of check standards. If acceptance criteria are not met, the problem is identified, fixed and a new calibration curve is generated.

9. METHOD PERFORMANCE

Accuracy and Precision. Single operator precision, overall precision and method accuracy are directly related to analyte concentrations and not affected by the sample matrices that have been analyzed using this method.

Method Detection Limits. Detection limits are established periodically, by analyzing the standard deviation of response to 7 replicates of a very low level standard.

10. REFERENCES

- Automated Sampler, Gradient Pump and Conductivity Detector Installation and Operation Manual (Dionex)
- Installation Instruction and Troubleshooting Guide for ASRS - I (4-mm)
- Installation Instruction and Troubleshooting Guide for the IONPAC AG 11 Guard Column and IONPAC AS 11 Analytical Column (DIONEX)

APPENDIX E
DETERMINATION OF PGDN DETECTION LIMITS AND EXTRACTION RECOVERY

Determination of PGDN Detection Limits and Extraction Recovery

The method detection limit (MDL) was established for PGDN. Seven replicate standards were prepared at a concentration of 0.68 mg/L. The samples were analyzed by HPLC and the standard deviations (SD) and percent relative standard deviation (% RSD) calculated. MDLs were evaluated from the following expression (Standard Methods, 1989).

$$MDL = 3.14\ (SD)$$

The MDL can be assumed to be equal to the Practical Quantitation Limit (PQL). The results of the MDL calculations are summarized in Table E-1.

Table E- 1. Results of Method Detection Limit Determination

Compound	Standard Conc. (ppm)	No. of Replicates	SD (ppm)	RSD (%)	MDL (ppm)
PGDN	0.68	7	0.03	3.9	0.09

The percent recovery of the solid phase extraction sample preparation was determined by preparing standards at 5 levels (Table E-2) using the solid phase extraction columns (as described in Appendix D). The same level standards were also prepared by direct injection of stock solutions (without solid phase extraction preparation). The standards were analyzed by HPLC as described in Appendix D. The areas of the two sets of standards were plotted and a linear regression performed on the data. The slope of the linear regression is the percent recovery (Figure E-1). The percent recovery of the sep-pak sample preparation was 90.4% and was not a function of the concentration of PGDN in the standard.

Table E- 2. PGDN Solid Phase Extraction (% recovery).

PGDN Concentration (mg/L)	Direct area/10^6	Sep-pak area/10^6
0.525	0.147352	0.190318
1.05	0.313761	0.29251
5.25	1.701388	1.577111
10.5	3.438547	3.107155
20	6.575354	5.931465

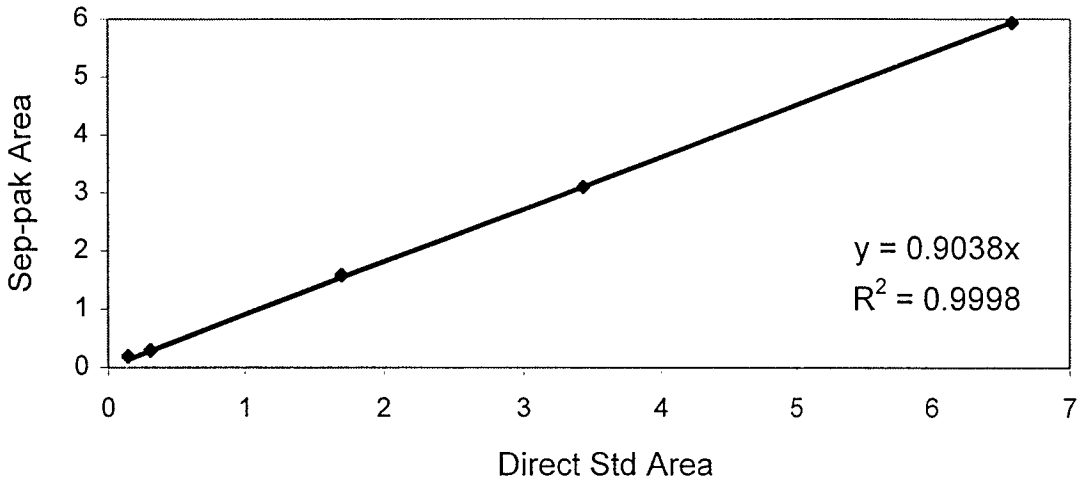


Figure E- 1. Percent Recovery Check for Sep-pak PGDN Standards.

APPENDIX F
RESULTS OF CONSERVATIVE TRACER (LiCl) EXPERIMENTS

Results of Conservative Tracer (LiCl) Experiments

Rationale for Tracer Study

High gas production rates (vol gas/vol reactor-day) were observed in the GAC-FBR used to degrade PGDN under anoxic conditions. During EP1, for example, a rate of 1.9 L gas/L-bed-d was observed (OLR = 10 Kg COD/m^3-d). In order to rule out by-pass due to the gas production as a reason PGDN was observed in the effluent, it was deemed necessary to perform a tracer study. Lithium chloride was chosen as the conservative tracer. The analytical procedure for Li determination is presented in Appendix D.

The hydraulic residence time (HRT) was calculated to be 7.2 minutes. The dispersion number Du/L was calculated to be 0.06 which indicates low dispersion (i.e., system can be essentially treated as a plug-flow reactor per pass). There was no evidence of by-pass flow. The effect of recycle of effluent and its mixing with raw influent water at ratios of 50:1, recycle:influent, make the entire system behave as a CSTR, however. This means that per pass through the reactor, the FBR behaves as essentially a plug-flow reactor despite the relatively high gas production rate.

Table F- 1. Trace Experiment on PGDN Reactor.

Date:	11/21/96
Operator:	Jing/Dan
Analysis:	Connie

HRT:	7.2
Offset:	0

103	259	1116	12672
st^2	s^2	D/μL	test s^2
5.885	0.114	0.060	0.113

The above is a trial and error determination of the dispersion in the reactor. The Dμ/L is a dimensionless dispersion number. Values are defined as follows:

0-plug-flow 0.002-small 0.025-intermediate 0.2-large infinity-CSTR

The value of 0.06 for the PGDN reactor is lower than observed for full-scale aerobic systems with no gas production. Results indicate low dispersion and little by-pass. Analysis method taken from Levenspeil, O., 1962, Chemical Reaction Engineering, John Wiley and Sons, Inc.

										F Curve Data	
Time (min)	Meas. Conc (mg/L)	Increase over Bdgnd (mg/L)	Time Less HRT for Recycle	Corres Conc	Conc Correct	Corrected Tracer C	t	t*C	t^2*C	Theta	C
0.00	0.26	0.01	-10.5	0.00	0.00	0.01	0	0.0	0.0	0.00	0.00
1.00	0.25	0.00	-9.5	0.00	0.00	0.00	1.00	0.0	0.0	0.14	0.00
2.00	0.25	0.00	-8.5	0.00	0.00	0.00	2.00	0.0	0.0	0.28	0.00
3.00	0.25	0.00	-7.5	0.00	0.00	0.00	3.00	0.0	0.0	0.42	0.00
4.00	0.29	0.04	-6.5	0.00	0.00	0.04	4.00	0.2	0.6	0.56	0.01
5.00	1.11	0.86	-5.5	0.00	0.00	0.86	5.00	4.3	21.5	0.69	0.13
5.50	1.62	1.37	-5	0.00	0.00	1.37	5.50	7.5	41.4	0.76	0.21
6.00	2.50	2.25	-4.5	0.00	0.00	2.25	6.00	13.5	81.0	0.83	0.35
6.50	2.86	2.61	-4	0.00	0.00	2.61	6.50	17.0	110.3	0.90	0.41
7.00	3.32	3.07	-3.5	0.00	0.00	3.07	7.00	21.5	150.4	0.97	0.48
7.50	3.56	3.31	-3	0.00	0.00	3.31	7.50	24.8	186.2	1.04	0.52
8.00	3.94	3.69	-2.5	0.00	0.00	3.69	8.00	29.5	236.2	1.11	0.57
8.50	4.11	3.86	-2	0.00	0.00	3.86	8.50	32.8	278.9	1.18	0.60
9.00	4.21	3.96	-1.5	0.00	0.00	3.96	9.00	35.6	320.8	1.25	0.62
9.50	4.31	4.06	-1	0.00	0.00	4.06	9.50	38.6	366.4	1.32	0.63
9.75	4.35	4.10	-0.75	0.00	0.00	4.10	9.75	40.0	389.8	1.35	0.64
10.00	4.48	4.23	-0.5	0.00	0.00	4.23	10.00	42.3	423.0	1.39	0.66
10.25	4.50	4.25	-0.25	0.00	0.00	4.25	10.25	43.6	446.5	1.42	0.66
10.50	4.54	4.29	0	0.00	0.00	4.29	10.50	45.0	473.0	1.46	0.67
10.75	4.59	4.34	0.25	0.00	0.00	4.34	10.75	46.7	501.5	1.49	0.68
11.00	4.71	4.46	0.5	0.00	0.00	4.46	11.00	49.1	539.7	1.53	0.69
11.25	4.73	4.48	0.75	0.00	0.00	4.48	11.25	50.4	567.0	1.56	0.70
11.50	4.88	4.63	1	0.00	0.00	4.63	11.50	53.2	612.3	1.60	0.72
11.75	4.97	4.72	1.25	0.00	0.00	4.72	11.75	55.5	651.7	1.63	0.74
12.00	5.21	4.96	1.5	0.00	0.00	4.96	12.00	59.5	714.2	1.67	0.77
12.50	5.53	5.28	2	0.00	0.00	5.28	12.50	66.0	825.0	1.74	0.82
13.00	6.04	5.79	2.5	0.00	0.00	5.79	13.00	75.3	978.5	1.81	0.90
13.50	6.18	5.93	3	0.00	0.00	5.93	13.50	80.1	1080.7	1.88	0.92
14.00	6.55	6.30	3.5	0.02	0.02	6.28	14.00	87.9	1231.0	1.94	0.98
15.00	7.11	6.86	4.5	0.45	0.44	6.42	15.00	96.3	1444.5	2.08	1.00
16.00	7.52	7.27	5.5	1.37	1.34	5.93	16.00	94.9	1518.1	2.22	0.92
17.00	8.13	7.88	6.5	2.61	2.55	5.33	17.00	90.6	1539.6	2.36	0.83
18.00	8.56	8.31	7.5	3.31	3.24	5.07	18.00	91.3	1643.6	2.50	0.79

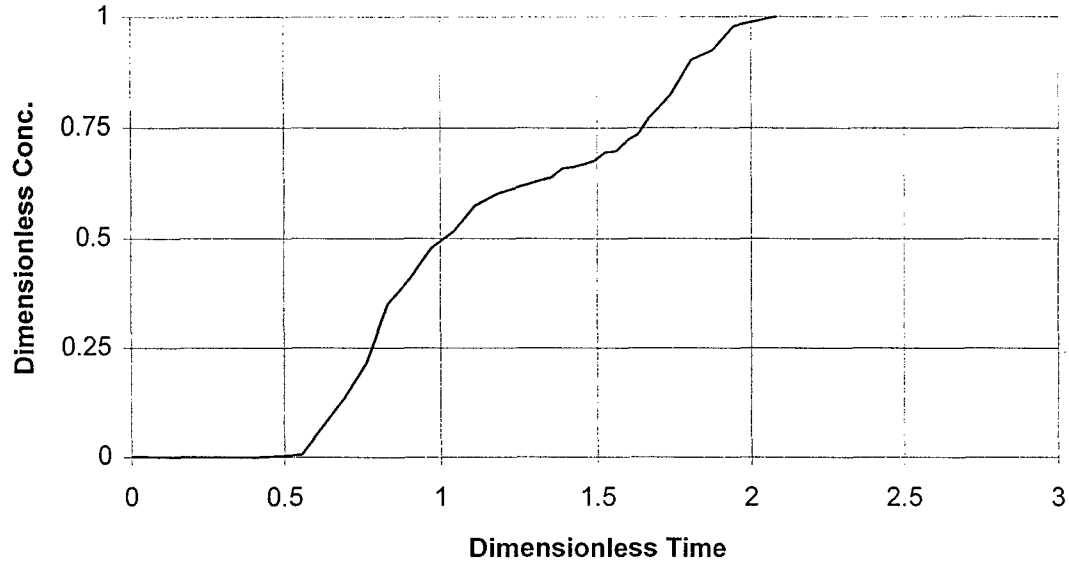


Figure F- 1. Dimensionless Curve.

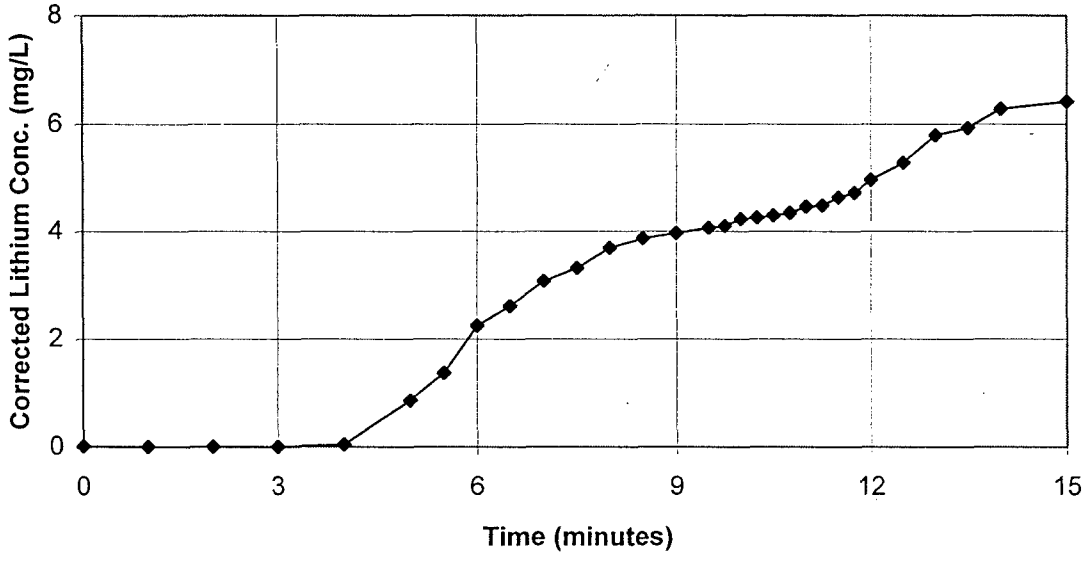


Figure F- 2. PGDN Fluidized Bed Reactor Tracer Experiment. (Bed Height - 307 cm; Overall Inlet Flow Rate - 14.0 ml/min w/o tracer; Flow Rate through Reactor = 1000 ml/min; pH = 8.76 with pH pump off; LiCl as tracer was used 9.7 ml/min in 18 minutes.)

APPENDIX G

SUMMARY OF REACTOR CONDITIONS AND TREATMENT RESULTS FOR A LABORATORY PILOT SCALE GAC-FBR TREATING SYNTHETIC MUNITIONS WASTEWATER

Table G- 1. Summary of Laboratory Pilot Scale GAC-FBR Treatment of PGDN in Biazzi Plant Wastewater and Synthetic Wastewater.

Evaluation Period	EP 1	EP 2	EP 3	EP 4	EP 5	EP 6	EP 7
Primary Substrate	**100% EtOH**	**100% EtOH**	**100% EtOH**	**100% EtOH**	**23% EtOH, 87% PG**	**100% PG**	**100% PG**
Wastewater Type	**Synthetic**	**Synthetic**	**Synthetic**	**Synthetic**	**Synthetic**	**Biazzi**	**Biazzi**
PGDN Conc. (mg/L)							
Feed	96	93	186	373	360	241	251
Effluent (Average ± SD)	0.5±0.2	0.9±0.3	1.8±0.2	4.3±1.1	7±1.6	3.8±0.4	1.8±0.9
PGDN Removal (%)	99.4	99.0	99.0	98.8	98.1	98.5	99.3
PGDN Removal Rates (Kg COD/m^3-d)	0.19	0.22	0.44	0.88	0.84	0.87	0.27
Primary COD/PGDN Ratio	52	21	9	4	14	12	40
Organic Loading Rate (Kg COD/m^3-d)							
Total	10.0	4.9	4.6	4.4	13.2	11.7	11.1
PGDN	0.19	0.22	0.44	0.89	0.86	0.88	0.27
PG	0.0	0.0	0.0	0.0	10.8	10.8	10.8
EtOH	9.8	4.6	4.1	3.5	1.6	0.0	0.0
Total COD/NO_3-N Ratio (<4 indicates excess NO_3)	0.6	0.2	0.2	0.2	0.6	1.0	3.2
Nitrate Loading Rate (Kg NO_3-N/m^3-d)	17.2	21.5	21.5	21.5	21.5	11.8	3.4
Effluent pH	7.7-8.1	8.0-8.2	8.0-8.3	8.0-8.3	7.5-7.6	7.5-8.0	7.9-8.7
Hydraulic Residence Time (hours)	9.7	7.8	7.8	7.8	7.8	5.1	17.4
Bed Parameters							
Bed Height (cm)	292	236	227	231	294	297	298
Bed Volume (liters)	6.2	4.8	4.6	4.7	6.4	6.6	6.6
PGMN Conc. (mg/L)							
Feed						5.2	20.5
Effluent						2.9	1.8
PGMN Removal (%)						44%	91%
PG Conc. (mg/L)							
Feed					2070	2270	7800
Effluent					8.0	8.2	17.8
PG Removal (%)					100%	100%	100%
Timing							
Days	67 to 93	101 to 112	115 to 127	135 to 144	281 to 285	286 to 294	295 to 317
Total Days Operation	27	12	13	10	5	9	23
Dates	2/24 to 3/22	3/30 to 4/10	4/13 to 4/25	5/3 to 5/12	9/26 to 9/30	10/1 to 10/9	10/10 to 11/1

Table G- 2. Conditions During Start-up, Maintenance, Transition, and Feed-Starvation Periods for a Laboratory Pilot Scale GAC-FBR.

Period Designation	SP 1	SP 2	MTP	MTP	MTP	MTP	MTP	MTP	MTP	MTP	FSP	FSP	FSP	FSP	FSP
Period Type	Start-up	Start-up	Maintenance	Maintenance	Testing	Testing	Transition	Transition	Transition	Transition	Feed	Starvation	Feed	Starvation	Feed
Primary Substrate	100% EtOH	100% EtOH	100% EtOH	65% EtOH, 35% PG	55% EtOH, 45% PG	100% EtOH	100% EtOH	75% EtOH, 25% PG	75-25% EtOH, 25-75% PG	100% PG	100% PG	None	100% PG	None	100% PG
Contaminant	None	PGDN	PGDN	PGDN	PGDN	PGDN	None	None	None	None	None	None	None	None	None
Organic Loading Rate (Kg COD/m^3-d)															
Total	4.0	10.0	10.0	5.2-10.3	10.1	9.9	10.2	10.3	10.3	10.1	10.8	0	10.8	0	10.8
PG	0	0	0	1.8-3.5	5.4	0.0	0	2.6	2.6-7.7	5.4	10.8	0	10.8	0	10.8
EtOH	4.0	9.8	9.8	3.4-6.8	4.3	9.5	10.2	7.7	7.7-2.6	4.3	0	0	0	0	0
PGDN	0	0.19	0.19	ca. 0.1	0.38	0.38	0	0	0	0	0	0	0	0	0
Total COD/NO_3-N Ratio (<4 indicates excess NO_3-N)	0.2	0.6	0.5	0.2-0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5		0.5		0.5
Nitrate Loading Rate (Kg NO_3-N/m^3-d)	17.2	17.2	21.5	21.5	21.5	21.5	21.5	21.5	21.5	21.5	21.5	0	21.5	0	21.5
Effluent pH	ca. 8	7.7-8.5	8.0-8.5	8.0-8.5	8.0-8.1	7.5-7.8	8.0-8.6	8.0-8.8	7.7-8.7	7.6-7.9	8.0-8.6	8.7-8.9	8.0-8.8	8.6-8.9	7.8-8.6
Hydraulic Residence Time (hours)	9.7	9.7	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	Reactor in 100% recycle	7.8	Reactor in 100% recycle	7.8
Bed Parameters															
Average Bed Height (cm)	213	248	231	231	234	228	252	252	248	277	309	308	308	313	307
Average Bed Volume (liters)	4.3	5.0	4.7	4.7	4.7	4.6	5.1	5.1	5.0	5.6	7.4	7.3	7.3	7.6	7.2
Timing															
Days	1-42	43-66	145-163	164-182	183-186	188-191	192-211	212-231	232-248	249-280	318-337	338-345	346-352	353-372	373-380
Total Days Operation	42	24	19	18	4	4	20	20	23	26	20	8	7	20	8
Dates	12/19-1/29	1/30-2/23	5/13-5/31	6/1-6/19	6/20-6/23	6/25-6/28	6/29-7/18	7/19-8/7	8/8-8/30	8/31-9/25	11/2-11/21	11/22-11/29	11/30-12/6	12/7-12/26	12/27-1/3

SECTION 3 - EVALUATION OF BIOLOGICAL TREATMENT OPTIONS FOR TRINITROBENZENE

Evaluation of Biological Treatment Options for Trinitrobenzene

Final Report

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May 1996

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COMMON ABBREVIATIONS

DNAB	dinitroaminobenzene
DNT	dinitromethylbenzene (dinitrotoluene)
DO	dissolved oxygen
GC	gas chromatography
HPLC	high performance liquid chromatography
HRT	hydraulic retention time
NDAB	nitrodiaminobenzene
ORP	reductive-oxidative potential
TAB	1,3,5-triaminobenzene
TAT	2,4,6-triaminotoluene
TNB	1,3,5-trinitrobenzene
TNT	2,4,6-trinitrotoluene
VSS	volatile suspended solids

EXECUTIVE SUMMARY

This report details results obtained from the bench-scale feasibility tests for biodegradation of trinitrobenzene (TNB) under aerobic and microaerophilic conditions. The major findings of the experiments are summarized as follows:

- TNB was completely removed in serum bottles inoculated with biological sludges under aerobic, microaerophilic, anoxic and anaerobic conditions at ambient temperature (21-22°C). Major transformation products observed included dinitromethylbenzene (dinitrotoluene-DNT), dinitroaminobenzene (DNAB) and nitrodiaminobenzene (NDAB).

- Complete biological removal of TNB was achieved in one 800-mL bench-scale, all-glass chemostats under aerobic and microaerophilic conditions using ethanol as a primary substrate to support growth.

- Under microaerophilic conditions, TNB was transformed to dinitroaminobenzene (DNAB), then to nitrodiaminobenzene (NDAB) prior to being removed. Degradation of NDAB required the addition of a supplemental nitrogen source (NH_4NO_3 or NH_4Cl).

- Under aerobic conditions, DNAB was the only observed TNB transformation product. This occurred in the absence of an additional nitrogen source.

- No intermediates were observed when an additional nitrogen source was supplied to the chemostat under aerobic conditions.

- Under aerobic conditions, approximately 10-15 mg ethanol/mg TNB removed was achieved in a batch-fed reactor. Complete removal of TNB over a 41-day period was achieved in a continuously fed reactor, with the ethanol/TNB consumption ratio at 50 mg/mg.

- Biological treatment significantly reduced toxicity caused by TNB. Microtoxicity assays indicated that no toxicity was observed in the aerobic and microaerophilic reactor effluents.

1. OBJECTIVES AND STRATEGY

1.1 Background

At many military sites, 2,4,6-trinitrotoluene (TNT) has been found in contaminated water, soil and sediments. TNT has been shown to cause liver damage and anemia in humans (Sax, 1963) and a mutagen by the Ames test (Won et al., 1974). Chemical oxidation of TNT by hydrogen peroxide or ozone or a combination of the two (peroxone) is one of the alternatives for TNT destruction. After oxidation, TNT is decomposed into several organic compounds which can be or may be further mineralized by biological process. Approximately 10% of TNT in groundwater was found to be transformed to 1,3,5-trinitrobenzene (TNB) after peroxone treatment (Hong et al., 1994). It is important to understand under what conditions TNB can be further degraded by natural microorganisms. In addition, 1,3,5-TNB is also found in red water discharged from TNT manufacturing process (Hao et al., 1994).

Due to the strong electron-withdrawing character of nitro-groups, TNB, like TNT, is electron deficient. According to theoretical examinations by Wagniere (1981), the nitro substituent of nitrobenzene withdraws 0.06 electrons from the benzene ring in the electronic ground state. The electron-withdrawing character of nitro-groups and thus the electron deficiency of the aromatic ring impede electrophilic attack by oxygenase of aerobic bacteria.

To our best knowledge, biodegradation or biotransformation of TNB has not been reported. However, significant progress has been made in biotransformation and biodegradation of TNT, 2,4-dinitrotoluene (2,4-DNT) and nitrobenzene. Under aerobic and nutrient-limiting conditions, a variety of fungi, particularly the white rot fungi *Phanaerochaete chrysosporium*, produces a variety of extracellular hydrogen peroxide-generating enzymes which are capable of degrading TNT and 2,4-DNT (Valli et al., 1992; Stahl and Aust, 1995). It was also demonstrated that 2,4-DNT and 2-nitrobenzene can be degraded by dioxygenases of *Pseudomonas* sp. (Spanggord et al., 1991). However, no aerobic bacteria capable of degrading 2,6-DNT has been reported (Rieger and Knackmuss, 1995).

Under strict anaerobic conditions, TNT can be transformed to triaminotoluene (TAT) and then degraded to easily biodegradable compounds by

sulfate reducing bacteria (Boopathy et al., 1993; Preuss et al., 1993) and *Clostridium* sp. (Crawford, 1995). It has been suggested that TNT can be treated using a continuous anaerobic-aerobic process (Rieger and Knackmuss, 1995). First, TNT is reduced to TAT, a highly reduced and reactive compound, under anaerobic conditions, and then TAT is degraded to compounds which are potential substrates for aerobic mineralization. The reduction of DNT to DAT and its subsequent degradation under aerobic conditions has been demonstrated (Berchtold et al., 1995). Transformation of TNT to amino-nitro intermediates and mineralization of ^{14}C-labeled TNT was reported in soil microcosm tests (Bradley et al., 1994).

It is also known that the gratuitous reduction of the nitro-group, in the biodegradation of nitrobenzene, does not require the establishment of special non-indigenous microorganisms. Substrates like glucose, alcohols or acetone are sufficient for activating the anaerobic biomass and supplying the reducing equivalents for the reduction of the nitro-group of nitrobenzene. After adoption of the microbial population, nitrobenzene is stoichiometrically reduced to aniline. In a subsequent aerobic process, aniline is mineralized by the indigenous aniline-degrading bacteria in activated sludge (Dickel et al., 1993). Complete conversion of TNT into 2,4,6-triaminotoluene (TAT) requires strict anaerobic conditions (E_h < -200 mV) (Funk et al., 1993).

The potential of reduction of TNB to triaminobenzene (TAB) has not been reported but would also be low. There is little to no literature available concerning biotransformation and degradation of TNB. If TNB could be transformed to TAB, it could likely be easily mineralized under aerobic conditions. The above two-stage process could be achieved in a microaerophilic or even aerobic reactor system. Due to diffusion limitations of oxygen, the center of activated sludge flocs or interior of biofilms in microaerophilic or aerobic reactors can be anaerobic when bulk liquid has measurable concentrations of dissolved oxygen (DO). Therefore, it is possible that reductive transformation and sequential aerobic degradation could occur within flocs or in biofilm in a single-stage reactor.

Rieger and Knackmuss (1995) reported that TAT underwent rapid autoxidation to generate dark polymers in the presence of oxygen. Little is known about the subsequent steps and the complex mechanism of polymerization. The polymers formed may be extremely stable, like humics. In the same way, reduced

transformation products of TNB, such as TAB, may also form stable polymers in the presence of oxygen. It was reported that TNT can be utilized as sole nitrogen source during anaerobic degradation of TNT by sulfate reducing bacteria (Boopathy et al., 1993; Preuss et al., 1993). It is not known if TNB can be utilized as a nitrogen source for microbial growth.

1.2 Objectives of this Study

The primary focus of this study was to investigate the feasibility of biological transformation and degradation of TNB. Feasibility of treatment was assessed by:

- TNB removal efficiency and rate,
- Formation of TNB transformation products,
- Reductive-oxidative condition requirements,
- Requirement of nitrogen source(s) other than TNB and
- Reduction in toxicity (as measured by Microtox®).

1.3 Experiments Conducted

The experiments conducted were separated into different tests that address one or more of the areas listed above. The specific tests conducted were:

- **Test 1**: Preliminary Serum Bottle Assays. Serum bottle assays were conducted under different conditions (aerobic, anoxic and anaerobic conditions) with different inocula. This test was used to examine environmental conditions under which TNB disappearance was observed.

- **Test 2**: Batch Reactor Test (T1). This test was designed to study time course of TNB removal with an added primary substrate (ethanol) under aerobic conditions. Both TNB and ethanol were added at discrete time intervals. The DO concentration was not controlled during this experiment.

- **Test 3**: Reactor Batch Fed with TNB and Continuously Fed with Ethanol (T2). This test was designed to study time course of TNB removal with continuously added primary substrate under aerobic conditions. The concentration of DO was maintained at a relatively stable level for this experiment.

- **Test 4**: Reactor Batch Fed with TNB and Continuously Fed with Ethanol in the Absence of Additional Nitrogen Source (T3). This test was conducted under both aerobic and microaerophilic conditions to examine whether TNB could be used as a source of nitrogen and if this actually accelerated degradation rates.

- **Test 5**: Continuous Reactor Feed Test without an Additional Nitrogen Source under Microaerophilic Conditions (T4). This test was designed to further study the requirements of an additional nitrogen source other than TNB.

- **Test 6**: Continuous Reactor Feed Test with Added Nitrogen Source under Aerobic Conditions (T5). This test was designed to examine if TNB removal was stable over a long-term period under the conditions identified previously as resulting in complete disappearance of TNB. A second objective was to determine whether there was any toxicity associated with the treated effluent.

2. MATERIALS AND METHODS

2.1 Batch Serum Bottle Tests

Batch serum bottle assays was performed using 158-mL glass serum bottles. Three different conditions (i.e. aerobic, anoxic (denitrification), and anaerobic) were examined, all at ambient temperature (21-23°C). The experimental matrix design for batch serum bottle tests is presented in Table 2-1. Each serum bottle contained 100 mL liquid medium plus 5 mL inoculum. The sludges used as inoculum were aerobic activated sludge from the East Lansing Wastewater Treatment Plant (East Lansing, Michigan) and anaerobic digested sludge from Jackson Municipal Wastewater Treatment Plant (Jackson, Michigan). After inoculation, the serum bottles were placed in a 200 rpm shaker. Liquid samples were taken two hours after incubation. Samples were also taken at 72, 120, and 168 hours, respectively.

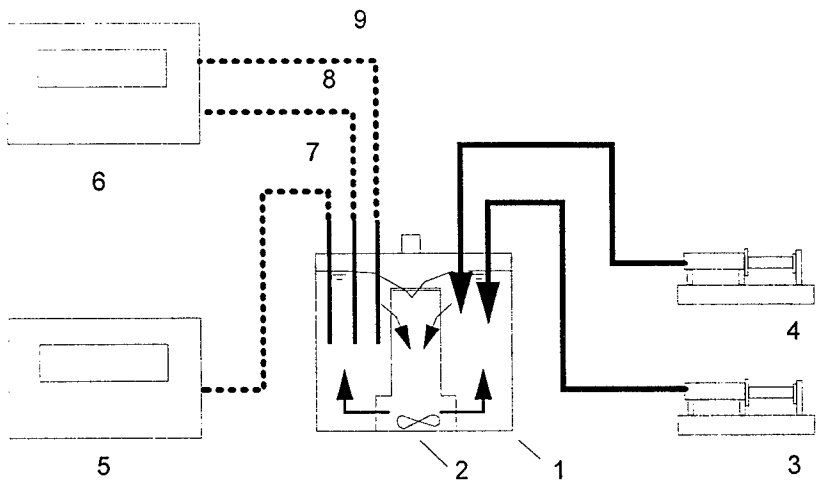
Table 2-1. Experimental Matrix for the Batch Serum Bottle Tests

No.	Condition	Inoculum	Sludge Conc.	Note
1	Aerobic	No		Air atmosphere
2	Aerobic	Aerobic activated sludge	1.0 g VSS/L	Air atmosphere
3	Anoxic	No		Nitrogen atmosphere 7 mM $NaNO_3$ in medium
4	Anoxic	Aerobic activated sludge	1.0 g VSS/L	Nitrogen atmosphere 7 mM $NaNO_3$ in medium
5	Anaerobic	No		Nitrogen atmosphere 0.3 mM Na_2S in medium
6	Anaerobic	Anaerobic digested sludge	1.0 g VSS/L	Nitrogen atmosphere 0.3 mM Na_2S in medium

2.2 Chemostat Reactor System and Tests

The laboratory reactor system used for this work is illustrated in Figure 2-1. This reactor system included an 1,800-mL all-glass reactor, a syringe pump (to feed TNB), a second syringe pump (to feed an ethanol solution), a pH probe and meter, and dissolved oxygen/ORP probes and meter. The reactor itself is illustrated in Figure 2-2. A Teflon-coated magnetic stir bar located within a center "well" in the reactor was driven by a magnetic mixer to achieve complete mixing, aeration and desired DO concentration. The changes in pH, DO and ORP were recorded automatically on a 30 minute basis.

All reactor tests were performed at ambient temperature conditions (21-23°C). The series of five experiments performed using the reactor system are summarized in Table 2-2. Five tests were performed with the reactor system. During the initial batch fed test (T1), the TNB and ethanol solutions were pulsed into the reactor. For the second and third reactor tests (T2 and T3), TNB was pulse fed to the reactor while ethanol was fed continuously using a syringe pump. During the continuous operation tests (T4 and T5), both TNB and ethanol solutions were fed continuously with syringe pumps. The dissolved oxygen concentration in the reactor was controlled by adjusting the mixing speed of the stir bar in the reactor and rate of air or oxygen addition to the reactor.



. Reactor
. Magnetic stir bar
. Syringe pump feeding TNB solution
. Syringe pump feeding ethanol solution
. pH meter
6. DO/redox meter
7. pH probe
8. DO probe
9. Redox potential prob

Figure 2-1. Reactor system used for treatment of synthetic TNB wastewater

Table 2-2. Experimental matrix conducted in the 1800 mL-reactor system

Test Designation	Condition	TNB Feed Method	Ethanol Feed Method	Nitrogen Source
T1	Aerobic	Pulse	Pulse	Not limiting
T2	Aerobic	Pulse	Continuous	Not limiting
T3	Aerobic/microaerophilic	Pulse	Continuous	Limited
T4	Microaerophilic	Continuous	Continuous	Limited
T5	Aerobic	Continuous	Continuous	Not limiting

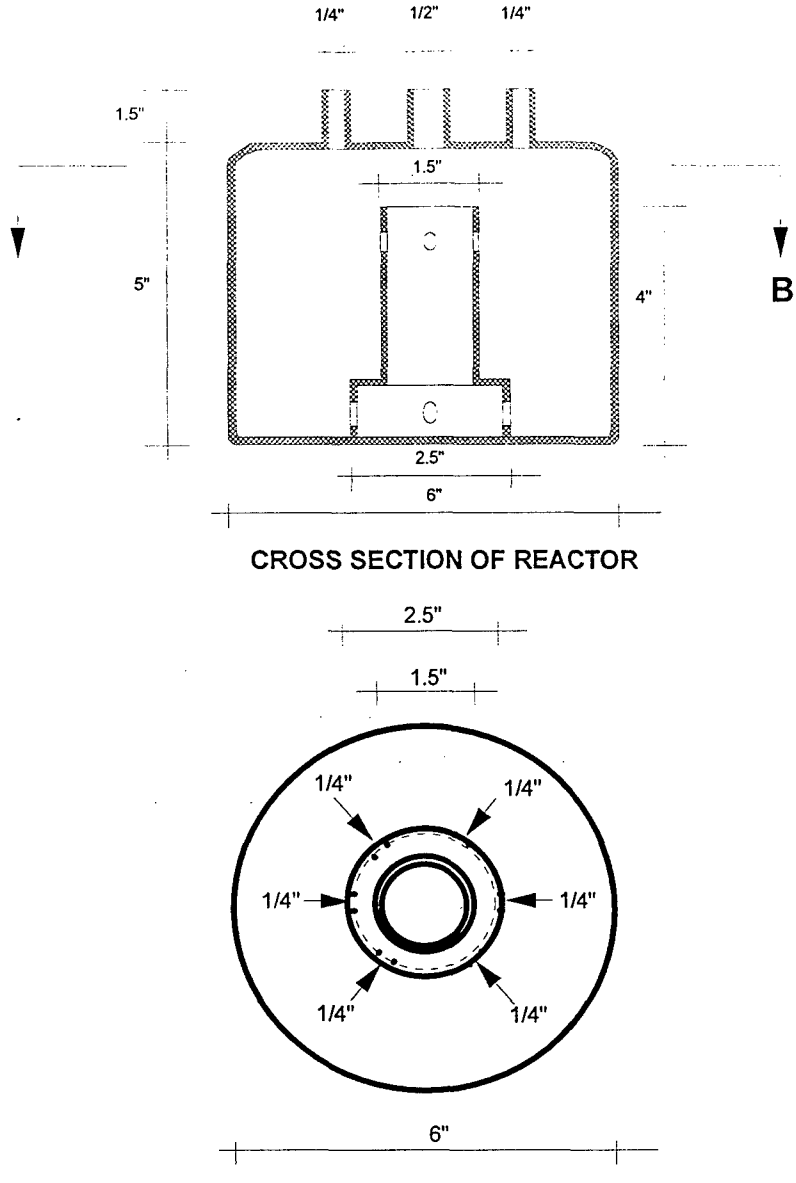


Figure 2-2. Design Details of the Laboratory Reactor System

In all tests, phosphorus was fed to excess and, therefore, not limiting. For tests T1-T3, potassium phosphate was used as a pH buffer (PO_4-P concentration >5 mM or 200 mg/L). During the continuous feed tests (T4 and T5), potassium phosphate was added with the ethanol stock solution. In tests T1 and T2, there was no nitrogen limitation; approximately 20 mg/L of NH_3-N was present initially. In tests T3 and T4, no additional nitrogen source was supplied. Distilled water was used to remove soluble nitrogen from the inoculum sludge. In test T5, ammonium chloride was added with the ethanol solution as nitrogen source. A trace mineral solution (5 mL) was added to the reactor to ensure trace nutrients were not limiting (Table 2-3).

Samples for analysis of nitroaromatic compounds (TNB and its transformation products) and ethanol were taken daily from the reactor using a 20 mL glass syringe. Samples for volatile suspended solids (VSS) analysis were taken periodically. Ethanol was analyzed immediately using chromatography while samples for nitroaromatics were extracted using methylene chloride, concentrated and stored for analysis which were performed in batches when a significant amount of samples had accumulated. Analytical methods used for the determination of nitroaromatics are provided below.

Table 2-3. Composition of Trace Mineral Solution

Component	g/L
$FeCl_2 \bullet 4H_2O$	1.5
$ZnCl_2$	0.07
$MnCl_2 \bullet 4H_2O$	0.1
H_3BO_3	0.06
$CoCl_2 \bullet 6H_2O$	0.19
$CuCl_2 \bullet 2H_2O$	0.002
$NiSO_4 \bullet 6H_2O$	0.024
$Na_2MoO_4 \bullet 2H_2O$	0.26

2.3 Analysis of N-aromatic Compounds

In the batch serum bottle tests, TNB and transformation products were screened using reverse phase HPLC separation in a C18 column. Individual products were identified following liquid-liquid extraction and gas chromatographic analysis with a mass spectrometer as the detector (GC-MS). For the reactor tests, TNB and its transformation intermediates and end-products were identified and quantified using GC-MS in the selective ion monitoring (SIM) mode.

The GC-MS system included a HP5890 Series II *Plus* GC equipped with a HP7673 GC/SFC autosampler, a temperature programmable Merlin Microseal injector, electronic pressure control (EPC) and a HP 5972 mass spectrometric detector. Separations were achieved using a 30 m, 250 µm ID, 0.25 µm film column, temperature programmed with constant flow EPC from 30 to 300 °C. The hardware was controlled using the HP G1034C MS ChemStation (Windows based) program. The MS was operated with a solvent delay of 3 minutes, followed by full scan data acquisition (10-650 AMU) in the electron impact (EI) mode at the rate of 3 scans/ sec for 60^+ minutes. Total ion chromatograms (TICs) were stored on the

computer and were available for real-time and subsequent qualitative analysis. The Wiley 138K Mass Spectral Database library (HP G1035A) was available for on-line probability based matching (PBM) forward and reverse searches.

Quantitative analysis was conducted by scanning samples in the MS for four characteristic ions in each group, in the SIM mode under EI conditions. Calibration curves were prepared using quantitative standards for the same compound or an isomer (if a standard with identical ring position was not available). 1,4-dichlorobenzene (d4) was used as the internal standard in the extracting solvent.

A methylene chloride (MeCl) based micro-extraction procedure was used for preparing the aqueous samples for GC-MS analysis. Samples (20 mL) from the serum bottles and the reactor were transferred to 30 mL glass centrifuge tubes using a 50 mL glass syringe fitted with 0.45µm PVDF syringe filters. Each centrifuge tube (with Teflon lined caps) contained 5 mL of MeCl with the internal standard. 10g of anhydrous sodium sulfate (*Baker Analyzed* pesticide residue grade) was added to the tubes, followed by vigorous shaking for 5 minutes. Following this extraction, the tubes were centrifuged at 7000 RPM for 20 minutes, to separate the MeCl/ Water emulsions. The MeCl layer was removed with a Pasteur pipette and passed through another Pasteur pipette packed with anhydrous Na_2SO_4 (to dry the solvent extract). The moisture free MeCl extracts were collected directly into two GC autosampler vials, one was transferred to the GC-MS and the other archived for future use.

Using the SIM mode, under conditions listed in Table 2-4, method detection limits (MDLs) of 12-45 µg/L (aqueous concentrations) were achieved for target compounds. Individual MDLs are listed in Table 2-5. It should be noted that MDLs for GC-MS/ SIM quantitative analysis are very dependant on SIM parameters. Examples are: the relative abundances of monitored ions within a given compound's mass spectrum, the number of ions monitored in each acquisition group, the scan rate and the detection threshold. It should also be noted that the rapid sample preparation protocol used in these experiments only yielded a concentration factor of 4X. All these parameters can be modified if lower MDLs are desired for specific compounds in the analysis, to meet site specific monitoring requirements.

2.4 Toxicity Reduction Evaluations (TREs)

Relative toxicity of TNB and its transformation products in aqueous solutions was determined using a bioluminescence based Microtox® System (Microbics Corp., Carlsbad, CA). A Microbics Model 500 analyzer was used and the assays were conducted using the extended range basic test protocol, with three controls and twelve 1:2 serial dilutions for each sample. Phenol was used as the positive-control benchmark for testing the validity of the results. Results, expressed as ECXXs, are obtained by observing the sample concentration that reduces light output in the test by XX%. The quantitative effect measured by the Microtox system, light loss, is a measure of the *rate* of biological activity, rather than a count of organisms affected (quantal data). The use of metabolic (respiratory) rate data, integrating the response of about 10^6 organisms, provides a level of confidence in the ECXX values (expressed as 95% confidence intervals) that is not available in conventional toxicity assays.

Table 2-4. GC-MS Characterization of TNB and its Transformation Products

Name	MF	MW	RT (min)	Characteristic Ions
*1,4-dichloro benzene-d4**	$C_6H_4Cl_2$	151	10.72	52, 78, 115, 150
o-nitro amino benzene	$C_6H_4(NO_2)(NH_2)$	138	22.50	65, 80, 92, 138
m-dinitro benzene	$C_6H_4(NO_2)_2$	168	23.57	30, 50, 76, 168
2,4-dinitro methyl benzene (DNT)	$C_6H_3(CH_3)(NO_2)_2$	182	25.65	30, 63, 88, 165
2,4-dinitro benzaldehyde	$C_6H_3(NO_2)(CHO)$	196	27.31	50, 75, 108, 120
1,3,5-trinitro benzene	$C_6H_3(NO_2)_3$	213	29.10	30, 75, 91, 213
2-nitro 1,4-diamino benzene	$C_6H_3(NO_2)(NH_2)_2$	153	30.83	53, 80, 107, 153
2,4-dinitro amino benzene	$C_6H_3(NO_2)_2(NH_2)$	183	35.62	52, 63, 91, 183
* *Internal Standard*				

2.5 Other Analytical Methods

Ethanol was determined using a Hewlett-Packard (HP) 5890A gas chromatograph equipped with a flame ionization detector. Separation was performed by using a 80/20 Carbopack B-DA/ 4% Carbowax 20M column (Supelco, Bellefonte, PA) at 110°C with helium as carrier. The injection was performed by using an HP autosampler. An injection volume of 100 µl was used. External calibration standards (5 point standard curves) were used for quantification.

DO and ORP determination was made using an Orion 960 Autochemistry System (Orion Research Inc., Boston, MA) equipped with an Orion O_2 electrode (Model 97-08-99) and Orion Comb. Redox electrode (Model 9678 BN). These probes were mounted directly in the reactor. The pH in the reactor was monitored using a Chemcadet pH meter/controller (Cole Palmer Instrument Company, Chicago, IL). This probe was also mounted in the reactor. Volatile suspended solid (VSS) was determined after the samples (40 mL each) were dried at 105°C overnight and then heated at 550°C for at least two hours, in accordance with *Standard Methods*.

2.6 Chemicals

1,3,5-trinitrobenzene (TNB) and other nitroaromatic compounds were obtained from Chem Service (West Chester, PA), as detailed in Table 2-5. Other reagent grade chemicals and solvents used in this study were obtained from Aldrich Chemical Company, (Milwaukee, WI), Sigma Chemical Company (St. Louis, MO.), or Malinckrodt, Inc. (Paris, KY).

Table 2-5. Quantitative Analysis for TNB and its Transformation Products

CAS #	Name	MDL (µg/L)	Purity	Source	Lot #
3855-82-1	*1,4-dichloro benzene-d4**	27	98%	Chem Service	144-80A
88-74-4	o-nitro amino benzene	41	99%	Chem Service	124-96B
99-65-0	m-dinitro benzene	40	99%	Chem Service	140-50A
121-14-2	2,4-dinitro methyl benzene (DNT)	45	99%	Chem Service	132-93A
528-75-6	2,4-dinitro benzaldehyde	18	100%	Chem Service	55-85H
99-35-4	1,3,5-trinitro benzene	12	99%	Chem Service	129-101A
5307-14-2	2-nitro 1,4-diamino benzene	31	100%	Chem Service	55-153A
97-02-9	2,4-dinitro amino benzene	28	98%	Chem Service	136-129B
* *Internal Standard*					

3. RESULTS

3.1 Batch Serum Bottle Tests

The feasibility of treating TNB biologically was first examined in serum bottles under three operating conditions: aerobic, anoxic (nitrate as electron acceptor), and anaerobic conditions. The test matrix is presented in Table 2-1. Each bottle received 8 mg/L of TNB. Bottles without microbial inoculum served as controls. Activated sludge obtained from the East Lansing Wastewater Treatment Plant was used as inoculum for the aerobic and denitrifying (anoxic) tests. Anaerobic digested sludge from Jackson Municipal Wastewater Treatment Plant was used as inoculum for the anaerobic tests.

Samples were analyzed for TNB and its transformation products, using qualitative GC-MS analysis in the full-scan electron-impact ionization mode. Additional samples were also screened by reverse-phase HPLC, using a UV detector. Results, represented by the total ion chromatograms (TICs) of the corresponding samples, are presented in Figures 3-1 through 3-8. Abiotic controls were extracted and analyzed along with the 24 hr samples from corresponding treatments.

3.1.1 Aerobic Conditions

Under aerobic conditions, neither transformation nor removal of TNB was observed in the abiotic control bottle, without addition of aerobic activated sludge (Figure 3-1). Biotransformation of TNB was observed in the bottles inoculated with activated sludge. After one hour of incubation, two predominant intermediate compounds (dinitrotoluene and dinitroaminobenzene) were observed, along with one minor intermediate (dinitrobenzaldehyde), concurrent with significant transformation of TNB (Figure 3-2). After 24 hours of incubation, TNB had disappeared completely, with small amounts of dinitrotoluene and nitrodiaminobenzene, while two isomers of dinitroaminobenzene appeared as predominant transformation products (Figure 3-3). Screening with HPLC-UV indicated that only dinitroaminobenzene remained after 72 hours, with small reductions in its concentrations after 120 and 148 hours.

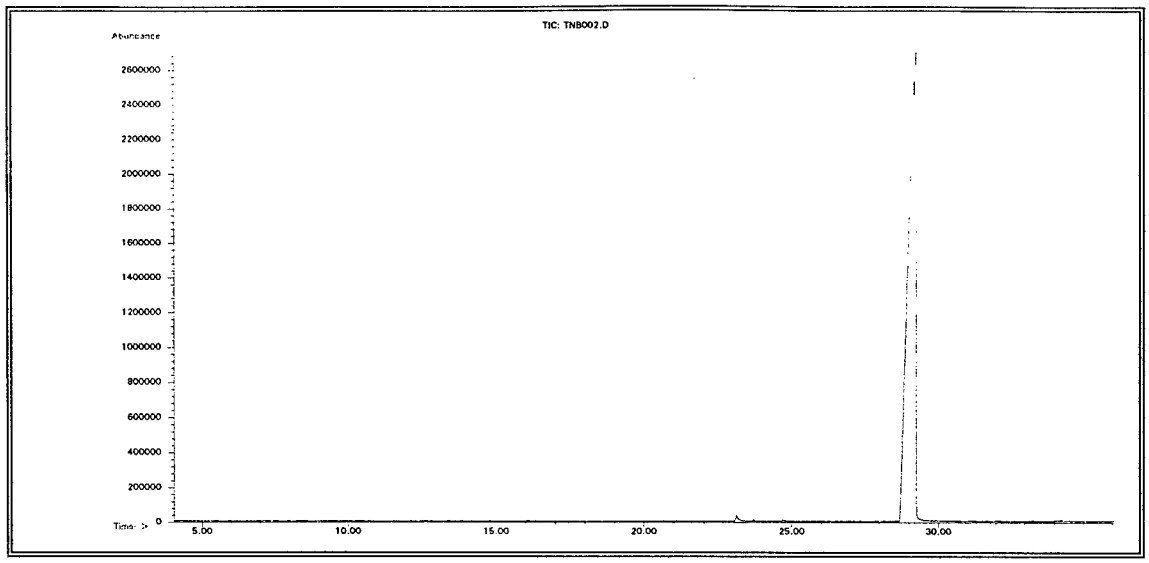


Figure 3-1. GC-MS Chromatogram of the Aerobic Abiotic Control

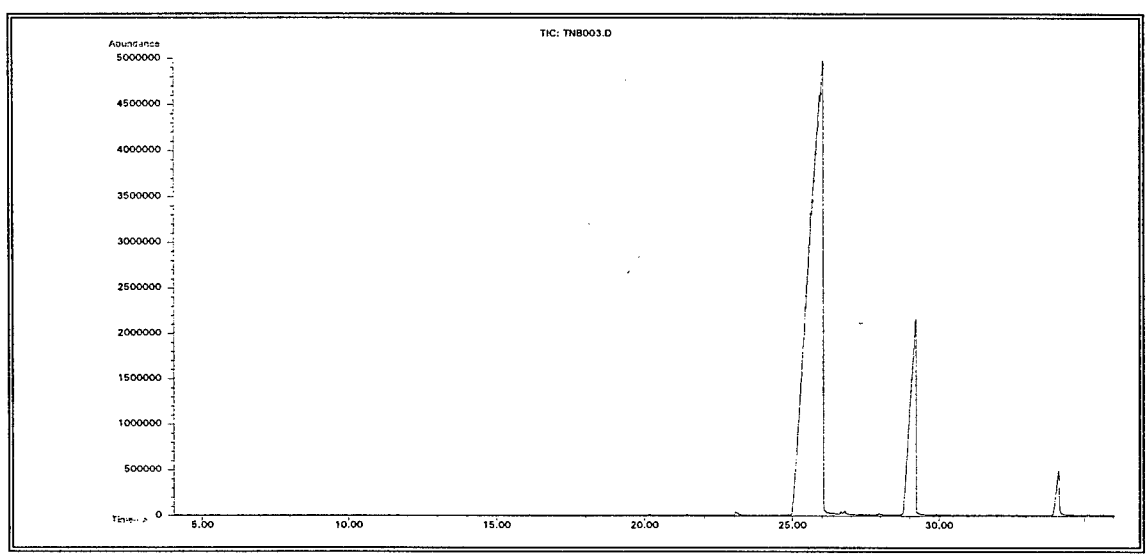


Figure 3-2. GC-MS Chromatogram of TNB's Aerobic Transformation Intermediates (1 hr)

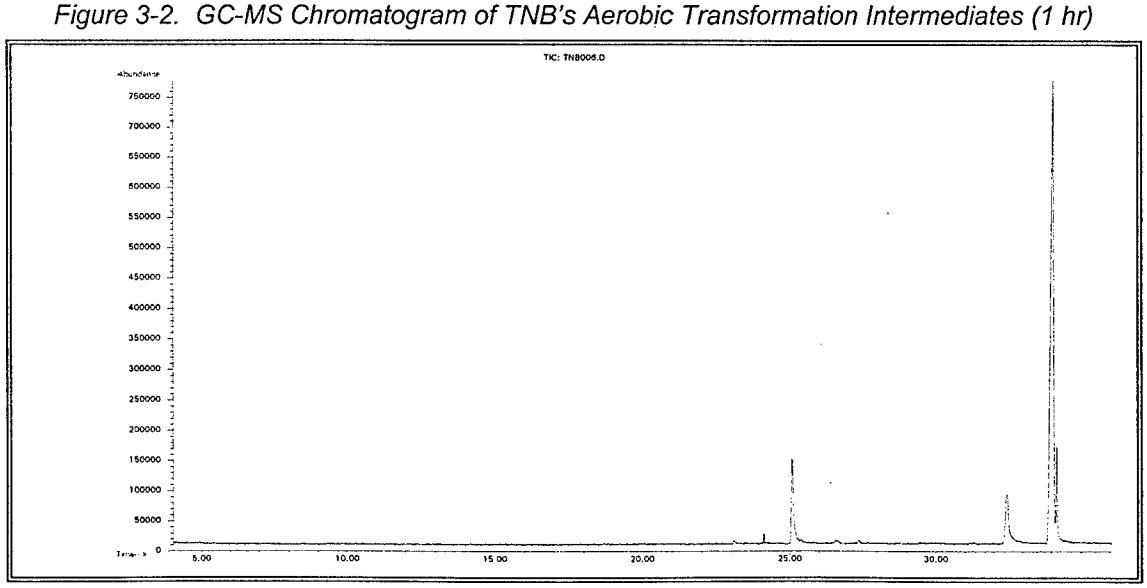


Figure 3-3. GC-MS Chromatogram of TNB's Aerobic Transformation Products (24 hr)

3.1.2 Anoxic Conditions

Under anoxic (denitrifying) conditions, neither transformation nor removal of TNB was observed in the control sample (Figure 3-4). Biotransformation of TNB was observed in the bottles with inoculum. The same intermediate products (dinitrotoluene and dinitroaminobenzene), as those observed under aerobic conditions, were observed after one hour incubation (Figure 3-5).

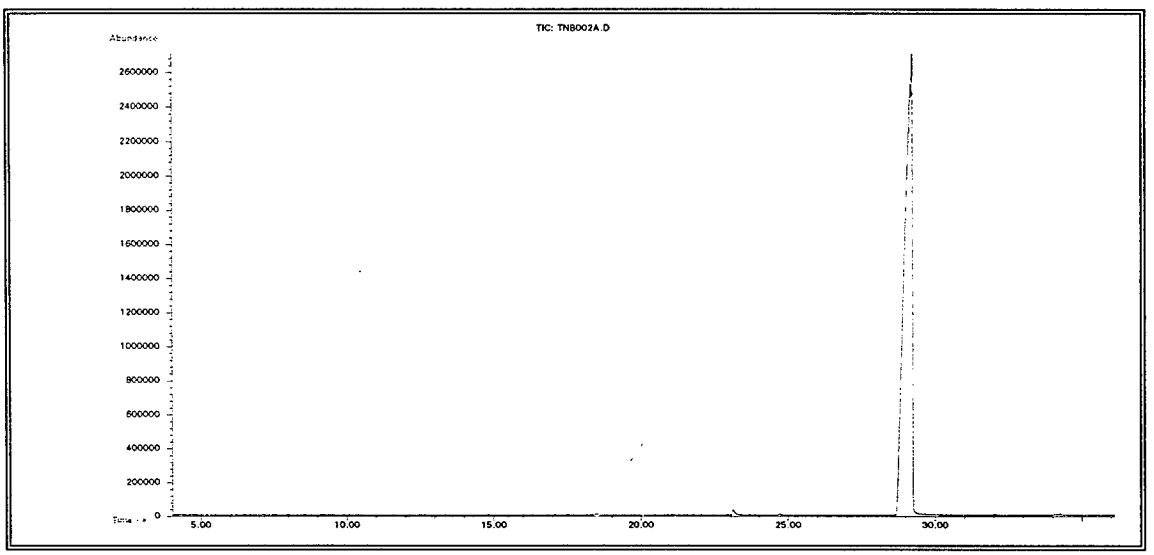


Figure 3-4. GC-MS Chromatogram of the Anoxic Abiotic Control

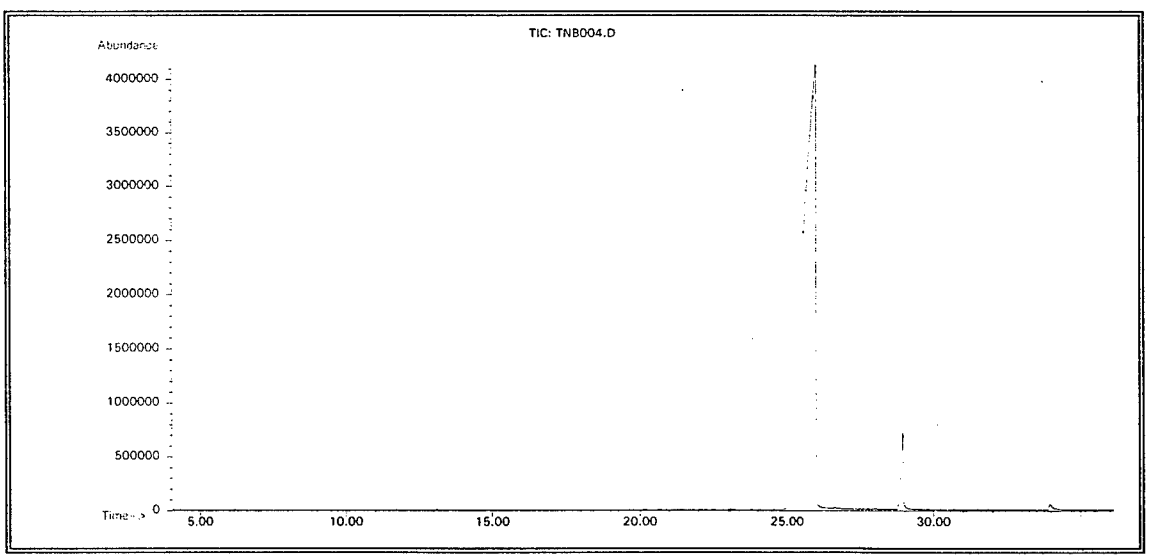


Figure 3-5. GC-MS Chromatogram of TNB's Anoxic Transformation Intermediates (1 hr)

After 24 hours of incubation, TNB had completely disappeared, but transformation products were still observed. The predominant product in the anoxic system was nitrodiaminobenzene, with small amounts of two isomers of dinitroaminobenzene (Figure 3-6). Further transformation of the nitrodiaminobenzene under anoxic conditions proceeded slowly, as observed by HPLC-UV screening after 120,168 and 216 hours.

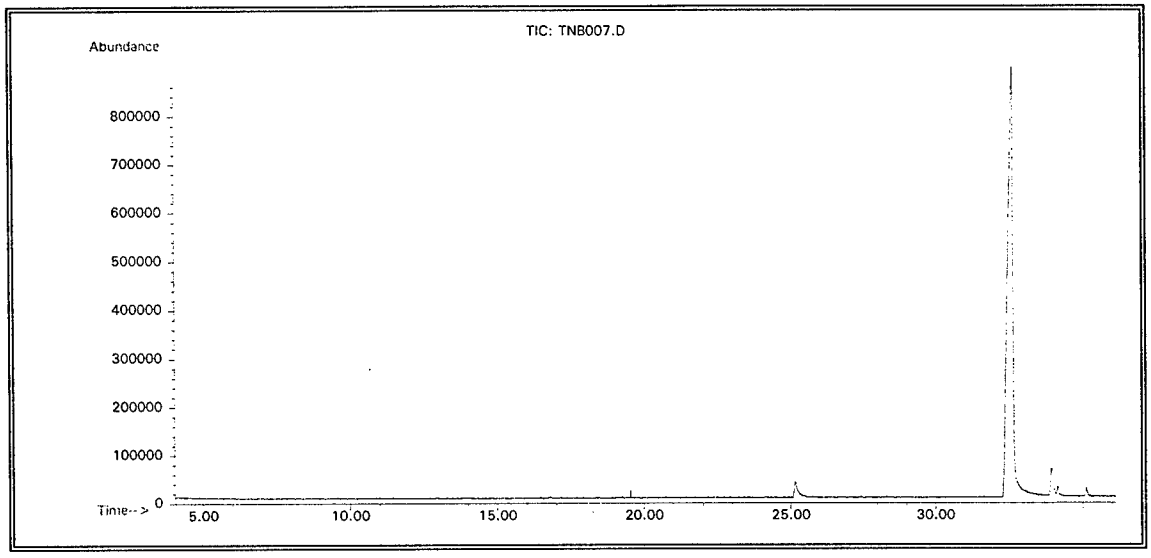


Figure 3-6. GC-MS Chromatogram of TNB's Anoxic Transformation Products (24 hr)

3.1.3 Anaerobic Conditions

Under anaerobic conditions, significant transformation of TNB was observed in the control bottle. After one hours, most of the TNB had been transformed, and small amounts of two transformation products were observed in HPLC-UV screening. More rapid transformation of TNB was observed in the presence of anaerobic digested sludge . Small amounts of TNB were observed in GC-MS analysis after 1 hour, along with trace amounts of dinitrotoluene (Figure 3-7).
TNB was completely transformed, with smaller amounts of dinitrotoluene remaining in the system after 24 hours (Figure 3-8). The predominant component in the chromatogram was elemental sulfur, probably the result of oxidation of the sodium sulfide that was added to establish anaerobic conditions. No TNB was observed in HPLC-UV screening of samples taken after 120 hours of incubation. These results indicate that TNB can be chemically transformed (sulfide used as the reducing compound) under anaerobic conditions. Between 72 and 168 hours of incubation, only very small amounts of dinitrotoluene persisted in the anaerobic system.

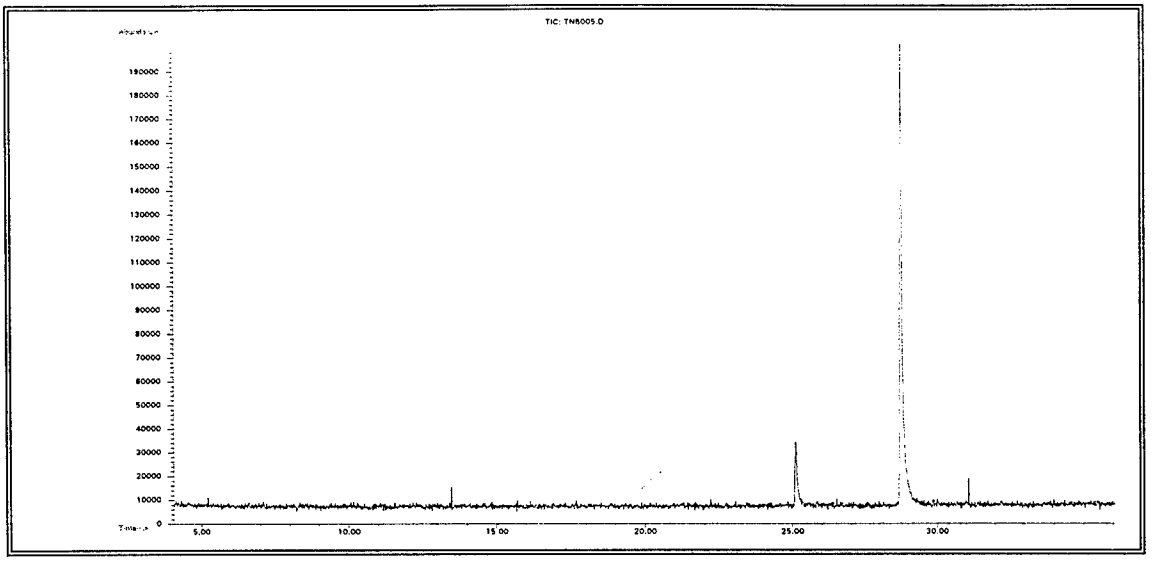


Figure 3-7. GC-MS Chromatogram of TNB's Anaerobic Transformation Intermediates (1 hr)

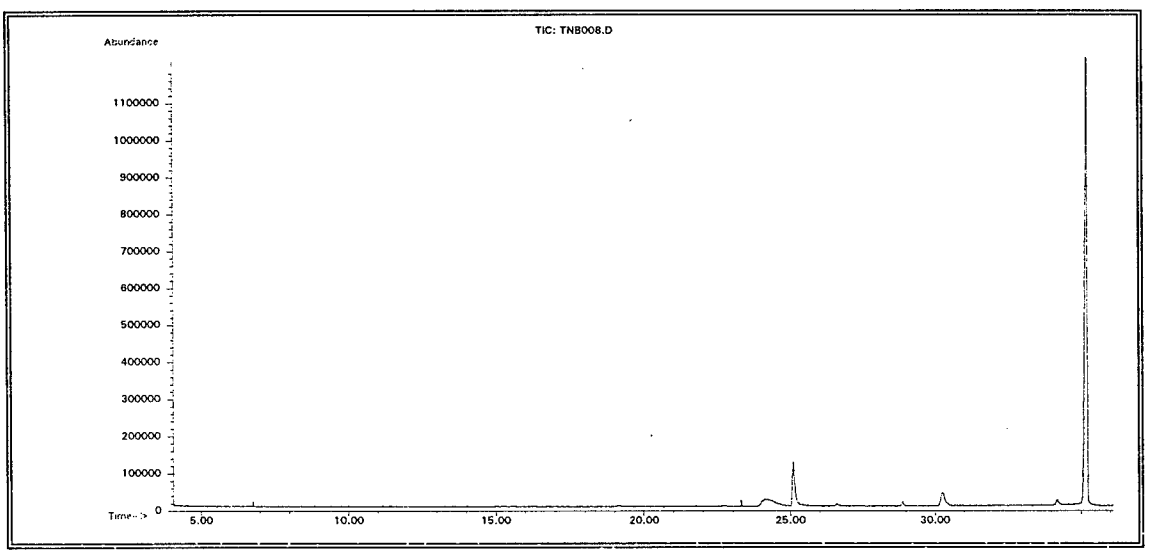


Figure 3-8. GC-MS Chromatogram of TNB's Anaerobic Transformation Products (24 hr)

In summary, TNB was observed to disappear under all three conditions tested, via biologically mediated activity. There was also significant abiotic conversion of TNB under anaerobic conditions. While significant amounts of transformation products persisted in aerobic (dinitro aminobenzene) and anoxic (nitro diaminobenzene) systems, gas chromatographable byproducts were minimal in the anaerobic system. It should be noted that the serum bottles could have been under nutrient limiting conditions, since no nutrients were added to these assays. A summary of all identified products is presented in Table 3-1.

Table 3-1. Summary of TNB Transformation Products Observed in Serum Bottles

Name	Aerobic			Anoxic			Anaerobic		
	Ctrl	*1 hr*	*24 hr*	*Ctrl*	*1 hr*	*24 hr*	*Ctrl*	*1 hr*	*24 hr*
o-nitro amino benzene	-	-	-	-	-	-	-	-	-
m-dinitro benzene	+	+	-	+	+	-	-	-	-
2,4-dinitro methyl benzene (DNT)	-	+++	++	-	+++	+	+	+	+
2,4-dinitro benzaldehyde	-	+	-	-	-	-	-	-	-
1,3,5-trinitro benzene	+++	++	-	+++	+	-	-	+	-
2-nitro 1,4-diamino benzene	-	-	++	-	-	+++	-	-	-
2,4-dinitro amino benzene	-	+	+++	-	-	+	-	-	-
- Not Present, + Present Trace, ++ Present Significant, +++ Present Predominant									

3.2 Chemostat Reactor Runs

Five tests were conducted using an 1,800 mL all-glass reactor system, to study biological removal of TNB under the conditions presented in Table 2-2. Because the biological process was envisioned to be a polishing treatment for peroxone treatment of TNT containing waters, no further tests using anaerobic conditions were performed. The wastewater expected to be produced from chemical oxidation (peroxone) treatment is likely to contain some dissolved oxygen, and exhibit a positive redox potential. Therefore, it was judged to be more cost-effective to treat the wastewater aerobically. TNB removal in the bench-scale reactor was examined under both aerobic and microaerophilic conditions (oxygen added but the DO concentration held at near zero).

3.2.1 Aerobic TNB Removal (pulse feed TNB and Ethanol) - T1

This experiment was conducted to gauge the requirement of a primary substrate (ethanol) for the TNB removal under aerobic conditions. The experimental results are presented in Figure 3-9, including the changes in the concentration of ethanol, TNB and DO. Ethanol and TNB were pulse fed to the reactor simultaneously on three occasions. The initial concentration of waste activated sludge used was approximately 600 mg VSS/L. The system pH was 6.9. The experiment was conducted over a 288-hour period (12 days).

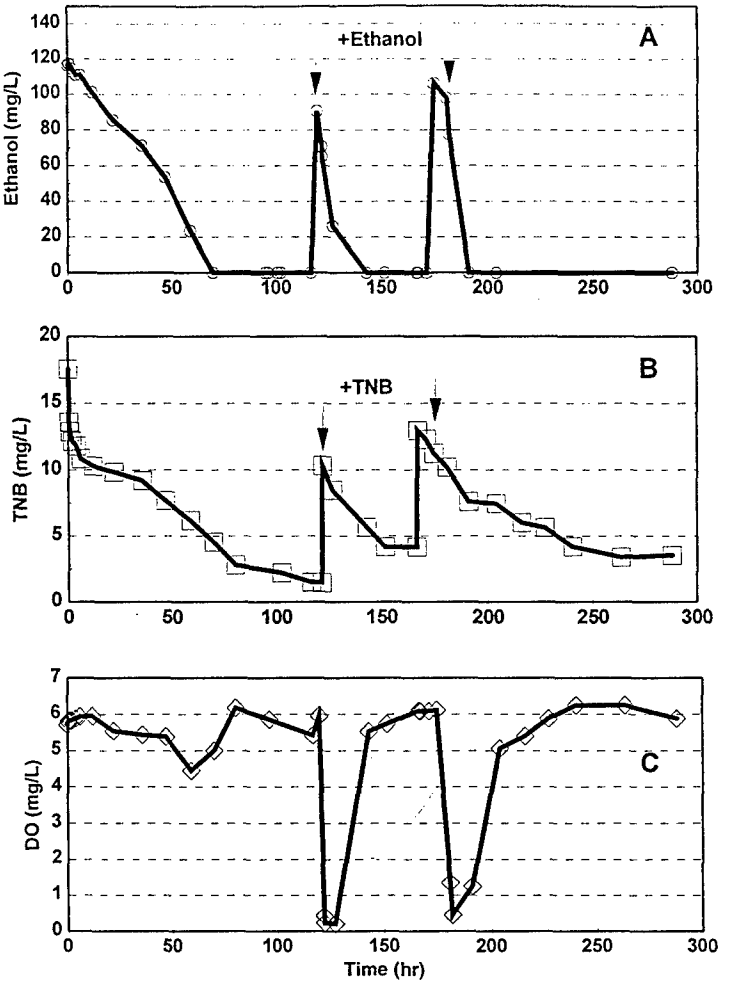


Figure 3-9. Aerobic Reactor Batch-fed with TNB and Ethanol

After ethanol (120 mg/L) was added to the reactor, it was degraded gradually (Figure 3-9a). Within the initial six hours, TNB had essentially disappeared (Figure 3-9b). This initial rapid removal was likely, in part, due to sorption of the TNB onto the inoculated activated sludge. Overall, however, TNB disappearance appeared to be associated with ethanol degradation. The ethanol removal rates and maximum TNB removal rates (in the presence of ethanol) are summarized in Table 3-2. The removal rate of TNB increased as the removal rate of ethanol increased. Rapid TNB disappearance occurred in the presence detectable ethanol concentrations in the reactor. When ethanol was depleted, the removal rate of residual TNB became quite slow. During this experiment, no transformation products were detected in the reactor mixed liquor. Nitrogen was not limiting.

Table 3-2. Removal Rates of Ethanol and TNB in the Batch Fed Aerobic Reactor

Pulse No.	Maximum Removal Rate (mg/L-hr)		Notes
	Ethanol	TNB	
1	2.4	0.14	Rate calculated by using the data from hours 22 to 59.
2	8.5	0.22	Initial rate is used as the maximum rate.
3	9.6	0.23	Initial rate is used as the maximum rate.
Ethanol consumed :TNB removed was 15 mg ethanol/mg TNB (31 mg COD/mg TNB).			

The DO concentration was strongly dependent on ethanol degradation, since ethanol was added to the reactor in pulses (Figure 3-9c). A rapid decline in DO concentration down to near zero occurred immediately after the second and third pulses of ethanol were added. After the added ethanol was consumed, the DO concentration increased to approximately 6.0 mg/L. Throughout the experiment, ORP in the reactor remained above zero, ranging from 20 to 110 mV. The lower ORP (20 mV) was detected after a few hours after ethanol was added. When the ethanol was consumed, the ORP increased gradually to ca. 100 mV as the DO concentration increased.

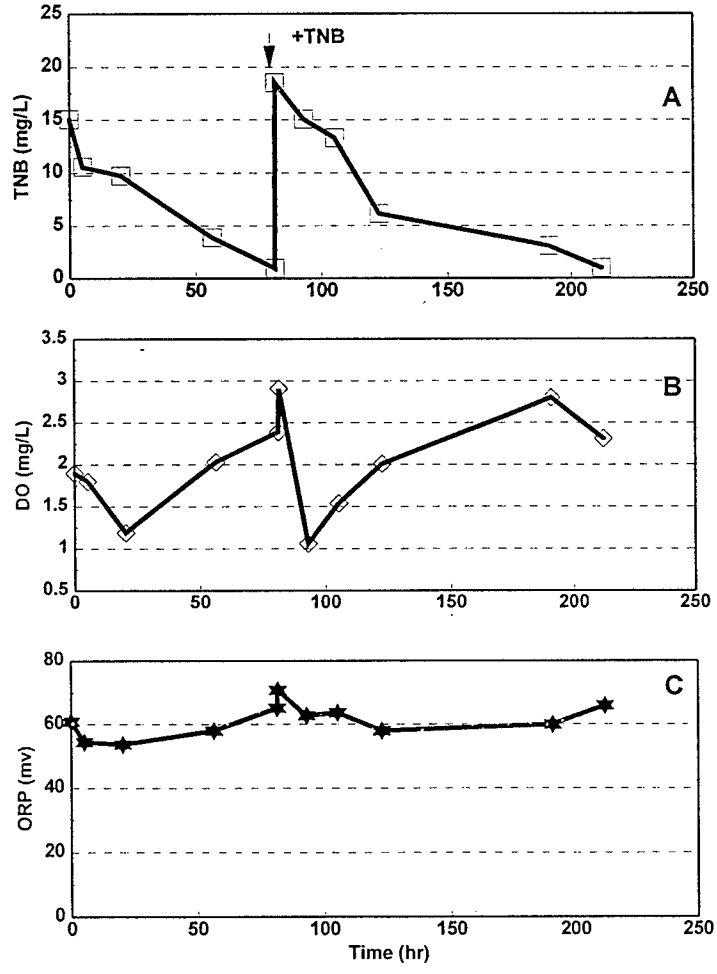
During this experiment, the average ratio between TNB removal and ethanol consumption was approximately 15 (mg ethanol/mg TNB) or 30 mg of COD/mg TNB. The results of this experiment indicate that TNB can be removed under aerobic conditions using ethanol as the primary substrate. Further, results indicate that without a primary substrate to provide reducing power, transformation of TNB was slow.

3.2.2 Aerobic TNB Removal (pulse feed TNB, continuous feed Ethanol) - T2

This experiment was conducted to investigate aerobic TNB removal at relatively stable DO and redox levels. In this experiment, an ethanol solution (50 g/L) was fed to the reactor continuously at a rate of 0.1 mL/hr, resulting in a volumetric ethanol loading rate of 70.6 mg/L-day or COD loading rate of 145 mg/L-d. The DO concentration was maintained between 1 and 3 mg/L. TNB was added in pulses manually.

Experimental results are presented in Figure 3-10, including the changes in TNB concentration, DO concentration and ORP. The experiment was conducted over a 216-hour period (9 days). The operational conditions and TNB removal

performance are summarized in Table 3-3. The ethanol concentration in the reactor was maintained below detection limits throughout. Initially, TNB (15 mg/L) was added to the reactor. This initial pulse was removed to a low level (0.9 mg/L) in three days (Figure 3-10a). A second pulse of TNB (18 mg/L) was then added. This TNB pulse disappeared with five days. The DO concentration in the reactor was maintained above 1.0 mg/L (Figure 3-10b) during these periods. When TNB was pulsed into the reactor, a decline in the DO level was observed. This may be due to DO consumption for degradation of TNB (or its transformation products). The ORP in the reactor was relatively stable (approximately 60 mV) during the entire experimental period. No transformation products from TNB were detected at any time during this experiment.



(Ethanol concentration in reactor was below detection limit)

Figure 3-10. TNB Removal in Aerobic Reactor (Batch TNB and Continuous Ethanol)

Table 3-3. Performance of Aerobic Reactor with TNB Pulse Feed and Ethanol Continuous Feed

Operating Conditions	
Ethanol loading rate (mg/L-day)	70.6 mg as ethanol /L-day or 145 mg TNB/L-day
pH	6.3-6.4
Temperature (°C)	22-23°C
Performance	
Total ethanol consumed (mg)	617
Total TNB removed (mg)	60
Ethanol/TNB consumption rate (as mg ethanol/mg TNB) (as mg COD/mg/TNB)	 10 20.6

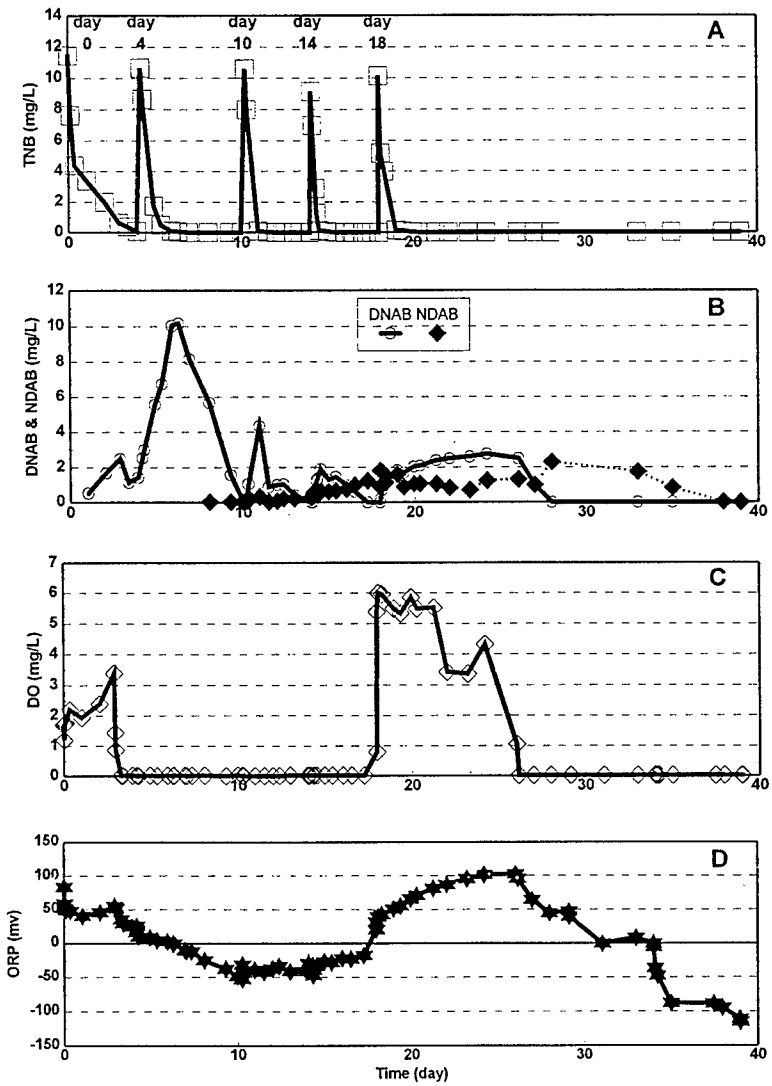
Based on the total amount of TNB removed and ethanol consumed during these experiments, the ratio of ethanol consumption to TNB removed was approximately 10 mg ethanol/mg TNB or 20.6 mg COD/mg TNB. The results of this experiment confirmed that TNB could be removed aerobically without accumulation of transformation products.

3.2.3 Aerobic and Microaerophilic TNB Removal (Nitrogen-limited Conditions) - T3

This experiment was conducted without the addition of a supplemental nitrogen source in order to determine if the nitrogen contained in the TNB could serve as a nitrogen source and support the microbial activity for TNB degradation. The experiment was conducted over a 40-day period. During this experiment, the reactor was operated alternatively under aerobic conditions (from day 0 to day 3, and from day 18 to day 26) and microaerophilic conditions (from day 4 to day 17, and from day 27 to day 40) to study the influence of ORP on the removal of TNB and its transformation products. Ethanol and potassium phosphate served as carbon (or energy) and phosphorus sources, respectively.

After quiescent settling, the supernatant of the activated sludge used in Test T2 was removed. The concentrated sludge (100 mL) was collected in a 2 liter glass beaker and then diluted with phosphate buffered distilled water (1000 mL) to remove residual dissolved nitrogen-containing compounds. After settling again, the concentrated sludge (150 mL) was added back to the reactor and mixed with phosphate buffered, distilled water up to a volume of 1700 mL. A trace mineral solution (10 mL) was added to the reactor. An ethanol stock solution (25 g/L) was

fed to the reactor continuously at a rate of 0.2 mL/hr. The resultant volumetric ethanol loading rate was the same as used in T2 (145 mg COD/L-d). The initial sludge concentration was 200 mg VSS/L. The desired DO concentration and ORP in the reactor were achieved by changing the mixing speed of stir bar and, therefore, aeration levels. The changes in TNB, TNB transformation products (i.e. dinitroaminobenzene (DNAB) and nitrodiaminobenzene (NDAB), DO and ORP are presented in Figures 3-11A-D, respectively. The summary of operational conditions used are presented in Table 3-4.



(Ethanol concentration in reactor was below detection limit)

Figure 3-11. Nitrogen Limited Aerobic Reactor (Batch TNB and Continuous Ethanol)

Table 3-4. Nitrogen Limited Aerobic Operation (TNB Pulse Feed and Ethanol Continuous Feed)

Day	Condition	pH	VSS concentration (mg/L)	TNB Addition
0-3	Aerobic	6.3-6.4	200-220	day zero
4-17	Microaerophilic	6.2-6.3	220-310	day 4, day 10, day 14
18-26	Aerobic	6.2-6.3	310-430	day 18
27-40	Microaerophilic	6.1-6.3	430-640	no addition

From day zero to day 3, the reactor was operated with a DO concentration of approximately 2-3 mg/L (Figure 3-11C) and ORP of 50 mV (Figure 3-11D). TNB was rapidly removed (Figure 3-11A), while the accumulation of DNAB, which was not observed under previous test conditions with added nitrogen source, was observed (Figure 3-11B).

On day 4, the reactor was switched to microaerophilic conditions (DO concentration near zero) by reducing the effective mass transfer rate of oxygen (through controlling the speed of the stir bar). Some gas bubble/liquid contact was visually evident and ORP in the reactor was still above zero, but declining. TNB (11 mg/L) was pulsed into the reactor. This pulse was observed to disappear rapidly. A significant amount of DNAB accumulated, reaching the highest level on day 6. At that time, the ORP was 3 mV. Subsequently, DNAB was removed to near zero as the ORP continued to decrease from near zero to -50 mV. During this period, only a trace amount of NDAB (0.03 mg/L) was observed.

On day 10, TNB was again added to the reactor (11 mg/L), with the ORP at -50 mV. After pulse addition, the TNB disappeared at an equally rapid rate (Figure 3-11a). DNAB was observed followed by the appearance of NDAB (Figure 3-11b). DNAB disappeared by day 13, but NDAB remained at a low concentration (0.4 mg/L). On day 14, TNB was again pulsed into the reactor (10 mg/L) and was quickly removed. DNAB appeared as a transformation product and then was removed. NDAB continuously increased up to 1.2 mg/L by day 17.

On day 18, the reactor was switched back to strict aerobic conditions in order to determine whether NDAB could be degraded under aerobic (and nitrogen-limiting) conditions. The DO concentration was increased to 5.4 mg/L and ORP to 31 mV. TNB was pulsed into the reactor again and its transformation productsoccurred rapidly. After 24 hours, no TNB was observed in the reactor. The DNAB concentration concurrently increased from below detection to 1.8 mg/L; NDAB increased to 1.6 mg/L. These two transformation products did not appear to be

transformed or degraded further under aerobic (nitrogen-limiting) conditions. DNAB and NDAB concentrations remained constant until day 27.

On day 27, the reactor was switched back to microaerophilic conditions. DO was near zero and ORP declined from 100 to -100 mV. Interestingly, DNAB was completely removed as the ORP declined to below zero. The concentration of NDAB increased up to 2 mg/L and then decreased to below detection. In total, twenty days were required for complete removal of these two compounds.

The results obtained from this test indicate that under both aerobic and microaerophilic conditions, transformation of TNB to DNAB and NDAB can occur under nitrogen limiting conditions. The transformation rate of these intermediates was slow compared to their rates of disappearance in the presence of an added nitrogen source.

3.2.4 Microaerophilic TNB Removal (Nitrogen-limited conditions) - T4

During experiment T3, it was observed that TNB was transformed to DNAB and NDAB under both aerobic and microaerophilic conditions but these two transformation products were readily removed only under reduced ORPs condition if no additional nitrogen source was added. The following experiment was conducted to investigate whether TNB and its transformation products were removed under microaerophilic conditions and low ORP (below -100 mV) in a continuously fed reactor without an added nitrogen source. TNB was continuously fed to the reactor used in the experiment T3. In order to reduce the initial biomass concentration, approximately one third of the sludge was removed from the reactor, resulting in a starting biomass concentration of 400 mg VSS/L in the reactor. The operational conditions used are summarized in Table 3-5.

From day 0 to day 44, the reactor was continuously fed TNB (200 mg/L) and ethanol (8.5g/L) solutions at a rate of 1.1 mL/hr. The TNB loading rate was 3.1 mg/L-day and ethanol loading rate was 128 mg/L-d (or 267 mg COD/L-d). The loading ratio of ethanol to TNB was 41 mg/mg. Approximately 52 mL of liquid was withdrawn from the reactor daily to maintain a constant liquid volume of 1,700 mL in the reactor. From day 45 to day 99, the reactor was fed with a more concentrated ethanol solution (50 g/L) and the same TNB solution (200 mg/L) at a rate of 0.1

mL/hr. Approximately 20 mL of liquid was withdrawn from the reactor every four days in order to maintain a constant liquid volume in the reactor.

Table 3-5. Nitrogen Limited Microaerophilic Operation (Continuous Feed of TNB and Ethanol)

Day	Ethanol Loading Rate (mg/L-day)	TNB Loading Rate (mg/L-d)	pH	VSS Conc. (mg/L)
0-44	128	3.1	6.4-6.5	from 400-280
45-99	70	0.28	6.2-6.5	from 280-400
From day 0 to day 44, HRT was 32 days; from day 45 to day 99, HRT was 354 days.				

The reactor system can be modeled as a continuous flow stirred tank reactor (CFSTR). If TNB was not degraded or transformed, the accumulated TNB concentration within initial 44 days can be calculated as:

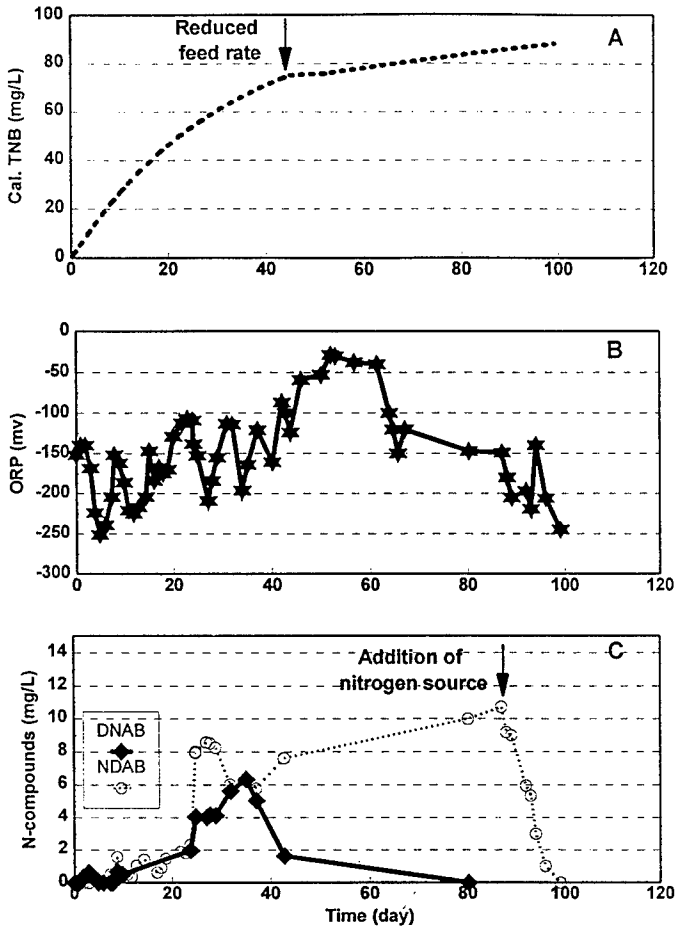
$$C_1 = 0.5C_{inf}\,[1-\exp(-Q_1 t/V)] \qquad (3\text{-}1)$$

where C_1 is TNB concentration in reactor at time t ($t \leq 44$ days); C_{inf} is TNB concentration of stock solution (200 mg/L); Q_1 is the total feed flow rate of TNB and ethanol solutions (52.8 mL/day from day 0 to day 44); and V is the liquid volume of the reactor (1700 mL). During the period from day 44 to day 99, the concentration can be calculated as:

$$C_2 = (0.5C_{inf}-C_{1,44})[1-\exp(Q_2(t-44)/V]+C_{1,44} \qquad (3\text{-}2)$$

where, C_2 is the TNB concentration in the reactor at time t ($t \geq 44$ days); $C_{1,44}$ is the TNB concentration calculated using equation (3-1) at t = 44 days; Q_2 is the total feed flow rate of TNB and ethanol solutions (4.8 mL/day). Based on the equations (3-1) and (3-2), the theoretical accumulation of TNB (with neither degradation nor transformation occurring) versus time is presented in Figure 3-12A.

Throughout the experiment, no accumulation of TNB was observed. This indicates that TNB was completely transformed. Otherwise, the TNB concentrations should have been in excess of 80 mg/L by the end of the experiment. The DO concentration was maintained near zero during the entire time and ORP below -100 mV (ranging between -100 mV and -250 mV) (Figure 3-12B). Appearance of TNB transformation products, DNAB and NDAB, was observed after three days (Figure 3-12C).



TNB and ethanol concentrations in reactor were below detection limits
(On day 83, NH_4NO_3 was added to the reactor and enhanced removal of DNAB).

Figure 3-12. Nitrogen Limited Microaerophilic Reactor (Continuous TNB and Ethanol)

NDAB concentration increased continuously to a peak concentration of 6 mg/L on day 35 and then decreased. NDAB concentration increased to 8 mg/L on day 23. On day 42, the DNAB and NDAB concentrations had declined to 1.8 and 7.6 mg/L, respectively. This result suggested that these intermediates were difficult to remove under the applied loading rates used. This may be attributable, in part, to the microbial growth rate being slower than microbial washout. The VSS concentration in the reactor did decrease over time (Table 3-5).

From day 44 to day 80, the feed of TNB and ethanol solution was reduced from 1.1 mL/hr to 0.1 mL/hr. In addition, the ethanol concentration of stock solution was increased to 50 g/L. This resulted in reduction of TNB loading rate from 3.1 mg/L-d to 0.28 mg/L-d and decrease in ethanol loading rate from 128 mg/L-d to 70 mg/L-d (Table 3-5). The hydraulic retention time (HRT) of the reactor was also increased from 32 days to 354 days. The objective was to study whether the TNB

transformed products, especially NDAB, could be removed by reducing TNB loading rate, and increasing the ethanol/TNB mass ratio from 41 mg/mg to 250 mg/mg. By day 80, the concentration of NDAB increased to approximately 10 mg/L. This indicates that the biodegradation and biotransformation of TNB is very poor under nitrogen limited conditions.

On day 83, a NH_4NO_3 solution was added to the reactor to supply 5 mg/L of nitrogen as N. This was done to examine the effect of an added nitrogen source. After the nitrogen source was added, the residual NDAB was completely removed within 12 days. These results confirmed that TNB can be completely removed via reductive transformation under microaerophilic and nitrogen-limited condition, but that the transformation products are difficult to degrade without the addition of a supplemental nitrogen source.

3.2.5 Aerobic TNB Removal in Continuously fed Reactor - T5

Experiments T1 and T2 provided evidence that TNB was removed under aerobic conditions without accumulation of transformation products. However, these experiments were performed over a short period of time. The following experiment was conducted to verify aerobic removal of TNB in the continuously fed reactor over a long operational period.

The reactor was inoculated with 200 mL of activated sludge from East Lansing Wastewater Treatment Plant and 100 mL of sludge from the reactor used in experiment T4. Tap water was used to dilute mixed liquor to 1,700 mL, resulting in a initial sludge concentration of 400 mg VSS/L. A trace mineral solution (1 mL) was added to the reactor. TNB solution (200 mg/L) and ethanol solution (10 g/L) were fed to the reactor at a rate of 0.65 mL/hr, resulting in a TNB loading rate of 1.84 mg/L-d and ethanol loading rate of 92 mg/L-d. HRT of the reactor system was 54 days. A combination of NH_4Cl, K_2HPO_4 and KH_2PO_4 were added into the ethanol solution as nitrogen and phosphorus sources, respectively (Table 3-6).

Table 3-6. Steady State Operation of Aerobic Reactor (Continuous Feed of TNB and Ethanol)

Ethanol Solution Content (g/L)	
Ethanol	10
NH_4Cl	1.9
K_2HPO_4	0.068
KH_2PO_4	0.132
TNB solution (mg/L)	**200**
Feed rate (mL/hr)	
TNB solution	0.65
Ethanol solution	0.65
Ethanol Loading Rate (mg/L-day)	92
TNB Loading Rate (mg/L-day)	1.84
HRT (day)	54
pH	7.0-7.2
VSS Concentration (g/L)	from 0.4 to 0.7

This experiment was performed over a period of 41 days. Approximately 31 mL of the mixed liquor were removed daily from the reactor for TNB analysis and for maintenance of constant liquid volume. If TNB were not transformed or degraded, the accumulated TNB concentration in the reactor could have been as the same as that calculated by using the equation (3-1) with Q_1= 31.2 mL/day (Figure 3-13A). For example, the TNB concentration would be 60 mg/L on day 41.

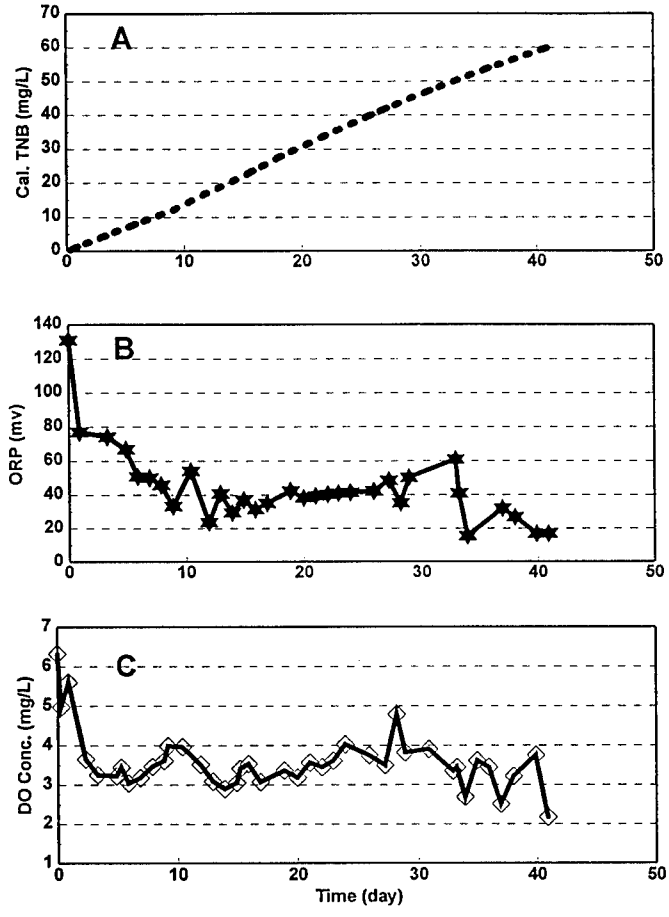
The changes in ORP and DO concentrations in the reactor are presented in Figures 3-13B and 3-13C, respectively. The reactor system was maintained under aerobic conditions with DO concentration above 2 mg/L and ORP greater than 20 mV. Neither TNB nor other TNB transformation products were detected in the mixed liquor throughout the experiment. After 41 days, the VSS concentration in the reactor was observed to have increased from the initial 400 to 700 mg VSS/L.

These results indicate that TNB can be completely removed under aerobic conditions when a primary substrate and nitrogen source are supplied. In this test, the ethanol/TNB consumption ratio was 50 mg ethanol/mg TNB or 103 mg COD/mg TNB.

3.3 Results of Toxicity Reduction Evaluations (Microtox®)

Rapid toxicity tests were conducted to determine the level of reduction in acute toxicity of the aqueous samples after continuous biological treatment in the reactor under both microaerophilic and aerobic conditions. One set of reactor samples were taken from at the conclusion of the microaerophilic treatment of TNB

(T4). The second set of samples was taken on day 41 after aerobic treatment (T5). Stock aqueous TNB solutions (80 mg/L) were used as the benchmark to establish relative reductions in toxicity.



(TNB and ethanol concentrations in reactor were below detection limits)
(No transformation products were detected during 41 days of operation)

Figure 3-13. Aerobic Reactor with Added Nitrogen Source (Continuous TNB and Ethanol)

Microtox results are calculated as effective concentrations for 50% light reduction (EC50), and commonly expressed as percentages of the test solution (TNB solution and the reactor samples). Thus, a higher EC50 would signify a sample of lower toxicity and vice-versa. To assist in intuitive interpretation of these assays, results are converted to arbitrary units called Toxicity Units (TU50), represented by:

$$TU50 = 100/EC50$$

Thus, higher TU50s correspond to higher sample toxicity. A summary of these test results is presented in Table 3-7.

Table 3-7. Summary of Results from Toxicity Reduction Evaluations Using Microtox™

Exposure Time (min)	TU50* (95% Confidence Interval Ranges)		Toxicity Reduction
	Raw TNB Water (80 mg/L)	Treated TNB Water	
5	23-24	4.5-5.5	77.6-80.0%
15	154-200	2-6	97.0-98.9%
30	790-843	2-7	99.1-99.7%
*TU = Toxicity Units			

It was apparent that TNB was a relatively slow acting, acute toxicant on the test organisms, with toxic response increasing over four fold from 15 to 30 minutes. EC50's for TNB correspond to aqueous concentrations of 3.5 mg/L (5 min), 400 µg/L (15 min) and 100 µg/L (30 min), respectively. Dramatic reductions in toxicity were measured in biologically treated samples. It should be noted that these sample (both from microaerophilic and aerobic reactors) were colored (yellowish brown) significantly, which would result in a false positive response in the serial dilutions. The TU50s for treated samples are conservative (high end) values, which were not corrected for color. In contrast to response from TNB, no effect of exposure time was observed, also an indication that much of this response could be form the sample color. The larger confidence intervals for treated samples are the result of the low TU50 values.

3.4 Discussion

3.4.1 Feasibility of Biotreatment of TNB

During this work, the biological removal of TNB under different conditions (i.e. aerobic, microaerophilic or anoxic, and anaerobic), were examined. TNB can be transformed under all these conditions. Technically, it is more practicable to treat the TNB wastewater under aerobic or microaerophilic conditions because wastewater containing TNB is produced by peroxone oxidation of TNT and may contain relatively high concentrations of dissolved oxygen from decomposition of the hydrogen peroxide and ozone. Results of toxicity reduction evaluations indicate that biological treatment virtually eliminates TNB toxicity completely. Both aerobic and microaerophilic conditions are acceptable for treatment of TNB containing wastewater.

The results of the continuous aerobic treatment experiments suggest that a TNB removal rate of at least 1.84 mg/L-d can be achieved without accumulation of any intermediates when VSS concentration ranged from 400 to 700 mg/L. The HRT or mean sludge retention time in the reactor was 54 days. This is similar to the sludge age in an aerobic biological fluidized bed reactor (FBR). In an aerobic FBR, the biomass concentration can easily reach 5-20 g VSS/L or more. Therefore, the aerobic FBR could remove a minimum of 60-200 mg TNB/L-day. Further experiments should be conducted to determine the actual TNB removal rates that can be achieved in a FBR system.

3.4.2 Primary Substrate and Nutrient Requirements

Ethanol was successfully used as primary substrate for the growth of microorganisms capable of removing TNB and its transformation products. During experiment T5, long-term continuous reactor test, TNB was completely removed using an applied ethanol/TNB ratio of 50 mg/mg. The ratio of ethanol/TNB could likely be considerably reduced based on the data obtained from batch-fed reactor tests (T1 and T2). Results of these tests indicate that an ethanol/TNB consumption ratio could be as low as 10-15 mg/mg under aerobic conditions. The optimum substrate/TNB ratio should be determined in future tests at pilot-scale. During peroxone treatment of TNT containing waters, production of unidentified products believed to be aldehydes and ketones was observed. It may be possible that these compounds could be used as the primary substrates, reducing or eliminating the need for addition of an external source of organic carbon such as ethanol.

Based on experimental results, in the presence of added nitrogen source (either ammonium or nitrate), TNB and TNB transformation products did not accumulate and any previously accumulated products were removed. Accumulation of TNB transformation products (DNAB and NDAB) was observed when there was no added nitrogen source, under both aerobic and microaerophilic conditions. This indicates that TNB or its intermediates cannot serve a sole nitrogen source for growth of microorganisms capable of removing these compounds. An additional source of nitrogen is necessary for TNB treatment. In addition, nutrients such as phosphorus, potassium and trace elements may also be needed.

3.4.3 Ultimate Fate of TNB

Under microaerophilic conditions and batch serum bottle assays, DNAB and NDAB were observed to be the primary intermediates of TNB transformation (see Sections 3.1. and 3.3.3). During aerobic treatment, DNAB was detected when a nitrogen source was not supplied (see Section 3.3.3). Based on the observed results, the biotransformation of TNB under both microaerophilic and aerobic conditions could follow the following sequence.

$$TNB \rightarrow DNAB \rightarrow NDAB \rightarrow ?$$

It is not known whether TAB was formed under the different conditions tested in this study (it was not detected under the full GC-MS scans in all serum bottle tests). Subsequently, fragment ions for TAB were not monitored for in SIM analysis of GC-MS samples from tests T1 through T5. It may be that rapid oxidation or instability of TAB resulted in its not accumulating in the system or that NDAB was degraded to some other compound(s). It can be inferred that NDAB can be attacked by oxygenases much easier than TNB and DNAB based on its molecular structure.

Formation of dinitrotoluene (DNT) was observed in the serum bottle tests. However, DNT was not detected during any of the chemostat reactor tests. While the pathway from TNB to DNAB and NDAB through DNT is not apparent, it is clear that DNT's transformation to other compounds was rapid even in the serum bottles. This might explain why no DNT was detected as an inetrmediate in the chemostats.

Triaminotoluene (TAT), the end-product of anaerobic transformation of TNT, is an electron-rich compound and should be readily oxidized by aerobic organisms. Rieger and Knackmuss (1995) reported that TAT underwent rapid autoxidation to generate dark polymers in the presence of oxygen. The polymers formed are thought to be extremely stable. Based on our observations, the supernatant of activated sludge system became much darker in color after one month of operation under either microaerophilic or aerobic conditions. This may be the result of humification of some TNB transformation products. Experiments designed to obtain a strict carbon balance by using radiotracer to determine whether CO_2 is produced from TNB degradation and fraction of TNB carbon bound to sludge and/or polymers would be required to answer these questions.

4. SUMMARY AND SUGGESTIONS FOR FURTHER STUDY

4.1 Summary

- TNB was removed to below detection in serum bottles inoculated with microbial sludge under aerobic, microaerophilic, anoxic and anaerobic conditions at ambient temperatures (21-22°C). Transformation products observed to accumulate include dinitrotoluene (DNT), dinitroaminobenzene (DNAB) and nitrodiaminobenzene (NDAB). TNB was also transformed to DNAB in anaerobic serum bottles via abiotic reactions. However, no further transformation and removal was observed.

- Complete biological removal of TNB was achieved in 1800-mL bench-scale, chemostat reactor systems under aerobic and microaerophilic conditions, using ethanol as a primary substrate to support the growth of microorganisms.

- Under microaerophilic conditions, TNB was transformed to dinitroaminobenzene (DNAB), then to nitrodiaminobenzene (NDAB). Degradation of NDAB required addition of a nitrogen source (NH_4NO_3 or NH_4Cl).

- Under aerobic conditions, DNAB was the only TNB transformation product observed in the absence of an added nitrogen source. No intermediates were observed when an additional nitrogen source was supplied under aerobic conditions.

- Approximately 10-15 mg ethanol/mg TNB removed was used during complete removal of TNB achieved in an aerobic, batch-fed reactor.

- Complete removal of TNB over a 41-day period was achieved in continuously fed reactor operated with an ethanol/TNB consumption ratio of 50 mg/mg.

- Biological treatment removed toxicity caused by TNB. Microtox™ assay results indicate that complete removal of toxicity was achieved under microaerophilic and aerobic conditions.

4.2 Suggested Work for Further Study

The following work is proposed to provide more necessary information for application of biotreatment process:

- Scale up of reactor test using aerobic fluidized bed reactor system with ethanol as primary substrate.

- Laboratory verification tests to identify the carbon flow during TNB transformation and mineralization by using [^{14}C]-labeled TNB.

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Treatment of Ketones in Groundwater Using the GAC-FBR Process

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1.0 INTRODUCTION

1.1 PROCESS DESCRIPTION

The fluidized bed bioreactor is a high rate, biological fixed-film treatment process in which the water to be treated is passed upwards through a bed of fluidized, fine-grained media, such as sand, granular activated carbon or ion exchange resins, on which contiguous films of microorganisms grow. As the water to be treated is passed upwards through the bed of media, biofilms attached to the media remove the organic pollutants from the water. Water is passed through the bed at a velocity sufficient to impart motion or fluidization of the media. This occurs when the drag forces caused by the liquid moving past the individual media particles are equal to the net downward force exerted by gravity (buoyant weight of the media).

Fluidization of fine grained media allows the entire surface of each individual particle to be colonized by bacteria in the form of a biofilm. Surface areas on the order of 300 m^2/m^3 of bed are common in fluidized bed reactor systems. This results in accumulation of biomass concentrations of up to 50,000 mg VSS/L of fluidized bed, which is an order of magnitude greater than the cell mass concentrations obtained in most other biological wastewater treatment processes. Fluidization is key to the ability of this process to concentrate active bacterial mass to high levels on small diameter media (<2 mm) without the clogging experienced with downflow filters. This superior ability to concentrate active bacterial mass in the reactor has considerable theoretical and kinetic advantages to the performance of the reactor. By manipulating the volume of media added to a system and the fluidization velocity, a great deal of control can be extended to the average biofilm thickness and mean cell retention time to optimize overall process performance. The conceptual advantages of biological fluidized bed reactor systems over conventional biological processes include:

- Large surface area for biomass attachment;
- High biomass concentrations;
- Ability to control and optimize biofilm thickness;
- Minimal plugging, channeling or gas hold-up; and,
- High mass transfer properties; maximum contact between biomass and substrate.

In the mid-1980s, it was recognized that the technology may have the potential of substantially reducing the cost of treating groundwater contaminated with industrial wastes. Currently, at thousands of contaminated sites in the U.S., interdiction wells are used to contain VOC pollutants in the subsurface; water that is pumped from these wells is usually treated with conventional air stripping processes and the effluent air is passed through a granular activated carbon (GAC) module to control VOC emissions. This conventional system of treating interdicted water with 10 ppm or less of VOCs can cost $1-3/1000 gallons, due mainly to the expense for GAC replacement/regeneration. This indicated that the opportunity for cost reduction lies in the use of biological treatment to destroy most of the pollutant mass instead of loading it on GAC. Yet it was also recognized that a bioprocess that was designed to replace this type of conventional treatment would have to achieve stringent removal capabilities. These included: 1) the ability to remove xenobiotic pollutants (chemicals foreign to biological organisms) at high efficiencies, 2) mobility, 3) the ability to handle a wide range of concentrations and loadings, and 4) resistance to process upsets due to sudden changes in influent concentration and composition. This pointed to the need for implementing the concept of integrating GAC into the biological fluidized bed reactor as the biomass carrier.

1.2 THE GAC-FBR PROCESS

The granular activated carbon fluidized bed reactor (GAC-FBR) process is a fluidized bed which employs GAC as the solid support for biofilm growth. The use of an adsorbent carrier offers three advantages. First, for xenobiotic pollutants which are non-polar, essentially complete contaminant removal occurs as soon as the system is commissioned due to adsorption. After rapid development of a mature biofilm, removal is due to biological degradation. Second, the effects of shock loads of pollutants and other perturbations may be buffered by the adsorptive capacity of the GAC resulting in a more stable, robust overall performance. Lastly, the process provides general removal of a broad range of pollutants whether they are biodegradable or not.

Over the past four years, EFX Systems, a spinoff company formed between the Michigan Biotechnology Institute (MBI) and Ecolotrol, Inc. of Westbury, NY, has pursued the application of the GAC-FBR for the cleanup of groundwater contaminated with gasoline, complex wastewaters and a number of process effluents. Laboratory and field-pilot data in this effort indicated that the GAC-FBR has the capability of removing >99% of the total VOCs from

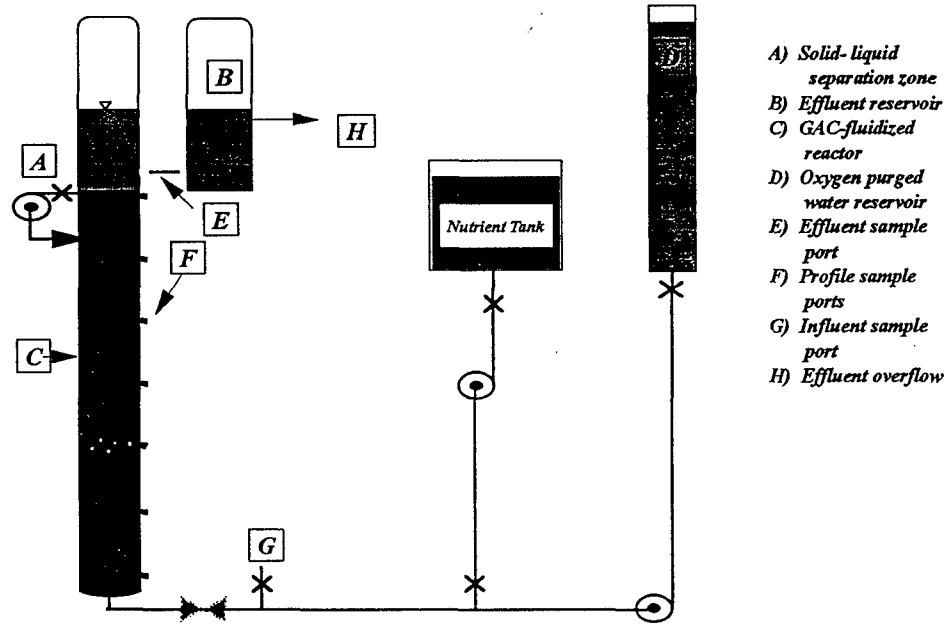
groundwater, with high removals of semi-volatile compounds as well. Full-scale systems are now in operation at field sites with groundwater flow rates as high as 4,000 gpm (5.8 million gallons/day).

2.0 MATERIALS AND METHODS

2.1 PILOT REACTOR

A two-inch diameter, all glass, fluidized bed reactor (FBR) using granular activated carbon (GAC) as the biomass support, was constructed and operated as a single pass system (no recycle), for testing the aerobic treatment of a mixture of acetone, methyl ethyl ketone (2-butanone), and methyl isobutyl ketone (4-methyl-2-pentanone). These ketones are often found at sites as groundwater contaminants. The ratio of the above components (Acetone:MEK:MIBK) in the contaminant mixture used was 8:1:1; target contaminant feed concentrations were 4.0 mg/L, 0.5 mg/L, and 0.5 mg/L, respectively. A schematic of the GAC-FBR system is presented in Figure 1.

Figure 1. Schematic of a Pilot Scale GAC-FBR for Treating Acetone, MEK and MIBK



Reactor construction and hydraulic testing were completed on 8/28/94. An initial charge of 2.72 Liters (L) GAC was added to the 7L working volume glass reactor. Prior to inoculation the system was flushed for 4 days to remove carbon fines and to rinse the GAC.

Nitrogen and Phosphorous were added at a ratio of COD:N: P of 100:5:1, respectively. The influent pH was maintained at approximately 7.0. The contaminant mixture (ketones) was added to the reactor influent using a syringe pump at an initial feed rate of 6.25 μL/min.

2.2 START-UP PROCEDURE

The GAC was inoculated using waste activated sludge obtained from the East Lansing Wastewater Treatment Plant. The system was originally inoculated with 240 mL of sludge added to the influent sampling port of the GAC-FBR with the bed fluidized. When the inocula was visible at the top of the fluidized bed, the flow was stopped and the bed was allowed to settle and remain static for approximately 30 minutes. This was done to increase the contact time of the sludge with the GAC and improve the likelihood of biomass adherence. Fluidization, nutrient delivery, and contaminant delivery were then resumed. This process was repeated three additional times over the ensuing six days with lesser amounts of sludge (60 to 120 mL).

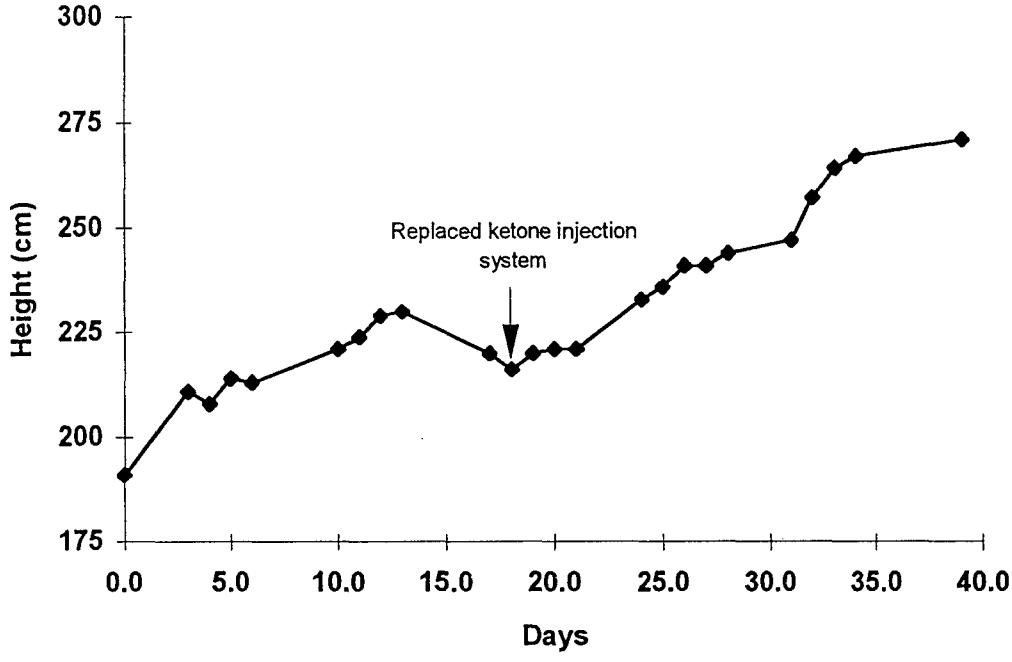
As a biofilm develops on GAC media, the GAC particles become more buoyant and the bed expands. Therefore, the bed height is an effective method to gauge the accumulation of biomass in the GAC-FBR. The change in the fluidized bed height during the inoculation period is shown in Figure 2.

The overall flow rate to the GAC-FBR was maintained at ca. 1000 mL/min. The nutrient flow rate was set at 15 mL/min.

A problem with the contaminant delivery system was encountered during the inoculation period. The contaminant delivery system developed a small leak. This resulted in low and intermittent feed of ketones to the system. Because of decreased loading of ketone, no significant biomass accumulation was evident during the initial 18 days. The original ketone injection system was subsequently redesigned. The new method of ketone injection was more reliable. Following the increase in ketone loading, bed height growth was rapid (see Figure 2).

During this treatability test, three steady-state periods at applied organic loading rates (OLRs) of 0.025 to 2.3 Kg COD/m^3-d were examined. The 0.025 Kg COD/m^3-d steady-state period was designed to test the viability of the GAC-FBR culture at extremely low OLRs, likely to be encountered at the end of a remediation.

Figure 2. Bed Height During Start-up Period



2.3 ANALYTICAL METHODS

Effluent and influent samples were analyzed for ketones using headspace gas chromatography. A detailed description of the analytical method used for this set of treatability experiments is given in Appendix A. The method detection limits (MDL) were established for each of the three ketones of interest, acetone, MEK, and MIBK. Seven replicate standards were prepared at concentrations equal to 10% of the target influent concentrations for each compound. The samples were analyzed by headspace gas chromatography (HSGC) and the standard deviations (SD) and percent relative standard deviation (%RSD) were calculated for each analyte. MDLs were evaluated from the following expression (APHA et al., 1989).

$$MDL = 3.14\ (SD)$$

Because there is no matrix interference between the three compounds, the MDL can be assumed to be equal to the Practical Quantitation Limit (PQL). The results of the MDL calculations are summarized in Table 1.

Table 1. Results of Method Detection Limit Determination					
Compound	**Standard Conc. (ppb)**	**No. of Replicates**	**SD (ppb)**	**RSD (%)**	**MDL (ppb)**
Acetone	40	7	4	10	12.6
MEK	5	7	2.5	50	7.9
MIBK	5	7	0.35	7	1.1

The concentration of dissolved oxygen in the reactor was measured using a YSI (Yellow Springs Inc.) polarographic electrode model 51B; the probe was air calibrated 15 min. prior to use. Temperature was also measured with the model 51B probe.

The pH of the influent and effluent samples were measured using a Cole-Parmer *Chemcadet* pH meter and a Sensorex model 450CD pH probe. The meter was calibrated daily with pH 7.0 and pH 4.0 buffers obtained from Fischer Scientific.

The concentration of solids attached to the GAC in the reactor is used as an indicator of the biomass concentration in the GAC-FBR system. This value was obtained by drying biomass coated GAC for 24 hours at 105°C. The mass of the dry, biofilm coated carbon was determined. The sample was then digested in 4N NaOH for 24 hours, and washed repeatedly with deionized water to remove the digested biomass. Once the GAC samples were rinsed, they were dried at 105°C and reweighed. Attached solids were determined to be the difference in the mass of the GAC plus biofilm after drying and the GAC after digestion and redrying.

3.0 RESULTS

As previously mentioned, acetone, MEK and MIBK are often present as contaminants in groundwater. These compounds cannot be easily removed by either air stripping or carbon adsorption. They are, however, readily oxidized biologically to CO_2 and water. The concentration of ketones observed in groundwater ranges from the µg/L to g/L range. For most sites, the concentrations are on the lower end of the spectrum (below 10 mg/L often less than 500 µg/L). There is some concern that at below 1 mg/L, it is not possible to maintain a viable biological process that will consistently be able to meet stringent effluent treatment criteria. The objective of this treatability work was to test the viability of the GAC-FBR process to treat low concentrations of the ketones of primary concern (acetone, MEK, and MIBK) at typical groundwater conditions.

3.1 STEADY-STATE PERIOD NO. 1

During Steady-State Period No.1 (10/25/94 to 11/23/94) the OLR was maintained at 2.3 Kg COD/m^3-d, which corresponded to target influent concentrations of 4.0 mg/L acetone, and 0.5 mg/L of both MEK and MIBK. The measured influent concentrations of each of these compounds during this period averaged 3087 µg/L, 335 µg/L, and 310 µg/L for acetone, MEK and MIBK, respectively. The calculated influent COD was 11.4 mg COD/L. Results for this steady-state period are summarized in Table 2.

Table 2. Summary of Results and Conditions for Steady-State Period No. 1

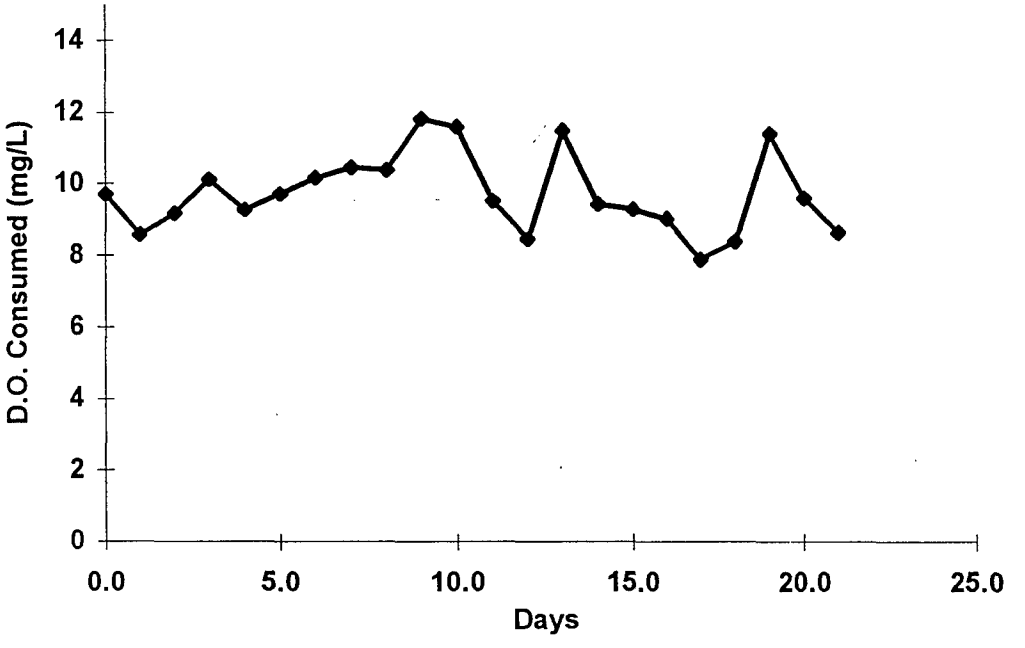
Compound	Influent Conc. (µg/L)	Effluent Conc. (µg/L)	% Removal
Acetone	3087 ± 1061	<12.6*	>99.2
MEK	335 ± 127	<7.9*	>96.9
MIBK	310 ± 128	<1.1*	>99.3

Dissolved Oxygen Influent (mg/L) = 11.0 ± 1.0	OLR = 2.3 Kg COD/m^3-d
Dissolved Oxygen Effluent (mg/L) = 1.3 ± 0.7	HRT = 7.0 min
Dissolved Oxygen Consumption (mg/L) = 9.7 ± 1.0	Temperature (influent) = 17.9°C
pH Range (influent) = 6.5-7.6	*Below method detection limits
pH Range (effluent) = 6.1-7.3	

The effluent concentrations for each of the three ketones tested were consistently below the MDL. This corresponds to greater than 99% removal for acetone and MIBK and greater than 96% removal for MEK.

The consumption of D.O. through the FBR during Steady-State Period No. 1 is shown in Figure 3. There was some variability in the feed of ketones to the system. The peaks in D.O. consumption observed in Figure 3 correspond to the peaks in the influent ketone concentrations. The average influent and effluent D.O. concentrations as well as the average influent and effluent pH values are presented in Table 2. The empty bed hydraulic retention time during this period was 7 minutes.

Figure 3. Dissolved Oxygen Consumption During Steady-State Period No. 1



3.2 STEADY-STATE PERIOD NO. 2

On 11/23/94 the OLR to the reactor was initially reduced by a factor of 10 to 0.23 Kg COD/m^3-d. The OLR to the reactor was maintained at this loading for several days and then reduced further by another order of magnitude. Sequential reduction in the rate of ketone addition continued until the target OLR was reached. During Steady-State Period No. 2 (12/6 - 12/12/94) the reactor was operated at an extremely low OLR to demonstrate viability of the GAC-FBR system at OLRs encountered at a number of sites, particularly near the end of a

remediation effort. Influent and effluent ketone samples were taken twice daily during this time. The target influent concentrations of each of the three compounds were 100 µg/L acetone, and 12.5µg/L each of MEK and MIBK.

To achieve a lower OLR, it was necessary to change to a smaller delivery syringe. After several days of use, it was noticed that this syringe had a defective plunger tip which allowed some of the contaminant solution to leak. This resulted in actual influent concentrations of 50 µg/L acetone and 4 µg/L of both MEK and MIBK. The influent concentration of MEK was below the MDL of 7.9 µg/L. As a result, the values for this compound have no statistical significance. It is clear from the chromatograms, however, that the small amount of MEK in the influent was removed to below detection limits. These influent ketone concentrations were approximately 50% of the target values; the actual OLR achieved during this steady-state period was 0.025 Kg COD/m^3-d. Results of this steady-state period are summarized in Table 3.

Table 3. Summary of Results and Conditions for Steady-State Period No. 2

Compound	Influent Conc. (µg/L)	Effluent Conc. (µg/L)	% Removal
Acetone	52 ± 9.0	<12.6*	>75.8
MEK	<7.9*	<7.9*	--
MIBK	4.1 ± 1.3	<1.1*	>73.2

Dissolved Oxygen Influent (mg/L) = 7.0 ± 0.3
Dissolved Oxygen Effluent (mg/L) = 3.7 ± 0.4
Dissolved Oxygen Consumption (mg/L) = 3.3 ± 0.4
pH Range (influent) = 7.0-7.7
pH Range (effluent) = 6.6-6.8

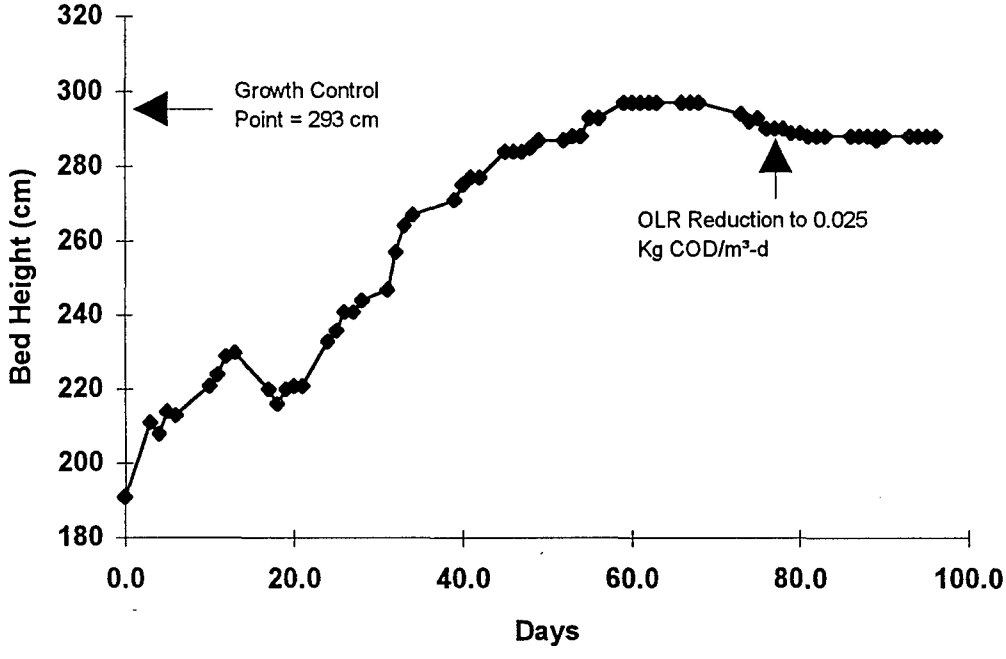
OLR = 0.025 Kg COD/m^3-d
HRT = 7.0 min
Temperature (influent) = 16.0°C
*Below method detection limit

The effluent concentrations for all three test compounds were below the MDL during the entire steady-state period. Unfortunately, the influent ketone concentration achieved wasn't substantially greater than the MDL. As a result it was not possible to determine the percent removal with any degree of confidence.

The low OLR did not affect the viability of the biomass in the system. A viable biofilm coating on the GAC was still visible with the naked eye. The only significant changes that occurred as a result of the lower OLR was a reduction in bed volume of approximately 0.41 L (9 cm. reduction in bed height), due to a reduction in biofilm depth. The bed height for the entire

reporting period is shown in Figure 4. The system biomass maintained the ability to degrade the ketones to below detection limits at this low OLR.

Figure 4. Bed Height During Entire Reporting Period



3.3 STEADY-STATE PERIOD NO. 3

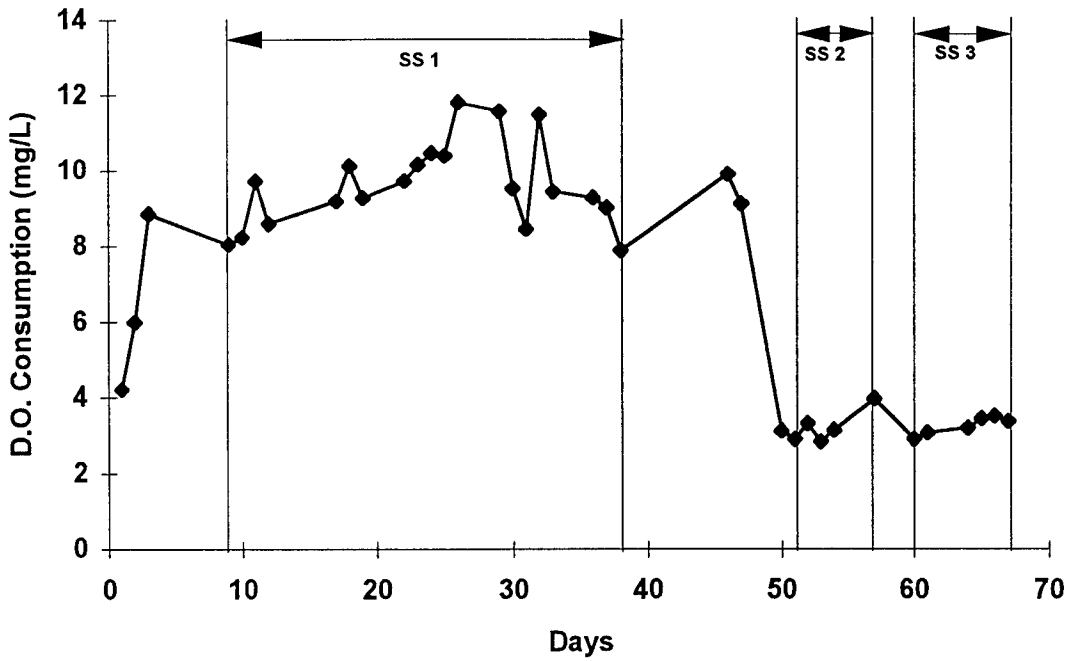
Steady-State Period No. 3 was conducted from 12/15/94 to 12/22/94. During this time the OLR was maintained at 0.23 Kg COD/m^3-d, which corresponded to target influent concentrations of 400 µg/L acetone, and 50 µg/L of both MEK and MIBK. Samples for analyzing the influent and effluent ketone concentration were taken twice daily. The measured influent concentrations averaged 302 µg/L acetone, 27 µg/L MEK, and 30 µg/L MIBK respectively. Results of this steady-state period are summarized in Table 4.

The effluent concentrations for all three test compounds were below the MDL for the entire steady-state period. This corresponds to removals greater than 95% for acetone and greater than 93% for MIBK. Because the influent concentration of MEK wasn't substantially greater than its MDL, the percent removal of this compound could not be calculated with any degree of confidence. The average D.O. consumption through the GAC-FBR during this period was 3.3 mg D.O./L.

Table 4. Summary of Results and Conditions for Steady-State Period No. 3			
Compound	**Influent Conc. (µg/L)**	**Effluent Conc. (µg/L)**	**% Removal**
Acetone	302 ± 142	<12.6*	>95.0
MEK	27 ± 16	<7.9*	>71.0
MIBK	31 ± 21	<1.1	>99.3
Dissolved Oxygen Influent (mg/L) = 6.4 ± 0.3 Dissolved Oxygen Effluent (mg/L) = 3.1 ± 0.3 Dissolved Oxygen Consumption (mg/L) = 3.3 ± 0.2 pH Range (influent) = 6.9-7.2 pH Range (effluent) = 6.4-6.8		OLR = 0.23 Kg COD/m^3-d HRT = 7.0 min Temperature (influent) = 15.6°C Reactor Biomass Conc. = 3.1 g TS/L *Below method detection limit	

Oxygen consumption through the GAC-FBR over the entire test period is presented in Figure 5. Note that as the OLR was decreased during Steady-State Period Numbers 2 and 3, oxygen consumption decreased to ca. 3.0 mg/L. This is considerably higher than would be anticipated for oxidation of the mass of ketones being added (less than 1 mg/L). The difference is likely due to a combination of endogenous respiration, energy for cell maintenance, perhaps some desorption and biodegradation of compounds adsorbed onto the GAC (i.e., by-products and polymers produced during ketone oxidation) or other dissolved organic carbon in the water being used. Oxidation of the GAC is also a potential oxygen sink.

Figure 5. Dissolved Oxygen Consumption During Entire Reporting Period



4.0 DISCUSSION AND SUMMARY

At OLRs ranging from 0.025 to 2.3 Kg COD/m^3-d, ketones (acetone, MEK and MIBK) were consistently removed to below analytical detection limits.

There was little decrease in the reactor bed height and, therefore, little loss of biomass when the OLR was decreased from 2.3 to ca. 0.025 Kg COD/m^3-d, a 100-fold reduction. The concentration of biomass in the GAC-FBR reactor in the subsequent steady-state period when the OLR was increased to 0.23 Kg COD/m^3-d was 3.1 g SS/L. During this period, there was essentially no change in the bed height. This circumstantially indicates that the biomass concentration at an OLR of 0.025 Kg COD/m^3-d was approximately equal to the 3.1 g SS/L observed at an OLR of 0.23 Kg COD/m^3-d. Values ranging from 3-5 g SS/L have been observed during previous experiments where the GAC-FBR was operated at low OLRs. The fact that this value was within previously observed biomass concentration ranges illustrates that a viable biomass population was retained in the reactor even at OLRs as low as 0.025 Kg COD/m^3-d. Because this viable population could be maintained at a low OLR, it is reasonable to assume that the same biomass concentration could be obtained if the system was continuously operated at an OLR of 0.23 Kg COD/m^3-d from the start. This, however, was not experimentally verified here. A complete presentation of all the data collected during this work is presented in Appendix B.

The results of this work clearly illustrate that the GAC-FBR is effective at degrading ketones most often encountered at contaminated sites. This was true at low feed concentrations and high volumetric throughput (7 min HRT). It was also shown that an active biomass could be sustained at extremely low OLRs and still retain the ability to completely degrade ketones.

APPENDIX A

Acetone, MEK, MIBK

Sampling and Storage - Samples are drawn from the influent and effluent ports continuously over a 2-3 minute time period to obtain a representative sample. The samples are collected in a 50 mL glass BOD bottle. The BOD bottles containing the sample are then sub-sampled (12 mL) using all glass, 20 mL syringes (one dedicated to each sampling location) and a 3 inch, 15 gauge needle. Syringes are rinsed with deionized water before sampling and pre-rinsed with sample prior to withdrawal of the 12 mL sub-sample volume. The samples are dispensed into 22 mL glass vials and sealed using Teflon coated septa and aluminum crimp cap seals. Each glass vial contains 7 g NaCl to improve the partitioning of volatiles into the gas phase. The samples are stored at 4°C for no longer than 72 hours before analysis.

Analysis - The ketones are analyzed directly in the sample vial headspace with a Tekmar Headspace Autosampler attached to a Varian 3600 Gas Chromatograph (GC). Separation is accomplished with a Supelco Inc. VOCAL column (60 m, 530 µm ID, 3.0 µm film thickness). Detection is by a flame ionization detector. The method parameters for both instruments were carefully optimized and are as follows:

Tekmar Headspace Autosampler

Platen temperature - 80°C	Sample equilibration time - 60 min
Valve temperature - 150°C	
Line temperature - 210°C	
Vial pressurization time - 0.40 min	Pressure equilibration time - 0.20 min
Loop pressurization time - 0.15 min	Loop equilibration time - 0.20 min
Inject - 0.30	
Vial pressurization - 7.0 psi	Transfer line back pressure - 15.5 psi

Varian 3600 Gas Chromatograph

Initial column temperature - 45°C

Initial column hold time - 6 min

Final column temperature - 200°C

Temperature ramp - 10°C/min

Column hold time - 2.5 min

Injector temperature - 250°C

Detector temperature - 250°C

Detector range - 12

External calibration procedures are used for quantification. Calibration check standards are prepared periodically from stock solutions and analyzed along with an equal number of blanks. Blanks are run to verify no sample contamination from the headspace apparatus. Curves are updated if response varies more than ± 15% from previous response.

Appendix B. Data from Steady-State Period No. 1

		DO (mg/L)				pH				Temperature °C			
Date	# of Days	Influent	Effluent	Uptake	Reservoir	Effluent	Influent	Reservoir	Bed Height (cm)	Influent	Effluent	Overflow (mL/min)	HRT (min
10/17/94	1	4.98	0.75	4.23		6.85	7.66		247	19.5	20.6	1160	6.03
10/18/94	2	8.04	2.06	5.98		8.12	6.67	9.08	257	19.5	20.2	1167	6.00
10/19/94	3	9.71	0.88	8.83	10.33	6.63	6.85	9.04	264	19.6	20.2	1160	6.03
10/20/94	4	10.92	2.88	8.04	10.69	6.34	6.82	8.67	267	19.1	19.7	1153	6.07
10/21/94	5	11.64	3.41	8.23	11.69	6.20	6.60	8.71	256	19.2	19.9	1015	6.90
10/24/94	8	In Kalkaska, No Data											#DIV/0!
10/25/94	9	11.67	1.96	9.71	11.68	6.31	6.71	8.49	271	18.8	19.2	1015	6.90
10/26/94	10	10.85	2.27	8.58	10.85	6.40	6.89	8.50	275	18.4	19.5	1015	6.90
10/27/94	11	11.00	1.82	9.18	11.00	6.63	6.94	8.83	277	18.2	19.2	1015	6.90
10/28/94	12	11.16	1.05	10.11	11.16	6.58	7.18	8.91	277	18.8	19.8	1000	7.00
10/31/94	15	10.05	0.78	9.27	10.05	6.34	6.82	8.74	284	18.8	19.1	1000	7.00
11/1/94	16	10.21	0.51	9.70	10.21	7.00	7.55		284	18.6	19.3	1000	7.00
11/2/94	17	11.03	0.88	10.15	11.03	6.23	6.61		284	18.1	19.3	1000	7.00
11/3/94	18	12.51	2.06	10.45	12.51	6.13	6.69	8.49	285	18.3	18.9	1000	7.00
11/4/94	19	11.09	0.71	10.38	11.09	6.45	6.79	8.63	287	18.8	20.3	1000	7.00
11/7/94	22	12.27	0.46	11.81	12.27	6.74	7.06		287	18.4	18.9	1000	7.00
11/8/94	23	11.99	0.41	11.58	11.99	6.75	7.05	8.86	288	18.1	19.2	1000	7.00
11/9/94	24	10.30	0.79	9.51	10.30	6.62	7.24	8.92	288	17.9	19.4	1000	7.00
11/10/94	25	10.54	2.09	8.45	10.54	6.42	6.89	8.74	293	17.5	19.1	1000	7.00
11/11/94	26	12.21	0.73	11.48	12.21	6.31	7.09	8.82	293	17.6	19.3	1000	7.00
11/14/94	29	10.40	0.98	9.42	10.40	6.27	6.97	8.63	297	17.8	19.3	1000	7.00
11/15/94	30	10.50	1.23	9.27	10.50	6.70	7.18	8.79	297	17.2	18.7	1000	7.00
11/16/94	31	10.45	1.45	9.00	10.45	7.26	6.62	8.87	297	17.2	18.9	1000	7.00
11/17/94	32	8.70	0.82	7.88	8.70	6.36	7.30	8.87	297	17.4	18.7	1000	7.00
11/18/94	33	9.68	1.31	8.37	9.68	6.26	6.86	8.53	297	17.9	18.9	1000	7.00
11/21/94	36	12.41	1.03	11.38	12.41	6.26	6.66	8.61	297	17.2	18.4	1000	7.00
11/22/94	37	11.82	2.25	9.57	11.82	6.44	6.90	8.59	297	16.6	18.0	1000	7.00
11/23/94	38	12.02	3.40	8.62	12.02	6.44	6.51	8.87	297	16.7	18.2	1000	7.00
SS #1 AVERAGES		11.04	1.32	9.72	11.04	7.26	7.55			17.9	19.1	1000	7.00
STANDARD DEV.		0.97	0.74	1.08	0.97	6.13	6.51					1000	7.00

Appendix B. Data from Steady-State Period No. 1

		Acetone (average)		MEK (average)		MIBK (average)		% Removal			
Date	# of Days	Effluent	Influent	Effluent	Influent	Effluent	Influent	Acetone	MEK	MIBK	Notes
10/17/94	1		4374		503		422				Start-up period
10/18/94	2		2683		306		258				
10/19/94	3		4244		493		435				
10/20/94	4										
10/21/94	5										
10/24/94	8										
10/25/94	9	<12.6	2904	<7.9	316	<1.1	288	99.6	97.5	99.5	Start ss#1
10/26/94	10	<12.6	2806	<7.9	309	<1.1	274	99.6	97.4	99.5	
10/27/94	11	<12.6	3945	<7.9	427	<1.1	390	99.7	98.1	99.6	
10/28/94	12	4.00	2093	<7.9	213	<1.1	183	99.4	96.3	99.2	
10/31/94	15										
11/1/94	16										
11/2/94	17	<12.6	2350	<7.9	242	<1.1	203	99.5	96.7	99.3	
11/3/94	18	<12.6	1789	<7.9	169	<1.1	142	99.3	95.3	98.9	
11/4/94	19	<12.6	3688	<7.9	410	<1.1	402	99.7	98.1	99.6	
11/7/94	22	<12.6	3444	<7.9	357	<1.1	340	99.6	97.8	99.6	
11/8/94	23	217.00	3413	<7.9	377	19.00	368	93.6	97.9	99.6	
11/9/94	24	<12.6	3944	<7.9	451	<1.1	421	99.7	98.2	99.6	
11/10/94	25	<12.6	3492	<7.9	382	<1.1	351	99.6	97.9	99.6	
11/11/94	26	<12.6	2988	<7.9	336	<1.1	303	99.6	97.6	99.5	
11/14/94	29	<12.6	652	<7.9	61	<1.1	46	98.1	87.0	96.7	
11/15/94	30	<12.6	2337	<7.9	253	<1.1	245	99.5	96.9	99.4	
11/16/94	31	<12.6	3133	<7.9	338	<1.1	294	99.6	97.7	99.5	
11/17/94	32	<12.6	3459	<7.9	389	<1.1	390	99.6	98.0	99.6	
11/18/94	33	<12.6	3073	<7.9	338	<1.1	330	99.6	97.7	99.5	
11/21/94	36	27.00	5662	<7.9	655	<1.1	612	99.5	98.8	99.8	
11/22/94	37	<12.6	1934	<7.9	171	<1.1	130	99.3	95.4	98.8	
11/23/94	38	<12.6	4625	<7.9	497	<1.1	488	99.7	98.4	99.7	End ss#1
SS #1 AVERAGES			3087		335		310	99.2	96.9	99.3	
STANDARD DEV.			1061		127		128	1.3	2.4	0.6	

Appendix B. Data from Steady-State Period No. 2

		DO (mg/L)				pH				Temperature °C			
Date	# of Days	Influent	Effluent	Uptake	Reservoir	Effluent	Influent	Reservoir	Bed Height (cm)	Influent	Effluent	Overflow (mL/min)	HRT (min
11/28/94	43	10.42	7.30	3.12	10.42	7.11	7.62	8.87	294	16.5	17.9	1000	7.00
11/29/94	44	15.00	12.10	2.90	15.00	6.58	6.87	8.78	292	16.1	17.9	1000	7.00
11/30/94	45	16.53	13.22	3.31	16.53	6.79	6.97	8.97	293	17.0	17.8	1000	7.00
12/1/94	46	13.38	6.91	6.47	13.38	6.92	7.41	8.83	290	16.4	18.2	1000	7.00
12/1/94	46								290	16.4	18.2	1000	7.00
12/2/94	47	12.95	10.08	2.87	12.95	6.81		8.81	290	15.8	16.9	1000	7.00
12/2/94	47	12.95	10.08	2.87	12.95	6.81		8.81	290	15.8	16.9	1000	7.00
12/5/94	50	9.18	6.38	2.80	9.18	6.74	7.24	8.55	289	15.7	17.8	1000	7.00
12/6/94	51	6.70	3.61	3.09	6.70	6.58	7.66	8.77	289	16.8	17.6	1000	7.00
12/7/94	52	6.56	3.39	3.17	6.56	6.64	7.21	8.64	288	16.5	18.0	1000	7.00
12/7/94	52	6.56	3.39	3.17	6.56	6.64	7.21	8.64	288	16.5	18.0	1000	7.00
12/8/94	53	7.21	3.24	3.97	7.21	6.68	7.17	8.60	288	15.9	17.0	1000	7.00
12/8/94	53	7.21	3.24	3.97	7.21	6.68	7.17	8.60	288	15.9	17.0	1000	7.00
12/9/94	54	7.05	3.95	3.10	7.05	6.74	6.99	8.52	288	16.4	17.2	1000	7.00
12/9/94	54	7.05	3.95	3.10	7.05	6.74	6.99	8.52	288	16.4	17.2	1000	7.00
12/12/94	57	7.30	4.60	2.70	7.30	6.82	7.18	8.52	288	14.6	17.2	1000	7.00
SS #2 AVERAGES		6.96	3.67	3.28	MAX-->	6.82	7.66			16.0	17.4		
STANDARD DEV.		0.28	0.44	0.42	MIN-->	6.58	6.99						

Appendix B. Data from Steady-State Period No. 2

		Acetone (average)		MEK (average)		MIBK (average)		% Removal			
Date	# of Days	Effluent	Influent	Effluent	Influent	Effluent	Influent	Acetone	MEK	MIBK	Notes
11/28/94	43										contaminant delivery problem; non-detect in inf and eff.
11/29/94	44										contaminant delivery problem; non-detect in inf and eff.
11/30/94	45										contaminant delivery problem; non-detect in inf and eff.
12/1/94	46	<12.6	357	<7.9	36	<1.1	34	96.5	78.1	96.8	
12/1/94	46	<12.6	180	<7.9	17	<1.1	20	93.0	53.5	94.5	
12/2/94	47	<12.6	182	<7.9	14	<1.1	10	93.1	43.6	89.0	
12/2/94	47	<12.6	117	<7.9	15	<1.1	21	89.2	47.3	94.8	
12/5/94	50	<12.6	262	<7.9	29	<1.1	36	95.2	72.8	96.9	
12/6/94	51	<12.6	59	<7.9	<7.9	<1.1	5	78.6		78.0	Start ss#2
12/7/94	52	<12.6	42	<7.9	<7.9	<1.1	3	70.0		63.3	
12/7/94	52	<12.6	44	<7.9	<7.9	<1.1	3	71.4		63.3	
12/8/94	53	<12.6	63	<7.9	<7.9	<1.1	5	80.0		78.0	
12/8/94	53	<12.6	60	<7.9	<7.9	<1.1	4	79.0		72.5	
12/9/94	54	<12.6	51	<7.9	<7.9	<1.1	5	75.3		78.0	
12/9/94	54	<12.6	56	<7.9	<7.9	<1.1	6	77.5		81.7	
12/12/94	57	<12.6	37	<7.9	<7.9	<1.1	2	65.9		45.0	
SS #2 AVERAGES			52				4	74.7		70.0	
STANDARD DEV.			9				1	4.7		11.4	

Appendix B. Data from Steady-State Period No. 3

		DO (mg/L)				pH				Temperature °C			
Date	# of Days	Influent	Effluent	Uptake	Reservoir	Effluent	Influent	Reservoir	Bed Height (cm)	Influent	Effluent	Overflow (mL/min)	HRT (min
12/13/94	58	6.00	3.20	2.80	6.00	6.31	7.18	8.51	288	15	17	1000	7.00
12/14/94	59	6.00	3.20	2.80	6.00	6.53	6.85	8.71	288	15	16.8	1000	7.00
12/15/94	60	6.39	2.84	3.55	6.39	6.35	6.66	8.71	287	16.7	17.2	1000	7.00
12/15/94	60	6.39	2.84	3.55	6.39	6.35	6.66	8.71	288	16.7	17.2	1000	7.00
12/16/94	61	6.26	2.94	3.32	6.26	6.43	6.93	8.38	288	16.1	16.6	1000	7.00
12/16/94	61	6.26	2.94	3.32	6.26	6.43	6.93	8.38	288	16.1	16.6	1000	7.00
12/19/94	64	6.30	2.93	3.37	6.30	6.84	7.15	8.06	288	14.9	17.2	1000	7.00
12/19/91	64	6.30	2.93	3.37	6.30	6.84	7.15	8.06	288	14.9	17.2	1000	7.00
12/20/94	65	6.32	3.31	3.01	6.32	6.53	7.10	8.19	288	15.4	16.8	1000	7.00
12/20/91	65	6.32	3.31	3.01	6.32	6.53	7.10	8.19	288	15.4	16.8	1000	7.00
12/21/94	66	6.67	3.16	3.51	6.67	6.68	6.95	8.43	288	15	16.9	1000	7.00
12/22/94	67	7.14	3.78	3.36	7.14	6.69	6.91	8.46	288	15.6	16.4	1000	7.00
SS #3 AVERAGES		6.44	3.10	3.34	MAX-->	6.84	7.15			15.6	16.9		
STANDARD DEV.		0.26	0.28	0.18	MIN-->	6.43	6.91						

Appendix B. Data from Steady-State Period No. 3

		Acetone (average)		MEK (average)		MIBK (average)		% Removal			
Date	# of Days	Effluent	Influent	Effluent	Influent	Effluent	Influent	Acetone	MEK	MIBK	Notes
12/13/94	58	no data	no data	no data	no data	no data	no data	no data			
12/14/94	59										contaminant delivery problem; needle connection bumped loose
12/15/94	60	<12.6	349	<7.9	30	<1.1	29	96.4	73.7	94.8	start ss#3; OLR 10x less than ss#1
12/15/94	60	<12.6	182	<7.9	14	<1.1	13	93.1	43.6	88.5	
12/16/94	61	<12.6	305	<7.9	22	<1.1	20	95.9	64.1	92.5	
12/16/94	61	<12.6	316	<7.9	24	<1.1	20	96.0	67.1	92.5	
12/19/94	64	<12.6	598	<7.9	66	<1.1	83	97.9	88.0	98.2	
12/19/91	64	<12.6	217	<7.9	18	<1.1	21	94.2	56.1	92.9	
12/20/94	65	<12.6	197	<7.9	17	<1.1	21	93.6	53.5	92.9	
12/20/91	65	<12.6	175	<7.9	13	<1.1	14	92.8	39.2	89.3	
12/21/94	66	<12.6	171	<7.9	20	<1.1	25	92.6	60.5	94.0	
12/22/94	67	<12.6	513	<7.9	47	<1.1	56	97.5	83.2	97.3	
SS #3 AVERAGES			302.3		27.1		30.2	95.0	62.9	93.3	
STANDARD DEV.			141.6		16.0		21.0	1.9	15.0	2.9	