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BIODEGRADATION OF NITROGLYCERIN AND PERCHLORATE IN PROPELLANT WASTEWATER

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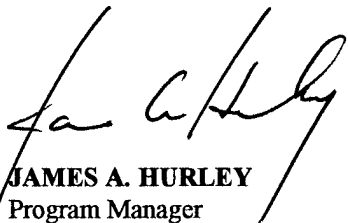
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13. ABSTRACT *(Maximum 200 words)*
This report describes studies on the degradation of nitroglycerin and nitroglycerin/perchlorate mixtures by an anaerobic mixed culture containing the perchlorate reducing bacterium HAP1. The final goal was to demonstrate the feasibility of biotreating a model hazard class 1.1 propellant (CYH) wastewater containing these compounds. The results showed that nitroglycerin rapidly disappeared under culture growth conditions. The disappearance appeared to be due to non-enzymatic chemical denitration resulting from the strong reducing potential generated in the medium. Nitroglycerin and nitrite were shown to inhibit perchlorate reduction. Both glycering and perchlorate were degraded in 10% CYH propellant wastewater. Perchlorate reduction in 25% and 50% CYH wastewater occurred only after nitroglycerin and nitrite were predegraded and the mixture culture was reinoculated. The other major components of the CYH wastewater, tricetin and resorcinol, were not inhibitory to perchlorate reduction.

14. SUBJECT TERMS
Ammonium Perchlorate, Chlorate, Chlorite, Anaerobic, Bioreduction, Propellants

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PREFACE

This report has been reviewed by the Public Affairs Office (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public including foreign nationals.

EXECUTIVE SUMMARY

A. OBJECTIVES:

The objectives of these studies were to; 1) demonstrate the anaerobic biodegradation of nitroglycerin and nitroglycerin/ammonium perchlorate mixtures associated with hazard class 1.1 propellant wastewaters, 2) define the products of anaerobic nitroglycerin degradation, 3) show the feasibility of biotreating a model class 1.1 (CYH) propellant wastewater for the removal of both nitroglycerin and perchlorate, and 4) determine if other components in CYH wastewater were inhibitory to perchlorate reduction.

B. BACKGROUND:

The disposal of solid rocket motors and propellant wastes are coming under increasingly stricter environmental requirements. To meet this challenge the United States Air Force has ongoing research in the physical, chemical, and biological destruction of these wastes. The two predominant types of solid propellant systems are designated as either hazard class 1.1 or 1.3. Class 1.3 motors contain primarily ammonium perchlorate as the energetic material whereas class 1.1 motors have less ammonium perchlorate but significant quantities of nitroglycerin, nitrocellulose, and HMX. Previous Air Force work has proven the feasibility of anaerobically bioreducing ammonium perchlorate in class 1.3 rocket motor wastewater to chloride salts. However, no equivalent work on class 1.1 rocket motor wastewater has been done. There have been reports on the biodegradation of nitroglycerin in aqueous wastestreams, but they dealt primarily with aerobic or microaerophilic degradation. Since both ammonium perchlorate and nitroglycerin are the major water soluble components of class 1.1 propellants, our focus was on the individual and combined anaerobic biotreatment of both compounds. While the metabolic pathway for perchlorate reduction has been demonstrated in previous work, the fate of nitroglycerin under anaerobic culture conditions and its effect on perchlorate reduction needed to be determined for biotreatment feasibility.

C.   SCOPE:

The investigation followed a sequential order with the initial work demonstrating the anaerobic degradation of nitroglycerin and the determination of intermediates formed during that degradation. The next step showed the anaerobic degradation of mixtures of ammonium perchlorate and nitroglycerin and the effects of nitroglycerin on perchlorate reduction. The third step demonstrated the degradation of ammonium perchlorate and nitroglycerin in various concentrations of aqueous extracted CYH propellant. The extract was representative of wastewater produced during water extraction and washdown of class 1.1 solid rocket motors. The final step showed the effects of other compounds present in CYH wastewater on perchlorate reduction. These compounds were nitrite, triacetin, and resorcinol.

D.   METHODOLOGY:

Conventional anaerobic microbiology techniques were used in all experiments. Culture manipulations and incubations were carried out in an enclosed anaerobic chamber. The bacterial culture used in testing was an anaerobic mixed sewage enrichment containing the perchlorate reducing bacterium HAP1. Nitrate esters were measured by HPLC. Anions beside perchlorate were measured by ion chromatography. Perchlorate and ammonia were measured with respective ion-selective electrodes attached to an ion analyzer.

E.   TEST DESCRIPTION:

Each test was setup with the addition of either nitroglycerin only, nitroglycerin and perchlorate, or CYH propellant aqueous extract, at the appropriate test concentrations to sterile anaerobic culture media. The tests were initiated by either inoculating the media with the perchlorate reducing mixed culture or adding the appropriate chemical reductant. The degradation of nitroglycerin and/or perchlorate was then followed over time. In some tests, the fate of nitrite, nitrate, and ammonia were also monitored.

F.   RESULTS:

Nitroglycerin was readily degraded at concentrations up to 700 ppm during the growth phase of the anaerobic culture. The nitroglycerin was converted to glycerol-1,3-dinitrate which was in turn completely removed. Similar results were obtained with culture medium which had been heat-killed (autoclaved) following culture growth and was maintained at its low redox potential. Glycerol-1-mononitrate could not be chromatographically resolved from the culture medium. Therefore it could not be conclusively shown to be the degradation product of glycerol-1,3-dinitrate under these culture conditions. The sequential reduction of nitroglycerin through glycerol-1,3-dinitrate to glycerol-1-mononitrate followed by complete loss was shown in simple buffered medium with the chemical reductant sodium hydrosulfite. The data from the autoclaved culture and sodium hydrosulfite experiments indicated that the degradation of nitroglycerin is a non-enzymatic chemical reaction mediated by the low redox potential generated during normal anaerobic culture growth and not direct biological metabolism.

The presence of nitroglycerin (100 ppm) produced a lag period for perchlorate reduction until the nitroglycerin was completely degraded. This indicated that nitroglycerin or its degradation intermediates were inhibitory to perchlorate reduction. The perchlorate reducing bacterium HAP1 did not degrade nitroglycerin in pure culture.

Aqueous extracted CYH wastewater was shown to be a complex mixture in which ammonium perchlorate, nitroglycerin, triacetin, resorcinol, nitrate and nitrite were the major components. Perchlorate (228 ppm) and nitroglycerin (35 ppm) were completely degraded in 10% CYH wastewater. In 25% CYH wastewater, nitroglycerin (89 ppm) was completely degraded but perchlorate (569 ppm) was not reduced. Similar results were demonstrated with 50% CYH wastewater. These data indicated that HAP1 was inhibited by some component(s) in the wastewater at higher concentrations. It was shown that predegradation of nitroglycerin and nitrite followed by reinoculation with the

HAP1 mixed culture allowed for perchlorate reduction to occur even in both 25% and 50% CYH wastewater.

Nitrite, which has been previously shown to be toxic to HAP1, was present in significant quantities in all nitroglycerin and CYH water samples used. Tests showed that nitroglycerin (460 ppm) and nitrite (140 ppm) together totally inhibited perchlorate reduction whereas nitrite (135 ppm) alone produced only an extended lag phase until it was degraded, at which point perchlorate reduction ensued. Neither triacetin (200 ppm) or resorcinol (200 ppm) or the combination of both inhibited perchlorate reduction.

G. CONCLUSIONS:

The rapid degradation of nitroglycerin in the presence of the anaerobic mixed culture indicated that biotreatment of this compound is highly feasible. The degradation of nitroglycerin appeared to be a result of abiotic denitration brought on by culture low redox potentials. The limiting factor for the treatment of nitroglycerin/perchlorate wastestreams appeared to be the inhibitory effect of nitroglycerin and nitrite on perchlorate reduction. It appeared that the growth of the mixed culture was responsible for the abiotic degradation of nitroglycerin and the removal of nitrite. These compounds were toxic to the perchlorate reducing bacterium HAP1, and following their elimination, HAP1 growth and subsequent perchlorate reduction proceeded in CYH wastewater samples.

H. RECOMMENDATIONS:

While the feasibility of biotreating class 1.1 propellant wastewaters containing nitroglycerin and ammonium perchlorate has been clearly demonstrated, there still remains several questions to be answered. In this study we showed the sequential denitration of nitroglycerin through glycerol-1,3-dinitrate and its subsequent removal by the anaerobic culture. However, due to analytical limitations we could not show that glycerol-1-mononitrate was formed from glycerol-1,3-dinitrate and then subsequently removed in the anaerobic culture as it was in the chemical reaction experiment with sodium hydrosulfite. Future work to develop new separation techniques for resolving

the mononitrate ester from the spent culture medium could help to better define this degradation pathway. We also could not determine the fate of the nitrogen moieties during the degradation. Since most environmental validation of treatment technologies require as complete a mass balance as possible, further experiments should be done to determine the final fate of both carbon and nitrogen from the biotreatment process. This could be accomplished by using radiolabelled nitroglycerin and monitoring for complete mineralization to carbon dioxide and inorganic nitrogen.

Along with a better understanding of the nitroglycerin endproducts, research into the continuous biotreatment of CYH wastewater should be undertaken. It is proposed that a two-stage anaerobic reactor system be tested. The first reactor would remove nitroglycerin and nitrite which are inhibitory to perchlorate reduction and the second reactor would remove perchlorate. A third aerobic reactor could be added to investigate the removal of COD, BOD and ammonia.

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SECTION I

INTRODUCTION

A.   OBJECTIVES

The Air Force has a current Statement of Operational Need (AFLC SON 003-90) for technologies to treat and dispose of solid rocket propellant wastes. Both nitroglycerin and ammonium perchlorate are major components in solid propellants. Previous work has demonstrated the feasibility of biotreating ammonium perchlorate in hazard class 1.3 propellant wastewater. However, no known work has been reported on the biodegradation of mixtures of nitroglycerin and perchlorate in hazard class 1.1 propellant wastewater. There are also other compounds present in class 1.1 propellant wastewater whose effects on perchlorate degradation are undefined. The objectives of this investigation were therefore:

1) Determine if nitroglycerin and perchlorate mixtures could be biodegraded anaerobically.

2) Identify and quantitate chemical intermediates formed from anaerobic nitroglycerin degradation.

3) Determine if actual class 1.1 (CYH) propellant wastewater could be biotreated for the removal of perchlorate and nitroglycerin.

4) Determine if other major compounds present in CYH propellant wastewater were inhibitory to perchlorate reduction.

B.   BACKGROUND

The United States Air force currently has over 100 million pounds of solid propellants stored or in use. Due to current demilitarization trends, arms treaty specifications, and more stringent environmental requirements, the Air Force is actively pursuing new and more efficient technologies for the disposal of solid rocket motors and propellants.

The two major solid rocket propellant designations are hazard class 1.1 and hazard class 1.3. Class 1.3 rocket propellants are typically composed of

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70% ammonium perchlorate, 17 % aluminum, and 13% organic binder. Class 1.1 rocket propellant is typically composed of 11% ammonium perchlorate, 28% nitroglycerin, 22% nitrocellulose, 11% HMX, 20% aluminum and small percentages of resorcinol, triacetin, and 2-nitrodiphenylamine.

The current methods for the disposal of both types of rocket propellant are open burning, open detonation or static firing. These methods generate large quantities of hydrochloric acid, chlorine, ammonia, nitrogen oxides, and undefined hydrocarbons liberated into the atmosphere. This uncontrolled release of pollutants is becoming heavily restricted under new federal and state environmental regulations and will most likely be banned in the future.

Two of the technologies being pursued by the Air Force for the disposal of rocket motors include; 1) high-pressure water washout of class 1.3 motors followed by recrystalization and reclamation of the solubilized ammonium perchlorate (Reference 11), and 2) cryogenic washout of class 1.1 motors followed by chemical destruction with supercritical water oxidation (Reference 8). Both technologies will probably have dilute aqueous wastestreams associated with them containing water soluble propellant components.

Aqueous wastestreams from class 1.3 washout water contain ammonium perchlorate at concentrations of 10% or less. Previous work has shown that ammonium perchlorate from these wastestreams can be readily biotreated in concentrations up to 10,000 ppm and the parameters and limitations on the biotreatment process are well defined (References 1,2,3,13). Aqueous wastestreams from class 1.1 washout processes contain a more complex mixture of water soluble organic and inorganic compounds than class 1.3 wastestreams. The major soluble compounds present are nitroglycerin, nitrite, nitrate, resorcinol, triacetin, and ammonium perchlorate. Nitroglycerin in particular represents a major problem in that it is both readily explosive in pure form (Reference 17) and toxic to a wide range of organisms in dilute solutions (References 14,20). The effects of these other compounds on perchlorate reduction and the potential degradation of nitroglycerin are key to any

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successful future biotreatment of class 1.1 propellants.

Because of its pharmacologic use as a vasodilator, nitroglycerin metabolism in mammalian systems has been heavily studied (Reference 20). However, there are relatively few reports in the literature devoted to the microbial degradation of nitroglycerin and its related nitrate esters. A bench scale aerobic reactor containing acclimated activated sludge was used to demonstrate that nitroglycerin was biodegraded to both glycerol-1,2-dinitrate and glycerol-1,3-dinitrate and then glycerol-2-mononitrate and glycerol-1-mononitrate respectively (Reference 18). The mononitrate esters were further degraded, presumably to glycerol. Similar biological denitration was observed with nitroglycerin desensitization product glycidyl nitrate (Reference 9). In a separate study the nitrate esters propylene glycol dinitrate, diethylene glycol dinitrate, triethylene glycol dinitrate and trimethylolethane glycol were biodegraded by a successive denitration process similar to that for nitroglycerin and which resulted in corresponding glycol formation (Reference 5). Various studies with pure fungal isolates also demonstrated sequential denitration of nitroglycerin to glycerol mononitrates as reported in a recent review (Reference 19). Nitroglycerin was shown to serve as a carbon source for bacterial growth by a culture acclimated from river water (Reference 15). In that study the glycerol di and mononitrate intermediates were not detected and were probably not rate limiting. In terms of biotreatment for actual industrial wastewaters containing nitroglycerin, there have been several studies on production streams from Ball Powder. Ball Powder is a double-based propellant and its associated wastewater typically contains nitroglycerin, nitrocellulose, ethyl acetate, dibutylphthalate, diphenylamine, and n-nitrosodiphenylamine. Initial work indicated that nitroglycerin at concentrations of 150 ppm or more was inhibitory to the overall performance of an aerobic pilot waste treatment system (Reference 4). Following work with the same wastestream process demonstrated that nitroglycerin was not a problem and was readily degraded at concentrations up to 250 ppm with proper culture acclimation and the presence

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of ethylacetate as a co-substrate (Reference 7). In that report the system degraded nitroglycerin both aerobically and anaerobically. Recent work on the enzymatic degradation of nitroglycerin has yielded a *Bacillus thuringiensis/cereus* and an *Enterobacter agglomerans* whose cell-free extracts are capable of degrading nitroglycerin to nitrite (Reference 16). Also in that report was data showing the active anaerobic degradation of nitroglycerin, propyleneglycol dinitrate, trimethyloethane trinitrate, and triethyleneglycol dinitrate by a treatment reactor seed culture. The culture degraded the compounds at concentrations as high as 2000 ppm with concomitant cumulative methane production and indicated that nitroglycerin is readily biodegraded anaerobically.

The organic binder components resorcinol and triacetin are readily dissolved in water and usually present in class 1.1 wastewater. Both resorcinol (Reference 12) and triacetin (Reference 6) have been shown to quickly biodegrade under anaerobic conditions. However, it is not known what effect these compounds will have on the organisms responsible for perchlorate or nitroglycerin degradation.

C.   APPROACH

The focus of the present study was to determine if nitroglycerin in class 1.1 wastewater is anaerobically biodegradable and if it and other water soluble components present have deleterious effects on perchlorate reduction in those wastewaters. The first objective was to define whether a mixed perchlorate reducing culture used in the treatment of class 1.3 wastewaters (References 1,2,3) was able to degrade nitroglycerin. Since the reduction of perchlorate is well defined using this culture, carrying out the degradation of nitroglycerin under the same conditions is very beneficial. The concentration ranges for nitroglycerin degradation were investigated to help predict future wastestream treatability limits. We also endeavored to determine what intermediates were formed from this process and if complete denitration of nitroglycerin was occurring as described in previous reports (References 15,18).

The second objective was to determine whether perchlorate reduction was inhibited by nitroglycerin. Perchlorate reduction by the bacterium HAP1 is strongly inhibited by compounds such as oxygen and nitrite (References 1,2) and the effects of nitroglycerin were unknown. Therefore it was necessary to determine whether HAP1's perchlorate activity was affected by the presence of nitroglycerin and at what concentrations.

The third objective was to determine if perchlorate and nitroglycerin were removed in actual class 1.1 propellant aqueous waste. The material tested was CYH propellant which is the primary energetic component of the Minuteman Stage 3 rocket motor and represents a significant waste source for the Air Force. Aqueous wastestreams containing this material can be generated through motor manufacturing, washout and refurbishment procedures.

Besides ammonium perchlorate and nitroglycerin, these aqueous streams have a variety of other chemical binder components as well as trace amounts of other explosives. The fourth objective was to determine the inhibitory effects of the three major compounds in CYH aqueous extract, other than nitroglycerin, on perchlorate reduction. These three compounds were nitrite, resorcinol, and triacetin. Because they are present in significant concentrations, knowledge of the effects of these individual compounds on HAP1 perchlorate reduction is a requisite for the operation of future biotreatment systems.

SECTION II
ANAEROBIC NITROGLYCERIN DEGRADATION

A. EXPERIMENTAL

1. Nitrate Esters

Standards and stock solutions of nitroglycerin in water or ethanol were provided by the Manufacturing Technology Department, Indian Head Division, Naval Surface Warfare Center, Indian Head, Maryland (Contact: Mr Douglas Elstrodt). Standards of glycerol-1,2-dinitrate, glycerol-1,3-dinitrate, and glycerol-1-mononitrate were synthesized by Steroids, Ltd., Chicago Technology Park Drive, Chicago, IL and provided in ethanol solutions from the Naval Surface Warfare Center, Indian Head, Maryland.

All nitrate esters were measured with a Hewlett Packard high-pressure liquid chromatograph equipped with a 250mm x 4.6mm Alltech Econoshpere column with 5μm C_{18} packing. The mobile phase was 50% acetonitrile - 50% water at a flow rate of 1 ml per minute. The esters were monitored with an ultraviolet detector set at 205 nm wavelength. Table 1 shows typical chromatographic retention times for nitroglycerin and its potential ester intermediates. All samples were centrifuged (14,000 X g) for 3 minutes to remove particulates and measured without dilution.

2. Nitrite and Nitrate

Standards and stock solutions of reagent grade sodium nitrate and sodium nitrite were used for all nitrite and nitrate analyses. Nitrate and nitrite were measured with a Dionex model DX-300 ion chromatograph equipped with an anion self-regenerating suppressor and conductivity detector. The separation was done on a Dionex AS11 anion column. The mobile phase was 10 mM sodium hydroxide at a flow rate of 1 ml per minute. Table 1 shows typical chromatographic retention times for nitrite and nitrate. Samples were centrifuged (14,000 X g) for 3 minutes and diluted appropriately in 18-megaohm or better high purity water.

TABLE 1. CHROMATOGRAPHIC RETENTION TIMES

COMPOUND	ANALYSIS METHOD	Retention Time (minutes)
Nitroglycerin	REVERSE PHASE HPLC	7.30
Glycerol-1,3-dinitrate	REVERSE PHASE HPLC	4.15
Glycerol-1,2-dinitrate	REVERSE PHASE HPLC	4.05
Glycerol-1-mononitrate	REVERSE PHASE HPLC	2.94
Nitrite	ION CHROMATOGRAPHY	2.50
Nitrate	ION CHROMATOGRAPHY	3.94

3.   Ammonia

Standards and stock solutions of reagent grade ammonium chloride were used for all ammonia analyses. Ammonia was measured by standard methods of addition using an Orion Research ammonia ion-selective electrode and EA 940 Ionanalyser.

4.   Test Culture Conditions and Media

The biological test culture was a mixed anaerobic sewage enrichment containing the perchlorate reducing bacterium HAP1 (References 1,2,3). In all tests the final medium composition per liter was: K_2HPO_4, 6 g; Na_2PO_4, 2 g; yeast extract (Difco) 10 g; Peptone (Difco) 10 g; resazurin, 0.001 g; the pH was adjusted to 7.1 with 5N HCl. Fifty ml aliquots were placed in 100 ml

serum bottles and autoclaved for 30 minutes. Following sterilization the bottles were placed in an anaerobic chamber (Coy Laboratory Products) containing an atmosphere of 10% hydrogen:10% carbon dioxide:80% nitrogen. The serum bottles were crimp sealed following 24 hour gas exchange to remove oxygen. Nitroglycerin was added to the medium at varying concentrations through 0.2 μM sterile filters (Sartorius). The serum bottles were wrapped in aluminum foil to prevent photodegradation. Test cultures were initiated with a 1% active inoculum and grown at 37°C in the anaerobic chamber. Degradation of nitroglycerin and nitrite were followed. Sterile controls contained identical medium but were not inoculated.

To determine nitroglycerin degradation by heat killed culture medium, the culture was grown for 24 hours in sealed serum bottles and then autoclaved for 30 minutes. Sterile nitroglycerin was added to the spent culture medium to a concentration of 100 ppm and degradation was monitored with time.

The medium used for abiotic chemical degradation tests was the same as stated above except no yeast extract or Peptone was present. Nitroglycerin was added at a concentration of 125 ppm. The chemical reducing agents, cysteine hydrochloride or sodium hydrosulfite, were added to a final concentration of 500 ppm and the medium was monitored for nitroglycerin loss and the subsequent formation of glycerol di and mononitrate intermediates. Controls had no reducing agent added. The environmental parameters were the same as described above for biological cultures. A similar experiment was carried out

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with a nitroglycerin test concentration of 443 ppm and the addition of 1200 ppm of sodium dithionite. The test medium was monitored for the production of nitrate, nitrite and ammonia as products of nitroglycerin degradation. All tests described were done in duplicate and data points are reported as the averages.

B.   RESULTS AND DISCUSSION

Nitroglycerin was readily degraded at concentrations as high as 700 ppm as shown in Figure 1. Culture growth was not noticeably inhibited nor was the initial degradation rate affected by increasing concentrations of nitroglycerin. Concurrent with the loss of nitroglycerin was the production of the intermediate, glycerol-1,3-dinitrate, which in turn disappeared with time as shown in Figure 2. The formation of the intermediate, glycerol-1,2-dinitrate, was not observed in any tests. Unfortunately, the potential intermediate, glycerol-1-mononitrate, could not be chromatographically resolved from the other compounds present in yeast extract and Peptone. If present, it was obscured in all test samples by large compound peaks formed from the fermentation of nutrient materials. There were intrinsic nitrate and nitrite impurities associated with the nitroglycerin used in all tests, however, no additional nitrite or nitrate was detected in the medium as nitroglycerin was degraded.

Nitroglycerin degradation also occurred in culture medium which had been autoclaved following normal growth as shown in Figure 3. The autoclaving procedure not only sterilized the medium but also presumably destroyed enzymatic activity. No color change in the redox indicator dye

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resazurin occurred, indicating the spent medium maintained its low redox potential during autoclaving. The typical measured redox potential for this culture grown under these conditions was in the range of -400 mV. We propose that the degradation of nitroglycerin is actually an abiotic reaction resulting from the biological lowering of the culture redox potential during normal growth. This theory is further supported by the demonstration of chemical degradation of nitroglycerin at low redox potentials and neutral pH. Nonbiological nitroglycerin degradation was observed in the presence of the chemical reductant sodium hydrosulfite. Sodium hydrosulfite undergoes oxidation to bisulfite in water and generates a standard redox potential less than -600 mV at pH 7.0 (Reference 10). As shown in Figure 4, sodium hydrosulfite added to a solution of nitroglycerin resulted in the sequential degradation of nitroglycerin through glycerol-1,3-dinitrate to glycerol-1-mononitrate which was in turn degraded. Glycerol-1,2-dinitrate was not detected during the test. The theoretical denitration of nitroglycerin to glycerol should result in the formation of nitrite as a by-product as shown in equation (1):

$$3Na_2S_2O_4 + 3H_2O + 3OH^- + C_3H_5N_3O_9 \rightarrow 6NaHSO_3 + C_3H_8O_3 + 3NO_2^- \quad (1)$$
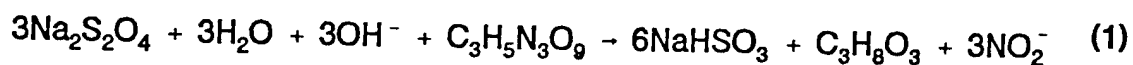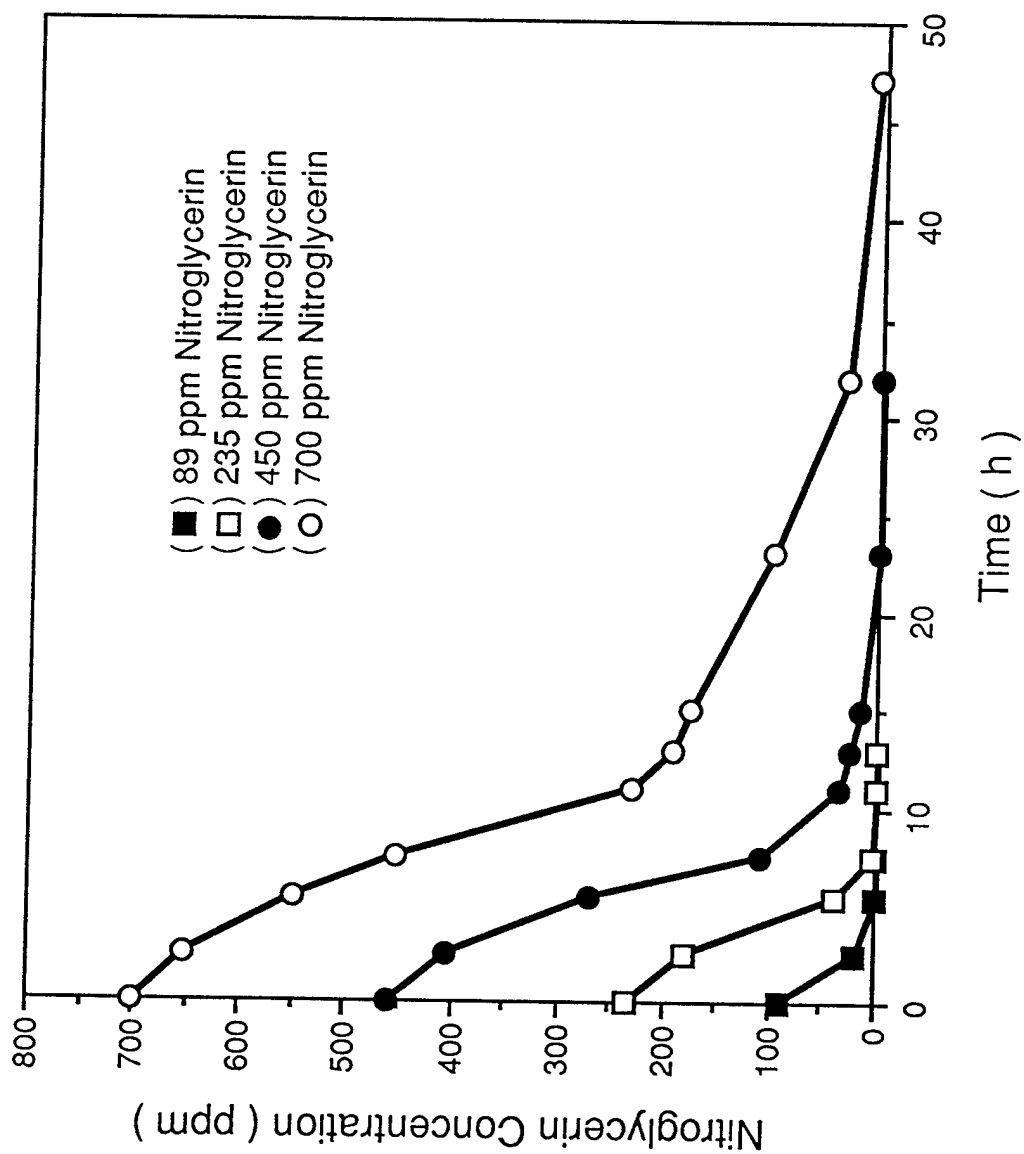
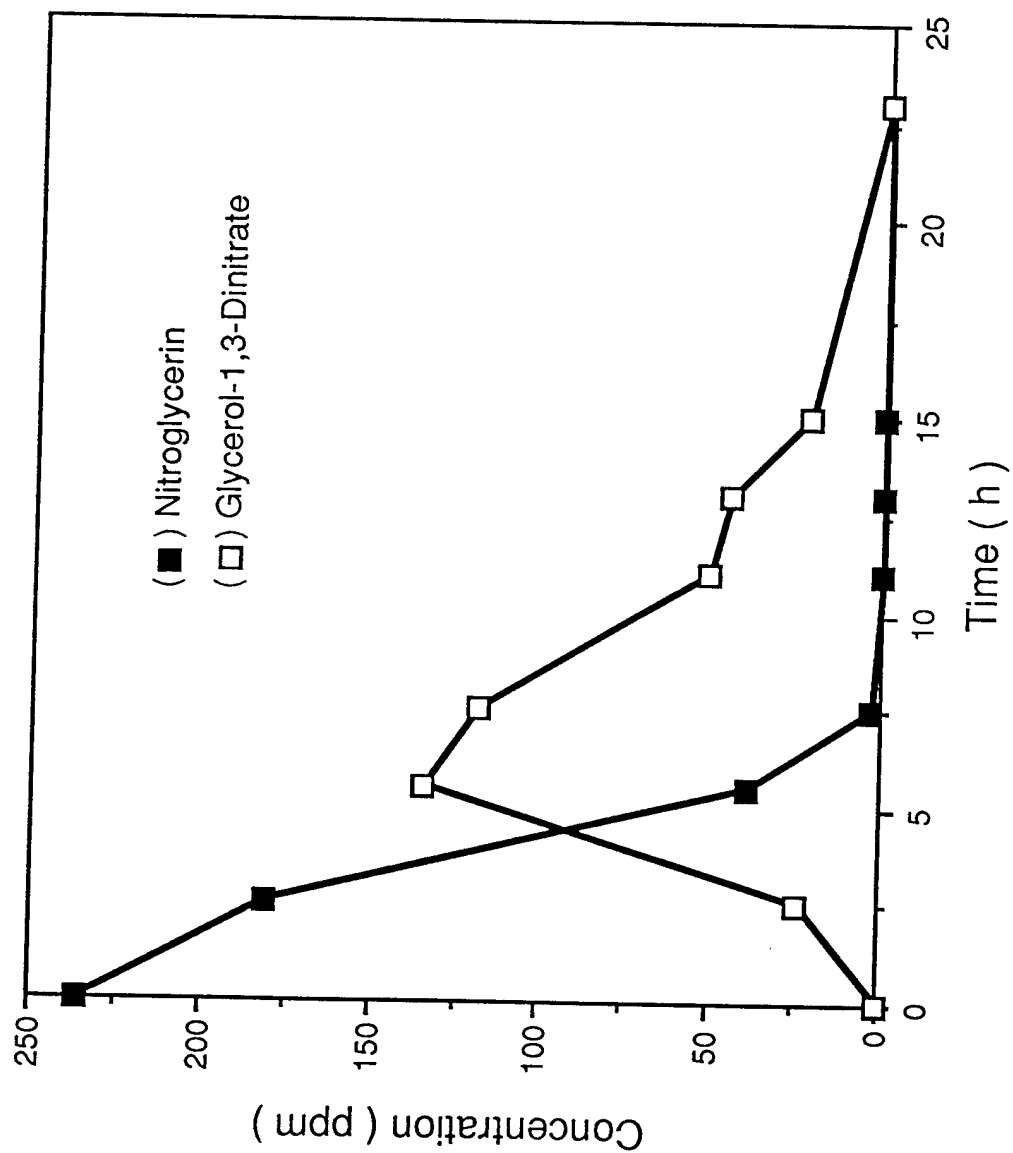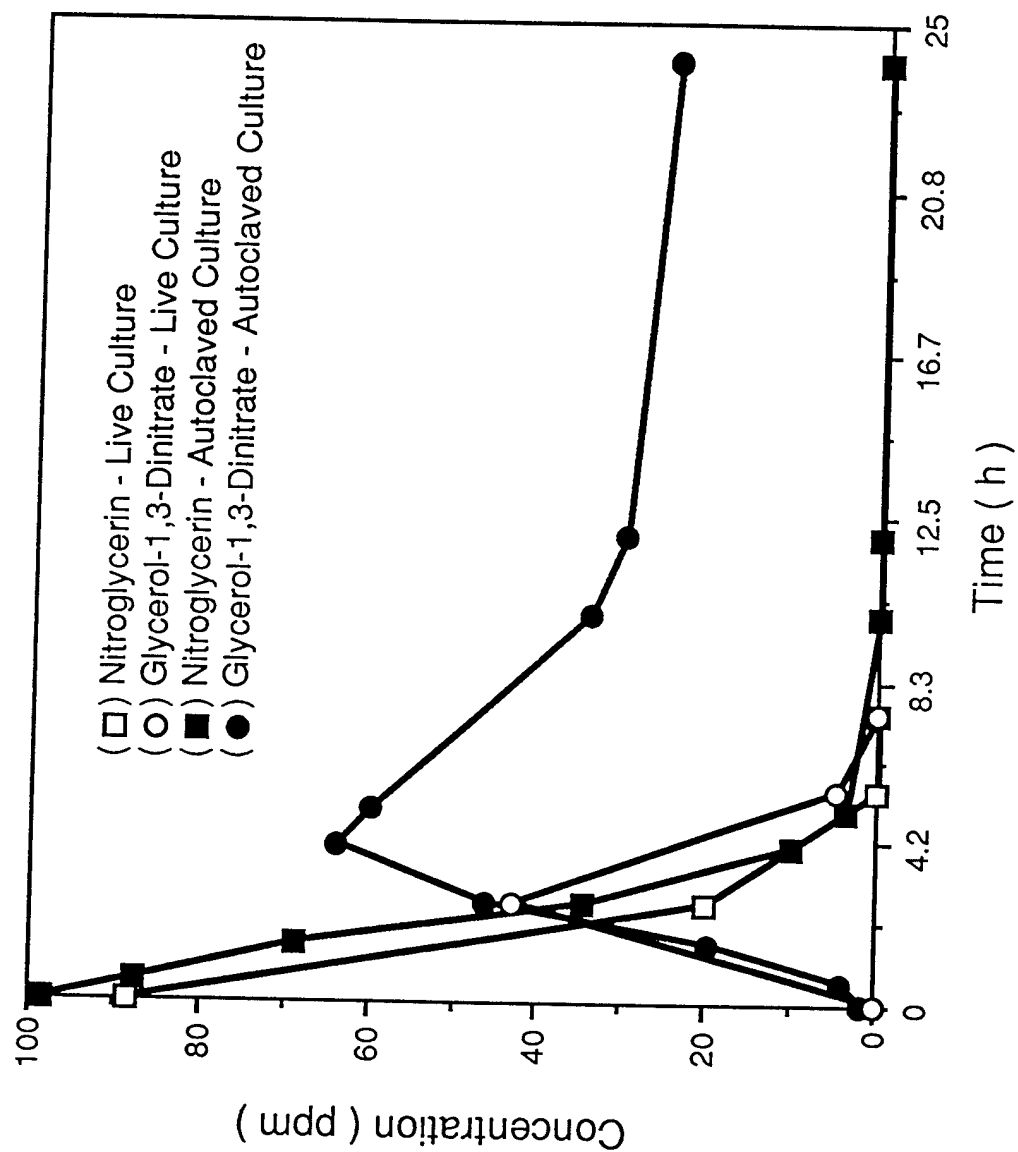Figure 1. Degradation of Nitroglycerin at Different Concentrations

Figure 2.   Degradation of Nitroglycerin and
Intermediate Glycerol-1,3-Dinitrate

Figure 3. Degradation of Nitroglycerin with Live and Autoclaved Culture

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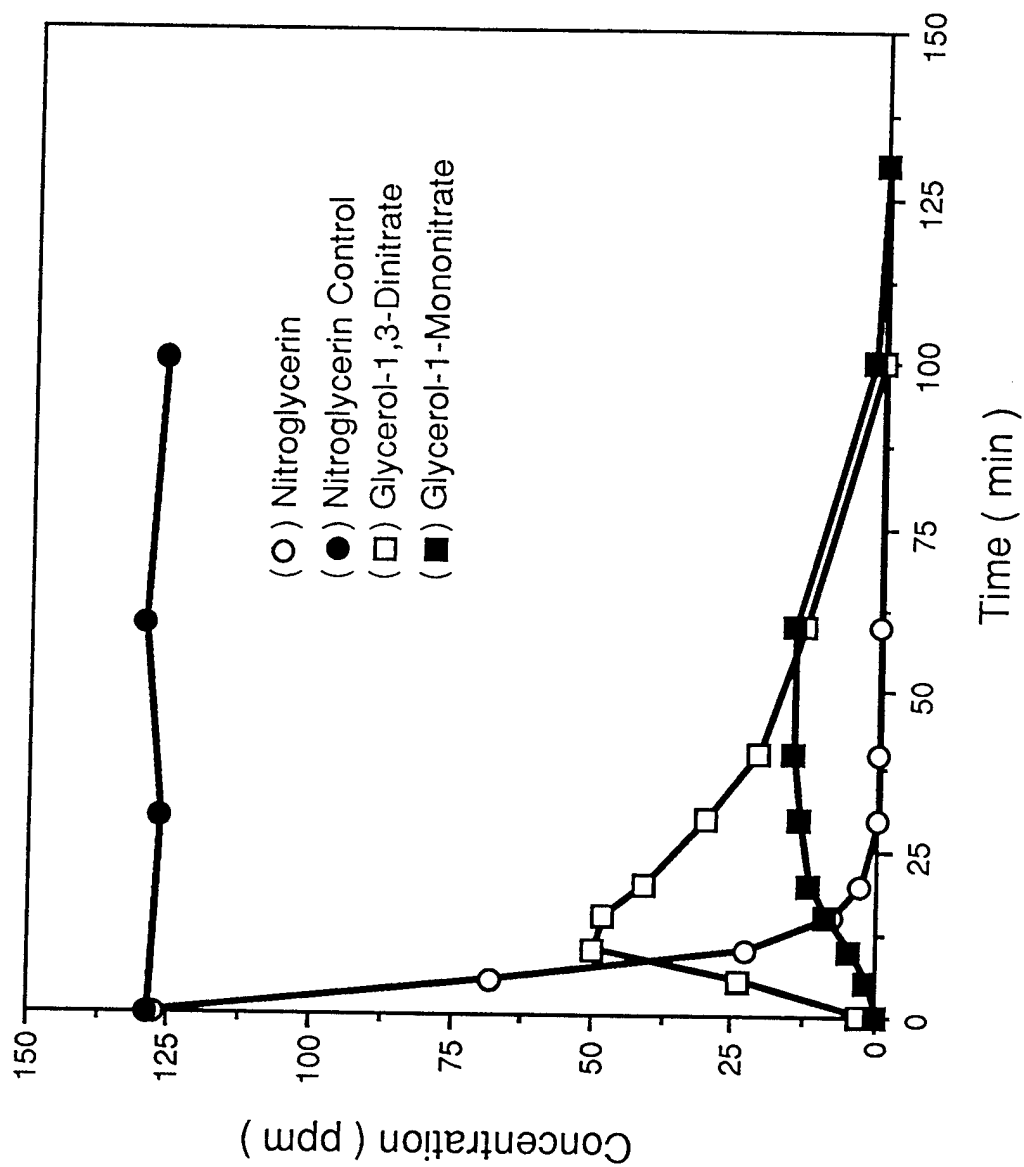
Figure 4.   Degradation of Nitroglycerin with Sodium Hydrosulfite

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Table 2 shows the results of an experiment in which we analyzed for the generation of nitrite or other nitrogen compounds after the chemical degradation of nitroglycerin.

TABLE 2. NITROGEN COMPOUNDS MONITORED DURING NITROGLYCERIN DEGRADATION WITH SODIUM HYDROSULFITE

COMPOUNDS	TIME 0 HOUR	TIME 5 HOURS
Nitroglycerin	443 ppm	0 ppm
Glycerol-1,3-dinitrate	0 ppm	19 ppm
Glycerol-1-mononitrate	0 ppm	180 ppm
Nitrite	285 ppm	290 ppm
Nitrate	77 ppm	81 ppm
Ammonia	None Detected	None Detected

If equation 1 is valid then the experimental test medium should have yielded 269 ppm of nitrite had all nitrate esters been completely degraded. The glycerol-1,3-dinitrate and glycerol-1-mononitrate remaining after 5 hours account for 59 ppm of nitrite leaving 210 ppm of nitrite unaccounted for. Since no significant increase occurred in any of the nitrogen compounds analyzed we do not know the mass balance for the reaction. It is possible that upon ester

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cleavage the nitrogen side chains were reduced to nitrogen gas or nitrous oxide, however, analysis for those compounds was not done in this study.

The presence of cysteine hydrochloride, which generates a standard redox potential of -210 mV at pH 7.0 (Reference 10), did not demonstrate nitroglycerin degradation. Therefore it seems possible that at a redox potential somewhere between -200 and -600 mV under the media conditions described nitroglycerin decomposes by sequential denitration. Figure 5 is the proposed pathway for the chemical reduction of nitroglycerin under highly reducing conditions.

Nitroglycerin   Glycerol-1,3-Dinitrate   Glycerol-1-Mononitrate   Glycerol

$$H-C-O-NO_2 \;\; H-C-O-NO_2 \;\; H-C-O-NO_2 \;\; H$$

Nitroglycerin:
$$H$$
$$H-C-O-NO_2$$
$$H-C-O-NO_2$$
$$H-C-O-NO_2$$
$$H$$

Glycerol-1,3-Dinitrate:
$$H$$
$$H-C-O-NO_2$$
$$H-C-OH$$
$$H-C-O-NO_2$$
$$H$$

Glycerol-1-Mononitrate:
$$H$$
$$H-C-O-NO_2$$
$$H-C-OH$$
$$H-C-OH$$
$$H$$

Glycerol:
$$H$$
$$H-C-OH$$
$$H-C-OH$$
$$H-C-OH$$
$$H$$

Glycerol-1,2-Dinitrate:
$$H$$
$$H-C-O-NO_2$$
$$H-C-O-NO_2$$
$$H-C-OH$$
$$H$$
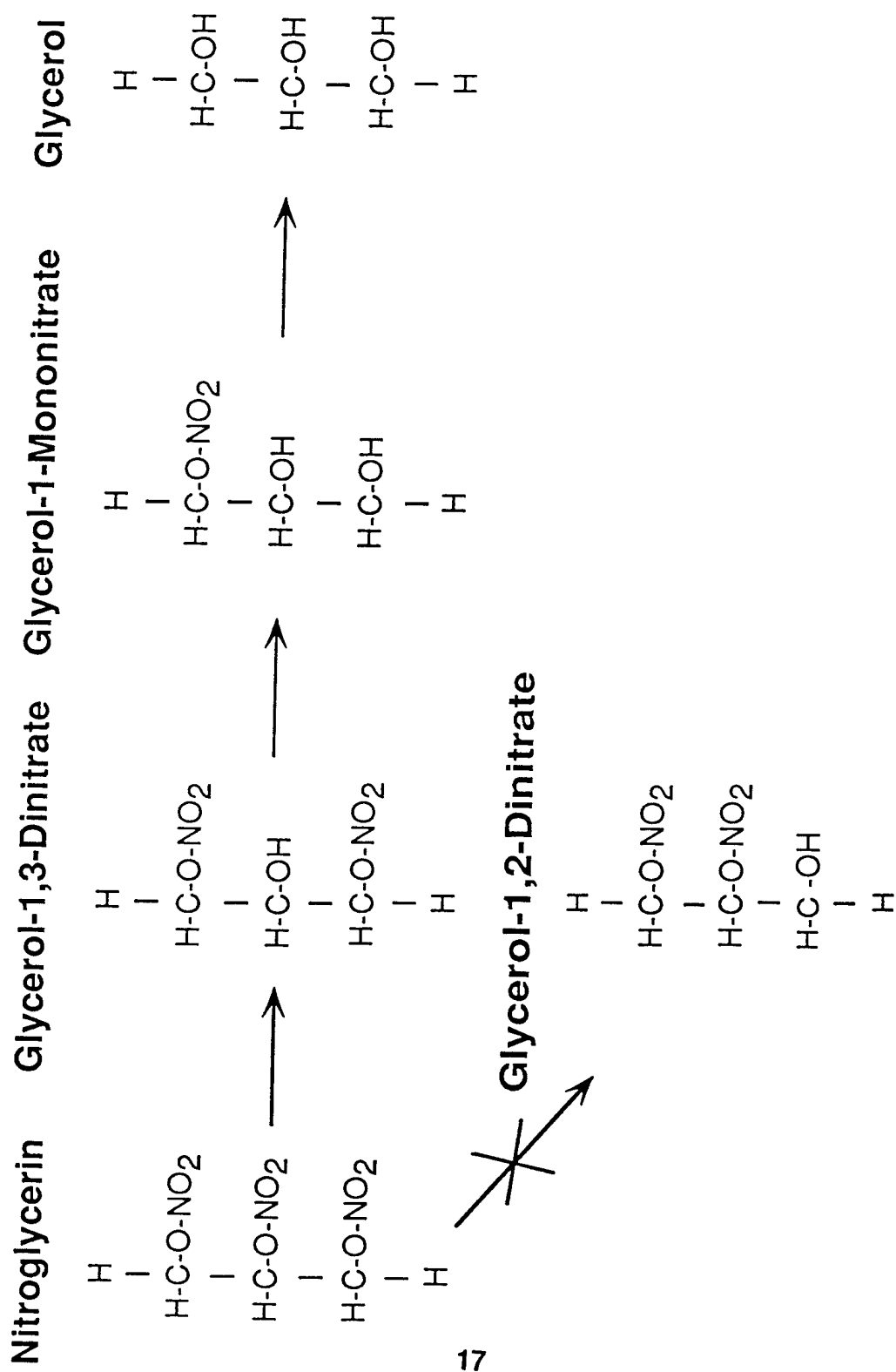
Figure 5.   Chemical Degradation of Nitroglycerin

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ANAEROBIC DEGRADATION OF AMMONIUM PERCHLORATE/NITROGLYCERIN MIXTURES

A. EXPERIMENTAL

1. Nitrate Esters.

Analysis procedures for these compounds were the same as described in Sections II.A.1.

2. Perchlorate

Standards and stock solutions of reagent grade ammonium perchlorate were used in all perchlorate experiments and analysis. Perchlorate was measured by standard methods of addition using an Orion Research perchlorate ion-selective electrode and EA 940 ionanalyser.

3. Test Culture Conditions and Media

Test cultures and media were the same as described in Section II.A.4 except that ammonium perchlorate was added at a concentration of 1.17 g per liter and controls in some experiments had no nitroglycerin added.

B. RESULTS AND DISCUSSION

Perchlorate reduction occurred in cultures amended with 100 ppm nitroglycerin but with a longer lag period than those without as shown in Figure 6. Although the lag period was longer, once the degradation initiated the rates were similar. Since the bacterium HAP1 is the only organism present that

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reduces perchlorate, this data indicated that HAP1 is inhibited by the presence of nitroglycerin and possibly the nitrate ester intermediates. Pure culture tests with HAP1 showed that it could not directly metabolize nitroglycerin nor could it grow in the presence of 100 ppm nitroglycerin. The other fermenting bacteria present in the mixed culture were responsible for lowering the redox potential of the medium enough to allow chemical denitration. The effects of glycerol-1,3-dinitrate on HAP1 were not investigated but in all nitroglycerin tests it was completely degraded before perchlorate reduction occurred. Since glycerol-1-mononitrate could not be chromatographically separated in the test medium, the presence of it during perchlorate reduction and any inhibitory effects could not be verified.
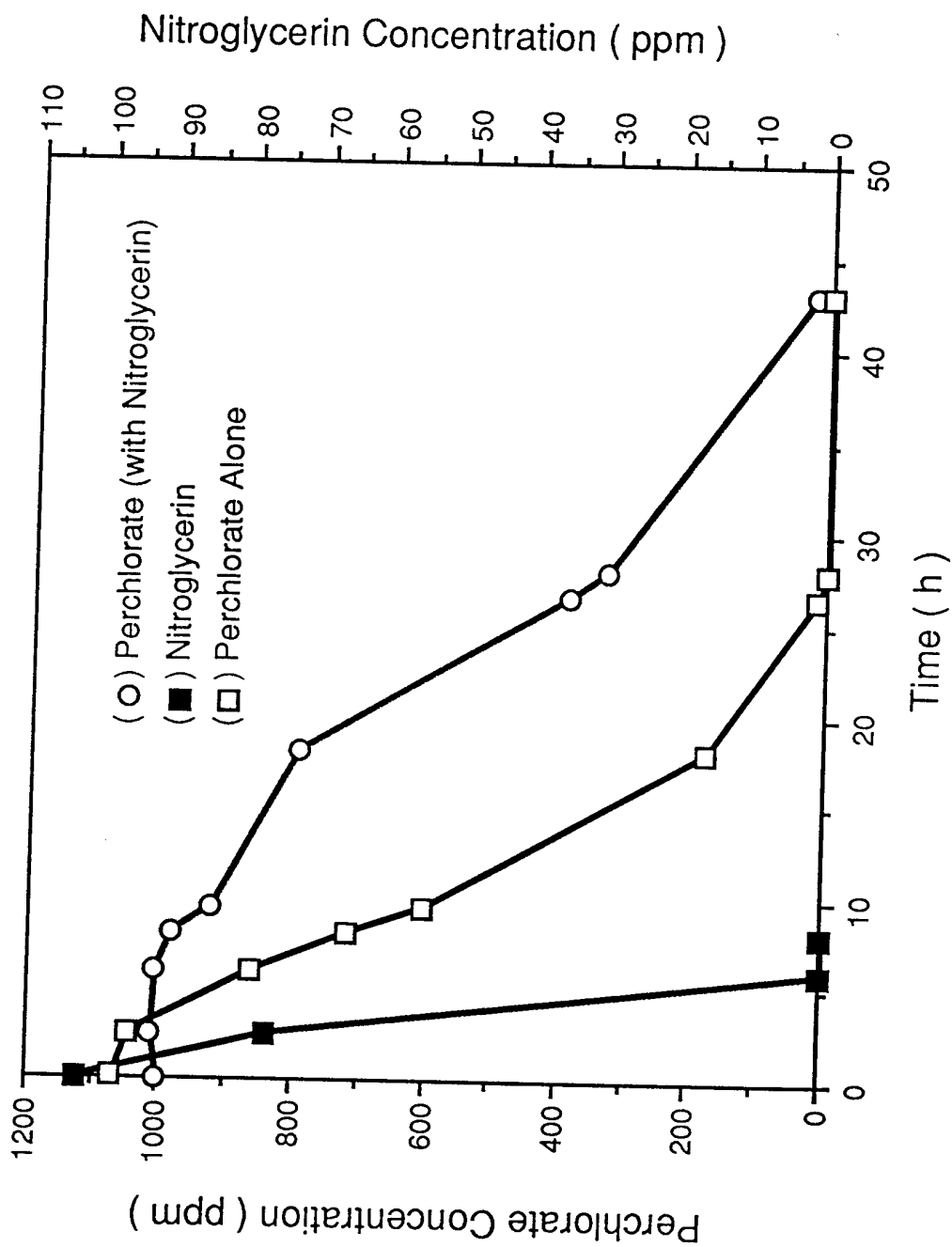
Figure 6. Bioreduction of Perchlorate Alone and Perchlorate with Nitroglycerin
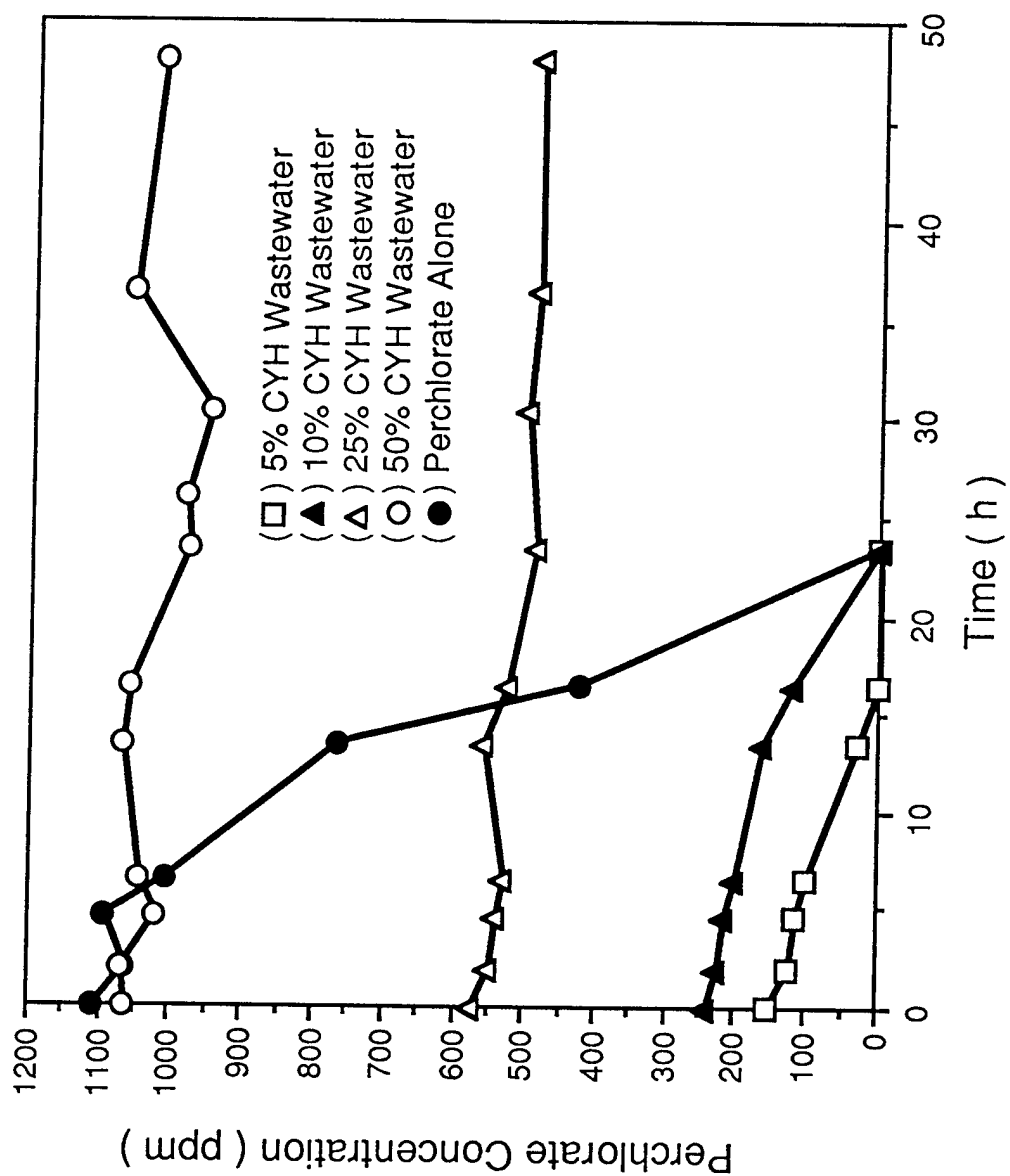
Figure 7. Bioreduction of Perchlorate in Different CYH Wastewater Concentrations

SECTION IV

ANAEROBIC DEGRADATION OF CYH PROPELLANT WASTEWATER

A.   EXPERIMENTAL

1.   Nitrate Esters and Perchlorate

Analysis procedures for these compounds were the same as described in Sections II.A.1. and Section III.2.A.

2.   CYH Propellant Water Extract Preparation

The CYH aqueous test extract was supplied by the Thiokol Corporation, Brigham City, Utah (Contact: Mr. Louis Cannizzo). The following is the preparation description provided with the sample by Mr. Cannizzo:

Two hundred grams of ground (10 mesh and 20 mesh) CYH propellant was stirred with 600 ml of water for 18 hours at 80°C. The water layer from the mixture was separated and an additional 600 ml of water was added to the remaining propellant. The new mixture was again stirred for 18 hours at 80°C. The water layer was again separated. The combined water layers were filtered through a piper filter under suction to give 1,120 mL of a slightly brown solution. The weight of the remaining propellant indicated that additional water remained absorbed on the propellant.

An aliquot of 50 mL was taken from the 1,120 mL of aqueous solution. This aliquot was extracted with chloroform (3 X 50 mL) and the water layer separated and evaporated to a constant weight. The weight of the residue

from the water layer was 1.096 grams. Proton NMR analysis of this residue indicated it contained 0.077 grams of resorcinol. Presumably the remaining material (1.019 grams) was ammonium perchlorate. The combined chloroform extracts were evaporated to give to a yellow oil (2.646 grams). Proton NMR analysis of this oil indicated that it contained nitroglycerin (0.17 grams), triacetin (0.26 grams) and chloroform (> 2.0 grams). The presence of trace quantities of unidentified organics (possibly partially hydrolyzed nitroglycerin and triacetin) was also evident in the NMR spectrum.

The above analysis gives a concentration of 20,400 ppm for ammonium perchlorate and a concentration of 3,400 ppm for nitroglycerin in the aqueous solution of 1,120 mL. To lower the concentration of nitroglycerin to the safety compliance level, 220 mL of the solution were diluted with distilled water to give a total volume of 880 mL. This lowered the theoretical ammonium perchlorate concentration to 5,100 ppm and the nitroglycerin concentration to 850 ppm.

Upon receiving the sample at Armstrong Laboratory/Tyndall Air Force Base, it was further diluted 1:1 with distilled water. The ammonium perchlorate and nitroglycerin concentrations at this dilution were determined to be 2277 ppm and 355 ppm respectively. The extracted material described is representative of wastewater that would be generated from aqueous class 1.1 washout and disposal procedures.

3. Test Culture Conditions and Media

CYH media of various concentrations were made in the same manner as nitroglycerin media described in Section II.A.4. Filter sterilized CYH wastewater was added as either 50%, 40%, 30%, 25%, 20%, 10%, or 5% by volume of the media and the bottles were crimp sealed. Controls had no CYH wastewater added but were amended with 1.17 g per liter ammonium perchlorate. Table 2 is a reference chart showing various initial component concentrations in our test media. The component concentrations used in other tests can be extrapolated from this table. Cultures were inoculated and grown as described in Section II.A.4.

TABLE 3. COMPOSITION OF CYH WASTEWATER CONCENTRATIONS TESTED

COMPONENTS	COMPONENT CONCENTRATION (PPM)					
	100% WASTE WATER	50% WASTE WATER	25% WASTE WATER	10% WASTE WATER	5% WASTE WATER	
AMMONIUM PERCHLORATE	2277	1138	569	228	114	
NITROGLYCERIN	355	177	89	35	17	
TRIACETIN	480	240	120	48	24	
RESORCINOL	139	69	35	14	7	
NITRATE	18	9	4	2	1	
NITRITE	142	71	36	18	9	
HMX	<1	-	-	-	-	
NITROCELLULOSE	<1	-	-	-	-	
ALUMINUM	TRACE	-	-	-	-	
2-NDPA	TRACE	-	-	-	-	
GRAPHITE	TRACE	-	-	-	-	
HYDROLYSIS PRODUCTS	TRACE	-	-	-	-	

B. RESULTS AND DISCUSSION

Perchlorate reduction occurred in 10% or less CYH wastewater but was inhibited at 25% or higher concentrations, as shown in Figure 7. Perchlorate and nitroglycerin concentrations in 25% CYH wastewater were less than that tested previously in Section II and perchlorate reduction was not expected to be totally inhibited based on those results. Therefore, it is possible that other components in the CYH wastewater, either individually or in tandem, inhibited perchlorate reduction. It can be seen from the perchlorate control that if there were not some inhibiting factor, perchlorate in the 50% wastewater would have been completely degraded in less than 25 hours. Increasing the perchlorate concentration by 1000 ppm in the various wastewater dilutions provided similar results with perchlorate reduction being terminated in 20-30% CYH wastewater as shown in Figure 8.

Nitroglycerin present was degraded in all wastewater concentrations at similar times as shown in Figure 9. Therefore, unlike perchlorate reduction the CYH components had no detectable effect on nitroglycerin removal.

Nitrite was intrinsically present in both CYH wastewater and nitroglycerin samples at concentrations of 0.3-0.4 mg nitrite per mg nitroglycerin present. Nitrite has been shown to inhibit both the growth of the bacterium HAP1 and perchlorate reduction (Reference 1,2). Figure 10 demonstrates that nitrite was removed in all wastewater concentrations within 5 hours. That was essentially the same time that nitroglycerin removal occurs. If nitroglycerin and/or nitrite were inhibiting HAP1's perchlorate reduction in the wastewater, then the addition of the HAP1 mixed culture after those compounds are removed should allow for perchlorate reduction to proceed. Figure 11 shows that in 25% wastewater, reinoculation after nitroglycerin and nitrite removal allowed for perchlorate reduction to proceed. Figure 12 shows similar results with a 50% wastewater culture. These results support the theory that the loss of perchlorate reducing activity in higher percentages of wastewater was probably

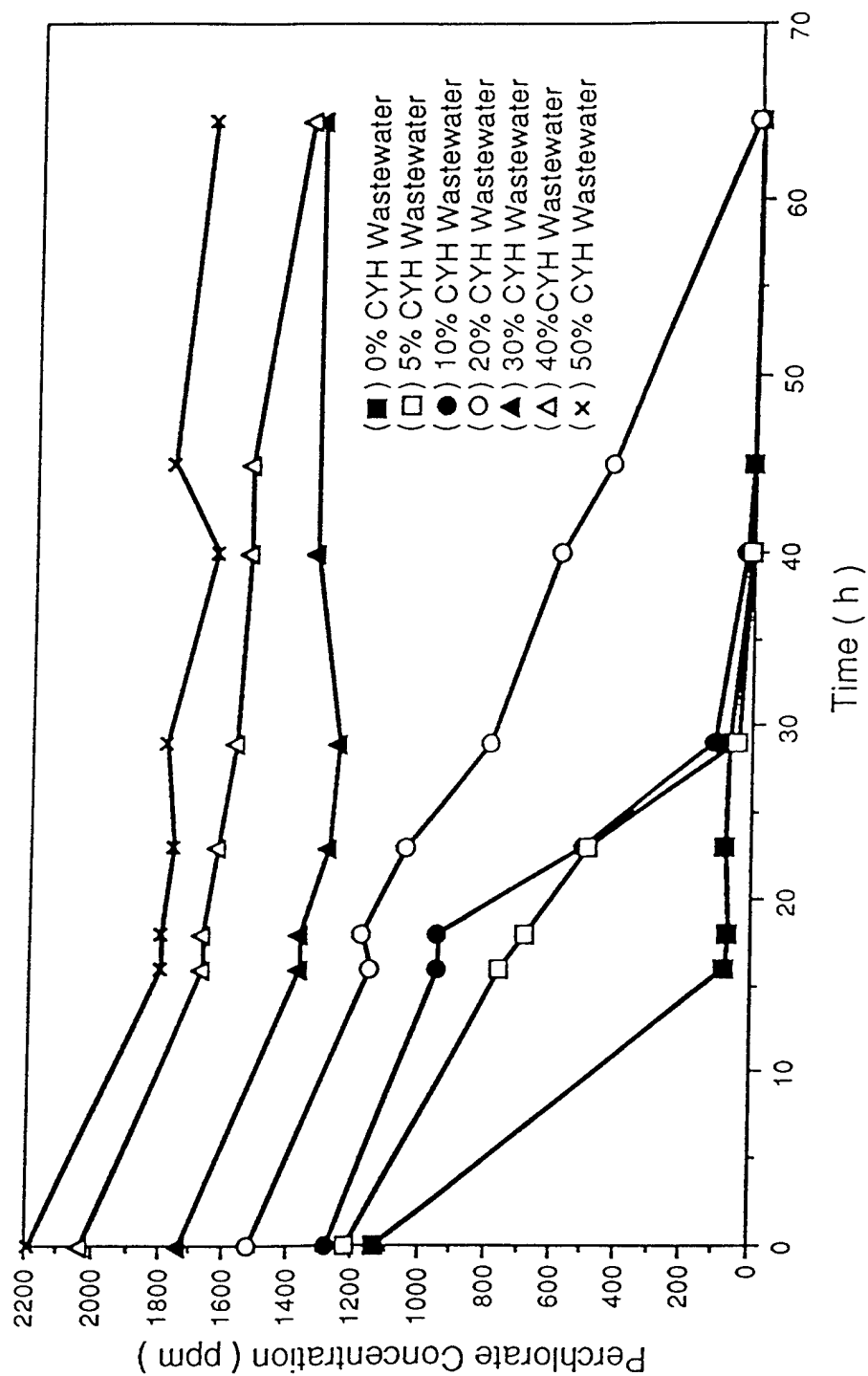a result of nitroglycerin and nitrite concentrations being sufficiently high to inhibit HAP1 in the culture.
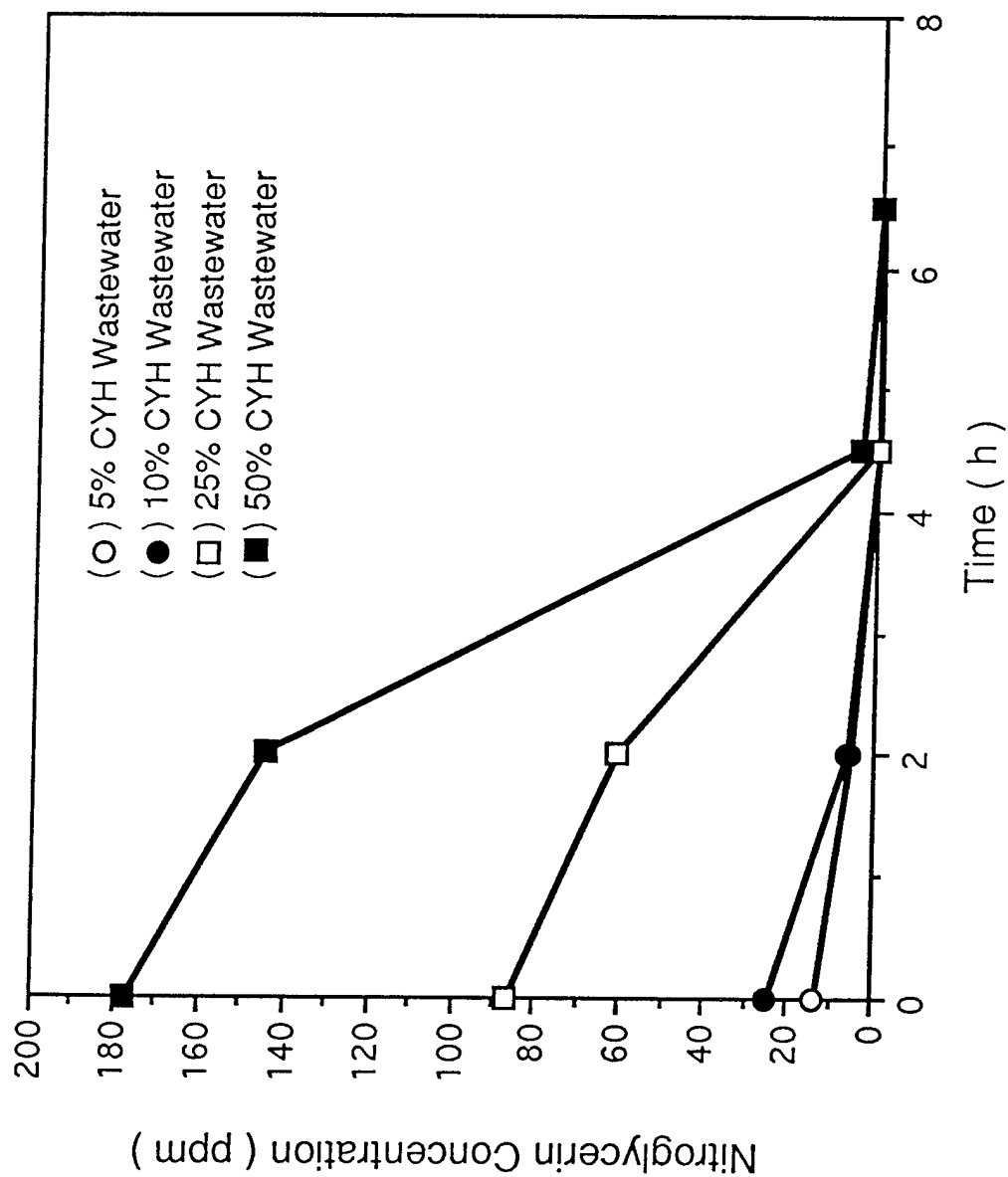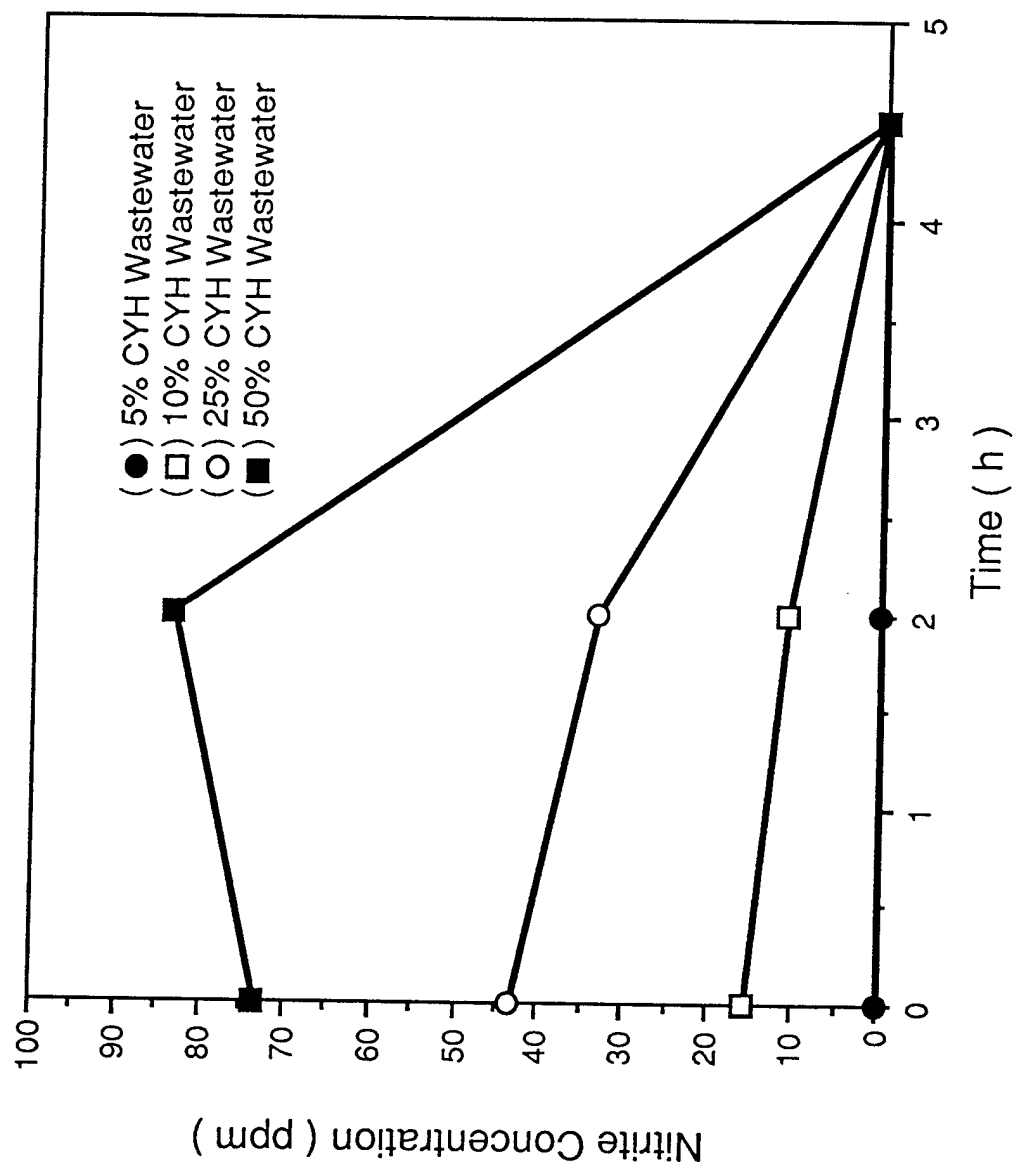
Figure 8. Bioreduction of Perchlorate in Different CYH Wastewater Concentrations Amended With 1000 ppm Perchlorate

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Figure 9. Degradation of Nitroglycerin in Different CYH Wastewater Concentrations

Figure 10. Degradation of Nitrite in Different CYH Wastewater Concentrations
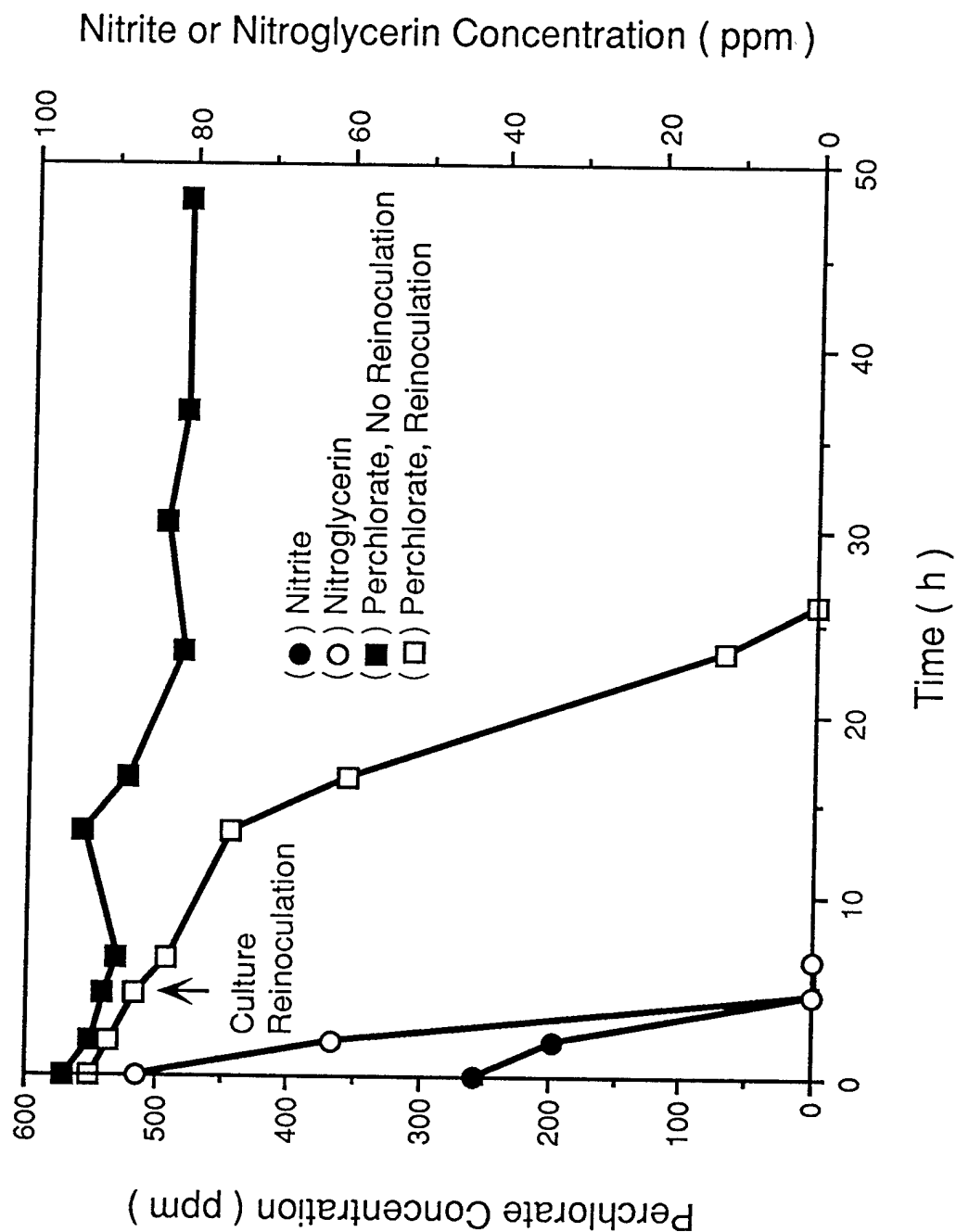
Figure 11. Bioreduction of Perchlorate in 25% CYH Wastewater After Nitroglycerin and Nitrite Degradation

Nitrite or Nitroglycerin Concentration (ppm)
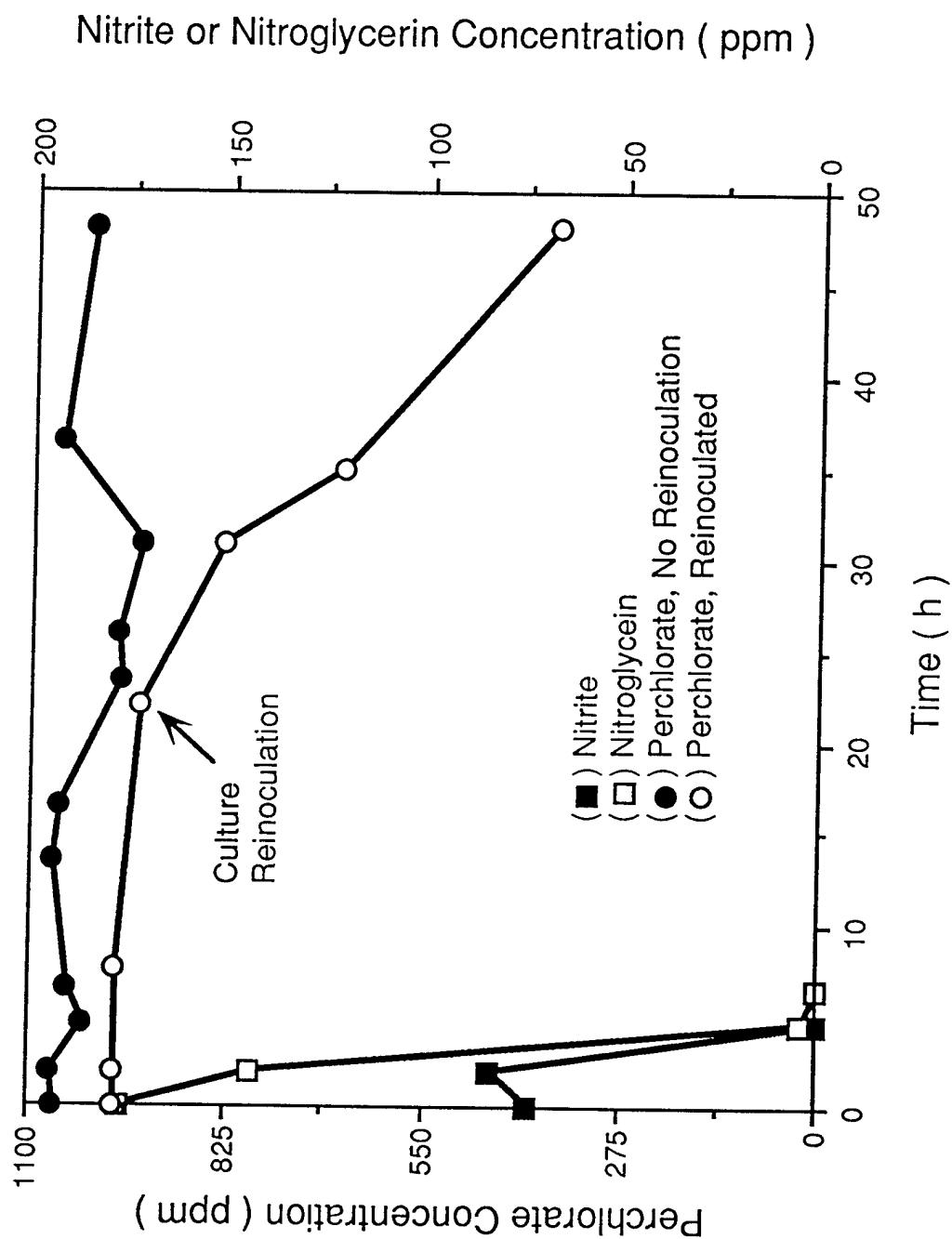
Figure 12. Bioreduction of Perchlorate IN 50% CYH Wastewater After Nitroglycerin and Nitrite Degradation

Time (h)

Perchlorate Concentration (ppm)

Culture Reinoculation

(■) Nitrite
(□) Nitroglycerin
(●) Perchlorate, No Reinoculation
(○) Perchlorate, Reinoculated

Figure 13 shows the results of an experiment to determine if nitroglycerin or the nitrite associated with it is the inhibitor of perchlorate reduction. Rates of reduction were compared between cultures containing nitroglycerin (460 ppm) plus nitrite (140 ppm) and nitrite (135 ppm) alone. The data indicated that while nitrite alone produces a lag phase before reduction occurred, nitroglycerin and nitrite, at the concentrations in this test, combine to terminate reduction permanently. Nitrite alone disappeared in less than 5 hours, whereas nitrite in the presence of nitroglycerin was only partially degraded after 25 hours. This suggests that nitroglycerin at high enough concentrations is an inhibitor of nitrite reduction.

The other major compounds in CYH wastewater were also tested for potential inhibitory effects on perchlorate reduction. The data in Figure 14 shows that neither triacetin (200 ppm), or resorcinol (200 ppm), or a combination of both, had any effect on the reduction rate.

The evidence indicates that nitroglycerin and nitrite present in CYH wastewater are readily degradable and must be removed before significant perchlorate reduction can occur. The other two major compounds, triacetin and resorcinol, do not affect reduction at typical concentrations. Other compounds present in trace quantities (HMX, nitrocellulose, 2-nitrodiphenylamine, and various hydrolysis products) were not tested in this study but may have an effect on perchlorate reduction.
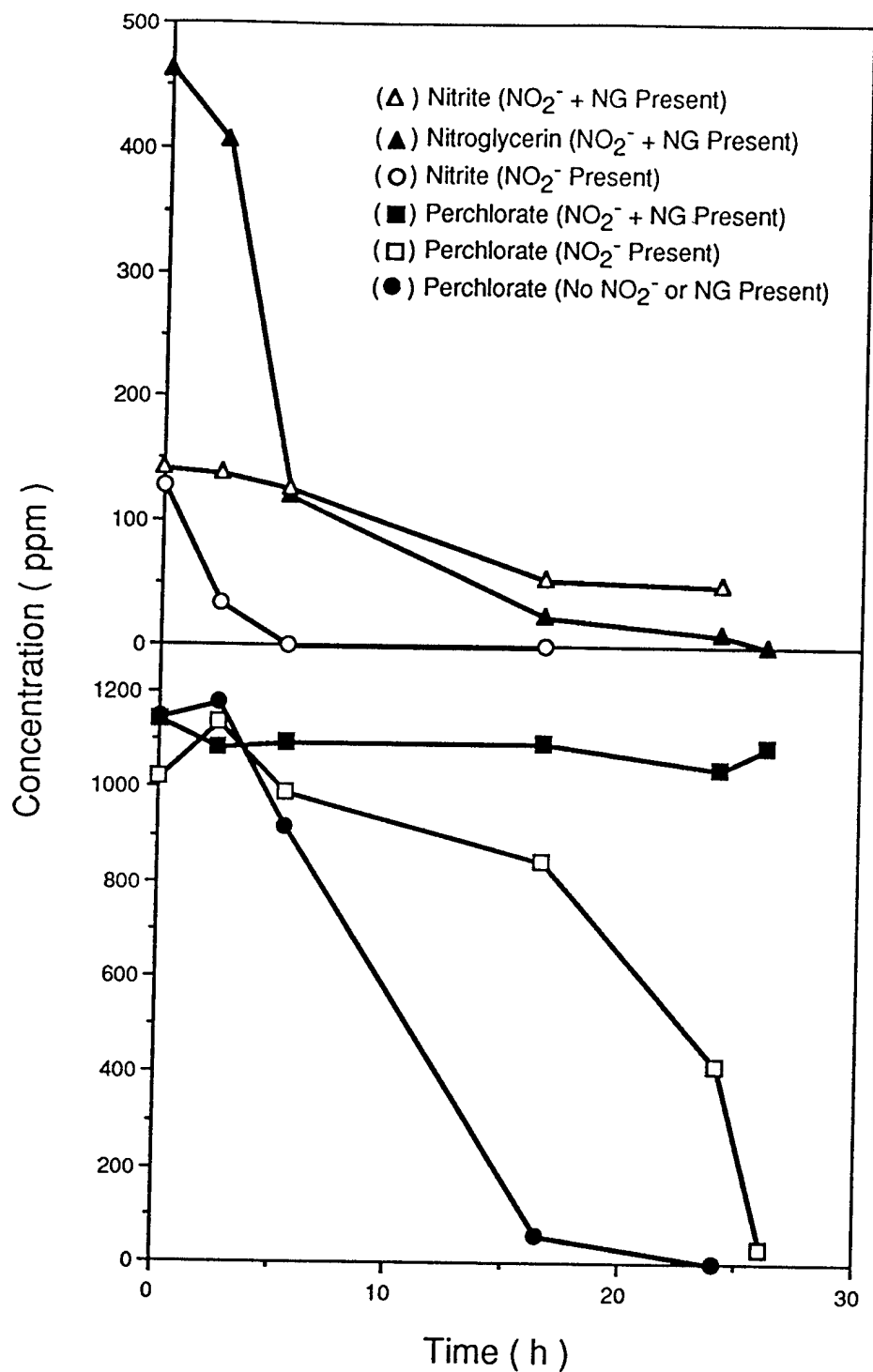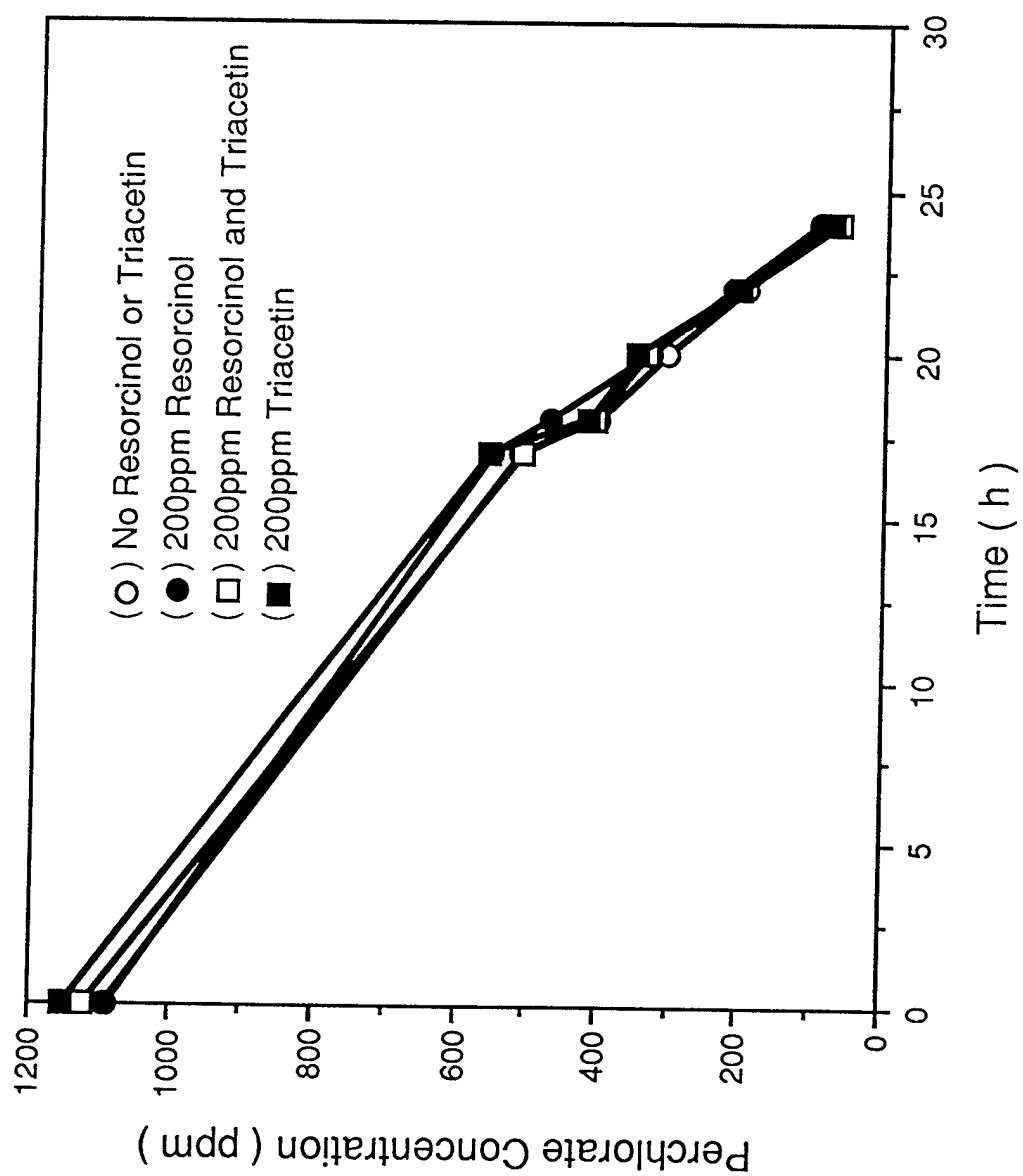
Figure 13. Comparison of Perchlorate Reduction in the Presence of Nitrite or Nitrite Plus Nitroglycerin

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Figure 14. Perchlorate Reduction in the Presence of Triacetin, Resorcinol, or Both

SECTION V
CONCLUSIONS

The rapid degradation of nitroglycerin in the presence of the anaerobic mixed culture indicated that biotreatment of this compound is highly feasible. The degradation of nitroglycerin appeared to be a result of abiotic denitration brought on by low culture redox potentials. The limiting factor for the treatment of nitroglycerin/perchlorate wastestreams appeared to be the inhibitory effect of nitroglycerin and associated nitrite on perchlorate reduction. The initial growth of the mixed culture was responsible for the abiotic degradation of nitroglycerin and the removal of nitrite. These compounds were toxic to the perchlorate reducing bacterium HAP1, and following their elimination, HAP1 growth and perchlorate reduction proceeded in wastewater tests.

SECTION VI

RECOMMENDATIONS

While the feasibility of biotreating class 1.1 propellant wastewaters containing nitroglycerin and ammonium perchlorate has been clearly demonstrated, there still remains several questions to answer. In this study we showed the sequential denitration of nitroglycerin through glycerol-1,3-dinitrate and its subsequent removal by the anaerobic culture. However, due to analytical limitations we could not show that glycerol-1-mononitrate was formed from glycerol-1,3-dinitrate and subsequently removed in the anaerobic culture as it was in the chemical reaction experiment with sodium hydrosulfite. Future work to develop new separation techniques for resolving the mononitrate ester from the spent culture medium could help to define this pathway. We also could not determine the fate of the nitrogen moieties during the degradation. Since most environmental validation of treatment technologies require as complete a mass balance as possible, further experiments should be done to determine the final fate of both carbon and nitrogen from the biotreatment process. This would probably best be accomplished using radiolabelled nitroglycerin and monitoring for complete mineralization to carbon dioxide and nitrogen gas.

With the data generated for this report, a modified version of the bioreactor system used for class 1.3 propellant disposal (Reference 2) should be designed and tested for class 1.1 wastewater treatment. Figure 15 shows a proposed continuous flow reactor system. The stage 1 anaerobic bioreactor, containing the mixed culture, would accept the diluted wastewater and carry out the degradation of nitroglycerin, nitrite and nitrate. The stage 2 anaerobic bioreactor, containing the mixed culture and HAP1, would accept the perchlorate bearing wastewater which is now free of compounds inhibitory to HAP1. Perchlorate reduction would be carried to completion in stage 2 and the wastewater would be passed through the stage 3 sludge removal system to the stage 4 aerobic bioreactor. Here an aerobic culture would metabolize organic

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material to lower the Biological Oxidation Demand (BOD) and Chemical Oxidation Demand (COD) as well as oxidize ammonia to nitrate. The wastewater would then be passed through a stage 5 sludge removal system and then passed on to a sewer or recycled back to the stage 1 reactor to be used as dilution water depending on regulatory effluent requirements. An anaerobic denitrifying bioreactor may be required after the stage 4 aerobic bioreactor to eliminate nitrate in the wastewater before sewering.

Development and testing of this type of system on a bench scale will provide scaleup and feasibility data for the large scale biotreatment of class 1.1 propellant wastewaters.
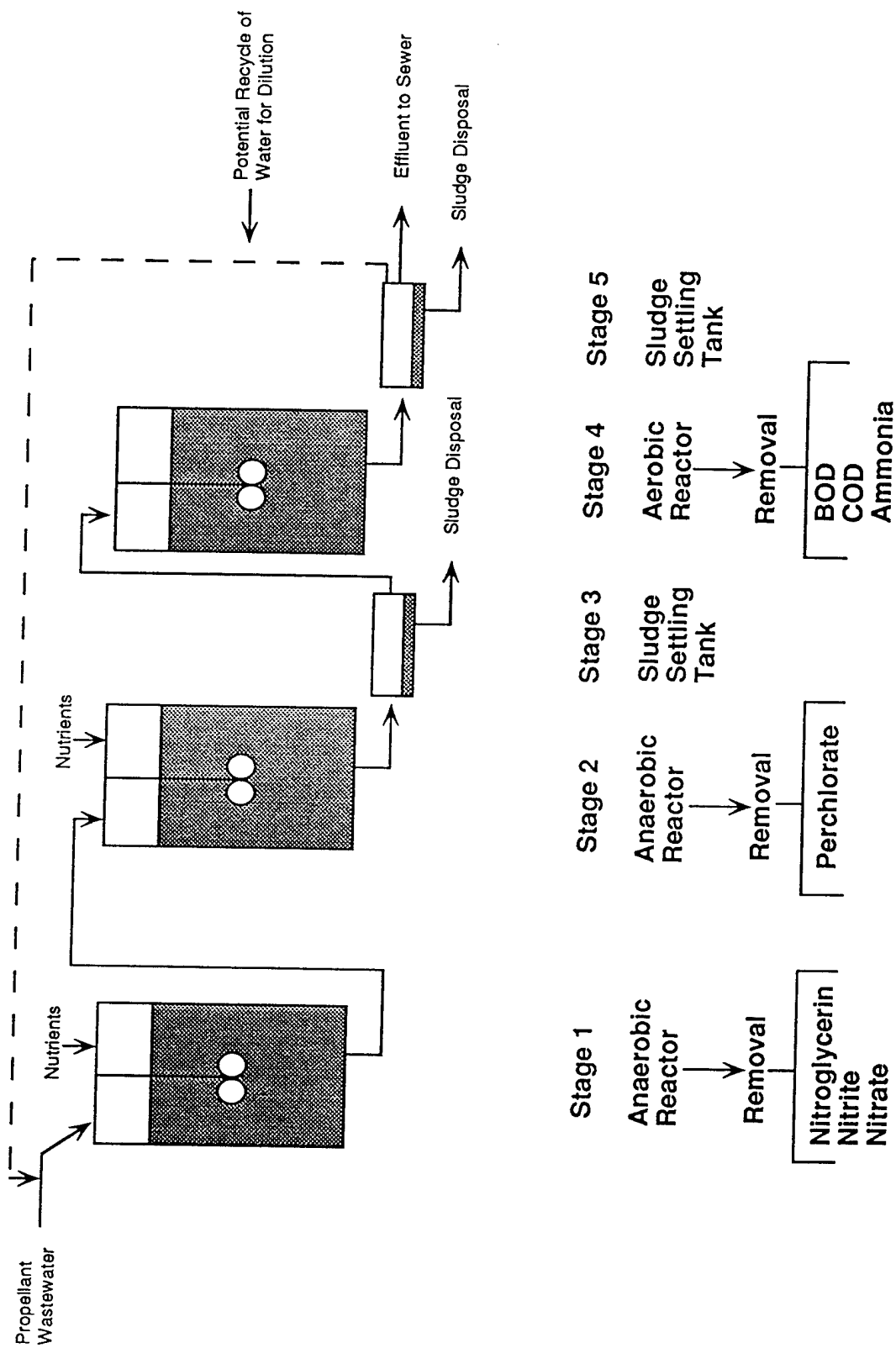
Figure 15. HAZARD CLASS 1.1 Propellant Wastewater Biotreatment System

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Anaerobic Reactor	Anaerobic Reactor	Sludge Settling Tank	Aerobic Reactor	Sludge Settling Tank
Removal	Removal		Removal	
Nitroglycerin Nitrite Nitrate	Perchlorate		BOD COD Ammonia	

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SECTION VII
REFERENCES

1.   Attaway, H. and M. Smith, <u>Reduction of Perchlorate by an Anaerobic Enrichment Culture</u>. Journal of Industrial Microbiology, vol 12, pp. 408-412, 1993.

2.   Attaway, H. and M. Smith, <u>Propellant Wastewater Treatment Process</u>. U.S. Patent 5,302,285, 1994.

3.   Attaway, H. and J. Shanahan, <u>Biodegradation of Ammonium Perchlorate Wastewater</u>. CPIA publication 600, pp. 341-347, 1993.

4.   Balasco, A.A., R.C. Bowen, I. Bodek, C.A. DeSantos, R.F. Machacek, J.M. Nystrom, S.L. Roberts, L.R. Woodland, <u>Ball Powder Production Wastewater Pilot-Scale Biodegradation Support Studies</u>. US Army Toxic and Hazardous Materials Agency, DAAK11-85-D-0008, 1989.

5.   Cornell, J.H., T.M. Wendt, N.G. McCormick, D.L. Kaplan and A.M. Kaplan, <u>Biodegradation of Nitrate Esters used as Military Propellants.</u> Science and Advanced Technology Laboratories, United States Army Natick Research and Development Laboratories, Technical Report NATICK/TR-81/029, 1981.

6.   Emde, R. and B. Schink, <u>Fermentation of Triacetin and Glycerol by</u> <u>*Acetobacterium sp.* No Energy is Conserved by Acetate Excretion.</u> Archives of Microbiology, Vol 149, pp. 142-148, 1987.

7.   Grasso, D. and H. Pesari, <u>Ball Powder Production Wastewater Biological Treatability Studies.</u> USATHMA, CETHA-TS-CR-90247, 1992.

8.   Hurley, J., <u>Large Rocket Motor Disposal Program</u>. The Military Engineer, August-September, pp. 19-22, 1994.

9.   Kaplan, D.L., J.H. Cornell, and A.M. Kaplan, <u>Biodegradation of Glycidol and Glycidyl Nitrate</u>. Applied and Environmental Microbiology, Vol 43, pp. 144-150, 1982.

10.   Ljungdahl, L.G., and J. Wiegel, <u>Working with Anaerobic Bacteria.</u> in Manual of Industrial Microbiology and Biotechnology, Eds. A.L. Demain and N.A. Solomon, pp. 84-96, 1986.

11.   Miks, K.F., <u>Solid Propellant Ingredient Reclamation and Reuse.</u> CPIA Publication 600, pp. 311-329, 1993.

12.   Schink, B., <u>Principles and Limits of Anaerobic Degradation: Environmental and Technological Aspects.</u> pp 771-846, in A. J. B. Zehnder (ed.), Biology of Anaerobic Microorganisms, Wiley-Liss, New York, 1988.

13.   Shanahan, J., H. Attaway, S. Harper, G. McDonald and M. Smith. <u>Biotreatment of Ammonium Perchlorate in Rocket Motor Wastewater.</u> In Implementation of Biotechnology in Industrial Waste Treatment and Bioremediation, Lewis Publishers, Chelsea, in press.

14.   Smith, J.G., <u>Water Quality Criteria for Nitroglycerin</u>. U.S. Army Medical Research & Development Command, Interagency Agreement No. 84PP4845, Contract No. DE-AC05-840R21400, 1986.

15.   Spanggord, R.J., <u>Environmental Fate Studies on Certain Munition Wastewater Constituents - Laboratory Studies.</u>, U.S. Army Medical Research and Development Command, Final Report DAMD17-78-C-8081, 1980.

16.   Stacy, J.R., M.K. Speedie, L. Geelhaar, G. Payne, W.Q. Sun, and R. Govind, Biodegradation of Nitrate Esters. CPIA Publication 600, pp. 87-154, 1993.

17.   Urbanski, T., Chemistry and Technology of Explosives - Volume II. pp. 32-61, Pergamon Press, 1990.

18.   Wendt, T.M., J.H. Cornell, and A.M. Kaplan, Microbial Degradation of Glycerol Nitrates. Applied and Environmental Microbiology, Vol 36, pp. 693-699, 1978.

19.   White, G.F., and J.R. Snape, Microbial Cleavage of Nitrate Esters: Defusing the Environment. Journal of General Microbiology, vol 139, pp. 1947-1957, 1993.

20.   Yinon, J., Toxicity and Metabolism of Explosives. pp. 81-117, CRC Press, 1990.