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DECOMPOSITION OF FOUR AMMONIUM NITRATE PROPELLANTS

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The biodegradability of four ammonium nitrate propellants, trimethylammonium nitrate (TMAN), isopropylammonium nitrate (IPAN), triethanolammonium nitrate (TEAN), and hydroxylammonium nitrate (HAN) was assessed in batch and continuous cultures under a variety of conditions. IPAN, TMAN, and TEAN were decomposed under aerobic conditions in batch and continuous cultures in a variety of media. Under anaerobic conditions in batch systems and denitrification conditions in continuous flow systems TMAN and TEAN were decomposed

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while IPAN was incompletely degraded. No significant build-up of intermediates was observed under any of the conditions studied. All three compounds were readily decomposed in soils at a variety of concentrations and in a range of different soil types. HAN was found to be chemically unstable down to a pH of 5.9. All four compounds tested negative in the ames screening test for mutagenicity. The contamination of stock solutions of TMAN and TEAN with nitrosamines may present the biggest hurdle to overcome in looking towards successful biological treatment of these propellants.

PREFACE

The ammonium nitrate derivatives are under evaluation as liquid monopropellants for Army applications such as their use as a substitute for nitrocellulose in ball powder mixtures. Follow on programs will most likely require substantial increases in the quantities of these propellants in production. Because of this expected increase, proper disposal guidelines, process water treatment, and the effects of spills on soils must be determined as part of the Army's program on pollution abatement.

This work was performed for US Army Toxic and Hazardous Materials Agency (USATHAMA) under work units 13214139000 and 23214139000. The results will provide the basis for recommendations on biological treatment as a method for disposing of these compounds. This effort has been carried out in direct support of activities at ARRADCOM, Ballistics Research Laboratory, Aberdeen Proving Ground, MD 21005.

We wish to thank John Knapten at BRL for his helpful communications. We wish to thank Jennifer Pierce and Scott Cowburn for their technical assistance.

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INTRODUCTION

Substituted ammonium nitrates are under study for use as liquid monopropellants by the Army. With expected increases in production of these compounds, the potential for biological treatment of waste waters from the production, loading, assembly and packing operations must be assessed as part of the Army's ongoing effort in pollution abatement. Similarly, because of the potential for soil contamination, the impacts of these compounds on the soil environment had to be addressed.

The major problem encountered at the outset of these investigations was the absence of suitable methods to directly quantitate low concentrations of the free amines (from the corresponding nitrate salts) in aqueous solutions. Analytical methods development constituted a large part of this effort and a separate report on this aspect has been issued.[1] We were unable to find information in the literature concerning the biodegradation of these propellants as interest in these compounds is relatively recent. However, information on the biodegradation of the corresponding free amines is available and assisted in our predictions of which metabolic intermediates to look for.

Figure 1 illustrates structures of the four compounds under study; in two cases the potential biotransformation pathways that might be expected are indicated. The potential formation of nitrosamines when these amines are present is a concern when denitrification occurs. This possibility has been discussed in a separate report.[2] Hydroxylamine, the corresponding free amine for the propellant nitrate salt is an intermediate formed

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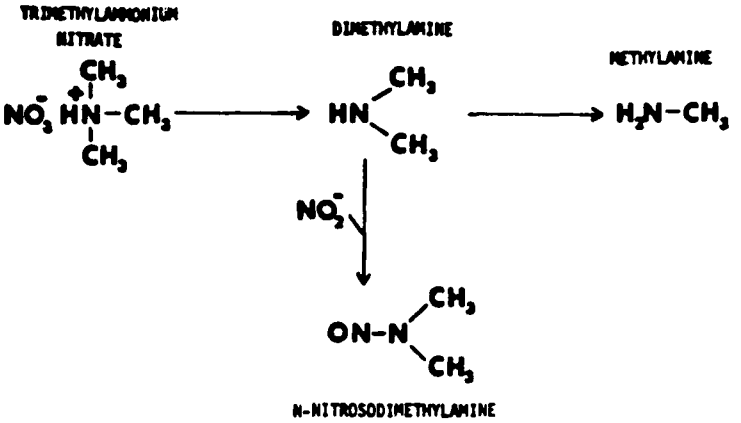
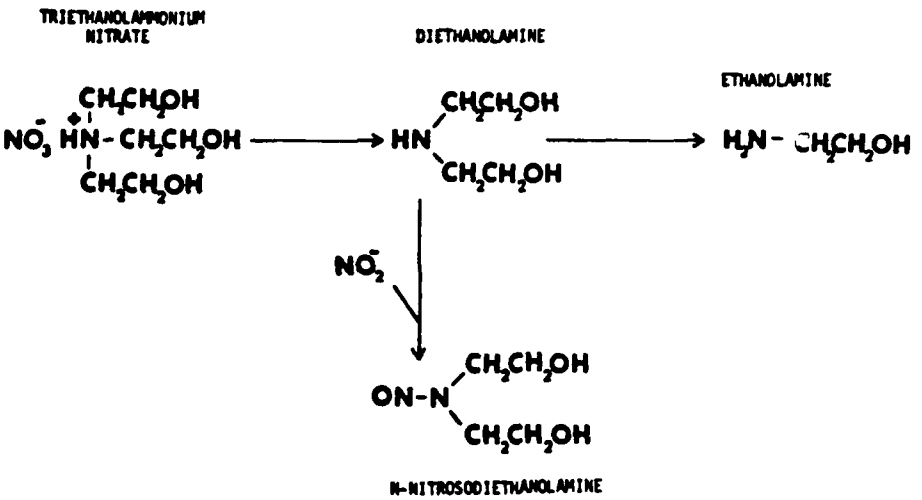
[1] D. J. Emerson, D. L. Kaplan, and A. M. Kaplan. Gas chromatographic method for direct measurement of trace levels of volatile aliphatic amines in aqueous samples. NATICK/TR-83/004. US Army Natick R&D Laboratories, Natick, MA 01760, 1982.

[2] D. L. Kaplan, S. Cowburn, and A. M. Kaplan. Formation of nitrosamines under denitrification conditions. NATICK/TR-83/030. US Army Natick R&D Laboratories, Natick, MA 01760, 1983.

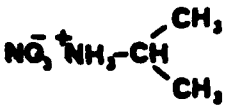
● **TRIMETHYLAMMONIUM NITRATE – pathway**



● **TRIETHANOLAMMONIUM NITRATE – pathway**



● **ISOPROPYLAMMONIUM NITRATE**



● **HYDROXYLAMMONIUM NITRATE**

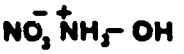


Figure 1. Structures and potential biotransformation pathways of the ammonium nitrate propellants.

during the microbial oxidation of ammonia to nitrate.[3]

The purpose of this investigation was to determine the biodegradability of the ammonium nitrate propellants in aqueous and soil systems under a variety of conditions. The identification of intermediates formed during the biotransformation of these compounds and screening the parent compounds for mutagenicity were also parts of this effort.

MATERIALS AND METHODS

Chemicals: Trimethylammonium nitrate (TMAN) (methyl-C-14), 1.5 mCi/mMole, 98% pure, isopropylammonium nitrate (IPAN) (isopropyl-1,3-C-14, 5 mCi/mMole, 98% pure, and triethanolammonium nitrate (TEAN) (ethanol-1,2-C-14), 5 mCi/mMole, 95% pure were purchased from California Bionuclear Corp., Sun Valley,CA. Hydroxylammonium nitrate (HAN); lot R149/151, TMAN; lot 128, IPAN; lot 85, and TEAN; lot 190, were provided by the US Army Ballistics Research Laboratory, Environmental Technology Division, Aberdeen Proving Ground, MD as 50 weight percent solutions in water.

Dimethylamine (DMA), methylamine (MA), diethanolamine, and ethanolamine were purchased from Eastman Kodak, Rochester, NY. N-nitrosodimethylamine (NDMA) was purchased from Aldrich Chemical Co., Milwaukee, WI. N-nitrosodiethanolamine (NDElA) was purchased from Columbia Organic Chemical Co., Columbia, SC.

Batch Cultures: A series of three batch system experiments were run to evaluate the degradation of IPAN, TEAN and TMAN, respectively, under aerobic and anaerobic conditions in a variety of culture conditions.

The first set of experiments was run with TMAN at a concentration of 100 ppm. Aerobic and anaerobic systems were run with the following media, nutrient broth, 4 g per L; basal salts (K_2HPO_4, 1.0 g per L; KH_2PO_4, 1.0 g

[3] J. M. Bremner and K. Shaw. Denitrification in soil. 1. Methods of investigation. J. Agric. Sci. 51: 22-39, 1958.

per L; $MgSO_4 \cdot 7H_2O$, 0.2 g per L; $CaCl_2$, 0.01 g per L; and NaCl, 0.01 g per L), basal salts with glucose (1.0 g per L), and basal salts with glucose (1.0 g per L), and $NH_4H_2PO_4$ (2.0 g per L). Controls consisted of sterile distilled water or one of these media with TMAN. Samples, 2-μL were withdrawn from these incubations and analyzed directly by gas chromatography (GC). Samples were taken seven times during the 18-day incubations. Aerobic systems contained 50 mL of media in 250 mL Erlenmeyer flasks incubated on a rotary shaker set at about 175 rpm. Anaerobic systems were unaerated and incubated in 125-mL screw top Erlenmeyer flasks filled with the media. The active flasks were inoculated with a mixed population of microorganisms.

The second set of batch experiments was run with 100 ppm TMAN, TEAN or IPAN, individually, in 125 mL Erlenmeyer flasks containing one additional medium to those used in the first set of experiments, basal salts with $NH_4H_2PO_4$ (2.0 g per L) without glucose. The basal salts media were adjusted to pH 6.8 before autoclaving. Flasks incubated under anaerobic conditions also contained 0.25 g per L sodium sulfide, which was added after autoclaving, to create reducing conditions. Aerobic and anaerobic incubations consisted of 50 mL and 125 mL media, respectively, and each incubation condition was run once. The active flasks were inoculated with cells harvested from the first batch experiment. The ammonium nitrates, 100 ppm, final concentration, were added along with the corresponding ^{14}C-labelled compound. In the case of TMAN, aerobic and anaerobic flasks received 0.23 μCi and 0.44 μCi, respectively. Aerobic and anaerobic incubations containing TEAN received 0.32 μCi and 1.11 μCi, respectively and similar incubations with IPAN received 0.22 μCi and 0.80 μCi, respectively. Volatile products were continuously collected in 1-N hydrochloric acid and 1-N sodium hydroxide traps. For aerobic systems, traps were changed 4 to 5 times during the first week and 1 to 2 times weekly thereafter up to 56 days for TMAN, 16 days for TEAN, and 37 days for IPAN. For anaerobic systems, traps were changed three times during the first week and 1 to 2 times weekly thereafter up to 31 days for TMAN, 35 days for TEAN, and 22 days for IPAN. On these 100 μL aliquots of the culture media were counted for radioactivity. At the termination of these experiments the flask

contents were centrifuged and filtered, and the resulting cell pellets, filter pads, and filtered broth were counted according to previously described procedures.[4] A 50:50 (v/v) dimethylsulfoxide: acetone mix, 25 mL per flask, was used to extract residual contents on flask walls and 1 mL of this extract was counted. Percent radioactivity within cells harvested from these systsms was determined by sonicating the cells for 1 minute at 50 watts using a Branson Sonifier Cell Disrupter (Danbury, CN), Model W185 with a 0.32-cm tapered microtip.

The third set of batch studies was conducted with 1,000 ppm of TMAN, TEAN, or IPAN in basal salts medium. In this study the ammonium nitrates served as the sole source of carbon and nitrogen. Anaerobic systems were incubated unaerated at room temperature in 500-mL Erlenmeyer flasks filled with medium and each contained 0.25 g per L sodium sulfide. Aerobic flasks contained 75 mL of medium in 250-mL Erlenmeyer flasks. Aerobic incubations were flushed with oxygen daily and incubated at 25°C on a rotary shaker at 75 rpm. Active systems under aerobic and anaerobic conditions were run in triplicate with one each of the corresponding sterile controls. Flasks contained 0.34 μCi of either ^{14}C-labelled TMAN or IPAN, or 1.37 μCi, of TEAN. Volatile products were continuously collected in sodium hydroxide and hydrochloric acid traps that were changed daily. Samples of the medium, 100 μL, were withdrawn daily and analyzed for radioactivity. Head space gases were sampled through septa in the flask stoppers and were analyzed for gases not trapped by either the acid or alkali.

A study involving a batch system was carried out to assess the potential for microbial decomposition of hydroxylammonium nitrate (HAN). The nutrient broth, basal salts, and water media were established at an initial pH 4.0 to eliminate chemical instability as a factor in the disappearance of the HAN (discussed later). Erlenmeyer flasks, 250 mL, were set up under aerobic (rotary shaker) and anaerobic (unaerated) conditions with 100 ppm HAN. Samples were withdrawn daily for four days and at days 7 and 10. Sterile

[4] D. L. Kaplan and A. M. Kaplan. 2,4,6-Trinitrotoluene-surfactant complexes, biodegradability, mutagenicity and soil leaching studies. NATICK/TR-82/006. US Army Natick R&D Laboratories, Natick, MA 01760, 1982.

control flasks under both aerobic and anaerobic conditions were run concurrently.

Continuous Systems: Continuous flow systems were run in eicher BioFlo Model C30 bench top chemostats (New Brunswick Scientific, New Brunswick, NJ) or in modified 500-mL Erlenmeyer flasks. The BioFlo systems used 1,500-mL reaction vessels and were run under anaerobic denitrification or aerobic conditions. Under aerobic conditions both vigorous stirring and forced aeration were used while under anaerobic conditions the medium was slowly stirred. The modified Erlenmeyer flasks were fitted with a 24/40 ground glass joint, an overflow tube, and a 35-cm-long glass tube, 5 mm I.D., suspended in the reaction vessel by a teflon adapter to deliver nutrient solution to the bottom of the reaction vessel. The total volume of the reaction vessel was about 500 mL. These vessels were run only under anaerobic denitrification conditions. Nutrient solution was delivered to the reaction vessel by a Rainin Rabbit peristaltic pump (Woburn, MA) and effluent was collected from the overflow line. In both types of systems, a series of in-line break tubes was used to prevent microbial contamination of the nutrient reservoir.

The continuous flow systems were operated with either TMAN, TEAN, IPAN, or DMA as sole or additional carbon and nitrogen sources. DMA is a potential decomposition product from TMAN (Figure 1). Solutions of DMA and TMAN were sterilized by passing through a 0.45 μm pore size membrane filter to sterilize solutions prior to addition to sterilized nutrient reservoirs to avoid losses due to volitization. TEAN and IPAN were added to the reservoirs prior to sterilization. All systems were run at room temperature and contained the following quantity of trace salts per liter of filtered lake water, $MgSO_4 \cdot 7H_2O$, 500 mg; NaCl, 50 mg; $CaCl_2$, 15 mg; $FeCl_3 \cdot 6H_2O$, 10 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $NaMoO_4 \cdot 2H_2O$, 1 mg. In addition to the trace salts the basal salts composition for aerobic systems contained per liter, $NH_4H_2PO_4$, 2.0 g; K_2HPO_4, 1.0 g; KH_2PO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2$, 0.1 g and NaCl, 0.1 g, unless otherwise indicated. The basal salts composition for anaerobic denitrification systems contained per liter,

KNO_3, 2.05 g; K_2HPO_4, 0.87 g; trace salts, 595 mg; and methanol, 1.4 mL; unless otherwise indicated.

The chronology of changes in media composition, retention time and concentration of ammonium nitrate propellant for the different continuous flow systems will be presented in tables contained in the results section. Flow rates, pH, oxidation reduction potential, nitrate concentration, methanol concentration, and the concentrations of the ammonium nitrates and their potential decomposition products were monitored weekly or more frequently. Samples, 5 to 10 mL of influent and effluent from the continuous systems, and 2 mL from the batch systems were taken one to four times weekly for analysis by GC or high performance liquid chromatography (HPLC). Samples for HPLC were centrifuged at 12,000 rpm for 4 minutes and the supernatant was passed through a Swinny stainless steel syringe-type filter holder containing a 0.45-μm pore size membrane filter.

Stability of Hydroxylammonium Nitrate (HAN): The stability of HAN was assessed over a range of hydrogen ion concentrations. Buffered solutions containing 50 ppm HAN were adjusted over a pH range of 1.0 to 6.9. Aliquots of these solutions were assayed spectrophotometrically over 28 days to determine residual concentrations of HAN.

Soil Incubations: The first soil study, which was carried out to determine the rates of decomposition of the ammonium nitrates at various concentrations was conducted with garden soil containing 6.7% organic matter by ignition and a pH of 5.5. The soil was incubated in 125-ml Erlenmeyer flasks closed with a gas tight trapping arrangement consisting of an adapter with a vial containing 1 mL 1 N sodium hydroxide. Each flask contained 24.3 g oven dry weight (odw) of sieved (3.35 mm pore size) soil and sufficient moisture to bring the soil up to field capacity. The sterile control flasks were autoclaved for 30 minutes on three consecutive days. Flasks contained ^{14}C-labelled IPAN, TEAN, or TMAN (0.23 μCi, 0.27 μCi, and 0.14 μCi per flask, respectively) diluted with the corresponding non-radioactive ammonium nitrate to give a final concentration of 50, 500, 5,000 ppm for each of the three compounds. The ammonium nitrate pro-

pellants were added as filter sterilized solutions. Each system was run in duplicate and the traps were changed three times the first week and weekly or every two weeks thereafter for the remainder of the experiment. Incubations ran for a total of 103 days.

The second set of experiments in soil was initiated in order to evaluate the effects of soil composition on the biodegradability of the ammonium nitrates. These experiments were run for a total of seven months in 125-mL Erlenmeyer flasks. These flasks were modified with two trapping tubes to allow for continuous collection of gaseous products in 1.0 mL of 1 N sodium hydroxide and separately in 1.0 mL 1 N hydrochloric acid. The flasks contained one of the following three matrices,(1) 20 g odw garden soil (pH 6.4, 42.5 percent organic matter by ignition, passed through a 3.35 mm pore size sieve) and 15 mL distilled water, (2) the same soil but flooded with 55 mL distilled water, or (3) sand, 70 g odw (Fischer Scientific Co., Fair Lawn, NJ, washed and ignited, pH 7.9, 0 percent organic matter) and 15 mL distilled water. Sterile flasks were autoclaved as described for the previous soil incubation experiment and a filter sterilized solution containing both the ^{14}C-labelled- and the unlabelled ammonium nitrate compound was added after autoclaving. Incubation flasks contained IPAN, 0.32 µCi per flask, TMAN, 0.17 µCi per flask or TEAN, 0.024 µCi per flask diluted to 100 ppm final concentration with the corresponding unlabelled ammonium nitrate. For the most part, acid and base traps were changed four times the first week of incubation, two or three times a week for the next three weeks, and once to twice per month for the duration of the experiment. Controls with 1 percent mercuric chloride were also included in this experiment, however incomplete inhibition of microbial activity was found under these conditions. All active systems were inoculated with a mixture of microorganisms from aerobic and anaerobic sewage sludge and soil, which were isolated in 0.85% KCl and filtered through Whatman filter paper. Flasks were incubated at room temperature throughout the experiment. Final pH and oxidation-reduction measurements were made on a Corning Model 130 pH meter with soil samples diluted with an equal volume of degassed, distilled, deionized water when necessary.

Colorimetric Assay for Hydroxylammonium Nitrate (HAN): The Spectrophotometric assay for hydroxylamine was based on the work of Frear and Burrell.[5] Stock 8-quinolinol, sodium carbonate, trichloracetic acid, and phosphate buffer; pH 6.8, were prepared according to their report. The assay was run with 1.0 mL phosphate buffer, distilled water and HAN (used instead of hydroxylamine) to 2.0 mL, 0.2 mL trichloracetic acid, and 1.0 mL 8-quinolinol. The mixture was gently swirled in a test tube and 1.0 mL sodium carbonate added. The tubes were vortexed for 3 seconds and placed in boiling water bath for 1 minute. After cooling for 15 minutes, the green color was quantified at 705 nm on a Perkin-Elmer Model Lambda 3 UV/VIS spectrophotometer. Standard curves in basal salts with and without glucose, nutrient broth, and distilled water were run at pH 4.0 due to the instability of HAN at more alkaline pH.

Thin-Layer Chromatography: Initial work on the ammonium nitrate propellants involved thin-layer chromatographic (TLC) analysis of TMAN, IPAN and TEAN samples from batch and continuous studies. TLC was run in a n-butanol, acetic acid, water (4/1/5) solvent system on cellulose plates. Spots were visualized with iodine vapors. The R_f's were 0.42, 0.47, and 0.58 for TEAN, TMAN, and IPAN, respectively.

TLC was also used to resolve TEAN from diethanolamine and ethanolamine. A solvent system consisting of chloroform and methanol (50/50) was used with both cellulose and silica plates. Low R_f's prompted the incorporation of ammonium hydroxide into the solvent to increase mobility. TLC runs in chloroform, methanol, ammonium hydroxide (47/47/6) resulted in R_f's of 0.40, 0.50, and 0.80 for ethanolamine, diethanolamine and TEAN, respectively. Spots were visualized with iodine vapors. Several other reagents were examined for visualization of the three compounds (cobalt II thiocyanate, ferricyanide potassium-ferric chloride, ninhydrin, and ninhydrin-cupric nitrate). However, only ethanolamine could be visualized with several of these reagents.

[5]D. S. Frear and R. C. Burrell. Spectrophotometric method for determining hydroxylamine reductase activity in higher plants. Anal. Chem. 27(10); 1664-1665, 1955.

Thin-layer electrophoresis (TLE)[6] was used in some preliminary work to determine whether or not these ammonium nitrates and their probable degradation products could be separated. A Desaga TLE Chamber with cooling block was used in conjunction with a Bio Rad Laboratories Model 500/200 Power Supply. The power supply was operated in a constant voltage mode at 450 volts using 20 cm plates. Commercially available thin-layer plates tried were: Eastman Chromagram: Alumina Sheets (with Fluorescent Indicator); Cellulose Sheets (with Fluorescent Indicator); Silica Gel (without Indicator), EM Reagents (Merck): Kieselguhr F-254; Whatman: KC_{18} reversed phase plates; LK5DR Linear-K silica gel. Electrolyte solutions tried were: 2 N acetic acid in 0.6 N formic acid; a mixture of 80 mL ethanol, 30 mL water, and 2 g sodium acetate with pH adjusted to 12 with sodium hydroxide; 0.05 M borax (pH 9.2); a mixture of 80 mL ethanol, 30 mL water, 4 g boric acid, and 2 g sodium acetate, pH adjusted to 6.8 with either glacial acetic acid or ammonia solution. Spots were visualized using ninhydrin spray for DMA and MA and Dragendorf's Reagent for TMAN. All electrolytes were run with each of the plates for 30 minutes. If spots were not apparent after spraying with indicator, 15 or 45 minute developing times were also tried. The 2 N acetic acid in 0.6 N formic acid in combination with silica gel plates for 30 minutes was the combination used as determined by less diffuse spots and mobility of the compounds.

Thin-layer sheets were placed on the cooling block in the developing chamber, electrolyte solution was poured into the solvent trough, and blotter paper wicks were positioned to provide the electrical connection to the thin layer. Electrolyte was then sparingly sprayed onto the thin layer to dampen it, a glass plate was placed over the thin layer to minimize volatilization of the electrolyte, and the chamber cover was put on. The thin layer was incubated for 15 minutes to allow it to fully equilibrate with the electrolyte, the chamber was re-opened, reference and sample solutions were spotted onto the wet layer, and the chamber was reassembled. The power supply was then turned on and the plate developed for the specified time. Development times ranged between 15 and 60 minutes. After

[6] G. Grassini. Thin-layer electrophoresis, thin-layer chromatography proceedings, Symp. Rome. 55-68, 1963.

development of the thin layer, the power was turned off and the plate dried and sprayed to visualize the compounds.

Gas Chromatography: CG analysis of TMAN (as the free amine), DMA, MA, IPAN (as the free amine), methanol, TEAN (as the free amine), DE1A and E1A was accomplished on a Hewlett Packard Model 5840A GC with a flame ionization detector (FID). A 3-m glass column packed with 10% Carbowax 20M and 2% KOH on 80/100 Chromosorb WAW and conditioned at 170°C with 45 mL per min nitrogen carrier flow for 24 to 48 hours was used for analysis of TMAN, DMA, MA, IPAN, and methanol. Details on the development of this method and conditioning requirements were previously described.[1] Operating conditions were the injector, oven, and FID temperatures of 150°C, 30°C, and 230°C, respectively. All sample injections were 2 μL, and injections of water were used to keep the column properly conditioned as described in the reference.

GC separation of TEAN (as the free amine), DE1A, and M1A was attempted with two packing materials, Tenax-GC and Dexsil (3% Dexsil 300 GC on Anakrom Q 90/100). Preliminary results suggested that oven temperatures in excess of 250°C would be necessary for effective separation since the boiling point of triethanolamine is 335°C. Separations at oven temperatures from 150°C to 325°C and at carrier flow rates from 15 mL per min to 70 mL per min were investigated to determine optimal parameters for separation. Both packings behaved similarly, but Tenax-GC was easier to recondition, and for this reason it was preferable to the Dexsil packing. The separation of TEAN (as the free amine) from DE1A and E1A occurred over wide limits within these two ranges, however, it was not possible to achieve maximum sensitivity for all three compounds under the same conditions due to peak spreading of TEAN. Therefore, TEAN and DE1A were determined with an oven temperature of 260°C while E1A was analyzed at an oven temperature of 180°C. For all three compounds the injector temperature was 280°C, the FID temperature was 380°C and carrier gas flowed at 60 mL per min. The use of Tenax-GC for analysis of ethanolamines has been previously described.[7]

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[7] N. C. Saha, S. K. Jain, and R. K. Dua. A rapid and powerful method for the direct gas chromatographic analysis of alkanolamines; application to ethanolamines. Chromatographia. 10(7): 368-371, 1977.

The detection limits, as calculated by the method of Hubaux and Vos[8] were as follows: TMAN; 11.4 ng or 5.7 ppm, DMA; 29.6 ng or 14.8 ppm, MA; 32.8 ng or 16.4 ppm, IPAN; 15.4 ng or 7.7 ppm, methanol; 6.0 ng or 3.0 ppm, TEAN; 175.8 ng or 87.9 ppm, diethanolamine; 201.4 ng or 100.7 ppm, and ethanolamine; 157.0 ng or 78.5 ppm.

GC analysis for head space gases for the third set of batch experiments with TEAN, TMAN, and IPAN incubated as the sole sources of carbon and nitrogen were determined on a Hewlett Packard Model 5880 GC with a thermal conductivity detector. The column was a 2.67-m-long by 0.32-cm-diam stainless steel column packed with Carbosieve S 120/140 mesh purchased from Supelco, Inc. (Bellefonte, PA). The oven temperature was programmed from 35°C to 250°C at 15°C per min and injector and detector temperatures were 150°C and 275°C, respectively. Helium carrier gas flowed at 30 mL per min and injections were 0.5 mL.

High Performance Liquid Chromatography: To determine whether nitrosamines might form during the the biological decomposition of these ammonium nitrate propellants under denitrification conditions, high performance liquid chromatography (HPLC) was used to quantitate NDMA and NDE1A in influent and effluent samples from continuous flow systems. Analyses were performed on a Water's Associates (Milford, MA) HPLC equipped with two Model 6000A solvent delivery pumps, a Model 441 detector set a 254 nm, a Model 720 system controller and a Model 730 data module. All nitrosamine analyses were run on a μBondapak C-18 reverse phase stainless steel column, 3.9 mm x 30 cm (Water's Assoc.).

NDMA was analyzed with water as mobile phase at a flow rate of 2.5 mL per minute. Injection volumes varied up to 200 μL, sensitivity varied from 0.02 to 0.005 absorbance units full scale (AUFS), the detection limit was 1 ppb (μg/L); and the retention time was 5.3 minutes. NDE1A was analyzed with water containing 0.1 M K_2HPO_4 as the mobile phase flowing at 1.5 mL per minute through two C-18 μBondapak reverse phase stainless steel columns (3.9 mm x 30 cm each) in tandem. Injection volumes varied up to 200 μL, sensitivity varied from 0.02 to 0.005 AUFS, the detection limit was 1 ppb, and the retention time

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[8]A. Hubaux and G. Vos. Decision and detection limits for linear calibration curves. Anal. Chem. 42: 849-855, 1970.

was around 7 minutes. Details on these methods and some further results of these analyses are presented in a separate report.[2]

Scintillation Counting: The acid base traps were counted for radioactivity in a Packard Tri Carb Model 3255 liquid scintillation counter with Aquasol-2-cocktail (New England Nuclear, Boston, MA). Sodium hydroxide traps were treated with Aquasol-2 and distilled water, while acid traps were treated only with the cocktail.

Nitrate Determination: Nitrate concentrations were determined either on an Orion (Cambridge, MA) ion-analyzer Model 901, using an Orion nitrate specific ion electrode (Model 93-07) and Orion double junction reference electrode (Model 90-02) or on a Corning (Medfield, MA) pH meter, Model 130.

Mutagenicity Studies: The Ames screening test for mutagenicity was performed according to standard procedures (6). TEAN, TMAN, IPAN, and HAN were tested with and without metabolic activation at 5, 50, 500, and 5,000 μg per plate against five strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537, and TA1538). The tests were run in triplicate.

RESULTS

The results of degradation of the ammonium nitrate propellants in three separate batch experiments are presented. In the results of the first set of experiments the concentration of TMAN in the different media was determined by GC, and the degradation was under anaerobic conditions as illustrated in Figure 2. To more effectively evaluate trends, the data presented has been transformed using moving averages (data points are averaged with the data points immediately before and after that sample point).

There is considerable variability in the data, but some general conclusions can be drawn. The concentration of TMAN in the sterile nutrient broth control, inoculated nutrient broth, basal salts with glucose, and basal

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[9] B. N. Ames, J. McCann, and E. Yamasaki. Methods for detecting carcinogens and mutagens with the Salmonella Mammalian-Microsome Mutagenicity Test. Mut. Res. 31: 347-364. 1975.

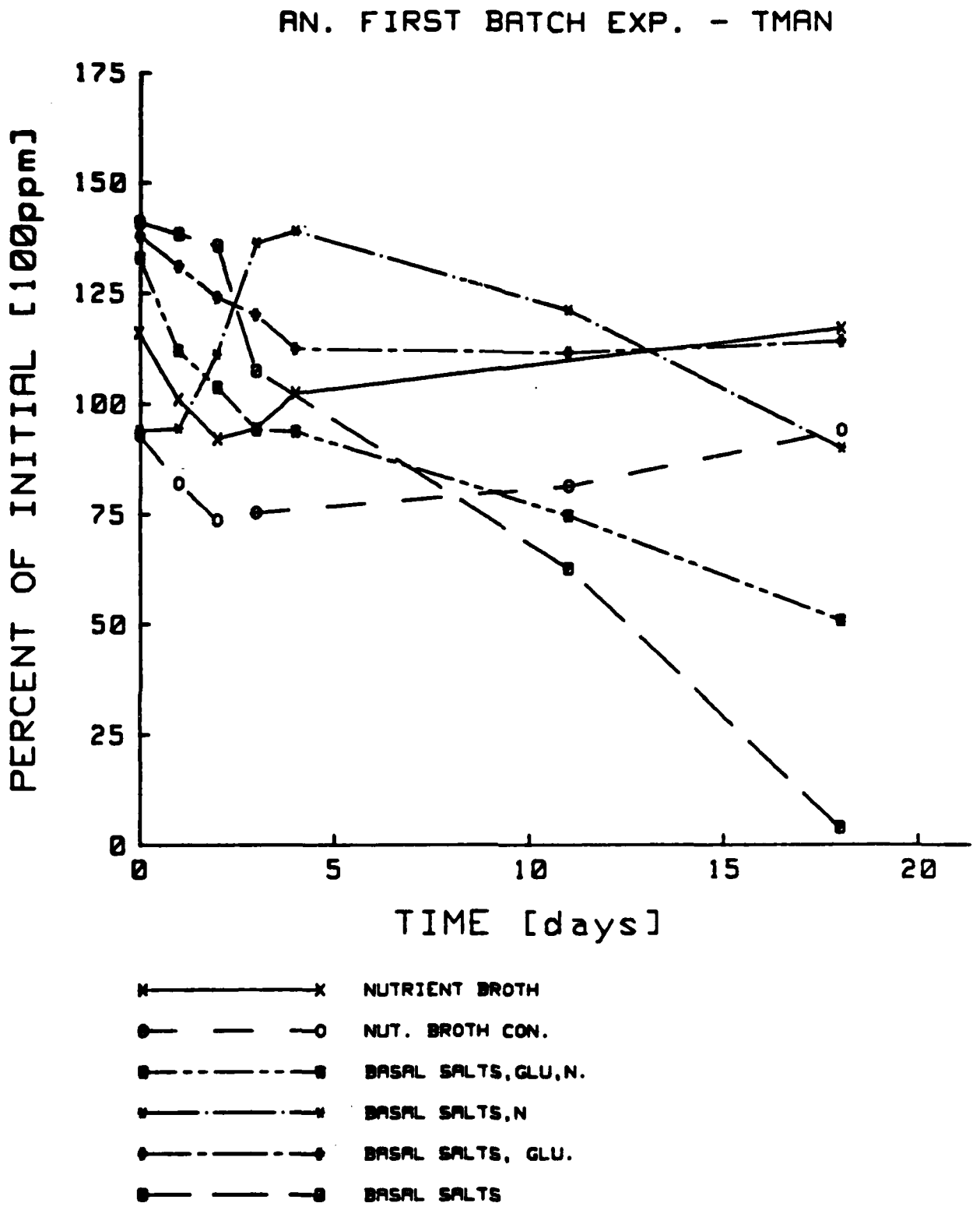

Figure 2. Decomposition of TMAN under anaerobic batch culture conditions (first experiment).

salts with nitrogen media were near initial levels by the end of the 18-day experiment indicating no degradation. In the inoculated basal salts medium, virtually all the TMAN had disappeared in this time frame, while in the basal salts medium with supplemental glucose and nitrogen, an intermediate level of degradation was found. The trend towards more efficient utilization of TMAN when it was present as the sole carbon source in the anaerobic environment is evident.

Under aerobic conditions (Figure 3), the concentration of TMAN was relatively stable in the two sterile controls (nutrient broth and basal salts with glucose and nitrogen). In the inoculated cultures, the concentration of TMAN in the basal salts medium with glucose had dropped to about 5 ppm by the end of the experiment, while intermediate levels of decomposition were found in the nutrient broth medium. After an initial decrease, there was little change in the concentration of TMAN in basal salts medium. The results in basal salts with nitrogen and basal salts with glucose and nitrogen were too variable to allow any conclusions to be drawn.

The variable nature of some of the results obtained in this experiment prompted the incorporation of ^{14}C-labelled ammonium nitrates in the remaining batch studies in order to elucidate the conditions under which optimal rates of biodegradation occur. Some of the variability seen in the first batch study may have been due to interference during GC analyses from volatile reaction products produced in the culture flask that exhibited similar retention times to TMAN or its metabolites. Another potential source of the variability may have occurred in aerobic cultures due to volatilization of the amines or their biotransformation products during aeration on the shaker.

The results from the second series of batch experiments run under aerobic and anaerobic conditions with ^{14}C-labelled TMAN, IPAN, and TEAN are presented in Figures 4, 5, and 6. The cumulative percent disintegrations per min (dpm) trapped represents the ^{14}C-labelled carbon dioxide recovered from the hydroxide traps throughout the experiments. Three curves representing different incubation conditions are presented for each

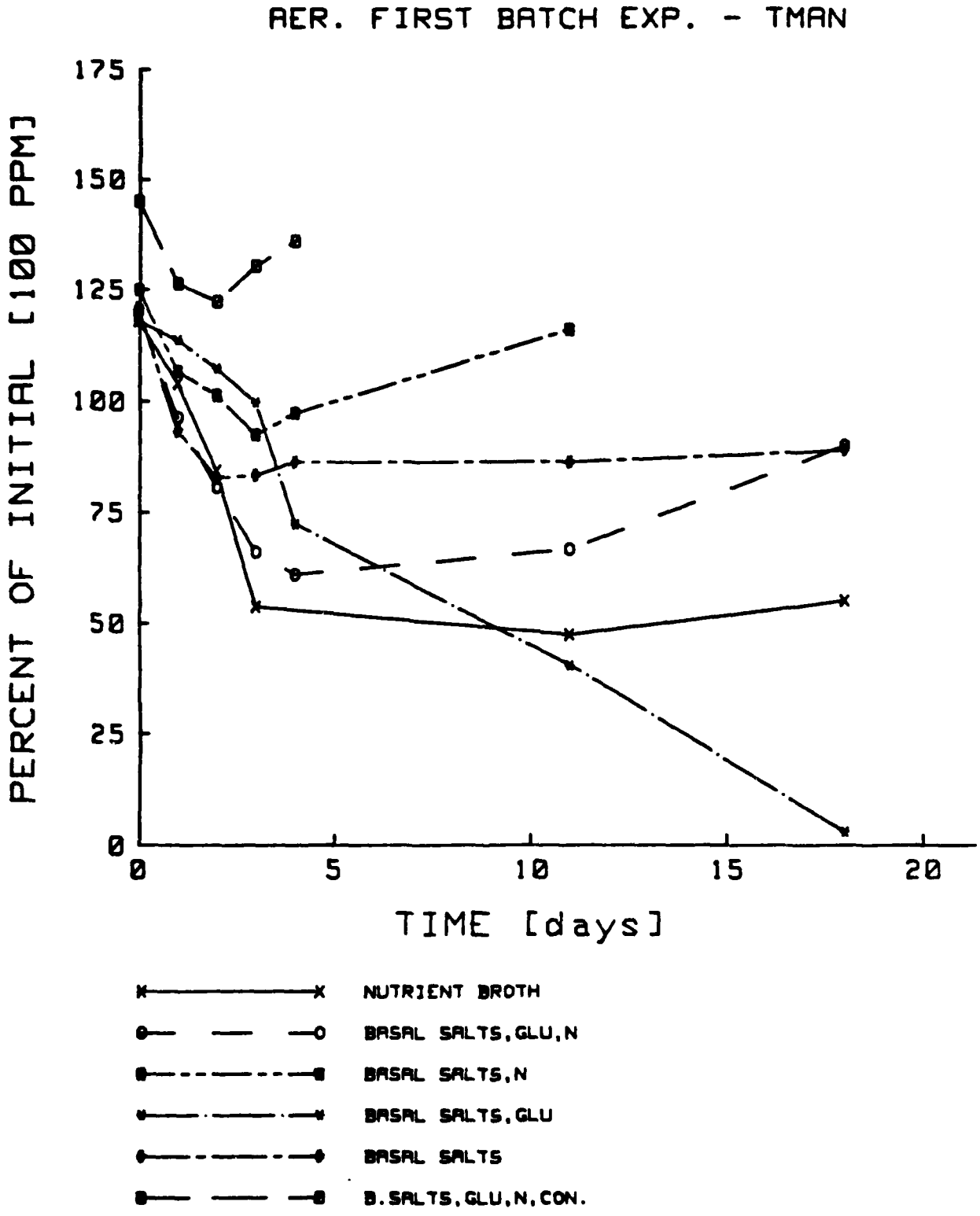

Figure 3. Decomposition of TMAN under aerobic batch culture conditions (first experiment).

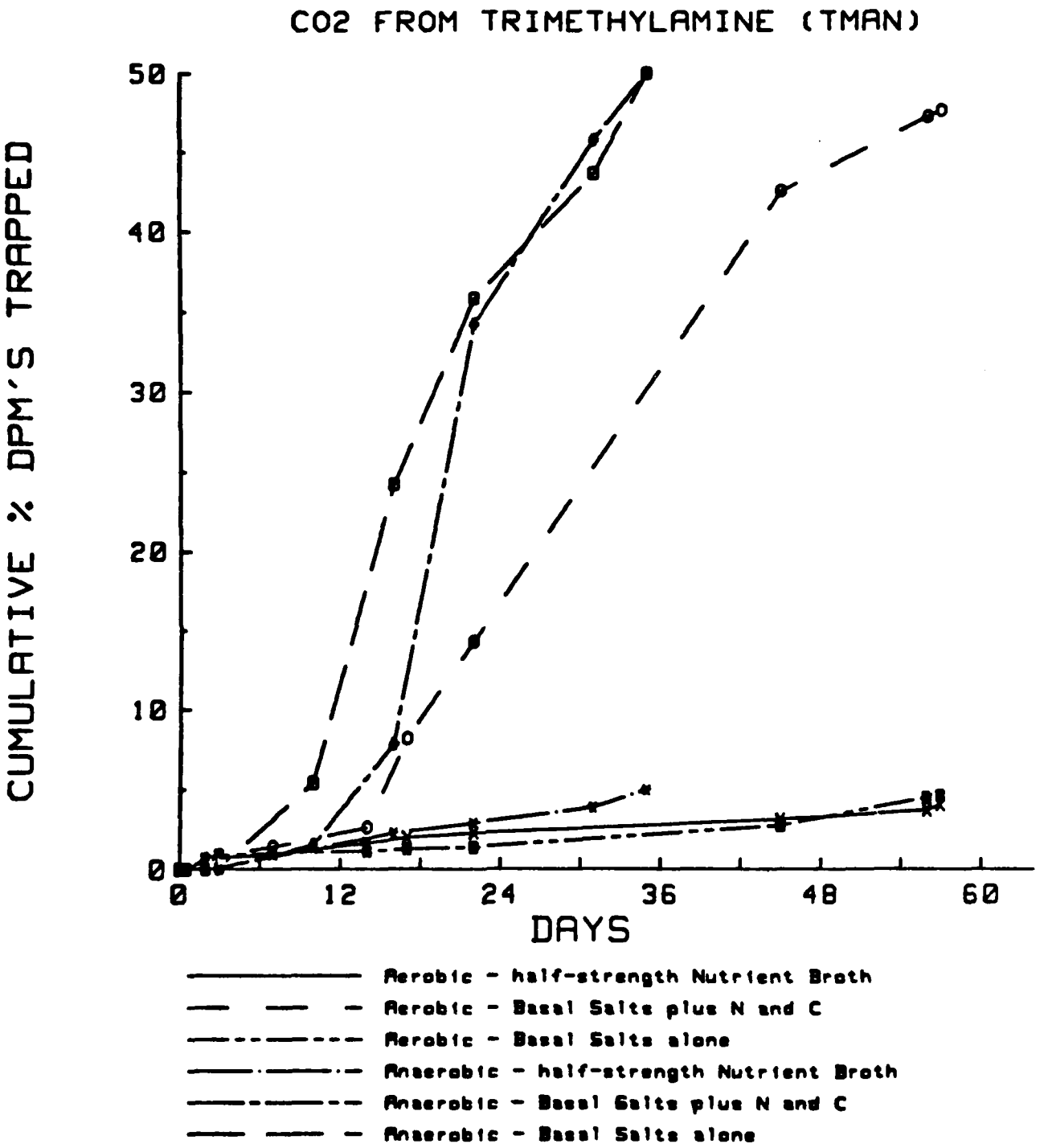

Figure 4. Decomposition of radioactively labelled TMAN under aerobic and anaerobic batch culture conditions (second experiment).

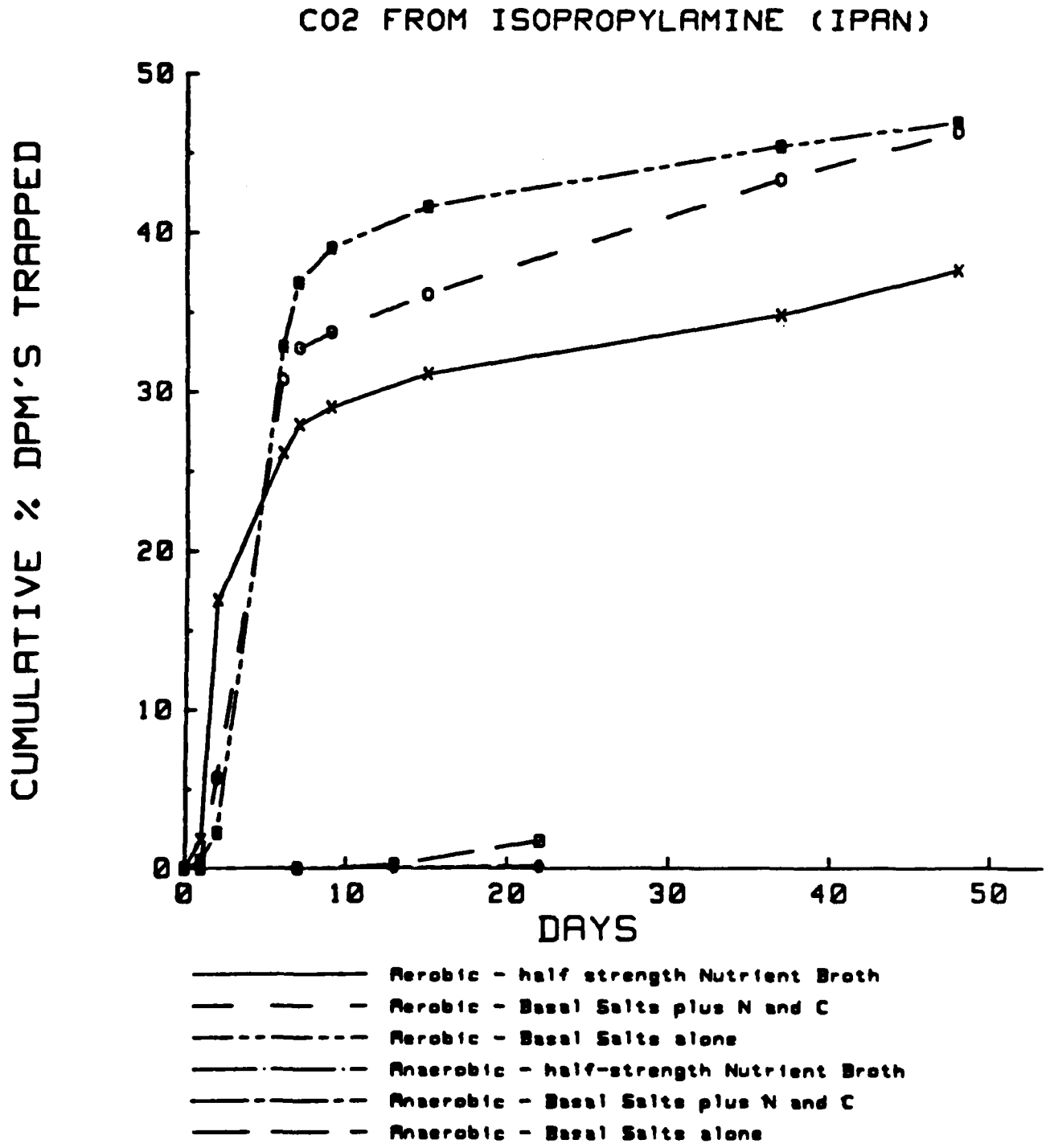

Figure 5. Decomposition of radioactively labelled IPAN under aerobic and anaerobic batch culture conditions (second experiment).

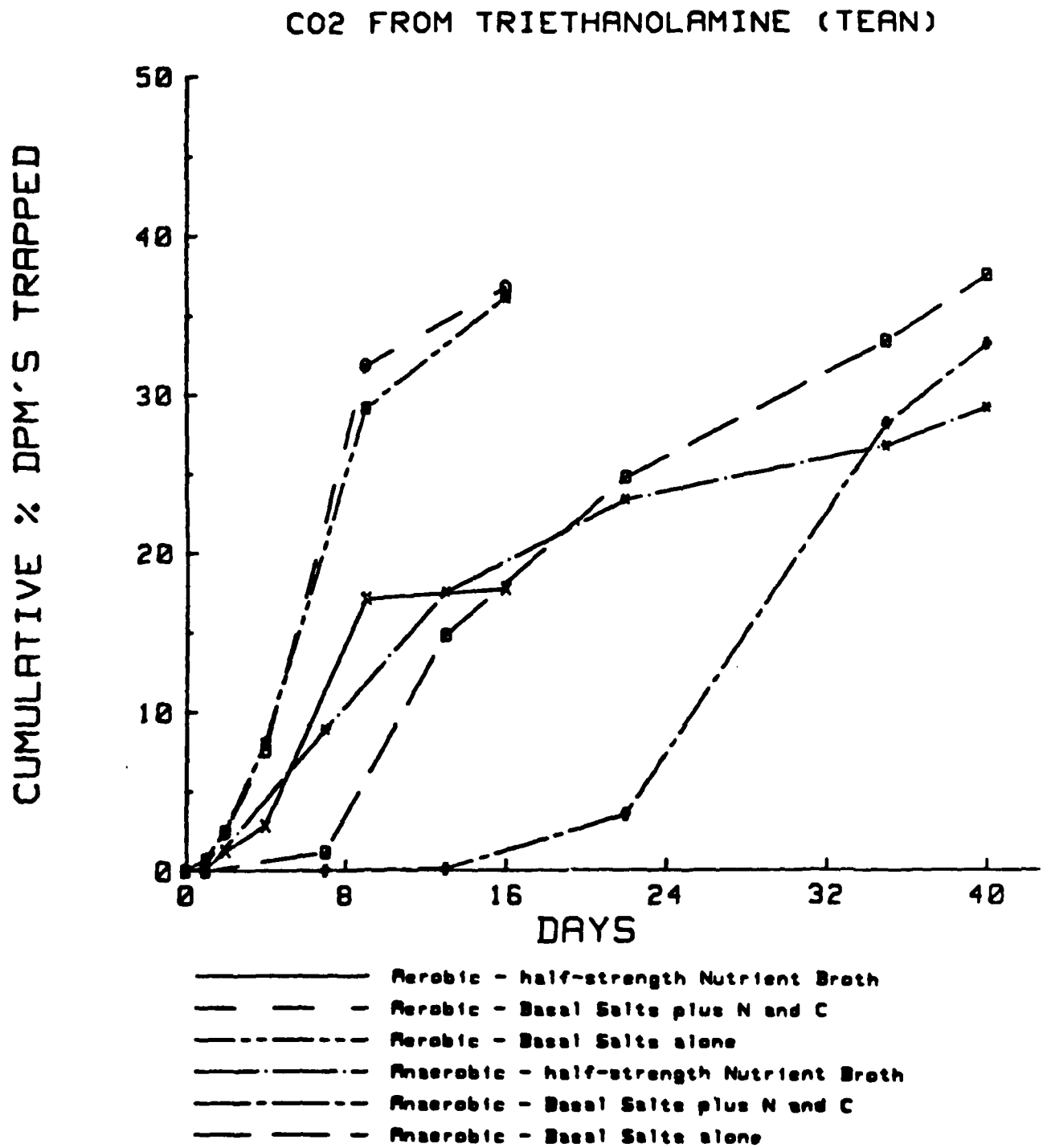

Figure 6. Decomposition of radioactively labelled TEAN under aerobic and anaerobic batch culture conditions (second experiment).

of the compounds under aerobic and anaerobic conditions. In Figure 4, significant rates of degradation of TMAN are achieved under aerobic conditions in basal salts supplemented with glucose and nitrogen, under anaerobic conditions in the same medium, and in basal salts alone. Considerably lower rates of decomposition are achieved in the remaining media. These findings support the results from the first set of batch experiments in that under anaerobic conditions higher rates of decomposition are achieved in media where TMAN is the sole source of carbon and nitrogen. In nutrient-rich media, the rates of decomposition are considerably reduced.

IPAN is degraded readily in all the aerobic culture media examined (Figure 5), ranging from a nutrient rich nutrient broth to a basal salts medium in which IPAN serves as the sole source of carbon and nitrogen. This is reflected in the rapid initial release of ^{14}C-labelled carbon dioxide. On the other hand, only very low rates of decomposition are achieved under the anaerobic incubation conducted under otherwise similar conditions.

In Figure 6, TEAN is shown to be biodegraded under all the conditions examined in both aerobic and anaerobic cultures. The most rapid rates of mineralization occurred under aerobic conditions with TEAN present as the sole source of carbon and nitrogen, or in basal salts supplemented with glucose and nitrogen. The level of $^{14}CO_2$ activity in alkaline traps (corresponding to the rates of mineralization) is inversely related to the level of residual radioactivity in the culture media as illustrated in Figure 7. The distilled water sterile controls showed unchanged levels of radioactivity in the medium under both aerobic and anaerobic conditions, while these levels decreased in the active flasks to varying degrees.

Table 1 summarizes the flask contents for the second batch experiment just described. The contents of each of these flasks was analyzed for radioactivity according to the procedure described in the methods section. In Tables 2, 3, and 4 the recoveries of radioactivity from anaerobic and aerobic flasks containing ^{14}C-labelled TMAN, IPAN, and TEAN are presented.

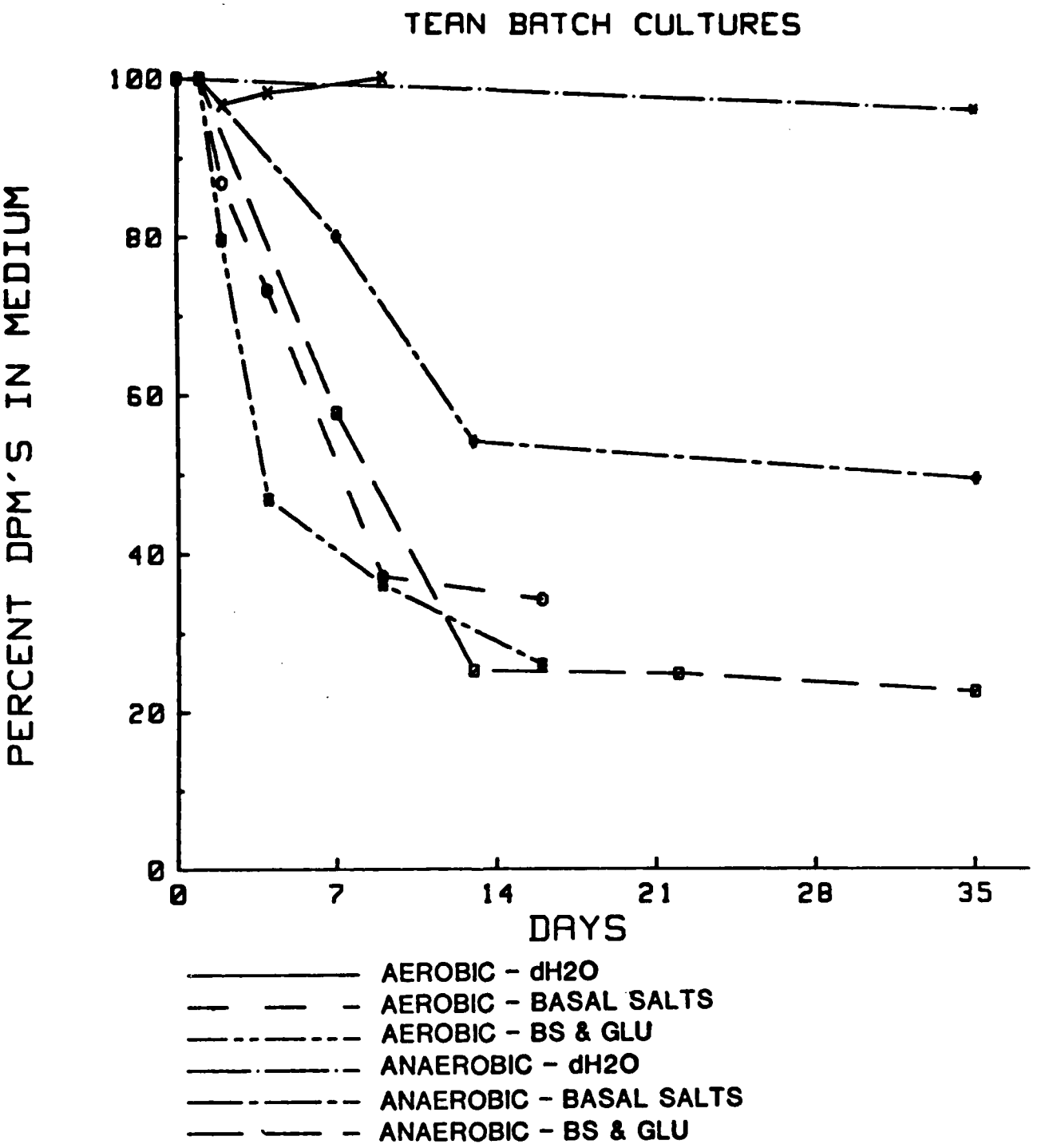

Figure 7. Residual radioactivity in the batch culture media incubated with radioactively labelled TEAN under aerobic and anaerobic conditions.

Table 1. Flask Contents in Second Batch Experiment under Aerobic and Anaerobic Conditions with Ammonium Nitrates (100 ppm)

Flask number	Nutrient broth (4 g/L)	Basal salts	Distilled water	Ammonium phosphate (2 g/L)	Glucose (1 g/L)	Cells
A. Aerobic						
1	+	−	−	−	−	+
2	+	−	−	−	−	−
3	−	+	−	+	+	+
4	−	+	−	+	−	+
5	−	+	−	−	+	+
6	−	+	−	−	−	+
7	−	−	+	−	−	−
8	+	−	−	−	−	−
B. Anaerobic						
1	+	−	−	−	−	+
2	+	−	−	−	−	−
3	−	+	−	+	+	+
4	−	+	−	+	−	+
5	−	+	−	−	+	+
6	−	+	−	−	−	+
7	−	−	+	−	−	−

In general, a negligible fraction of the total radioactivity was recovered in the acid traps; in the incubations with TMAN, this amounted to a few tenths of a percent of the total counts. An exception was the nutrient broth incubation of TMAN under anaerobic conditions when 17.5% was recovered in the acid. In the incubations with IPAN and TEAN the amounts recovered in these traps were no higher than 0.2 percent. Similarly little of the radioactivity was in the cellular biomass from the media, with the exception of some higher levels near seven percent from the total harvested from the incubations with IPAN under anaerobic conditions. Since the sterile control incubations were extracted and prepared in the same manner as the active systems, in some cases trace levels of radioactivity were recovered in the fraction corresponding to the pellet. Significant amounts of radioactivity were present in the broth after the experiment was terminated and the cells removed. This remaining radioactivity accounts for compounds incompletely

Table 2. Percent Recoveries of ^{14}C-labelled Products under Aerobic (top) and Anaerobic (bottom) Conditions with ^{14}C-labelled TMAN in the Second Set of Batch Experiments

A. Aerobic

	Flask Number[1]							
	1	2	3	4	5	6	7	8
Base[2]	3.9	0	45.7	37.1	51.8	43.6	0.1	0
Acid	0	0	0.1	0.1	0.2	0.1	0	0
Broth	94.1	94.5	22.9	35.8	132.9	43.3	83.5	94.6
Cells	1.6	0	0	0	0	0	0	0
Total[3]	98.0	94.5	68.6	72.9	65.9	87.0	83.6	94.6

B. Anaerobic

	Flask Number[1]						
	1	2	3	4	5	6	7
Base[2]	4.0	0.2	47.6	5.5	22.3	3.9	0.3
Acid	17.5	0.2	0.4	0.6	0.4	0.3	0.2
Broth	72.4	102.0	31.5	86.6	66.3	88.6	100.0
Cells	0.9	0	0	0	0.6	0	0.1
Total[3]	93.9	102.4	79.5	92.7	89.0	92.8	100.5

[1] Refer to Table 1 for flask contents.

[2] Total trapped during experiment plus that trapped within 24 hours after acidification of the culture vessel.

[3] Total recovery calculated by adding all items above, excluding the percent recovery from the cells. Recoveries obtained from rinsing the dried, empty flasks with the DMSO-acetone mix were not significant.

Table 3. Percent Recoveries of ^{14}C-labelled Products under Aerobic (top) and Anaerobic (bottom) Conditions with ^{14}C-labelled IPAN in the Second Set of Batch Experiments

A. Aerobic

	Flask Number[1]							
	1	2	3	4	5	6	7	8
Base[2]	0.1	0	0.2	0.1	0.3	1.7	0	0
Acid	0	0	0	0	0	0	0	0
Broth	99.9	100.0	96.7	97.1	96.0	93.5	100.0	100.0
Total[3]	100.0	100.0	96.9	97.2	96.3	95.2	100.0	100.0

B. Anaerobic

	Flask Number[1]						
	1	2	3	4	5	6	7
Base[2]	37.6	0.3	46.3	48.6	46.0	47.0	0.1
Acid	0.2	0	0.1	0.1	0.1	0.1	0.1
Broth	33.4	90.2	22.5	21.5	26.7	26.6	102.0
Cells	6.9	0.8	5.5	1.2	4.5	4.1	0.1
Total[3]	71.2	91.3	68.9	90.2	72.8	73.7	102.2

[1] Refer to Table 1 for flask contents.

[2] Total trapped during experiment plus that trapped within 24 hours after acidification of the culture vessel.

[3] Total recovery calculated by adding all items above, excluding the percent recovery from the cells. Recoveries obtained from rinsing the dried, empty flasks with the DMSO-acetone mix were not significant. No significant counts were recovered from the cells in the anaerobic flasks.

Table 4. Percent Recoveries of ^{14}C-labelled Products under Aerobic (top) and Anaerobic (bottom) Conditions with ^{14}C-labelled TEAN in the Second Set of Batch Experiments

A. Aerobic

	Flask Number[1]							
	1	2	3	4	5	6	7	8
Base[2]	26.7	0	28.1	33.6	34.3	33.3	0	0
Acid	0.1	0	0	0.1	0.1	0.1	0	0
Broth	45.0	98.0	39.7	24.0	20.8	43.7	96.9	100.0
Cells	2.5	0	0	0	4.7	0	0	0
Total[3]	71.8	98.0	67.8	57.6	55.1	77.0	96.9	100.0

B. Anaerobic

	Flask Number[1]						
	1	2	3	4	5	6	7
Base[2]	17.7	-[4]	36.7	40.4	38.2	36.1	0.6
Acid	0	-	0	0	0	0	0
Broth	63.1	-	18.1	29.7	26.0	34.2	102.0
Total[3]	80.8	-	54.8	70.1	64.2	70.2	102.6

[1] Refer to Table 1 for flask contents.

[2] Total trapped during experiment plus that trapped within 24 hours after acidification of the culture vessel.

[3] Total recovery by adding all items above excluding the percent recovery from the cells. Recoveries obtained from rinsing the dried empty flasks with the DMSO-acetone mix were not significant.

[4] Flask 2 became contaminated early in the experiment.

mineralized. The pattern of recovery of radioactive material from the flasks corresponds with the general pattern of mineralization evident in Figures 4, 5, and 6.

The third set of batch experiments was run with each of the ammonium nitrate propellants incubated individually at 1,000 ppm in basal salts medium as the sole source of carbon and nitrogen. Figure 8 illustrates the results from these incubations, and each point on the curves is the average of three replicates. In all cases the compounds were degraded more rapidly under aerobic batch culture conditions than anaerobic conditions. For each of the three ammonium nitrates the total recoveries of radioactivity in the alkaline traps approached 45 percent in the aerobic systems and between about 5 and 15 percent in the anaerobic systems. The lag prior to rapid onset of mineralization was shorter for TEAN and longest for TMAN under the aerobic conditions. The corresponding control flasks in this study are not illustrated because no significant levels (less than 0.1 percent of the total) of radioactivity were recovered in acid or base traps during the experiment. The change in radioactivity in the culture broth of the active aerobic cultures is illustrated in Figure 9 and clearly shows the drop in residual activity as the compounds are degraded. The decrease is earliest for TEAN while all flask contents appear to be inhibitory to further activity after about 60 percent had been lost from the medium.

The total recoveries of ^{14}C-labelled products formed during the incubations and fractionated during the extraction procedure are presented in Tables 5 and 6. The broth in the sterile controls accounts for 100 percent of the radioactivity. Low total recoveries are evident for the active flasks with considerably lower totals for the anaerobic incubations compared with the aerobic flasks. An unexplainable phenomenon seen during the extraction process was the recovery of higher amounts of radioactivity from the broth after it had been centrifuged and filtered than before this manipulation. This phenomenon was found with TMAN and IPAN sterile control incubations under anaerobic conditions. To account for the low total recoveries from some of the active incubations, head space gases were analyzed. No significant amounts of carbon dioxide or methane

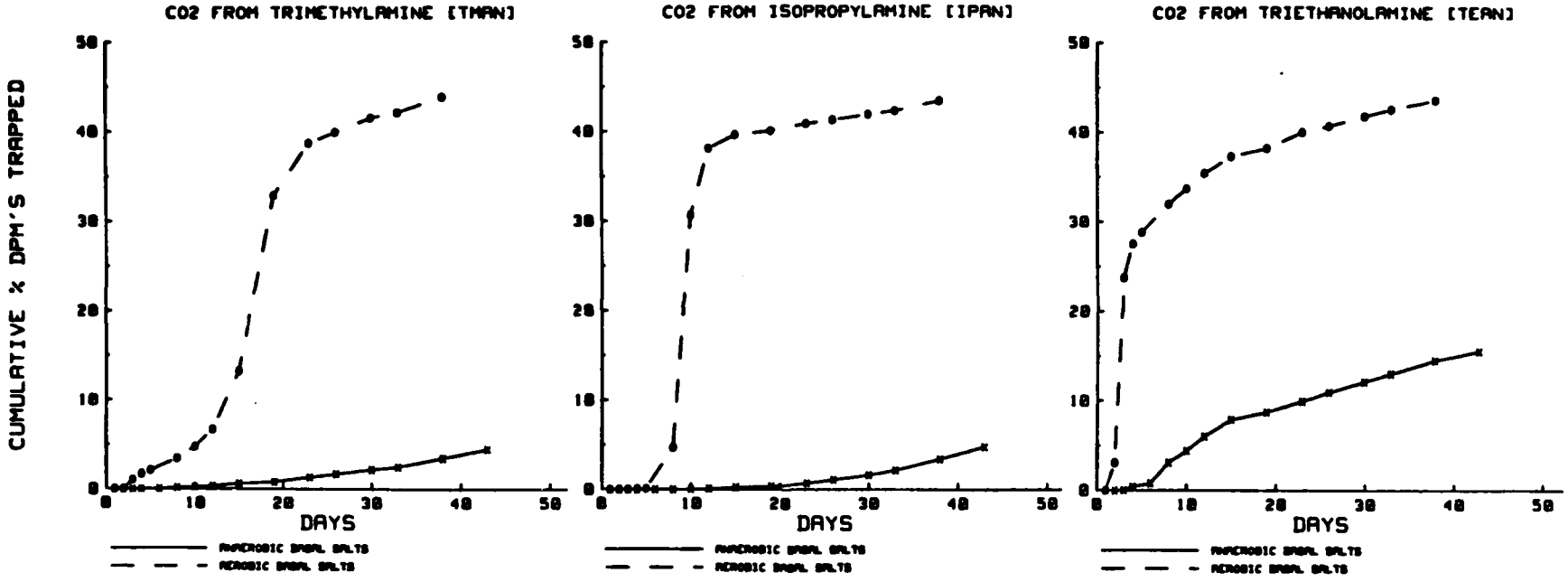

Figure 8. Decomposition of radioactively labelled TMAN, IPAN, and TEAN under aerobic and anaerobic batch culture conditions as the sole source of carbon and nitrogen (third experiment).

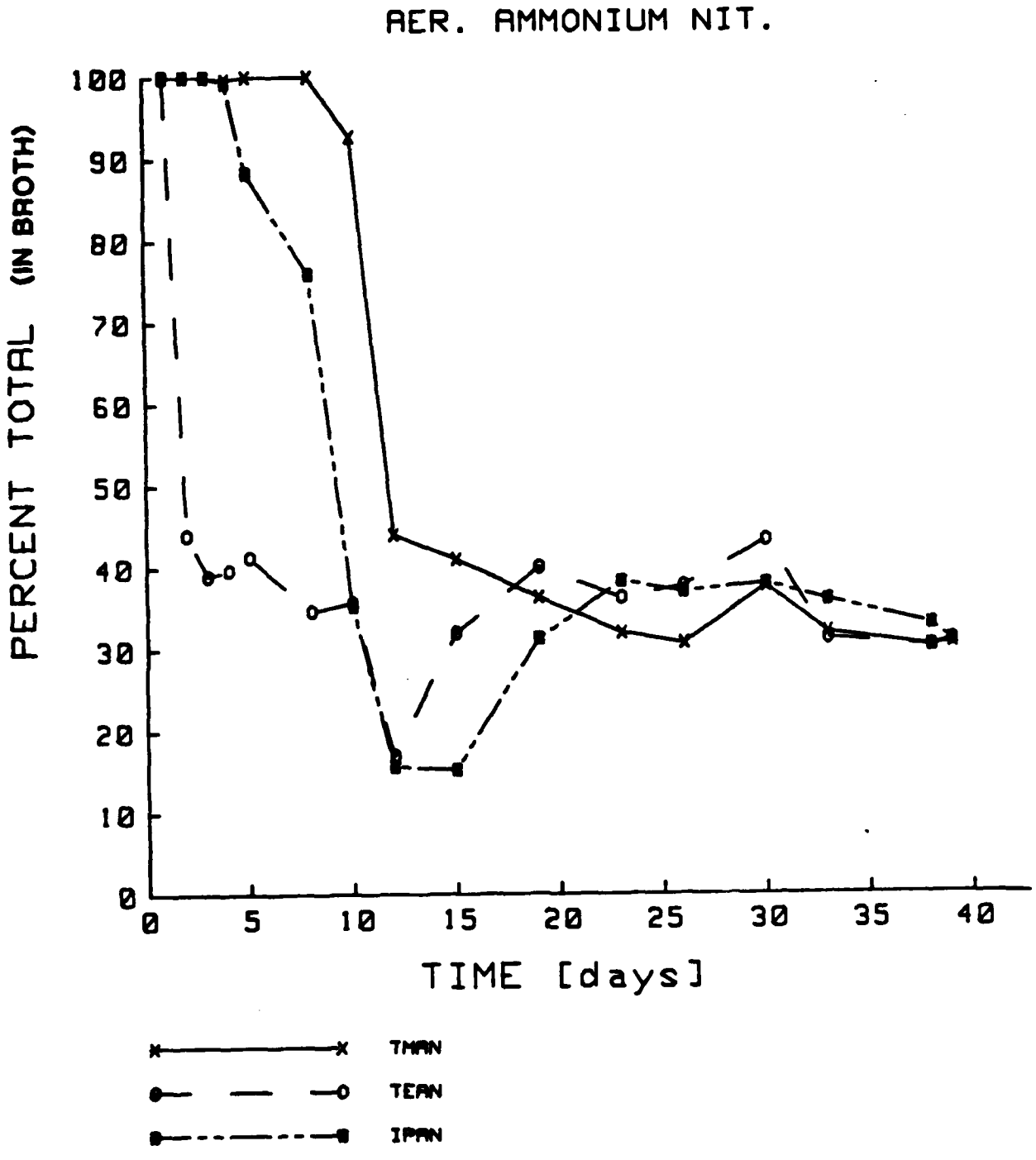

Figure 9. Residual radioactivity in the broth from aerobic batch cultures incubated with radioactively labelled ammonium nitrates (third experiment).

were recovered that would account for the low recovery of carbon in these incubations.

Table 5. Total Percent Recoveries of ^{14}C-labelled Materials from Aerobic Flasks in the Third Batch System Experiment

	TMAN (active)	TMAN (sterile)	IPAN (active)	IPAN (sterile)	TEAN (active)	TEAN (sterile)
N	3	1	3	1	3	1
Base	43.7	0.0	43.7	0.0	46.5	0.0
Acid	0.0	0.0	0.0	0.0	0.0	0.0
Filter	1.5	-[1]	1.3	-	1.1	-
Filtrate	12.5	-	16.1	-	10.2	-
Flask[2]	0.6	-	1.3	-	0.4	-
Cells	3.9	-	5.8	-	7.1	-
Broth[3]	30.7	100.0	31.0	100.0	30.3	100.0
Total[4]	74.4	100.0	74.7	100.0	76.8	100.0

[1] Not determined

[2] DMSO-acetone extract

[3] Prior to filtration

[4] Sum of acid, base, and broth totals

Table 6. Total Percent Recoveries of ^{14}C-labelled Materials from Anaerobic Flasks in the Third Batch System Experiments

	TMAN (active)	TMAN (sterile)	IPAN (active)	IPAN (sterile)	TEAN (active)	TEAN (sterile)
N	2	1	3	1	3	1
Base	11.1	0.0	19.8	0.0	19.1	0.0
Acid	0.0	0.0	0.0	0.0	0.0	0.0
Filter	0.5	0.5	1.1	1.1	1.3	0.3
Filtrate	14.2	149.0	10.9	133.0	15.7	75.4
Flask[1]	0.8	0.1	0.3	0.1	1.7	0.1
Cells	2.1	-	4.0	0.2	0.5	0.2
Broth[2]	19.2	100.0	16.2	100.0	28.3	100.0
Total[3]	30.3	100.0	36.0	100.0	47.4	100.0

[1] DMSO-acetone extract

[2] Prior to filtration

[3] Sum of acid, base, and broth totals

A batch study containing HAN, 100 ppm in media at pH 4.0, was conducted under aerobic and anaerobic conditions and the results are presented in Table 7. The results indicate no significant difference in the concentration of the HAN between inoculated flasks and sterile control flasks during the 10 day incubations. Growth was not extensive under these acidic conditions.

System changes, measurements of pH, denitrification efficiency and degradation of the ammonium nitrates and methanol in the continuous flow systems are presented in Tables 8 to 15. In general, denitrification systems ran efficiently when C/N ratios and retention times were suitably adjusted for each of the systems. The pH of the effluents from the denitrification systems were in the alkaline range as expected in a successfully operative denitrification system. The degradation of the ammonium nitrates was monitored by GC. The percent degraded represents the ratio of effluent to influent concentrations of amine.

Table 7. Average Concentration[1] (ppm) of Hydroxylammonium Nitrate during the 10 Day Incubation under Different Conditions

	Medium			
	Nutrient Broth	Basal Salts	Basal Salts & Glucose	Water
Aerobic				
Active	90.0 ± 12.6	76.7 ± 5.1	61.7 ± 2.9	90.5 ± 5.7
Sterile	97.2 ± 4.5	77.7 ± 3.1	57.3 ± 7.7	90.3 ± 5.9
Anaerobic				
Active	92.0 ± 7.0	74.0 ± 3.6	55.0 ± 3.3	84.7 ± 4.0
Sterile	91.8 ± 7.1	71.4 ± 5.4	53.8 ± 3.4	81.1 ± 3.0

[1]Concentrations determined for days 1, 2, 3, 4, 7, and 10.

Table 8. Chronology of Changes and Results of Analysis for the Anaerobic Denitrification Continuous Flow System with TMAN (100 ppm)

Time (days)	Retention time (days) $\bar{x} \pm 1$ S.D.	Media Composition	pH $\bar{x} \pm 1$ S.D.	Denitrification (%) $\bar{x} \pm 1$ S.D.	Degradation TMAN (%) $\bar{x} \pm 1$ S.D.	Degradation Methanol (%) $\bar{x} \pm 1$ S.D.
0-55	4.6 ± 0.8	basal salts[1]	8.6 ± 0.2	99.0 ± 0.0	99.7 ± 0.8	92.4 ± 5.4
56-74	2.1 ± 0.5	-[2]	8.4 ± 0.2	74.3 ± 3.0	99.5 ± 0.6	82.8 ± 7.1
75-91	1.6 ± 0.5	-	8.4 ± 1.0	57.0 ± 24.0	99.5 ± 1.1	60.7 ± 3.1
92-98	5.5 ± 1.2	-	8.5 ± 0.0	93.0 ± 0.0	N.D.[3]	N.D.
99-130	4.7 ± 0.9	basal salts[4]	9.0 ± 0.2	83.2 ± 10.8	99.8 ± 0.5	N.A.[5]

[1] KNO_3 4.93 g, methanol 4.1 mL. Ratio of grams of total organic carbon to grams of total nitrogen as nitrate (C/N) = 1.8.

[2] No change

[3] No data

[4] Sodium acetate 6.96 g per L instead of methanol as the electron donor.

[5] Not applicable

Table 9. Chronology of Changes and Results of Analysis for the Anaerobic Denitrification Continuous Flow System with TMAN; Effect of Concentration

Time (days)	Concentration TMAN (ppm)	Retention time (days) $\bar{x} \pm 1$ S.D.	pH $\bar{x} \pm 1$ S.D.	Denitrification (%) $\bar{x} \pm 1$ S.D.	Degradation TMAN (%) $\bar{x} \pm 1$ S.D.	Degradation Methanol (%) $\bar{x} \pm 1$ S.D.
0-31	100	3.0 ± 0.4	7.5 ± 0.3	79.3 ± 12.6	88.1 ± 4.2	89.0 ± 7.9
32-39	200	5.3 ± 0.7	7.2 ± 0.3	79.6 ± 10.8	N.D.[2]	N.D.
40-60	2,000	4.4 ± 0.9	7.4 ± 0.3	7.0 ± 16.0	16.5 ± 9.9	99.8 ± 0.4

[1] Medium was basal salts but with K_2HPO_4 6.96 g per L, C/N = 1.5.

[2] No data

Table 10. Chronology of Changes and Results of Analysis for the Anaerobic Denitrification Continuous Flow System with IPAN (100 ppm) [1]

Time (days)	Retention time (days) $\bar{x} \pm 1$ S.D.	pH $\bar{x} \pm 1$ S.D.	Denitrification (%) $\bar{x} \pm 1$ S.D.	Degradation IPAN (%) $\bar{x} \pm 1$ S.D.	Degradation Methanol (%) $\bar{x} \pm 1$ S.D.
0-64	4.3 ± 0.7	8.1 ± 0.5	99.0 ± 0.0[2]	32.1 ± 19.2	95.6 ± 5.9
65-97	8.7 ± 1.5	8.2 ± 0.1	98.9 ± 0.4	42.8 ± 5.0	100 ± 0.0

[1] Medium was basal salts, C/N = 1.5.

[2] Denitrification was 59.3 ± 13.7 percent for the first 34 days and 99.0 ± 0.0 after that period.

Table 11. Chronology of Changes and Results of Analysis for the Anaerobic Denitrification Continuous Flow System with TEAN

Time (days)	Concentration TEAN (ppm)	Retention time (days) $\bar{x} \pm 1$ S.D.	pH $\bar{x} \pm 1$ S.D.	Denitrification (%) $\bar{x} \pm 1$ S.D.	Degradation TEAN (%) $\bar{x} \pm 1$ S.D.
0-29	1,000	9.3 ± 1.1	8.0 ± 0.1	56.0 ± 0.0	N.D.[2]
30-76	-[3]	4.1 ± 0.4	7.9 ± 0.1	95.3 ± 4.7	31.8 ± 17.0
77-100	-	9.5 ± 1.0	7.6 ± 0.0	99.0 ± 0.0	60.8 ± 20.8
101-161	500	7.6 ± 0.9	7.8 ± 0.1	94.3 ± 4.2	63.3 ± 16.5

[1]Medium was basal salts, C/N = 1.5.

[2]No data

[3]Unchanged

Table 12. Chronology of Changes and Results of Analysis for the Anaerobic Denitrification Continuous Flow System with DMA (100 ppm)

Time (days)	Retention time (days) $\bar{x} \pm 1$ S.D.	Media Composition	C/N	pH $\bar{x} \pm 1$ S.D.	Denitrification (%) $\bar{x} \pm 1$ S.D.	Degradation DMA (%) $\bar{x} \pm 1$ S.D.	Degradation Methanol (%) $\bar{x} \pm 1$ S.D.
0-18	3.6 ± 0.2	basal salts[1]	11.3	7.9 ± 0.1	84.6 ± 6.9	100 ± 0.0	47.7 ± 9.3
19-33	5.2 ± 0.4	basal salts[2]	5.8	7.9 ± 0.1	74.6 ± 9.2	99.9 ± 0.2	47.0 ± 8.5
34-55	2.5 ± 0.5	-[3]	-	8.3 ± 0.2	27.4 ± 34.5	46.7 ± 10.8	37.0 ± 20.0

[1] K_2HPO_4 3.48 g, methanol 10.55 mL, trace salts 298 ng.

[2] K_2HPO_4 6.96 g, KNO_3 4.04 g, trace salts 595 mg.

[3] Unchanged

Table 13. Chronology of Changes and Results of pH Analysis for the Aerobic Continuous Flow System with TMAN (100 ppm)

Days	Retention time (days) $\bar{x} \pm 1$ S.D.	Media Composition	pH($\bar{x} \pm 1$ S.D.)	Degradation TMAN (%) $\bar{x} \pm 1$ S.D.
0-44	4.0 ± 0.5	nutrient broth (4 g/L)	8.1 ± 0.2	99.4 ± 0.9
45-79	2.4 ± 1.0	-[1]	8.2 ± 0.3	100.0 ± 35.6
80-93	1.0 ± 0.1	-	8.1 ± 0.0	51.5 ± 35.6
94-114	5.4 ± 1.7	basal salts	7.3 ± 1.1	93.5 ± 9.2
115-135	2.6 ± 0.5	-	6.0 ± 0.0	99.3 ± 1.2
136-177	4.2 ± 0.3	basal salts[2]	6.7 ± 0.1	100.0 ± 0.0

[1]Unchanged

[2]Without $NH_4H_2PO_4$

Table 14. Chronology of Changes and Results of pH Analysis for the Aerobic Continuous Flow System with IPAN (100 ppm)

Days	Retention time (days) $\bar{x} \pm 1$ S.D.	Media Composition	pH ($\bar{x} \pm 1$ S.D.)	Degradation IPAN (%) $\bar{x} \pm 1$ S.D.
0-25	4.2 ± 0.4	nutrient broth (4 g/L)	8.9 ± 0.1	110.0 ± 0.0
25-56	2.0 ± 0.2	-[1]	8.8 ± 0.0	86.5 ± 13.3
57-74	1.1 ± 0.2	-	8.6 ± 0.1	42.7 ± 23.8
75-95	4.7 ± 0.3	basal salts	6.2 ± 0.0	100.0 ± 0.0
96-116	2.8 ± 0.3	-	6.1 ± 0.2	94.0 ± 4.2
117-137	4.4 ± 0.2	basal salts[2]	6.5 ± 0.4	97.0 ± 4.2

[1] Unchanged

[2] Without $NH_4H_2PO_4$

Table 15. Chronology of Changes and Results of pH Analysis for the Aerobic Continuous Flow System with TEAN

Days	Concentration TEAN (ppm)	Retention time (days) $\bar{x} \pm 1$ S.D.	Media Composition	pH ($\bar{x} \pm 1$ S.D.)	Degradation TEAN (%) $\bar{x} \pm 1$ S.D.
0-10	1,000	4.0 ± 0.2	nutrient broth (4 g/L)	8.6 ± 0.0	91.5 ± 7.8
11-48	-[1]	8.7 ± 1.2		8.9 ± 0.1	78.0 ± 6.1
49-81	500	8.3 ± 1.9	-[1]	8.6 ± 0.1	77.5 ± 17.0
82-111	-	8.0 ± 0.7	basal salts + glucose (1 g/L)	6.6 ± 0.3	50.3 ± 16.7
112-147	-	5.8 ± 0.3	basal salts	6.3 ± 0.2	58.0 ± 31.1

[1]Unchanged

Under anaerobic denitrification continuous flow conditions TMAN, 100 ppm, was efficiently degraded (Table 8) at 4.6, 2.1, and 1.6-day retention times. This was also the case when sodium acetate was substituted for methanol as the electron donor. With methanol in the system, its efficiency of utilization and the denitrification efficiency decreased as the retention time was shortened. Thus, even when the denitrification efficiency was adversely effected by shortened retention times, TMAN was still effectively degraded.

In Table 9, the effect of increasing concentrations of TMAN on the system as just described is shown. At 100 ppm, denitrification and TMAN degradation efficiencies were relatively high, while upon increasing the concentration of TMAN 20-fold to 2,000 ppm there was a significant reduction in denitrification efficiency and TMAN degradation but not in methanol utilization.

IPAN (Table 10) was incompletely degraded in the denitrification system at a 4.3-day retention time, and there was little improvement as a result of lengthening the retention time to 8.7 days (Table 10). Denitrification efficiency and methanol utilization were high throughout the operation of this system.

TEAN, 1,000 ppm, was not efficiently degraded at a 4.1 retention time, while higher percentages of decomposition were achieved at longer retention times. (9.5 day and 7.6-day retention times with 1,000 ppm and 500 ppm TEAN, respectively)(Table 11). In all instances except for the initial acclimation period, denitrification efficiency was high.

DMA was effiencntly degraded at 3.6 and 5.2-day retention times, while at the shorter retention time, 2.5 days, decomposition was incomplete (Table 12). Denitrification efficiency and utilization of methanol were adversely affected by excessive carbon to nitrogen ratios.

Results from aerobic continuous flow systems are presented in Tables 13-15. TMAN (Table 13) was readily degraded at 4.0 and 2.4 day retention

times but efficiency decreased at a 1.0 day retention time in nutrient rich solution. In basal salts medium both with and without supplemental nitrogen TMAN was effectively decomposed. The basal salts system without nitrogen represents a culture medium in which TMAN serves as the sole source of carbon and nitrogen.

In Table 14 in nutrient rich solution, IPAN was completely degraded at a 4.2-day retention time and progressively less efficiently degraded as the retention time was shortened. In basal salts medium with and without supplemental nitrogen IPAN was readily degraded.

TEAN was effectively decomposed at a 4.0-day retention time and somewhat less efficiently degraded as the retention time was lengthened at both 1,000 ppm and 500 ppm (Table 15). TEAN was incompletely metabolized when present in a basal salts medium both with and without supplemental glucose.

In Figure 10, results of NDMA analysis of the TMAN continuous flow denitrification system are presented. Influent and effluent samples were monitored for the nitrosamine by HPLC and low ppb levels were present in influent and effluent samples. For the most part, concentrations were slightly higher in the effluent. The system changes corresponding to this graph show in Table 8.

The stability of HAN at different hydrogen ion concentrations is presented in Figure 11. At pH 6.9 and pH 5.9 HAN was unstable, while at pH 4.9 and below HAN was stable under these conditions over the 28-day study. The initial work on utilizing a colorimetric assay for HAN determinations was unsuccessful due to the instability of HAN at the pH recommended in the assay procedure for hydroxylamine. Once standards were maintained at pH 4.0 there were no further difficulties.

The results from the soil incubations with IPAN, TMAN, and TEAN are presented in Figures 12-18. The recoveries of ^{14}C-labelled carbon dioxide in base traps and volatile products in acid traps for active and sterile systems are detailed. In some instances the data is summarized

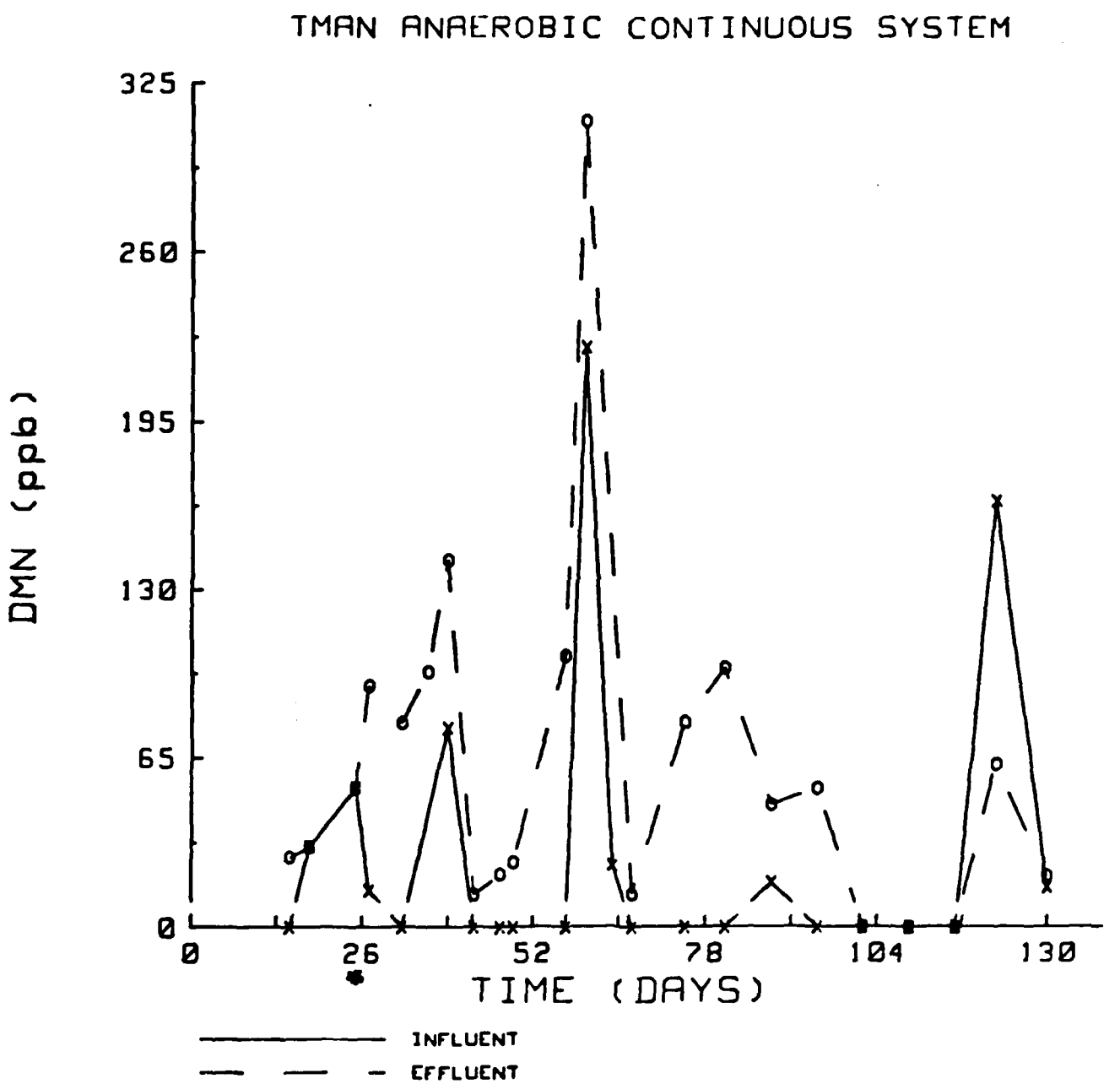

Figure 10. Concentrations of NDMA in influent and effluent samples from the anaerobic continuous flow denitrification system with TMAN.

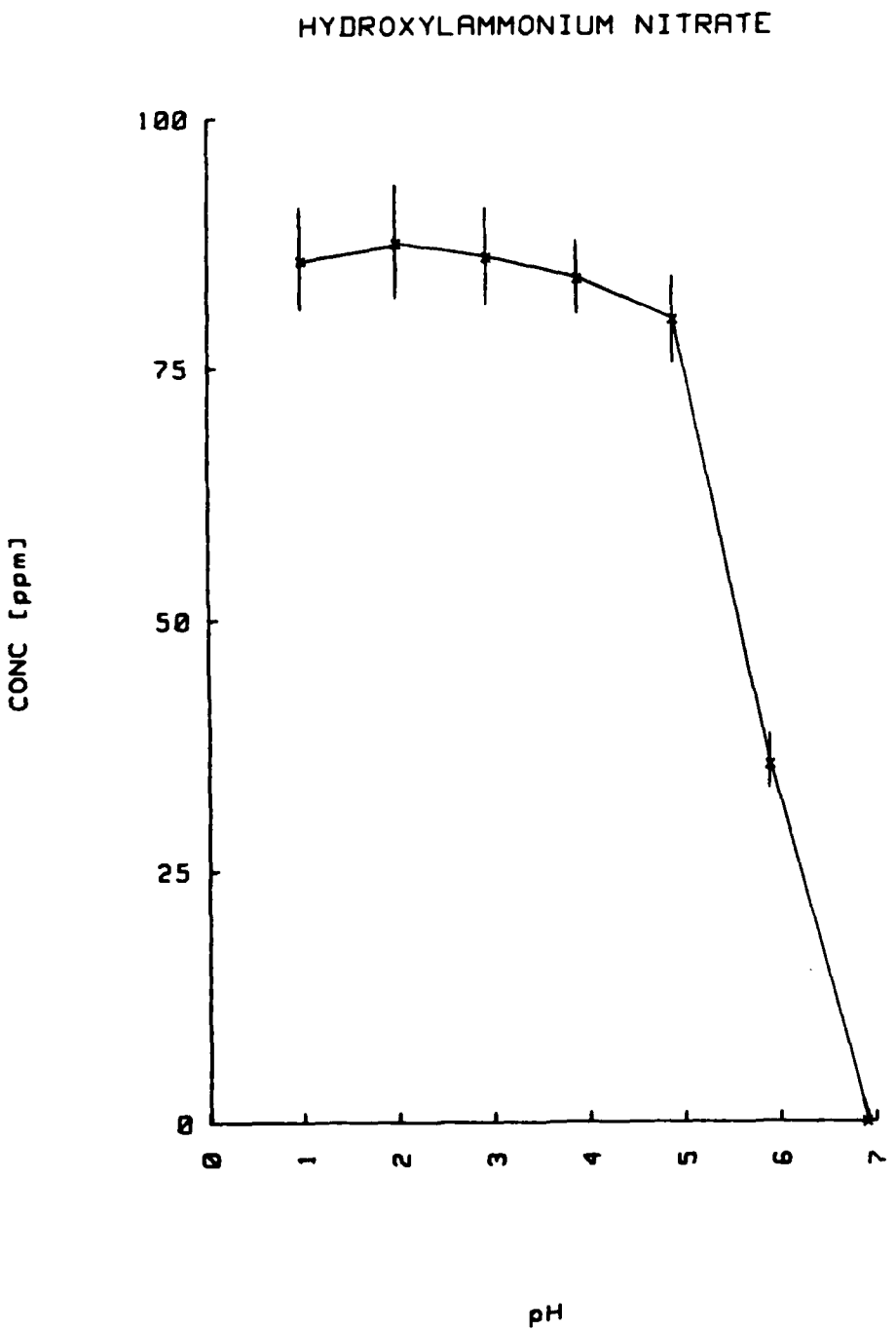

Figure 11. Stability of HAN at different hydrogen ion concentrations ($\bar{x}$ ± 1 S.D.).

by month instead of the daily figures. Final measurements of pH from the second set of soil incubations are listed in Table 16. Final oxidation-reduction measurements indicated aerobic conditions in all flasks.

Table 16. Final pH Readings (Average of Duplicates) from ^{14}C-labelled Soil Incubations with Ammonium Nitrate Propellants

Contents	TMAN	IPAN	TEAN
1. Soil			
-active	6.2	6.2	6.4
-sterile	6.1	5.9	6.0
2. Soil (flooded)			
-active	6.8	6.6	6.3
-sterile	6.2	6.3	5.7
2. Sand			
-active	5.0	5.0	5.0
-sterile	6.5	6.5	6.6

In the first set of soil incubations the effect of concentration of the ammonium nitrates on rates of mineralization in soil was examined. It is clear from Figures 12-14 that at concentrations of 50, 500, and 5,000 ppm the total percent degraded was not significantly different for each of the three components (Figure 15). At 5,000 ppm the initial rates of degradation were somewhat lower for TMAN and IPAN, but this lag was no longer evident after the first month. Only trace amounts of radioactivity were recovered in alkali traps from the control flasks during the study (Figures 12-14). The total percent degradation after 103 days was between 45 and 60 percent for all of the active flasks.

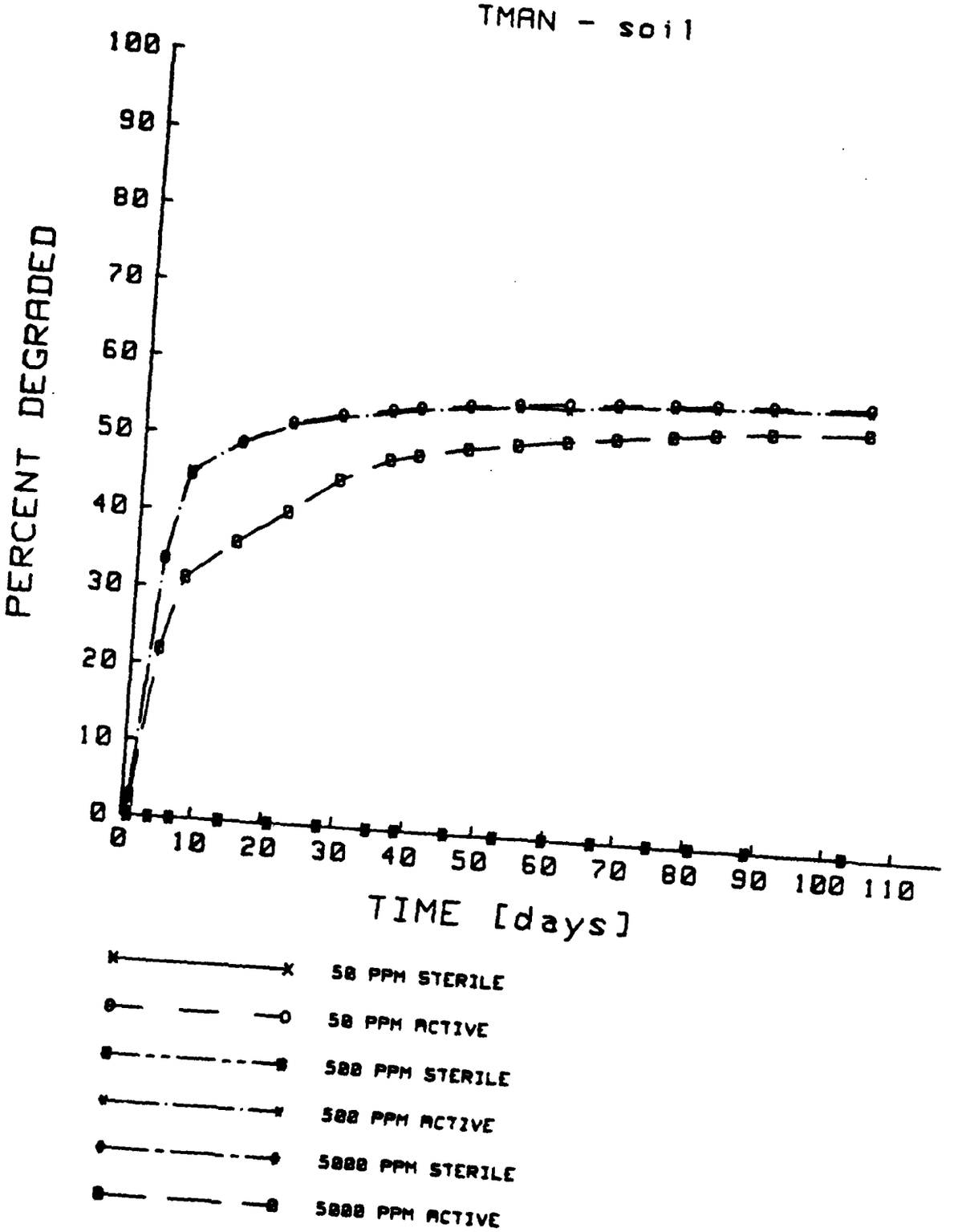

Figure 12. Effect of concentration of radioactively labelled TMAN on rates of mineralization in soil.

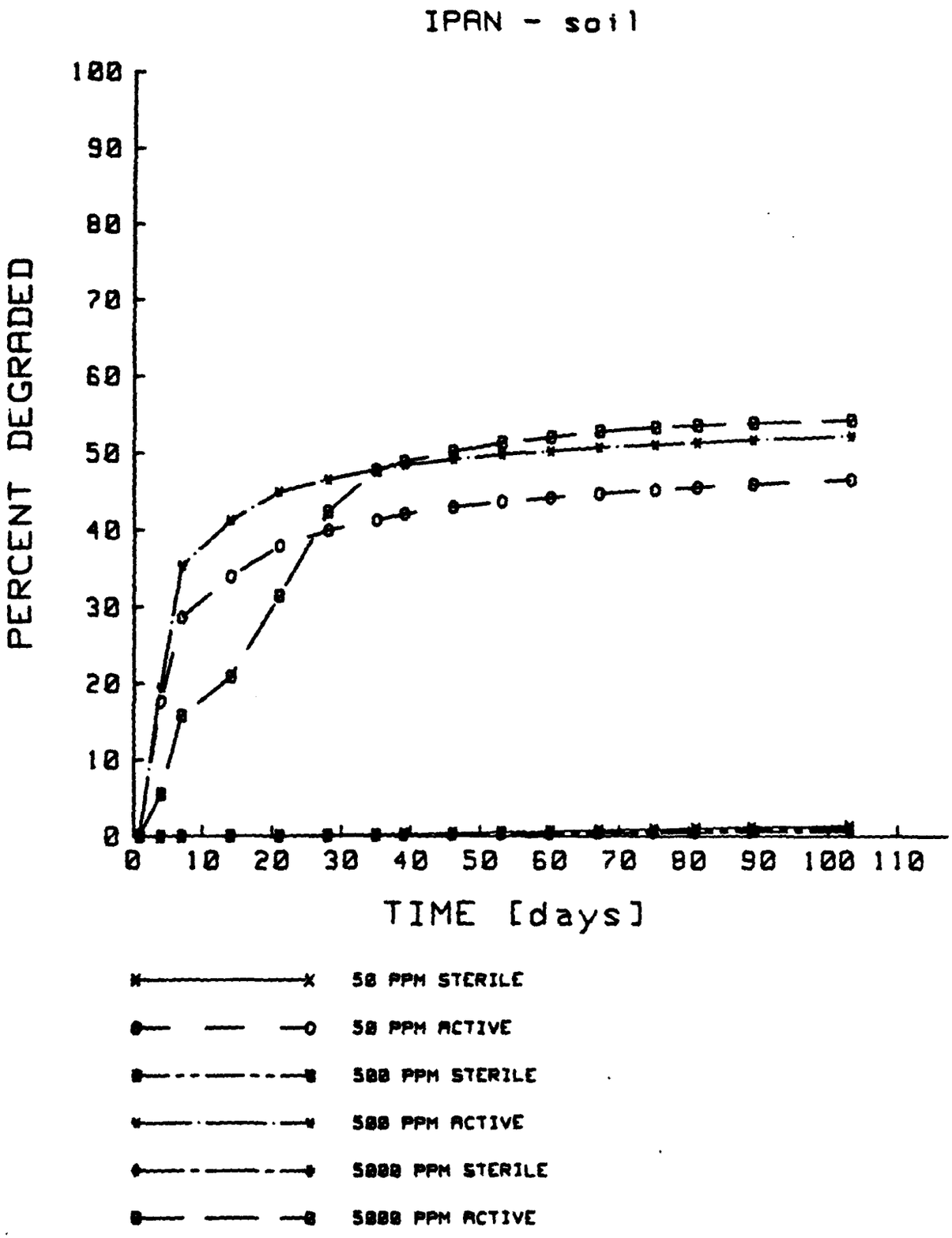

Figure 13. Effect of concentration of radioactively labelled IPAN on rates of mineralization in soil.

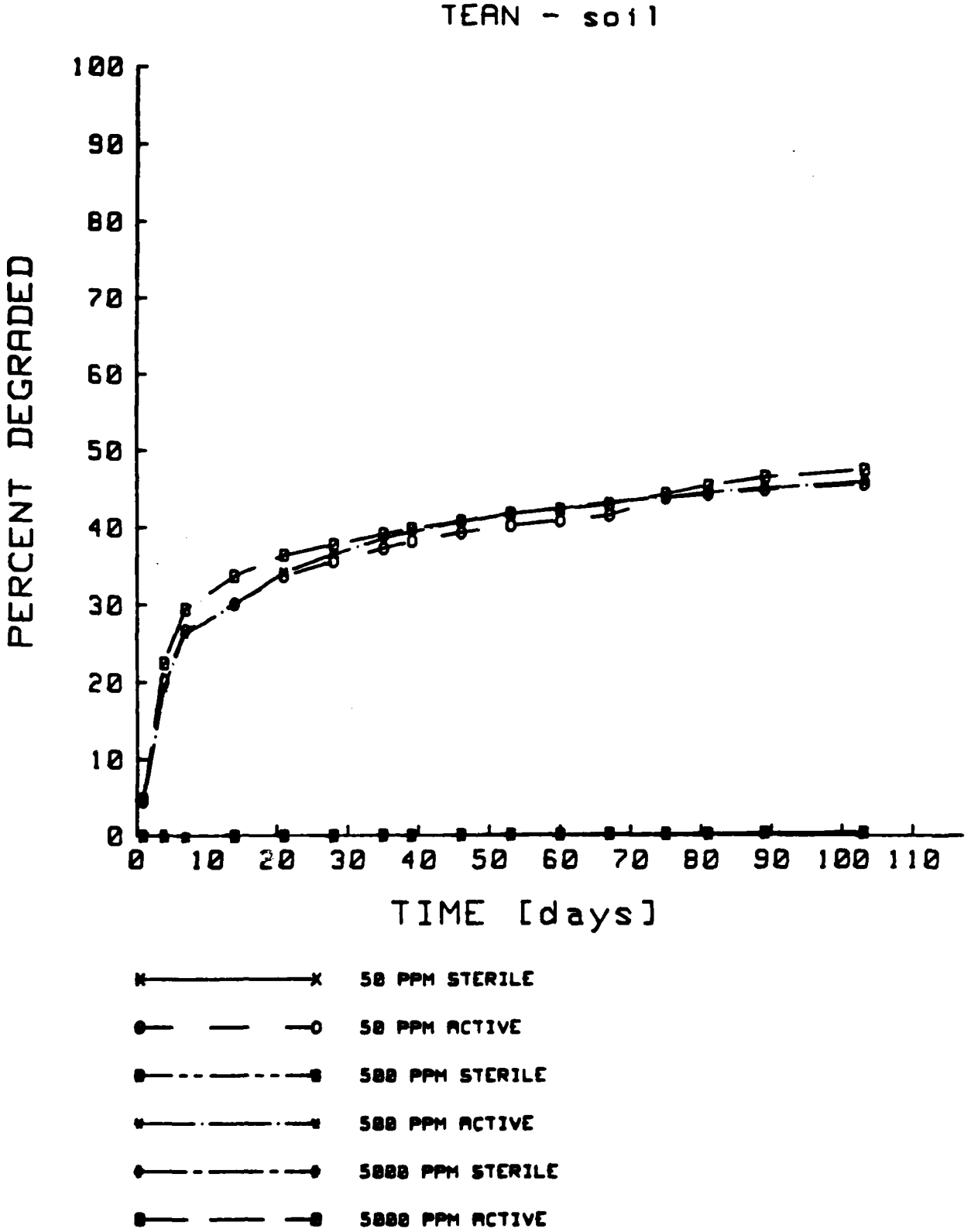

Figure 14. Effect of concentration of radioactively labelled TEAN on rates of mineralization in soil.

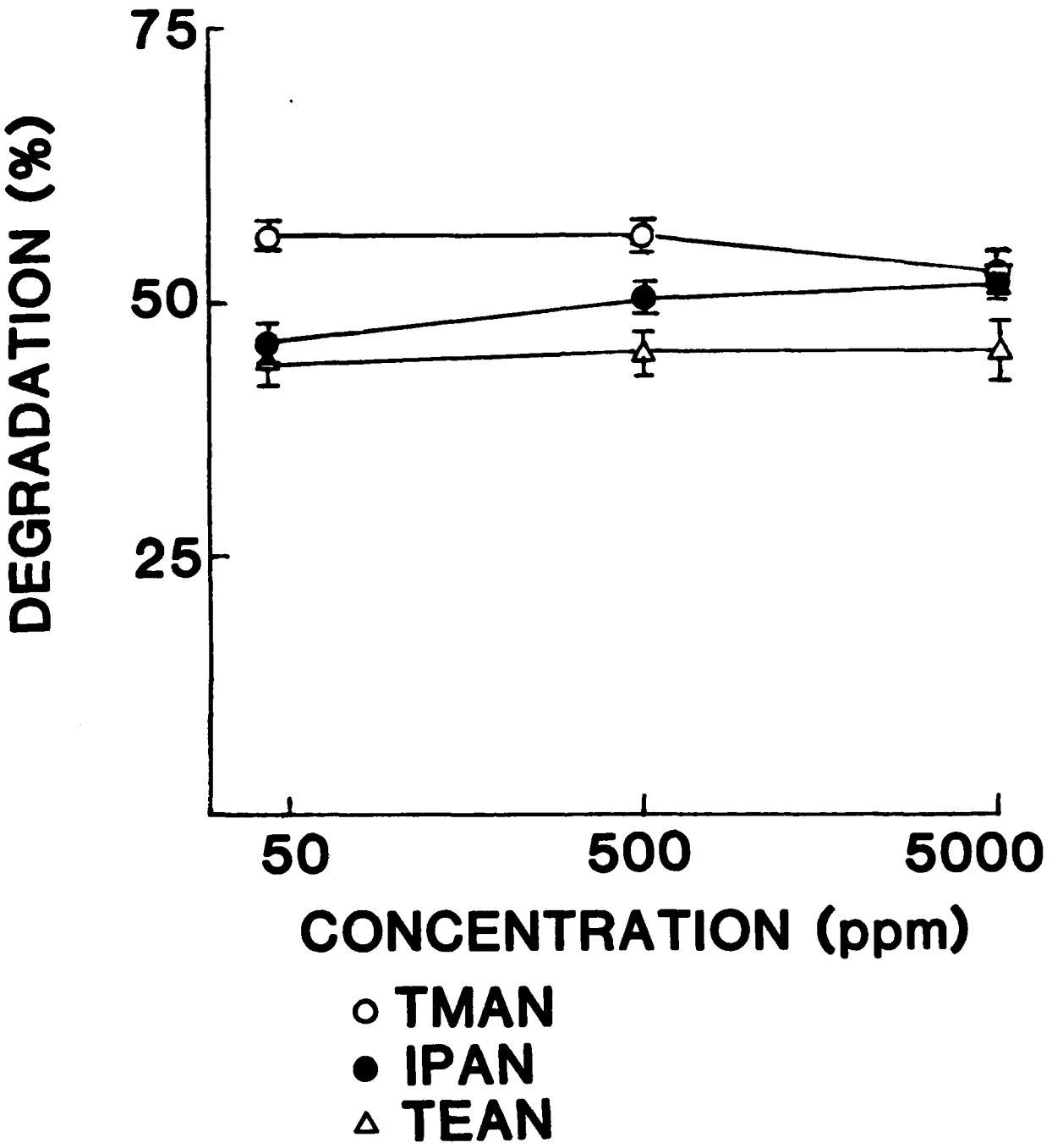

Figure 15. Effect of concentration of ammonium nitrates on total percent degradation ($\bar{x} \pm 1$ S.D. for days 60-103).

In the second set of soil incubations the effect of soil conditions on rates and total degradation of the ammonium nitrates over seven months was examined. Table 17 presents the radioactivity recovery data from base traps. The data is summarized and illustrated in Figures 16-18 for the alkali trap figures. There was a rapid initial rate of degradation during the first month and a relatively gradual increment thereafter in all the active systems. At most, 4.2% of the total counts were released into the acid traps from the active systems during the seven months and considerably lower amounts from the sterile controls. A total release of between 53.7 and 74.5 percent was found for all the active flasks during the study under the different soil conditions. The initial rates of degradation of TMAN were highest in soil, lower in flooded soil, and lowest in sand; however, by the end of the incubation period the total percentages were within 5 percent. A similar pattern of initial reaction rate was found in the soil flasks incubated with IPAN. After seven months there was a higher total release from the flasks containing flooded soil.

The results with TEAN are illustrated in Figure 18 and Table 17. Initial rates of release of $^{14}CO_2$ were similar under all three conditions while total release was highest in the sand medium and lowest in the soil.

Thin-layer electrophoresis, as described in methods section, was evaluated at as a potential method for analysis of these amines. There was about a 30 percent loss of TMAN during an electrophoretic run using these conditions as determined by seeding with radioactively labelled TMAN. This is probably due to volatilization despite the acidic electrolyte. Because of this problem, further development of this method was not undertaken and the GC methodology was used for quantitative assessment of degradation.

The results of Ames mutagenicity tests of the four ammonium nitrate propellants are presented in Table 18. The results indicate no evidence for mutagenic activity, as all mutations rates fall within background levels. It was noted, however, that HAN was toxic to the Salmonella typhimurium at the higher concentrations tested.

Table 17. Decomposition of ^{14}C-labelled Ammonium Nitrates in Soils

Cumulative counts (percent) recovered in sodium hydroxide

Contents	N	Months 0.3	0.7	1	2	3	4	5	6	7
TMAN[1]										
-soil	2	59.1	63.4	65.4	68.4	69.7	70.4	71.3	72.1	72.6
-soil (flooded)	2	40.1	58.6	64.5	68.8	70.6	71.3	72.2	73.0	74.5
-sand	2	7.6	28.1	46.1	57.2	62.2	65.8	66.8	67.5	68.2
IPAN[2]										
-soil	2	43.5	54.0	55.9	59.2	60.4	62.1	62.7	62.8	63.4
-soil (flooded)	2	36.4	60.6	63.3	67.4	69.2	70.8	73.2	73.3	74.0
-sand	2	12.6	49.7	52.0	58.2	59.0	60.5	60.9	63.3	63.6
TEAN[3]										
-soil	2	31.3	36.3	40.4	47.9	49.3	51.1	52.5	52.7	53.7
-soil (flooded)	2	34.1	41.8	47.7	55.7	57.2	59.5	60.9	61.2	62.1
-sand	2	33.6	40.5	46.6	63.1	64.5	66.4	67.7	68.9	69.9

[1] 0.17 μCi per flask, sterile controls released 0.1, 1.4, and 2.1% in soil, flooded soil, and sand, respectively, over the 7 months. Less than 0.8% of the total was collected in acid traps in both active and sterile systems.

[2] 0.32 μCi per flask, sterile controls released 0.5, 1.3, and 0.4% in soil, flooded soil, and sand, respectively, over the 7 months. Less than 2.4% of the total was collected in acid traps in both active and sterile systems.

[3] 0.024 μCi per flask, sterile controls released 1.9, 0.3, and 0.2% in soil, flooded soil, and sand, respectively, over the 7 months. Less than 4.2% of the total was collected in acid traps in both active and sterile systems.

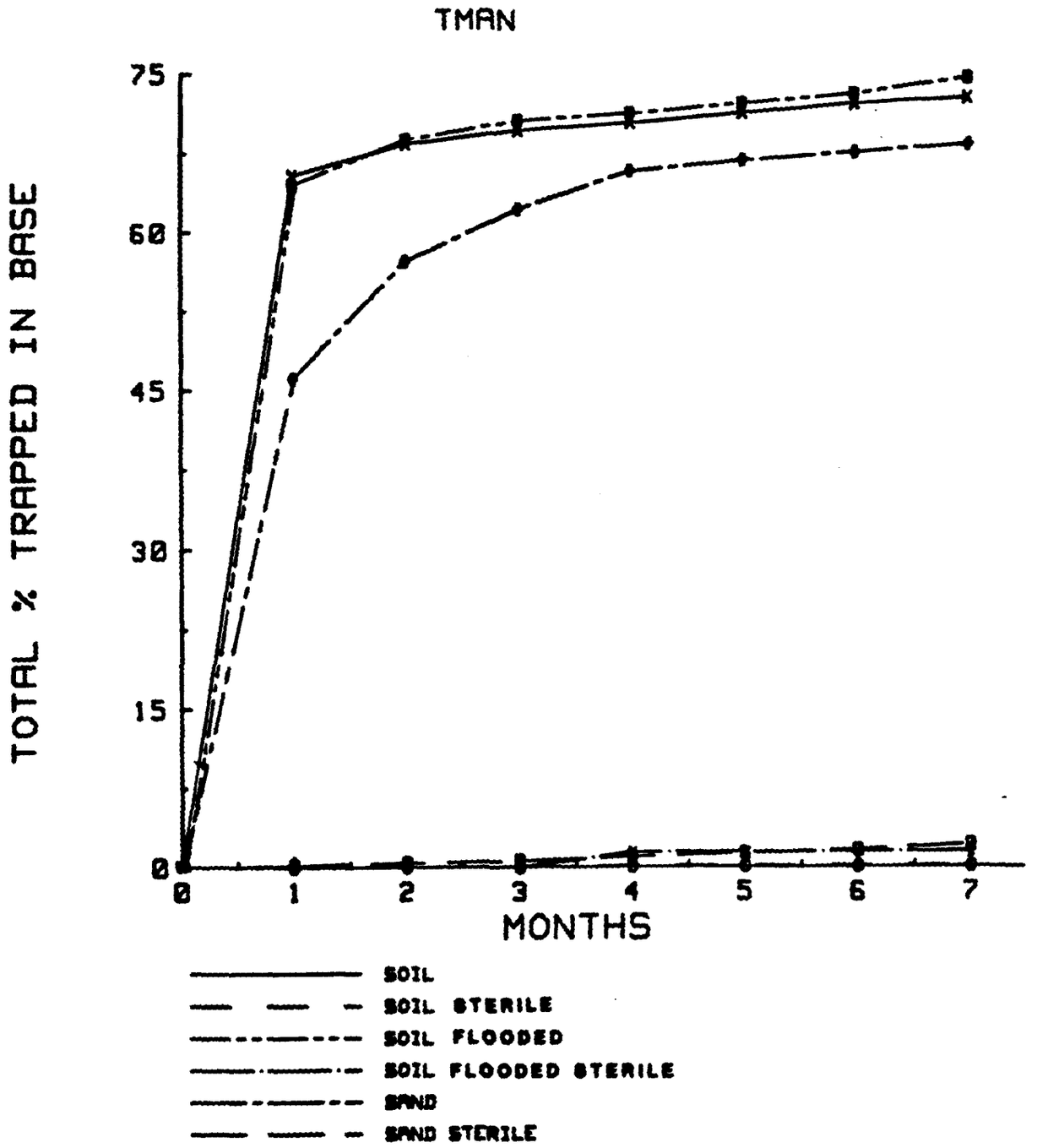

Figure 16. Effect of soil conditions on rates of mineralization of radioactively labelled TMAN.

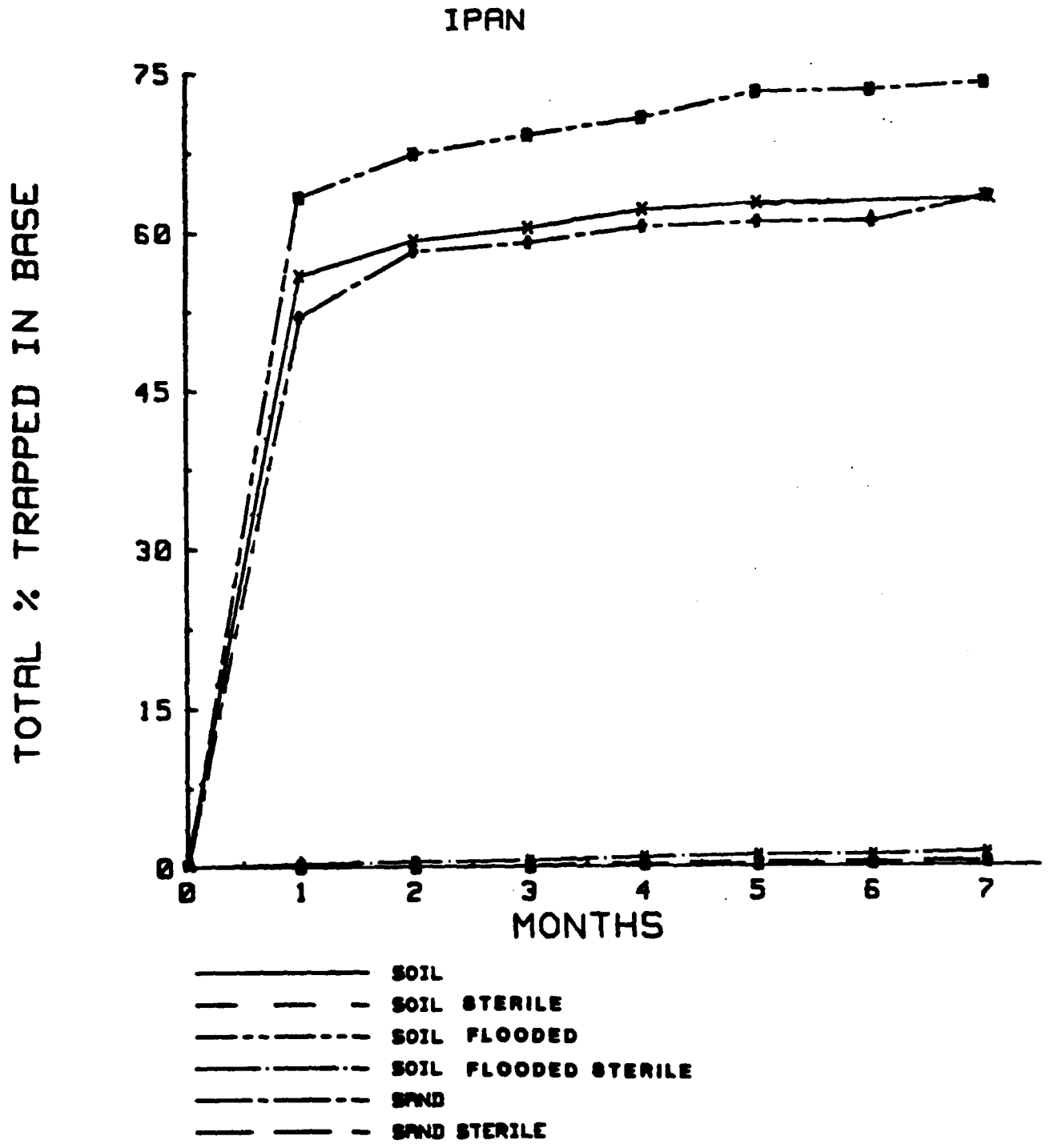

Figure 17. Effect of soil conditions on rates of mineralization of radioactively labelled IPAN.

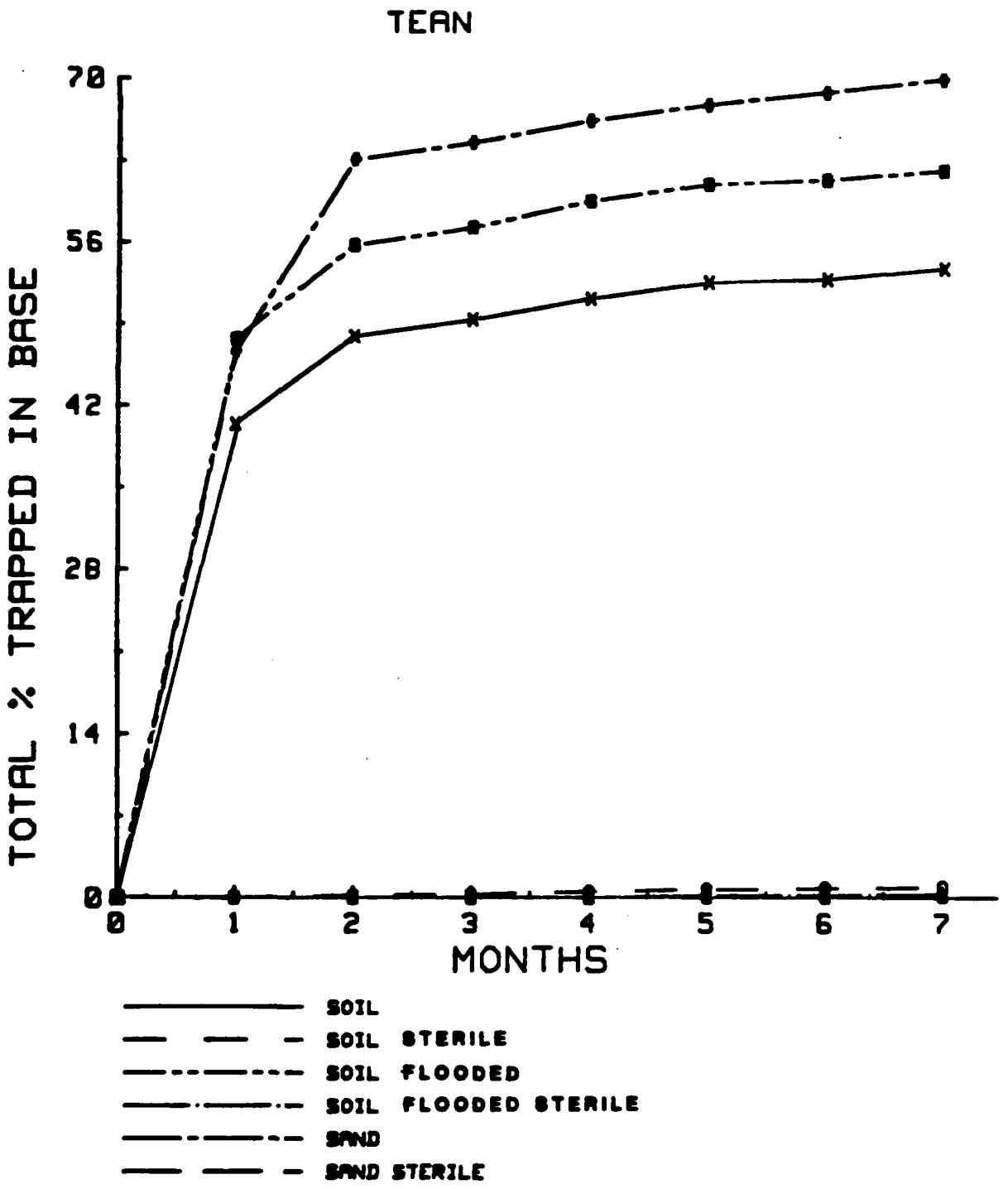

Figure 18. Effect of soil conditions on rates of mineralization of radioactively labelled TEAN.

Table 18. Results of Ames Screening Test for Mutagenicity with TMAN, IPAN, TEAN, and HAN

AMES DATA
Summary

COMPOUNDS - HAN, IPAN, TMAN, TEAN

COMPOUNDS	Metabolic Activation	µg per plate	Histidine Revertants Per Plate (X ± 1 S.D.) TA1535	TA1537	TA1538	TA98	TA100
1. Negative Controls	-	0	9±1	5±2	11±4	21±7	95±5
	+	0	12±1	4±0	14±2	18±2	120±7
2. Positive Controls							
sodium azide	-	1	210±10	-	-	-	343±93
9-aminoacridine	-	150	-	1787±503	-	-	-
4-nitrophenylenediamine	-	10	-	-	1028±211	-	-
2-nitrofluorene	-	50	-	-	-	885±68	-
2-anthramine	+	2	111±15	195±11	1402±44	1197±85	1839±190
3. HAN	-	5000	T[1]	T	T	T	T
	-	500	7±3	4±2	8±3	9±3	93±15
	-	50	10±2	9±1	9±2	19±2	92±12
	-	5	13±5	8±5	11±1	14±4	122±18
	+	5000	T	T	T	T	T
	+	500	7±3	4±2	6±5	15±4	115±50
	+	50	9±2	7±3	13±4	18±6	94±8
	+	5	8±3	8±1	15±6	21±2	138±26
4. IPAN	-	5000	11±1	5±0	11±3	14±6	107±3
(water)	-	500	11±3	4±1	10±2	16±4	111±20
	-	50	14±3	6±4	15±4	15±2	111±9
	-	5	15±4	5±2	8±3	18±6	127±58
	+	5000	6±3	4±4	21±11	15±3	89±6
	+	500	10±2	5±2	12±8	15±7	99±16
	+	50	11±2	7±5	13±3	17±3	130±9
	+	5	12±4	3±1	20±6	5±0	114±14
5. TMAN	-	5000	6±4	6±3	13±2	14±3	107±2
(water)	-	500	14±1	6±3	10±2	14±5	106±6
	-	50	11±1	5±4	13±2	14±5	106±26
	-	5	18±4	7±3	11±3	18±4	112±13
	+	5000	6±4	9±1	14±1	18±10	111±15
	+	500	9±4	7±3	14±1	18±2	105±13
	+	50	9±3	5±1	18±3	16±5	107±9
	+	5	11±4	6±3	13±2	25±5	95±13

DISCUSSION

The results from the biological studies with TEAN, TMAN, and IPAN indicate that all three compounds are biodegradable. Under aerobic conditions they are decomposed, and there is no evidence for the formation of significant concentrations of intermediates. Under anaerobic denitrification conditions TEAN and TMAN were biodegraded, while IPAN was only partially decomposed. As under aerobic conditions, there is no indication that significant formation of intermediates occurred. For the most part, intermediates were detectable only if the system was perturbed, for instance when the nutrient reservoir was changed. Under aerobic batch conditions all three ammonium nitrates are mineralized in a minimal medium in which they are the sole source of carbon and nitrogen. These findings suggest that development of a biological approach to treat process waters containing these compounds would be a feasible one. The system should have a degradative capability over a wide range of environmental conditions.

In organic poor or organic rich soils, or in flooded soils at concentrations up to 0.5%, the three compounds are readily mineralized. As in most batch systems, the initial rates of decomposition are very rapid with a subsequent leveling off of activity and gradual release of carbon dioxide. This was true for the aqueous and soil batch systems studied. Inhibitory effects develop in these systems which prevent 100 percent mineralization. Similarly, incorporation of some of the material into microbial biomass would account for a slow turnover and subsequent release of the ^{14}C-label.

HAN was found to be chemically unstable above a pH of about 5.9. This indicates greater instability for the nitrate salt of hydroxylamine than the free amine. The free amine was reported to be unstable above pH 6.8 in the work of Frear and Burrell.[5] Reports in the literature indicate that hydroxylamine rapidly disappears from soils through a number of chemical

reactions.[10,11] Reactions with inorganic and organic soil components include some of those presented in Figure 18. With a demonstrated instability above pH 5.9 and with the numerous potential reactions in soils, it would be expected that HAN would not persist under most environmental conditions. The limited biological study on HAN at low pH did not demonstrate biodegradability.

REACTIONS WITH INORGANIC COMPONENTS

1. $2MnO_2 + 2NH_2OH \longrightarrow 2MnO + N_2O + 3H_2O$
2. $4Fe^{+3} + 2NH_2OH \longrightarrow 4Fe^{+2} + N_2O + 4H^+$
3. $2Fe^{+3} + 2NH_2OH \longrightarrow 2Fe^{+2} + N_2 + 2H_2O + 2H^+$

REACTIONS WITH ORGANIC COMPONENTS

1. $R_2C{=}O + NH_2OH \longrightarrow R_2C{=}NOH + H_2O$

Principal products - fixation of N into soil organic matter

Figure 19. Some reactions with soil inorganic and organic components which transform hydroxylamine (from Nelson, 1978).

Results of the Ames test indicated that none of the four ammonium nitrate propellants was mutagenic but that HAN was toxic at the higher concentrations.

Hyphomicrobium sp. was the predominant microorganism identified in culture vessels from the denitrification systems with methanol as the principal carbon source. Pathways have been established in the literature for the

[10] Brenner, J. M., A. M. Blackmer, and S. A. Waring. Formation of nitrous oxide and denitrogen by chemical decomposition of hydroxylamine in soils. Soil Biol. Biochem. 12: 263-269, 1980.

[11] Nelson, D. W. Transformations of hydroxylamine in soils. Proc. Indiana Acad. Sci. 87: 409-413, 1978.

degradation of some amines[12] corresponding to the nitrate salts examined in this study. (Trimethylamine is metabolized successively to dimethylamine and methylamine.)

The presence of NDMA and NDElA in TMAN and TEAN stock solutions, respectively, may present the major difficulty in using a biological treatment approach to alleviate potential pollution hazards associated with these compounds (Table 19). If the source of the nitrosamines can be determined, corrective modifications during manufacture could be taken to alleviate this problem. Current research on the biodegradability of nitrosamine will clarify some of these concerns, while some of these areas have already been addressed.[2] Studies on the biological degradation of nitrosamines have produced variable findings. A 6.1 basic research program, funded through USATHAMA, to evaluate the biodegradation of NDMA in aqueous and soil systems, is currently underway here at Natick Laboratories.

Table 19. Nitrosamine Contamination of Ammonium Nitrate Propellants

1. TRIMETHYLAMMONIUM NITRATE
   2.8 ppm N-nitrosodimethylamine
2. TRIETHANOLAMMONIUM NITRATE
   8.4 ppm N-nitrosodiethanolamine
   6.2 ppb N-nitrosomorpholine
3. ISOPROPYLAMMONIUM NITRATE
   none detected

The effects of higher concentrations of these ammonium nitrates on biological activity should also be evaluated to further evaluate the potential for biological treatment. With the results to date, and with the two qualifications just described (nitrosamine contamination and high concentrations) there appears to be good potential for biological treatment of these compounds.

[12] W. Harder and M. M. Attwood. Biology, physiology and biochemistry of Hyphomicrobia. Adv. Microb. Physiol. 17: 303-359, 1978.

CONCLUSIONS

Trimethylammonium nitrate (TMAN), triethanolammonium nitrate (TEAN), and isopropylammonium nitrate (IPAN) were mineralized under aerobic conditions in nutrient rich solutions as well as when present as the sole source of carbon and nitrogen. Under anaerobic conditions TMAN and TEAN, but not IPAN were mineralized. No significant concentrations of intermediates (dimethylamine, methylamine, diethanolamine and ethanolamine) were identified during the biotransformation of the ammonium nitrates. All three compounds were readily mineralized from soils under a variety of conditions. HAN was found to be chemically unstable at pH 5.9 and above. All four compounds were negative in the Ames screening test for mutagenicity.

Based on the results of this study, biological treatment is a potentially viable route for removal of ammonium nitrate propellants from waste streams. However, TMAN and TEAN were found to be contaminated with N-nitrosodimethylamine and N-nitrosodiethanolamine, respectively. This contamination problem must be addressed in order to alleviate this nitrosamine hazard and permit a biological treatment approach to be utilized.

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12. W. Harder and M. M. Attwood. Biology, physiology and biochemistry of Hyphomicrobia. Adv. Microb. Physiol. 17: 303-359, 1978.