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OXIDATION/BIODEGRADATION OF SOLID PROPELLANTS USED IN LEGACY CHEMICAL ROUNDS

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14. ABSTRACT Nitrocellulose based compounds are the primary ingredients historically used as solid rocket and mortar propellants. These compounds were mass produced for many years and stored in bulk or configured into chemical and high-energy munitions. With the planned destruction of the U.S. chemical agent inventory, the associated propellant charges and the now antiquated propellants in storage for use in high energy rounds are awaiting disposal. Many of these propellants were manufactured over 40 years ago and are of questionable reliability. Reuse of these propellants is unlikely due to advances in more modern formulations and the economics of converting them into more usable materials. Traditional open burn/open detonation of these compounds is under pressure from more stringent environmental regulations. Biotreatment is seen by environmental and citizen groups as a friendly alternative for destruction of hazardous wastes. This report describes laboratory study where peroxone and biotreatment were successfully used to degrade neutralized propellants to near surface water regulatory requirements.					
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PREFACE

The work described in this report was authorized under Project No. 5EA1CA/ER33, Oxidation/Biodegradation of Solid Propellants. The work was started in October 2003 and completed in September 2005.

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CONTENTS

1.	INTRODUCTION	9
2.	STUDY OBJECTIVES.....	11
3.	METHODS	12
3.1	Hydrolysate Preparation.....	12
3.2	Biofeed Preparation	12
3.3	Peroxone Treatment.....	13
3.4	Immobilized Cell Bioreactors.....	14
3.5	Treatment Strategies	15
3.5.1	Treatment Strategy 1.....	15
3.5.2	Treatment Strategy 2.....	15
3.6	Process Monitoring.....	16
3.7	Analysis for VOCs and SVOCs.....	16
3.8	Toxicity Monitoring.....	17
3.9	Identification of Bacterial Cultures.....	17
4.	RESULTS	17
4.1	Strategy 1	17
4.1.1	Process Monitoring for 3-Hr Peroxone Treatment of Biofeed	17
4.1.2	Process Monitoring.....	20
4.2	Strategy 2	20
4.2.1	6-Hr Peroxone Treatment of Biofeed	20
4.2.2	Process Monitoring.....	22
4.3	Results for VOC and SVOC Analysis	23
4.4	Toxicity Monitoring.....	26
4.5	Bacterial Species Identification	28
5.0	CONCLUSIONS.....	29
5.1	Ability to Degrade Propellant Hydrolysates.....	30
5.2	Ability to Oxidize Compounds Resistent to Biodegradation.....	32
5.3	Ability of Combined Treatment to Detoxify Effluents.....	32
5.4	Measure the Removal of Nitrogen Compounds.....	32
6.	SUMMARY	33
	LITERATURE CITED	35

FIGURES

1.	Photograph of Extruded M1 Pellets.....	13
2.	Photograph of M8 Sheets.....	13
3.	Photograph of ICB Setup.....	14
4.	Typical ICB Laboratory Packing Material	14
5.	Comparisons of ICB/AOP Treatment Strategies	16
6.	Strategy 1 M1 Peroxone Biofeed Treatment Results for COD, NO ₂ , and NO ₃ Assays	18
7.	Strategy 1 M8 Peroxone Biofeed Treatment Results for COD, NO ₂ , and NO ₃ Assays	19
8.	Strategy 1 M-28 Peroxone Biofeed Treatment Results for COD, NO ₂ , and NO ₃ Assays	19
9.	Strategy 1 Overall Biotreatment Results of All Propellants for COD and and Total Nitrogen Assays.....	20
10.	Strategy 2 M1-2 Peroxone Biofeed Treatment Results for COD, NO ₂ , and and NO ₃ Assays.	21
11.	Strategy 2 M8-2 Peroxone Biofeed Treatment Results for COD, NO ₂ , and and NO ₃ Assays.	21
12.	Strategy 2 M28-2 Peroxone Biofeed Treatment Results for COD, NO ₂ , and NO ₃ Assays.	22
13.	Strategy 2 Overall Biotreatment Results of all Propellants for COD and Nitrogen Assays.....	23
14.	Strategy 1 Microtox EC50 Results for Major Treatment Steps of All Three Study Propellants	27
15.	Strategy 2 Microtox EC50 Results for Major Treatment Steps of All Three Study Propellants	27

TABLES

1.	Composition of Study Propellants before Neutralization	12
2.	Composition of Wolin Salts Micronutrient Solution	13
3.	Summaries of Combined Positive Results for VOC and SVOC Analysis of M1 Biofeed and Effluents from Both Treatment Strategies	24
4.	Summaries of Combined VOC and SVOC Analysis of M1 Biofeed and Effluents from Both Treatment Strategies	24
5.	Summaries of the Tentatively Identified Compounds for the M1 ICBs	25
6.	Summaries of the Tentatively Identified Compounds from the M28 ICBs	26
7.	Listing of the Bacteria Cultured from the M28 Immobilized Cell Bioreactor	29
8.	Summary Results for COD and Total Nitrogen across both Stages of Bioreactor Treatment	30
9.	Results for Averaged Nitrogen Content in ICB Biofeed and Effluent Streams	33

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OXIDATION/BIODEGRADATION OF SOLID PROPELLANTS USED IN LEGACY CHEMICAL ROUNDS

1. INTRODUCTION

Nitrocellulose based compounds are the primary ingredients historically used as solid rocket and mortar propellants. These compounds were mass produced for many years and stored in bulk or configured into chemical and high-energy munitions. With the planned destruction of the U.S. chemical agent inventory, the associated propellant charges and the now antiquated propellants in storage for use in high energy rounds, is awaiting disposal. Many of these propellants were manufactured over 40 years ago and are of questionable reliability. Reuse of these propellants is unlikely due to advances in more modern formulations and the economics of converting them into more usable materials. Traditional open burn/open detonation of these compounds is under pressure from more stringent environmental regulations. Biotreatment is seen by environmental and citizen groups as a friendly alternative for destruction of hazardous wastes.

The U.S. Army's Alternative Technology and Assembled Chemical Weapons Assessment (ACWA) Program has effectively demonstrated the use of biological treatment for destruction of chemical agents removed from chemical rounds stored at Pueblo Chemical Depot. The same neutralization/biological treatment schemes used for chemical agents have not worked well for destruction of the propellants removed from these chemical rounds. Mixed bacterial cultures in immobilized cell bioreactors grown on hydrolyzed mustard agent were unable to degrade or detoxify the hydrolyzed propellant feeds under similar treatment conditions. Alternative biotreatment schemes for disposal of propellant charges were proposed but never attempted for propellants specific to the Pueblo site. While a neutralization/biodegradation solution has been approved for full-scale design at the Pueblo Chemical Depot site for destruction of agent containing munitions, an alternative method for destroying the potentially contaminated propellants has not been decided.

Propellant charges that are configured into agent containing chemical rounds are being removed and stored for later destruction. The eventual destruction of these propellants will be the responsibility of the Department of Defense (DoD). Present baseline technology for this destruction would be incineration. Any new incineration program would meet with opposition from the public and environmental groups. However, these same groups have endorsed the use of neutralization followed by biodegradation for destruction of chemical rounds at Pueblo Chemical Depot and bulk containers at the U.S. Army Aberdeen Proving Ground (APG).

Cellulose nitrate or nitrocellulose (NC) is a fibrous solid that has many industrial applications including varnishes, ink bases and adhesives. Nitrocellulose is produced by the nitration of cellulose using nitric and sulfuric acids. Nitrogen content in prepared NC ranges from 11.5 to a maximum of 14.5%. Nitrocellulose in a lower nitrated form is commonly used in commercial applications, while NC with 12.5-13.5% nitration is used primarily as energetic

material like rocket and gun propellant. The highly nitrified NC in a dry state is capable of detonation by shock, abrasion, and sparks. The production of NC produces waste in industrial wastewater known as NC fines. Historically, this NC fines has found its way into the environment and contaminated soils and water in areas near production facilities. Nitrocellulose production in the U.S. is now closely regulated, and the release of untreated pink-water is prohibited.

Studies have previously found nitrified, cellulose-based propellants to be difficult to treat¹ biologically without preliminary caustic.² Jones et al. found that soil microorganisms isolated from previously contaminated production facilities show activity for degradation of NC and other widely used propellants and explosives. The activity demonstrated was low and not conducive to a stand-alone biological treatment scheme. Additionally, the potential for agent contamination of propellant charges that were removed from agent containing projectiles necessitates the clearance of these propellants from any agent contamination. Therefore, a process similar to agent neutralization is required. Christodoulatos and Su found that NC could be dissolved and broken down in a heated reaction with varying concentrations of sodium hydroxide ranging up to 25%. The process is exothermic; so during treatment, the temperature is increased slowly and monitored closely. Once at temperature, the NC breaks down in minutes and produces nitrite and nitrates in approximately a 3:1 ratio.

The propellants associated with assembled chemical rounds consist of mixtures of mostly NC with other conventional high-energy explosives like nitroglycerine (NG), dinitrotoluene (DNT), and diphenylamine (DPA).^{3,4} The neutralization products of these compounds together in a mixture are not as clear-cut. Bunte and Krause⁵ examined the products of five propellants produced during alkaline pressure hydrolysis and found in addition to nitrite and nitrate, in concentrations lower than previously reported, mixed carboxylic acids and solid residues of DPA, centralite, and dibutylphthalate.

In addition to the more complex mixture of the propellant recipes, propellant that was configured with rockets and projectiles include metals and other impurities from the long and close association with metal projectile housings and associated rinsing water from metal parts clean out. Complete characterization of the compounds in the propellant hydrolysate is difficult due to the sheer number of products found. Analytical reporting routinely lists many unknowns or unquantifiable compounds or compounds are partially identified as unknown acids, alcohols, or alkanes. Measures of a processes success become a measure of removal of total nitrogen compounds, total VOC, SVOC, carbon, Toxic Leachate Procedure (TCLP), or detoxification.

The use of ozone combined with peroxide and/or ultraviolet light has been used to destroy toxic industrial chemicals in contaminated ground and drinking water supplies. These techniques, collectively called Advanced Oxidative Processes (AOP), use hydroxyl radicals generated in-situ to oxidize and destroy contaminants. Kuo et. al.⁶ successfully demonstrated peroxone oxidation as a method to remove toluene and 2,4,6 trinitrotoluene commonly found in process wastewater from explosives production facilities. Beltran et. al.⁷ successfully destroyed nitro aromatic hydrocarbons using ozone in water, ozone combined with peroxide, and UV radiation. Beltran noted the broad effect of oxidation rates on nitrobenzene and dinitrotoluene in surface waters as opposed to ultra pure laboratory water and the effect of hydroxyl radical

scavengers like carbonates that interfered with NB and DNT oxidation. Therefore, the longer the list of organic and inorganic compounds in the original neutralization process, the greater the unpredictability in component interactions during neutralization and oxidation processes and subsequent biotreatment.

The U.S. Army Environmental Center (AEC) has conducted a groundwater treatment demonstration study at the Cornhusker Army Ammunition Plant⁸ to remove explosives contamination. This demonstration used a peroxone oxidation system to remove mixed explosive contaminants to below regulatory levels. In this study and each of the previous works, AOP was used to destroy or remove contaminants to regulatory levels, where the starting concentration was in the milligram/liter range.

Previous studies were conducted using water of a higher quality than our study in that there were fewer compounds to complicate the neutralization/oxidation process. Additionally, the neutralization processes discussed were designed for optimal propellant neutralization. In our study, the neutralization process closely resembled that of the chemical agents of interest because the process goal was to validate that the propellant was agent free not to optimize propellant destruction. In this study, we will use solutions of hydrolyzed propellants in the range of 60,000 parts per million (ppm). The AOP processes will be employed to make degradable the more recalcitrant components of the concentrated hydrolysate so that biotreatment may be successful where it had previously performed poorly.⁹

2. STUDY OBJECTIVES

- Demonstrate the ability of a mixed microbial consortium cultured from activated sludge to degrade propellant hydrolysates.
- Demonstrate the ability of combined ozone and peroxide treatment to oxidize compounds resistant to biotreatment and render them more easily biodegradable.
- Measure the ability of the combined oxidative and biological treatment to detoxify the propellant materials based on the Microtox Assay.
- Measure the removal the excessive nitrogen compounds inherent from the breakdown of NC based propellants, and screen the final treated effluents for potential release to surface waters or to a municipal wastewater treatment system.

In this study, the utility of ozone combined with peroxide treatment will be examined for its ability to detoxify and breakdown mixed nitrogen and NC compounds that were previously shown resistant to biodegradation. Three hydrolyzed propellants, M1, M8, and M28, removed from assembled chemical rounds were treated with combined ozone and peroxide (peroxone) and treatment in immobilized cell bioreactors. Another goal of the study is to remove nitrogen compounds known to be present to levels that may allow discharge of the biotreated effluents to surface waters or a wastewater treatment system.

3. METHODS

3.1 Hydrolysate Preparation.

Solid propellants in this study were produced in elongated pellets, Figure 1 and M8 sheets, Figure 2 for configuration into chemical rounds. In this study, we examined hydrolyzed propellants M1 and M8 that were removed from chemical rounds. The propellants were hydrolyzed at 6.7 % (weight/volume) loading in 6% NaOH/water solution. The mixture was hydrolyzed at 90 °C for 4 hr before cooling and coarse filtering. These hydrolysates were treated with ozone and peroxide (peroxone) to reduce their toxicity to biological cultures. Two separate treatment schemes were used to evaluate the effectiveness of the AOP treatment. Immobilized Cell Bioreactors were used to compare biotreatability of the peroxone treated and untreated hydrolyzed propellants. Table 1 lists the general recipe for the M1, M8 and M28 propellants prior to hydrolyzation.

Table 1. Composition of Study Propellants before Neutralization

	M1 (% wt/wt)	M8 (%wt/wt)	M28 %wt/wt)
Nitrocellulose	84.0	52.15	60.0
Nitroglycerine		43.0	23.8
Triacetin			9.9
Dinitrotoluene	9.0		
Dibutylphthalate	5.0		
Diethylphthalate		3.0	
Dimethyl phthalate			2.6
Lead Stearate			2.0
2-nitrodiphenylamine			1.7
Potassium nitrate		1.25	
Diphenylamine	1.0		
Lead carbonate	1.0		
Ethyl centralite		0.60	

3.2 Biofeed Preparation.

Biofeed was prepared by diluting 200 mL of propellant hydrolysate in 1-L of distilled water. Biofeed to be peroxone treated also initially contained 55 mL of 30 % hydrogen peroxide solution. Before feeding to the ICB reactor, 10 mL sulfur free wolins salts 100x solution and 0.06 g sodium phosphate dibasic was added to 1 L of biofeed. Wolin salts is a solution published by E.Z. Wolin et. al.¹⁰ used to provide media micronutrient requirements for biological cultures. The recipe for sulfur free Wolin salts is provided in Table 2 below.

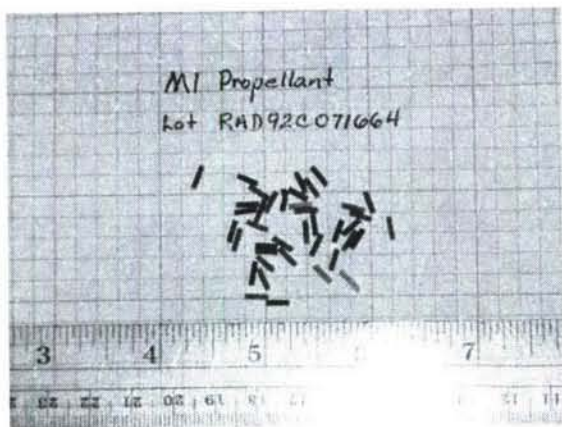


Figure 1. Photograph of Extruded M1 Pellets

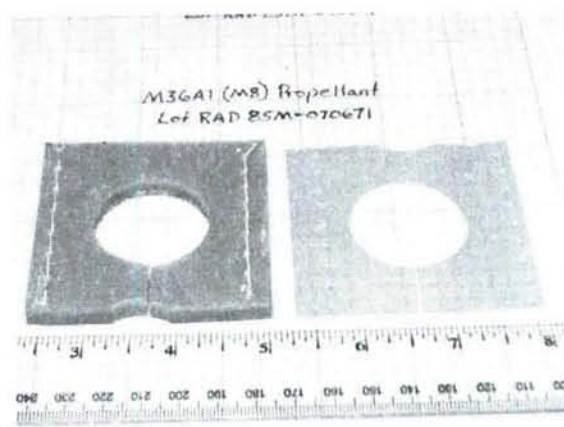


Figure 2. Photograph of M8 Sheets

Table 2. Composition of Wolin Salts Micronutrient Solution

Compound	WEIGHT Per Liter (g)
Nitrilotriacetic acid	3.00
NaOH	Enough to allow Nitrilotriacetic acid to dissolve
MgCl ₂ 4H ₂ O	6.95
MnCl ₂	0.66
FeCl ₂	0.23
CaCl ₂ 2H ₂ O	0.07
CoCl ₂ 6H ₂ O	0.10
ZnCl ₂	0.06
H ₃ BO ₃	0.02
Na ₂ MoO ₄ 2H ₂ O	0.01
CuCl ₂ 2H ₂ O	0.01

3.3 Peroxone Treatment.

The hydrolyzed propellants were diluted to a concentration of 200 mL/L as feed to the bioreactors. This biofeed solution was either fed to the bioculture directly or first treated with peroxone. Hydrogen peroxide (55 mL of 30%) was initially added to each liter of solution to be treated. An additional 12 mL of peroxide was added after 2 hr during Strategy 2 treatments.

Ozone was generated at approximately 1.25 g/hr using an Ozonology, Inc. (Northbrook, IL) Labzone L100 ozone generator. The ozone was reacted with the biofeed in a

1.5-L glass reaction vessel through an aeration stone. The reactor vessel was fitted with ports to allow addition of peroxide and removal of timed samples. The reactor vessel was held in place on a Barnstead stir plate. The biofeed in the reactor was stirred vigorously to increase ozone interaction and transfer to solution. The peroxone reaction took place at room temperature (22 °C).

The Labzone produces ozone by passing oxygen supplied from a compressed gas cylinder through a corona discharge. Ozone in the presence of peroxide produces hydroxyl radicals that then oxidize the components of the propellant hydrolysate. Timed samples were periodically removed from the liquid reactor for analysis. Upon completion, the oxidized solution was pH adjusted to below 9.0 when required with HCl, and any remaining peroxide was removed by the addition of catalase.

3.4 Immobilized Cell Bioreactors.

The Immobilized cell bioreactors are 600-mL glass vessels with a single port top and bottom for input/output. The top is open but fitted with butyl rubber stoppers. The stoppers are ported to allow pH monitoring and control, feed and nutrient addition, and exhaust gas release to a trap. The pH was controlled in one direction only with 0.5N HCl.

Immobilized cell bioreactors provide support material for bacterial cells to attach themselves. Numerous support materials are available for targeted applications. The support material employed in this study was expanded foam blocks (Figure 3). The support material is simply poured into the immobilized cell bioreactors before startup; the culture will attach itself as it grows. An expanded nylon spacer is also added to allow nutrient circulation throughout the reactor. The ICB, which are operated aerobically, are plumbed to allow air addition to the bottom of the reactor and allow mixing within the reactor. An ICB that is operated anoxically requires an additional circulation system. In our study, an external circulating pump cycled every 7 min removing media from near the bottom of the reactor and adding it back to the top. The recirculating line also served as a pickup for the bio-feed loop. The ICB media and ICB bio-controllers are shown in Figure 4 below.



Figure 3. Photograph of ICB Setup

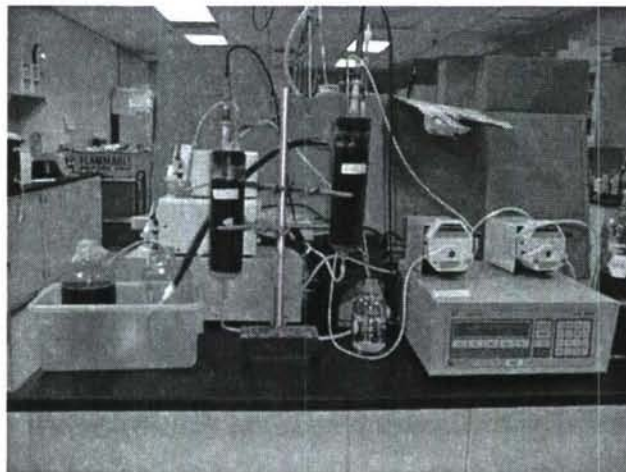


Figure 4. Typical ICB Laboratory Packing Material

The bioreactors were inoculated with sludge from the publicly owned Back River wastewater treatment works (Essex, MD). The inoculum was centrifuged, and the clarified supernatant was added to the reactors. Approximately 10% sludge (original treatment plant sample concentration) by media volume was then added to each reactor.

Stage 1 of the reactors was operated at a 5-day hydraulic residence time (Hrt). Initial feed concentration started at 25% of the 200mL/L hydrolysate feedstock and was ramped up over time to 100%. Stage two of the reactors were operated at a 10-day Hrt. Stage 1 was intended to degrade the hydrolyzed feed as best possible and remove excess nitrogen in the form of nitrite and nitrate through denitrification. The reactors were operated anoxically to encourage denitrification. Additional treatment was applied to the biofeed in the second stage. In this study, this included either peroxone treatment or an added carbon source or both to breakdown more recalcitrant compounds and to increase metabolic activity and denitrification..

3.5 Treatment Strategies.

Two treatment strategies were tested for oxidation and biotreatment of the hydrolyzed propellants.

3.5.1 Treatment Strategy 1.

Strategy 1 consisted of treating the propellant biofeed initially with mixed biocultures seeded with activated sludge in the ICB without peroxone treatment. This step uses the carbon compounds available to the bioculture to drive metabolism that can denitrify the nitrogenous media compounds under anoxic conditions. The effluents from the first biotreatment stage is filtered to remove biomass and subjected to peroxone treatment for 3-hr. A secondary biotreatment was then used to treat the now oxidized nitrocellulose compounds left untreated by the first stage biotreatment. In discussions in this paper, ICB reactors and their samples will be designated as Strategy 1 or 2 by the extension, for example, M1-1 will be propellant M1, treatment Strategy 1 and M1-2 will be treatment Strategy 2.

3.5.2 Treatment Strategy 2.

Strategy 2 consisted of pre-treating the same concentration of biofeed for 6-hrs in the peroxone reactor. The media would also receive a secondary biotreatment that includes addition of carbon as glucose to increase metabolism and denitrification. The two strategies are represented in Figure 5 below.

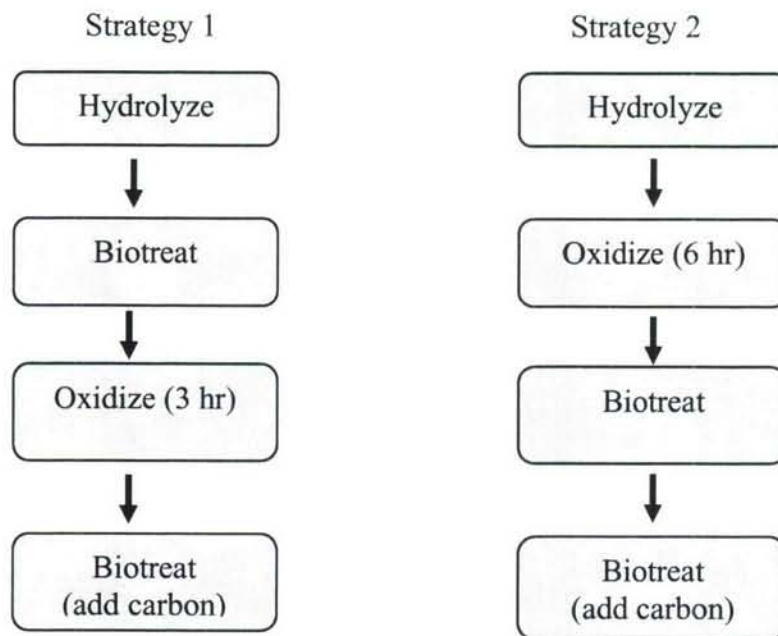


Figure 5. Comparisons of ICB/AOP Treatment Strategies

3.6 Process Monitoring.

Periodic samples from peroxone treatments, biofeed and bioreactor effluent analyzed for Chemical Oxygen Demand (COD), ph, phosphate, nitrate and nitrite levels. These tests were conducted in-house using Hach assays kits and a Hach DR/2010 spectrometer. Process monitoring was accomplished using the following methods:

- Nitrate, Chromotropic Acid Method, Hach No. 10020
- Nitrite, Ferrous sulfate Method, Hach No. 8153
- Ammonia TNT, Salicylate Method, Hach No. 8150
- Chemical Oxygen Demand, Reactor Digestion Methon, Hach No. 8000
- Phosphorus (orthophosphate) Amino Acid Method, Hach No. 8178

3.7 Analysis for VOCs and SVOCs.

Analysis for volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs) were accomplished using an off-site analytical services lab. Analysis was accomplished using EPA methods SW827C for SVOCs and SW8260B for VOCs. Biofeed and peroxone treatment samples for VOC and SVOC were furnished by complete batch process. Due to volume requirements bioreactor effluent samples were collected and analyzed as

composite samples. Biofeed and peroxone treatments were analyzed as treated. Effluent samples were centrifuged and filter sterilized prior to shipment.

3.8 Toxicity Monitoring.

Timed peroxone and biotreatment samples were monitored for toxicity using the Microtox (MTX) assay. The MICROTOX assay exposes a bioluminescent marine bacterium (*Vibrio fischeri*) to a sample of unknown toxicity and measuring the change in light output, indicating metabolic activity. Data was analyzed with the MTX Test Protocol software to determine the EC₅₀ (the effective concentration causing a 50% reduction in light output). The Microtox assay has been proved to be a good measure of substrates toxicity to biocultures used in earlier studies.^{6,7}

3.9 Identification of Bacterial Cultures.

The degradation studies for M1 and M8 propellants were conducted simultaneously. The M-28 study was conducted after completion of M8. The M1 and M8 cultures were used to seed the M-28 Strategy 1 and 2 ICB's, respectively. A small quantity of fresh activated sludge was added to each ICB with the conditioned inoculums. Near the end of the M28 study, samples of the culture were isolated for bacterial identification.

Biomass suspension was extracted from the M28 process 1 and 2 reactors, and plated raw material. All incubation steps occurred at ambient temperature, approximately 27 °C, using tryptic soy agar (Difco). From this original culture, all visually differentiable colony morphologies were isolated, transferring each to achieve culture purity. Isolates were grown on TSA slants, and shipped for GC-FAME analysis (MIDI Labs, Newark DE).

4. RESULTS

4.1 Strategy 1.

4.1.1 Process Monitoring for 3-Hr Peroxone Treatment of Biofeed.

In Strategy 1 the biofeed is prepared with 200 mL of the propellant hydrolysate per liter of bio-feed. The biofeed was administered to the culture over a 30-min period, once per day. Representative samples of the feed and effluents were taken after initial acclimation and biomass ramp-up period when the reactors were considered to be at steady state. These samples were assayed for chemical oxygen demand (COD), nitrite, nitrate, and phosphate concentrations. The levels of nitrogen compounds are significant in that they are principal breakdown products of the propellants nitrocellulose base, Dinitrotoluene, Nitroglycerine, and mixed nitrogen compounds. These compounds before neutralization are fairly toxic to aquatic species. When not completely removed during biotreatment they are closely regulated pollutants, or nutrients when considered for release to surface waters or waste water treatment systems. Figure 6 below represents results of these assays for COD, nitrate and nitrite during Strategy 1 biofeed stage 1 and the 3-hr peroxide/ozone (peroxone) treatment of M1 propellant.

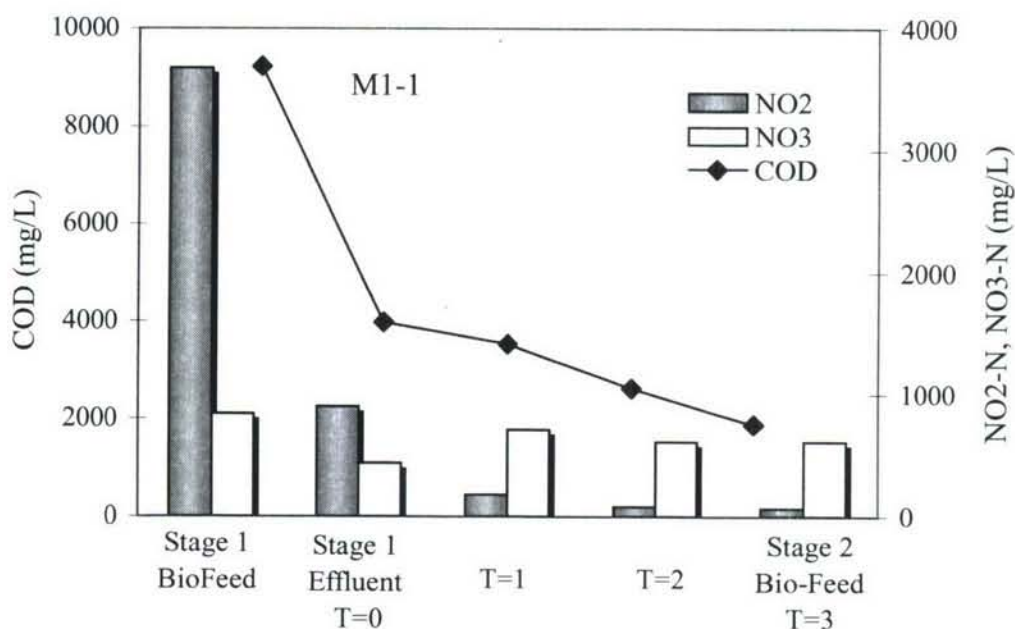


Figure 6. Strategy 1 M1 Peroxone Biofeed Treatment Results for COD, NO₂, and NO₃ Assays

The stage-1 biotreatment greatly decreased media COD and nitrite concentrations. In an anoxic culture nitrite and nitrate oxygen is used as an electron donor during metabolism of available carbon in place of dissolved oxygen. Therefore, metabolism of the available COD results in removal of nitrite and nitrate (denitrification) and liberation of nitrogen gas.

The peroxone treatment removes additional COD and more recalcitrant carbon remaining after stage 1 biotreatment. Available nitrite is oxidized to nitrate. The nitrate must be removed during the stage-2 biotreatment process to yield nitrogen levels below regulatory discharge requirements. Discharge requirements differ by state consult specific state permitting regulations.

Figure 7 displays results of COD and nitrogen assays for M8 propellants during Strategy 1. COD removal during initial biotreatment seems greater in the M8 biotreatment than in M1. From Figure 14, the greater detoxification of M8 media is also apparent following biotreatment 1.

Despite the initial higher toxicity of M8 biofeed, the M8 appears to be more treatable with stage 1 biotreatment than the M1. Treatment of stage 1 biotreatment effluents with peroxone further detoxifies and removes more recalcitrant COD from the media but has little effect on total nitrogen. Further treatment is required to remove excess nitrogen, a closely regulated nutrient in surface and wastewaters.

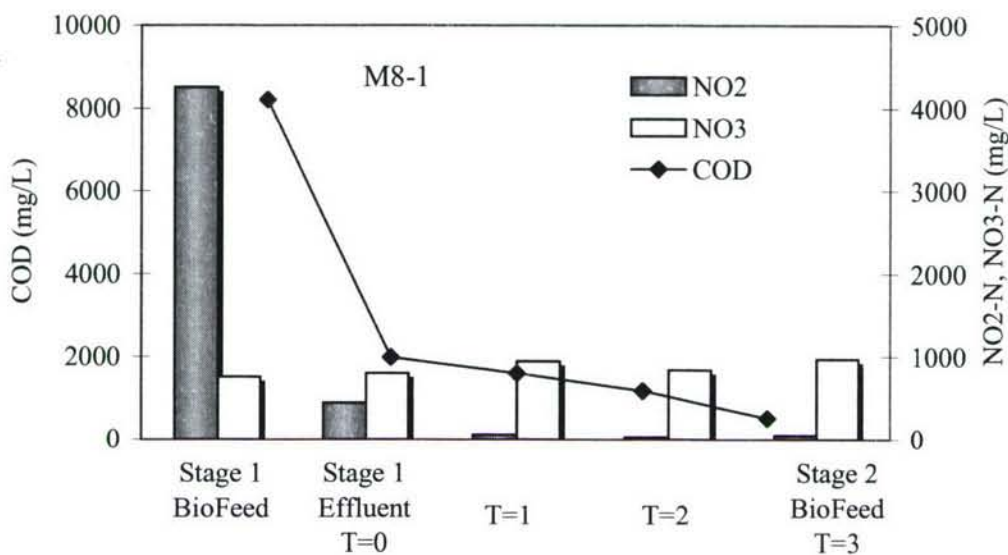


Figure 7. Strategy 1 M8 Peroxone Biofeed Treatment Results for COD, NO₂, and NO₃ Assays

The M28-1 showed a similar treatment profile. COD and total nitrogen were significantly reduced during the first stage biotreatment, even more so in the case of total nitrogen than with M1 and M8. Peroxone treatment further reduced COD and converted nitrite to nitrate.

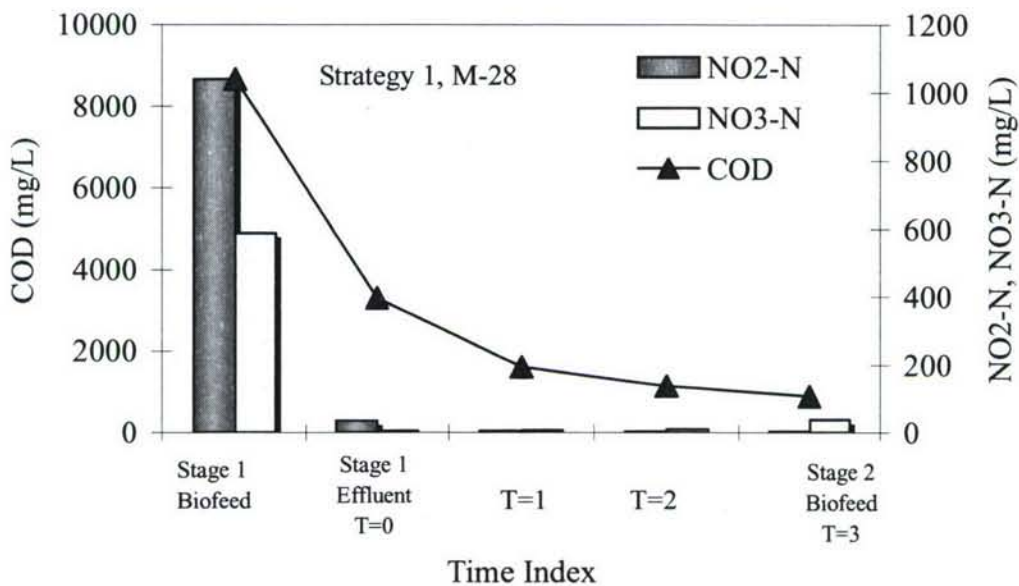


Figure 8. Strategy 1 M-28 Peroxone Biofeed Treatment Results for COD, NO₂, and NO₃ Assays

4.1.2 Process Monitoring.

In Figure 9 below are represented the process monitoring results for all reactors during using the Strategy 1 approach. The results shown are an average for the steady state period of 30-45 days of analysis performed three times per week. At each stage of the process the COD is consumed first using biotreatment, then by peroxone treatment from around 8000 mg/L in the original biofeed to approximately 1000 mg/L as stage 2 biofeed. It is advantageous that during Strategy 1 treatment the more easily degraded compounds are removed by biomass that in the end saves on operational costs during the peroxone treatment process. During the final biotreatment carbon was added exogenously to increase denitrification. The precise balance of carbon was difficult to maintain resulting in a slight COD increase in M1 and M-28 between stage 2 biofeed and effluent.

4.2 Strategy 2.

4.2.1 6-Hr Peroxone Treatment of Biofeed.

Biofeed in Strategy 2 was prepared the same as in Strategy 1, however, Strategy 2 biofeed was treated for 6-hr with peroxone prior to any biotreatment. Figures 10 through 12 represent the effect of the 6-hr peroxone treatment on biofeed COD, nitrite and nitrate levels. Data represented is averaged results over four-peroxone biofeed treatments.

During the oxidation process easily oxidizable COD is removed and media nitrite is converted to nitrate. This is similar to conversions in the Strategy 1 peroxone treatment except that in Strategy 2, the COD that is easily broken down during biotreatment is probably removed before more recalcitrant compounds. For the samples analyzed, starting nitrogen levels are also higher than with Strategy 1.

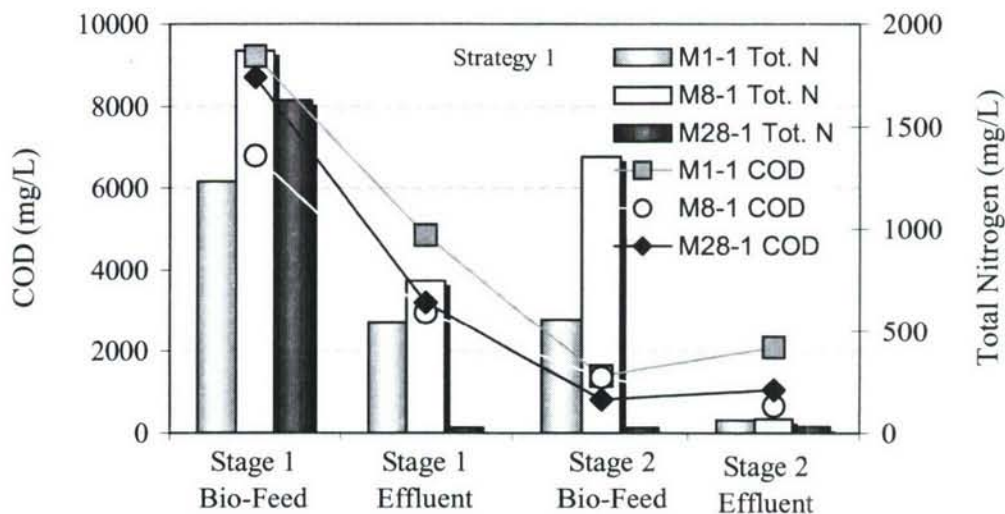


Figure 9. Strategy 1 Overall Biotreatment Results of all Propellants for COD and Total Nitrogen Assays

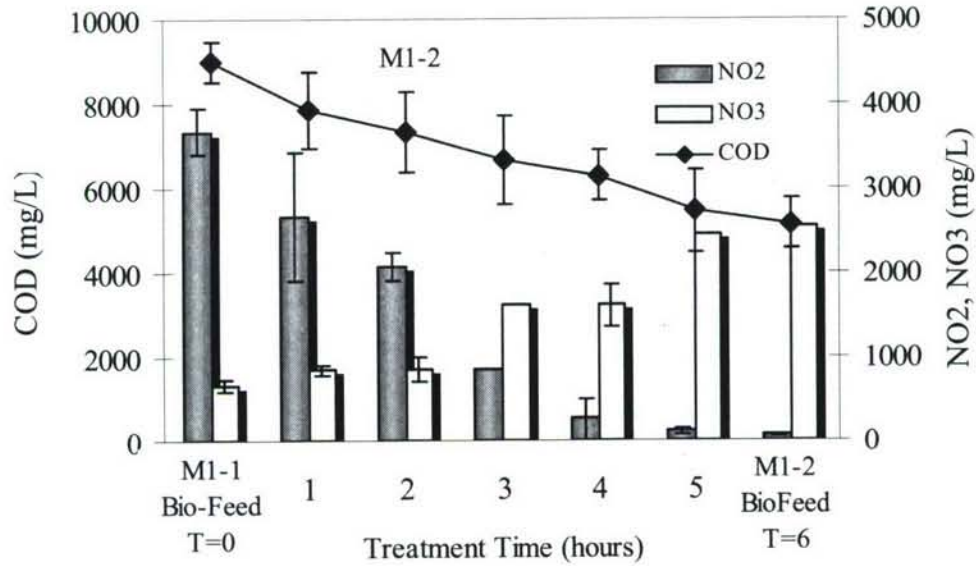


Figure 10. Strategy 2 M1-2 Peroxone Biofeed Treatment Results for COD, NO₂, and NO₃ Assays

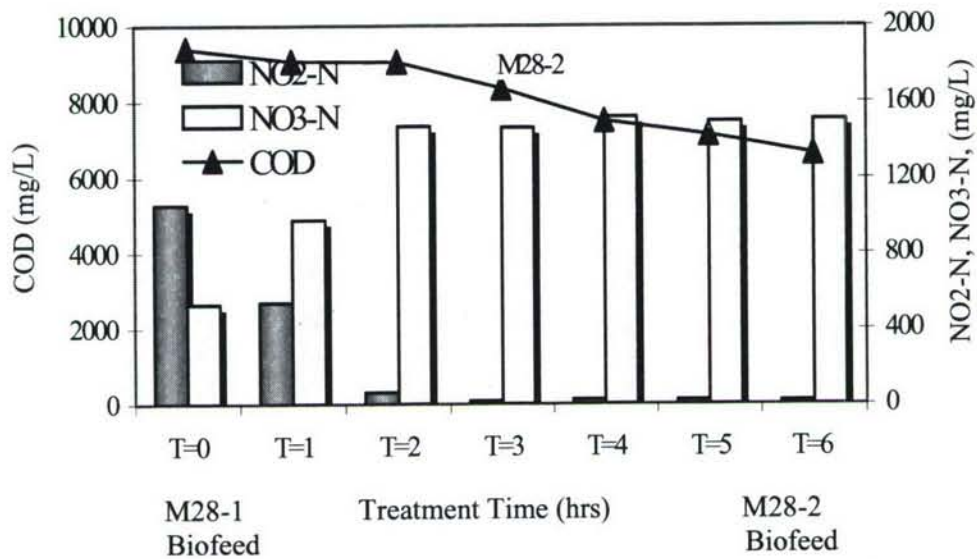


Figure 11. Strategy 2 M8-2 Peroxone Biofeed Treatment Results for COD, NO₂ and NO₃ Assays.

During the 6-hr peroxone treatment, COD is lowered, and the initially higher levels of nitrite are converted to nitrate. The increased nitrate levels required additional denitrification to get below allowable discharge limits. The addition of an exogenous carbon source in the secondary biotreatment stage boosts metabolism and oxygen requirements, thus increasing the rate of denitrification. Additionally, the nitrate-nitrogen represents a greater denitrification challenge than nitrite ion. In Figures 10-12 the removal of COD and conversion of nitrite to nitrate during the 6-hr peroxone treatment is easily recognizable.

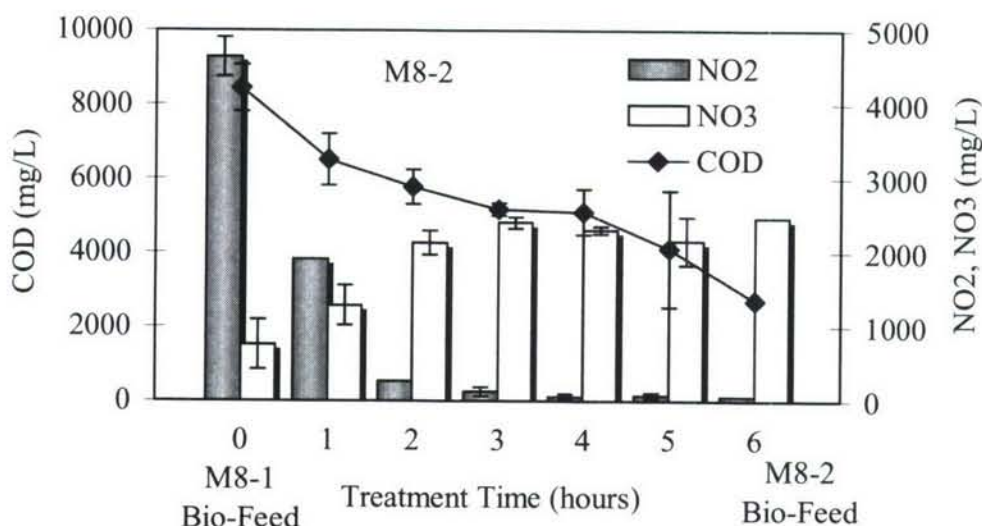


Figure 12. Strategy 2 M28-2 Peroxone Biofeed Treatment Results for COD, NO₂, and NO₃ Assays

The COD removal and oxidation of nitrite to nitrate occurs more quickly during M8 and M28 peroxone treatment than it does in M1 media. Even though the original biofeed recipe contains 200 mL/L hydrolysate, the original COD and nitrogen concentrations are higher because no biological pretreatment was done. Because media carbon is lost as CO₂ during peroxone treatment, the Strategy-2 approach may require more exogenously added carbon than Strategy 1 to remove higher nitrogen levels. Added peroxone treatment is also required to oxidize more recalcitrant compounds into easily biodegradable media. The advantage to Strategy 2 may be a decrease in media handling once biotreatment is started.

4.2.2 Process Monitoring.

The process monitoring results for Strategy 2 are represented below. Data represented are averages from samples taken over a 30-45 day steady state period from analysis performed three times per week. Stage 1 bio-feed concentrations are consistently lower than in Strategy 1 due to the loss of carbon as CO₂ during peroxone treatment. COD levels generally decline across each stage of the treatment process. Carbon levels are elevated in the M1 and M8 final effluents from difficulty controlling exogenously added carbon. Total nitrogen including NO₂, NO₃, and NH₃ decrease steadily and are near discharge limits.

Biofeed was prepared as described in Section 2. Strategy 1 biofeed was first processed through the ICB and residual biomass removed by centrifugation prior to 3-hr peroxone treatment. Strategy 2 biofeed was peroxone treated for 6-hr prior to feeding to the ICB. Any residual peroxide was removed prior to sending for analysis or administering to the ICB. Zande Environmental Services for VOC and SVOC using EPA methods SW8270C and SW8260B analyzed the treated feeds and collected bioeffluents. Each sample was analyzed for over 140 compounds. The positive results of these analyses are listed in Table 3 and 4 below. Due to sample size requirements for the EPA methods M8, VOC/SVOC analysis was not performed. Results are grouped by material type for comparison.

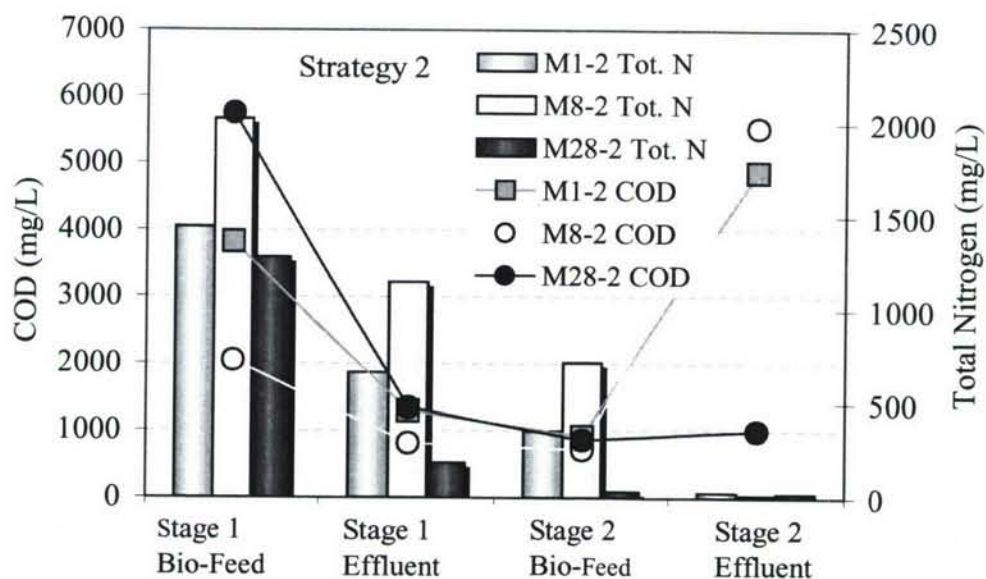


Figure 13. Strategy 2 Overall Biotreatment of all Propellants for COD and Nitrogen Assays

Table 3. Summaries of Combined Positive Results for VOC and SVOC Analysis of M1 Biofeed and Effluents from Both Treatment Strategies

Compound ($\mu\text{g/L}$)	M1 Untreated Biofeed	M1-1 Stage 1 Effluent	M1-1 Final Effluent	M1-2 6-hr treated Biofeed	M1-2 Final Effluent
Di-n-butyl phthalate	47.1	24.6	11.3	13.3	-
Nitrobenzene	1200	-	-	59.1	-
2,4-dinitrophenol	990	-	-	-	-
2,4 Dinitrotoluene	430	-	-	4990	-
2-Nitrophenol	151	-	-	-	-
4,6-Dinitro-2-methylphenol	1120	-	-	-	-
4-Nitrophenol	417	-	-	-	-
Benzoic Acid	997	-	-	-	150
Chloroform	35	-	-	-	-
Diethyl Ether	47	-	-	-	-
Benzene	160	-	-	-	-

Table 4. Summaries of Combined VOC and SVOC Analysis of M1 Biofeed and Effluents from Both Treatment Strategies

Compound (μL)	M28 Untreated Biofeed	M28-1 3-hr Treated Biofeed	M28-1 Final Effluent	M28-2 6-hr Treated Biofeed	M28-2 Final Effluent
Nitrobenzene	158.0	10.4	-	121	-
2,4-dinitrophenol	735.0	-	-	-	-
2-Nitrophenol	407.0	-	-	-	-
4-Nitrophenol	67.3	-	-	-	-
Azobenzene	30.9	-	-	-	-
Benzoic Acid	315.0	-	-	-	-
Chloroform	27.0	-	-	-	-

Effluent from each of the reactors was collected over a 30-45 day steady state period and after biomass removal a composite sample was sent for analysis.

Analytical reporting also included tentatively identified compounds with probability of correct identification and quantization >70%. In some cases, the compounds identified were not expected. It is fairly routine for separation and identification of compounds to be difficult when analyzing a complex mixture. Many of the peaks detected are not identified but from previous readings are breakdown products of the nitrocellulose to include various carboxylic acids, alcohols and alkanes most of which are easily biodegraded. While not weighted heavily for discussion in this study, these tentative findings are for the M1 and M28 propellant ICB's are presented in Table 5 and 6 below.

Table 5. Summaries of the Tentatively Identified Compounds for the M1 ICBs

Sample ID VOCs	Most Abundant VOCs (μ L Estimated)	Total Estimated Conc. (μ L)	Most Abundant SVOCs (μ L Estimated)	Total Estimated Conc. (μ L)
M1 Untreated Feed	Unknown 653 1-Butanol 211 1,4 Dioxane 23	888	Unknown 8050 1-methyl-2-nitro-Benzene 1871 dinitro-N-phenyl-Benzamine 406 Formic Acid 348	22,690
M1-1 Stage 1 Effluent	1,4-Dioxane 5 Decanal 2	12	Methyl-nitro-benzenamine 980 Methyl-nitro-benzene 74	2,500
M1-1 Effluent	Unknown 72 1,4-Dioxane 2	75	Methyl-nitro-benzenamine 287	553
M1-2 6-Hr Oxidized Feed	Unknown 196 Butanal 66 1,4 Dioxane 23	305	(11) Unknowns 7674 Methyl nitro-benzene 173	8,304
M1-2 Effluent	1,4 Dioxane 23	23	Diisooctyl adipate 736 Unknown 49	1,200

Table 6. Summaries of the the Tentatively Identified Compounds from the M28 ICBs

Sample ID VOCs	Most Abundant VOCs ($\mu\text{g/L}$ Estimated)	Total Estimated Conc. ($\mu\text{g/L}$)	Most Abundant SVOCs ($\mu\text{g/L}$ Estimated)	Total Estimated Conc. ($\mu\text{g/L}$)
M28 Untreated Feed	1,4 Dioxane 7293 (2)Unknowns 4221 Tetradecadien 1834 Decanal 1075	20,616	Phenyl-Cyclopenta pyridazine 2394 Dimethyl Phenanthroline 1000 Phenyl-Benzotriazole 618	7,657
M28-1 3-hr treated	1,4-Dioxane 25 Heptanal 18	43	Furfural 26 Heptanol 17 Unknown 14	221
M28-1 Effluent	1,4-Dioxane 2	2	Acridinamine 89 Benzimidazol 55 Unknown 39	288
M28-2 6-hr feed	Unknown 15 1,4 Dioxane 2	19	(8) Unknowns 55,978 bis-2-propanol 566 Unknown 480 Acridinamine 455	61,866
M28-2 Effluent	1,4-Dioxane 2	2	N, N-diethylcarbanilide 143 N-N-nitroso-2-Propanamine 15 (6) Unknowns 178	756

4.4 Toxicity Monitoring.

The toxicity of the initial propellant biofeed, effluent, and treatment intermediaries were monitored using the MICROTOXTM Assay. The results of the assay are significant in that they predict the relative toxicity of the compound under study to microorganisms. That information can gauge the progress of the oxidation process and predict, with limitations, the success of the ICB cultures ability to survive exposure to the compound and potentially degrade it.

In this specific assay, the luminescent bacteria are suspended in treatments of the biofeed or effluent and a light output reading is measured after 5 min. Measurements can also be taken at different times. This relative toxicity value is represented at the concentration of the compound or compounds in the media that causes a 50 % decrease in light output by the luminescent bacteria *Vibrio fischer*. The 50 % decrease in activity is a standard measure used in accessing environmental toxicity known as an Environmental Concentration 50 (EC₅₀). It is a calculation based on the amount of material in the organism's environment, but not necessarily in the organism. It is related to other common toxicity measures like a Lethal Dose 50 (LD₅₀) often used to represent a dose or concentration that causes 50 % death in laboratory animals that inhale or inject an amount of contaminant that is related to its body weight.

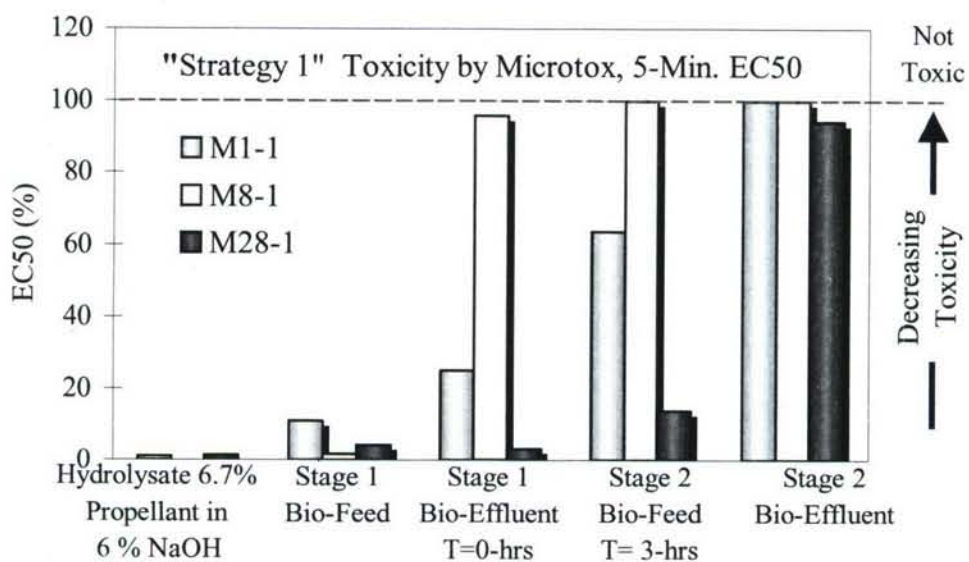


Figure 14. Strategy 1 Microtox EC50 Results for Major Treatment Steps of all Three Study Propellants

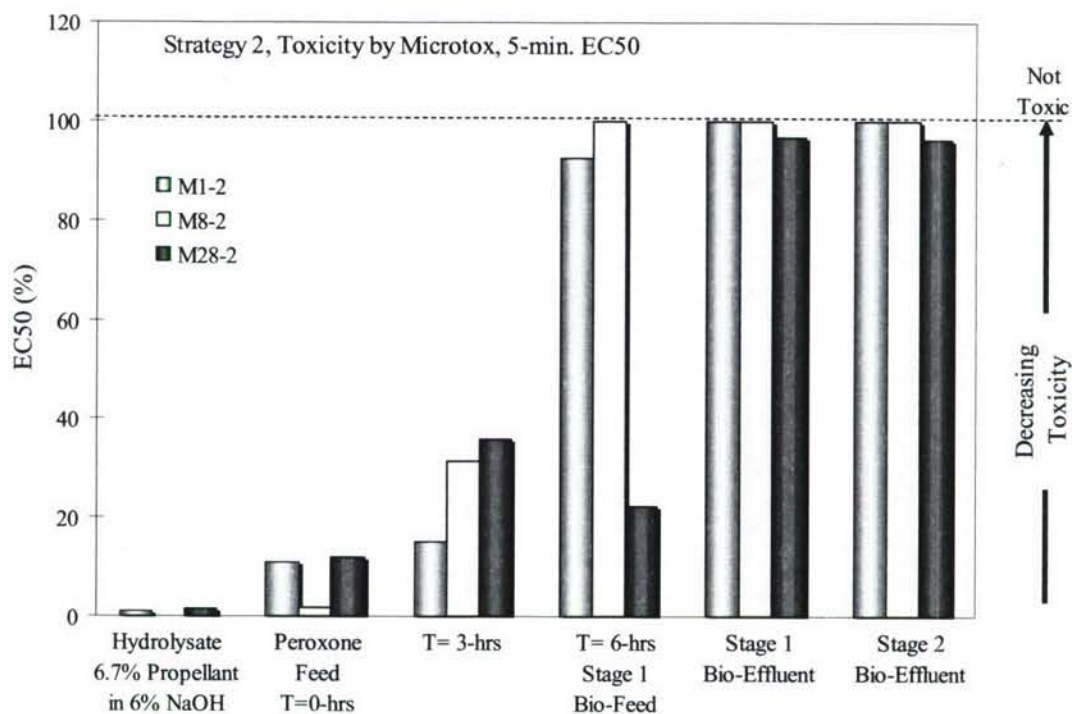


Figure 15. Strategy 2 Microtox EC50 Results for Major Treatment Steps of all Three Study Propellants.

In each strategy the toxicity of the media based on the Microtox 5-min EC₅₀ decreased across the treatments until the final effluent is considered to be non-toxic to the point of being considered more of a nutrient than a toxic compound if released into the environment.

4.5 Bacterial Species Identification.

Biomass was removed from the two ICBs used in the two treatment strategies during the M-28 treatment study. Strains deemed visually different were repeatedly isolated on TSA to obtain pure cultures. Microbial ID, Inc. (Newark, DE) performed identification using GC fatty acid analysis. Identification is based on a similarity analysis of the fatty acid composition based on known samples from the Microbial ID library.

General Guidelines for Similarity Index.

- Strains with a single match of at least 0.6 SI or 6.00 with more than a 0.100 distance from the second choice are good species matches.
- A SI between 0.400 and 0.600, with good separation from others listed may be a species match, indicating an atypical strain. In some cases, a gram stain or biochemical test will confirm or eliminate possible species.
- Values lower than 0.400 or several choices with similar values suggest that the sample species is not in the database. Those listed provide the most closely related species entries found in the current database.

Several species were removed and identified from ICB M28-1. Several of these species were only tentatively identified as probable matches, the most likely species is listed first. Only one species was isolated and identified from ICB M28-2. The species identified are listed in Table 7 below.

All of the species identified are described in the Bergy's¹¹ manual of systematic Bacteriology. Most are aerobic, gram-negative rods or cocci that can be commonly found in soil, surface waters and domestic wastewater and have the ability to reduce nitrite, nitrate or both under anoxic conditions. The identification of these bacteria does not indicate their relative abundance in the culture or that they played a major role in the overall denitrification process. In fact *Photobacterium luminescens* does not appear to reduce either nitrite or nitrate but was able to survive in the more toxic Strategy 1 ICB, thrive on the carbon sources presented, and successfully compete with the other culture bacteria.

The presence of the staphylococcus species is somewhat suspicious. These gram-positive bacteria are commonly found on human skin. While it is possible they may find their way into a municipal wastewater treatment system and survive the fairly toxic Strategy 1 biofeed, it is unlikely they had a major contribution to the degradative process and are more likely a contaminant introduced during the isolation or identification process.

Table 7. Listing of the Bacteria Cultured from the M28 Immobilized Cell Bioreactor

Sample Source	Sample Number	Similarity Index	Genus Species
ICB M28-1	Sample 1A	0.771	<i>Kluyvera cryocrescens</i>
		0.704	<i>Enterobacter cloacae</i>
		0.678	<i>Photobacterium luminescens</i>
		0.676	<i>Enterobacter aerogenes</i>
	Sample 1B	0.901	<i>Alcaligenes faecalis</i>
	Sample 2	0.894	<i>Pseudomonas stutzeri</i>
		0.807	<i>Pseudomonas mendocina</i>
	Sample 3	0.895	<i>Staphylococcus epidermidis</i>
		0.896	<i>Staphylococcus capitis</i>
ICB M28-2	Sample 1	0.925	<i>Paracoccus denitrificans</i>

Several of the species have been used previously in degradative studies. *Pseudomonas stutzeri* strain KC was identified as a microbe that can transform carbon tetrachloride to carbon dioxide. The species was enriched from aquifer material from beneath Seal Beach Naval Air Station, California by Craig Criddle.¹² Nivinskas et. al.¹³ has also described the role of *Enterobacter cloacae* NADH in the degradation of nitro aromatic compounds. *Paracoccus denitrificans*, commonly found in sediment and waste water, has been used in more generic denitrification processes involving fixed-film biotreatment of municipal wastes. The point being that bacteria commonly found in wastewater systems and soils can be readily adapted to perform the degradative process and denitrification required to dispose of these hydrolyzed propellants as outlined in our treatment strategies. The identification of these bacteria in our ICB cultures should not be surprising, and the success of this type process is probably not limited to using the cultures identified here.

5. CONCLUSIONS

This objective of this study was to demonstrate the combined degradative potential of oxidation and biodegradation of hydrolyzed propellants. This study was conceived partially because of a previous failure to demonstrate this capability, partially because suggested process modifications to the previous study were not pursued, and finally because oxidative work conducted in similar studies showed the potential of the combined oxidation/biodegradation approach. Also, at the initiation of this study there was still a need to destroy propellants that are potentially contaminated with chemical agents. Finally an environmentally friendly cost effective alternative to open burn/open detonation is still required for many antiquated propellant materials sitting in storage.

From Section 2, the study objectives are repeated below:

- a. Demonstrate the ability of a mixed microbial consortium cultured from activated sludge to degrade propellant hydrolysates.
- b. Demonstrate the ability of combined ozone and peroxide treatment to oxidize compounds resistant to biotreatment and render them more easily biodegradable.
- c. Measure the ability of the combined oxidative and biological treatment to detoxify the propellant materials based on the Microtox Assay.
- d. Measure the removal of excessive nitrogen compounds inherent from the breakdown of nitrocellulose based propellants and screen the final treated effluents for potential release to surface waters or to a municipal wastewater treatment system.

5.1 Ability to Degrade Propellant Hydrolysates.

The ability of the suggested strategies to degrade propellant hydrolysates was measured several ways:

- a. The ability to remove propellant breakdown products based on COD measurement, which measures the combined chemically oxidative load of the mixture of compounds.
- b. The ability to remove nitrogen compounds that are typical of hydrolysates of nitrocellulose based explosives.
- c. The ability to remove specific chemicals typical of the propellant hydrolysate process identified here as VOC, SVOC compounds.

A summary of COD and total nitrogen removal across both stages of the dual strategy comparison is presented in Table 8.

Table 8. Summary Results for COD and Total Nitrogen across both Stages of Bioreactor Treatment

Propellant Material	COD Biofeed (mg/L)	COD Stage 1 (% removal)	COD Consumption (mg/Day/L)	Total N Feed (mg/L)	Total N Stage 1 Consumption (mg/Day/L)	Total N Consumption (mg/Day/L)
M1-1	9226	47.4	341.9	1232.2	58.3	45.7
M8-1	6799	56.5	300.2	1869.4	87.8	55.3
M28-1	8713	63.2	430.4	1627.6	125.1	70.3
M1-2	3824	66.0	197.3	1446.5	61.0	78.4
M8-2	2045	60.0	95.9	2022.4	68.2	62.4
M28-2	5757	76.6	344.6	1284.7	85.6	49.25

Averaged COD and total nitrogen summaries are an average based on a 30 to 50-day, steady state period. Biofeed COD levels were consistently higher for Strategy 1 approach, which did not include peroxone pre-treatment. First stage COD removal efficiency was slightly better in the Strategy 2 approaches, perhaps due to breakdown of more recalcitrant compounds. COD removal is only calculated across the stage 1 treatment because COD in the form of glucose was eventually added in stage 2. The maximum COD removal efficiency, 90 %, was measured across reactor M8-1, where very little glucose was added. Ninety percent or greater COD removal can be expected with proper carbon control, a factor that was complicated by mechanical control failures throughout the study. The rate of COD removal is considered good, as compared to the previous study using aerobic cultures and no peroxone treatment where COD removal was 30% and 64% for M1 and M8 biofeeds, respectively.

Total nitrogen removal was also good across each reactor set in the study. In an anoxic culture, denitrification turns the oxidized nitrogen states into nitrogen gas as nitrate and nitrite act as electron donors. The rate of denitrification measured in milligram per day per liter of reactor volume ranged from 91.4 to 156.8 mg/day/L. The majority of denitrification occurs in the first stage of biotreatment. Nitrogen removal efficiency was good across all reactors in the study. Nitrogen removal is discussed further below. COD and total nitrogen profiles are also represented in Figures 9 and 13.

VOC and SVOC analysis results were previously presented in Section 3, Table 3 and 4. Findings from this analysis are separated into two groups, those with positive identification and those with tentative identification and estimated concentration. Due to the high number of low level compounds in the hydrolysates, separation and positive identification can be a challenge. From Table 3 it is clear that the biotreatment is able to remove most of the identified compounds except for Di-n-butyl phthalate. The Strategy 1 M1 analysis, 3-hr treatment and subsequent stage 2 biotreatment, was able reduce it by over 50% to 11.3 μL . The 6-hr peroxone pretreatment eliminated the number of confirmed contaminants to three, 2,4 Dinitrotoluene (4990 μL), Nitrobenzene(59.1 μL), and Di-n-butyl phthalate (11.3 μL). While the later two compounds were reduced from their untreated levels, the dinitrotoluene was dramatically increased. However, all three were completely consumed in subsequent biotreatment but allowed breakthrough of benzoic acid. Benzoic acid was in the initial biofeed and eliminated during peroxone treatment. Benzoic acid can be naturally occurring in the environment and is a byproduct in the bacterial breakdown of toluene.

In Table 7, analysis of M28 compounds nitrobenzene is the only compound confirmed after the 3-hr and 6-hr peroxone treatments. In each case it was eliminated from Strategy 1 and 2 final effluents.

Tables 4 and 8 summarize the tentatively identified compounds. It is difficult to closely characterize tentatively identified compounds, and therefore, estimated concentrations. In each strategy, there is a drastic reduction in the total estimated concentration of each class of compounds from untreated biofeed to final effluent. In each case there are fairly low (1 mg/L) total estimated concentrations of compounds.

5.2

Ability to Oxidize Compounds Resistant to Biodegradation.

The ability of peroxone to oxidize recalcitrant compounds is apparent in the Strategy 1 approach. Bioeffluent from the stage 1 biotreatment is further treated with peroxone for 3-hr prior to final biotreatment. The compounds remaining in the bioeffluent after stage 1 would be those more difficult to degrade. In this study, those compounds and the ability to further oxidize was measured generically using the change in COD across the peroxone treatment. The decreases in COD across the 3-hr peroxone treatment after the initial biotreatment were 71, 54 and 74% for M1, M8 and M28, respectively. COD decreases are represented in Figures 9 and 13.

Based on the analytical data from this study it is difficult to determine if the compounds eliminated by peroxone would have also eventually, given sufficient time, been eliminated by the bioculture. From Tables 3 and 4, specific compounds eliminated by peroxone were also eliminated by biotreatment, although the conversion to one of the tentatively or unidentifiable compounds makes the specific route and level of elimination difficult. Suffice it to say that the concentration of specific identified compounds and tentatively identified compounds from Tables 4 and 6 demonstrate effective breakdown and removal of VOC and SVOC compounds by either peroxone or biological treatment. The total VOC and SVOC whether specifically identified or not are near the 1 mg/L range in the final effluent.

5.3

Ability of Combined Treatment to Detoxify Effluents.

The toxicity of the compounds in the bioreactor and peroxone reactor feeds and effluents were presented in Section 4. Figures 14 and 15 clearly represent the toxicity decrease in the propellant media as it progresses through each of the two treatment strategies. For every material, the 5-min EC₅₀ increased, indicating a decrease in toxicity, across peroxone and biotreatment processes from starting lows below 10% to effluents with no measurable toxicity at 100% concentration. Using the process described in this study, the media went from being more waste characteristic to that of a nutrient solution, where nutrient levels must be monitored to permit discharge. It seems the decrease in toxicity was very easy for these compounds. The same compounds treated aerobically and without any peroxone treatment produced effluents with Microtox EC₅₀'s of approximately 2% and 35 % for M1 and M8, respectively, in the previous study by Guelta and DeFrank.⁹

5.4

Measure the Removal of Nitrogen Compounds.

The three propellant compounds degraded in this study (M1, M8, and M28) are all nitrocellulose based compounds produced from the nitration of cellulose. The hydrolysis of these compounds and added peroxone treatment produces a rich soup of nitrogen compounds. Once detoxified, they could serve as a nutrient supply for many bacterial and algae species. The releases of high nitrogen liquid effluents to surface waters are closely regulated.

Table 9 below lists feed and effluent nitrogen levels as measured using the Hach test kit methods described in Section 3. Nitrogen is listed in four major forms: ammonia (which is present in the hydrolyzed propellant but is also added as a nutrient during the process), nitrite,

nitrite, and nitrate-nitrogen (which represents the nitrogen content for total nitrogen calculations that is in the respective nitrite or nitrate form in solution). The form of the nitrogen is relevant for regulatory compliance, which may stipulate the nitrogen form and total nitrogen limitations.

Table 9. Results for Averaged Nitrogen Content in ICB Biofeed and Effluent Streams

Material/ Treatment	Feed Concentration (mg/L)			Effluent Concentration (mg/L)			
	NH3	NO2	NO3-N	NH3	NO2-N	NO3-N	Total N
M1-1	7.1	2832.5	473.6	5.4	39.2	16.0	60.6
M8-1	28.9	4275.0	558.0	5.0	17.2	46.8	69.1
M28-1	23.6	3594.4	526.7	21.7	7.5	0.9	30.1
M1-2	10.5	222.5	1293.6	4.7	14.4	11.2	30.2
M8-2	31.6	269.0	1953.6	1.7	8.1	5.2	15.0
M28-2	5.3	795.2	1092.9	16.8	4.9	1.0	22.7

From Table 9, bio-effluents are much lower than biofeed and peroxone reactor feeds. The bio-feeds for the Strategy 2 treatments are clearly higher in nitrate as available nitrite is converted to nitrate during oxidation. The numeric conversion from nitrite to nitrite-nitrogen is approximately 3:1 as expressed in the effluent nitrogen levels. Permissible regulatory limits for nitrogen release to surface waters vary from state to state and also within permitted applications. The nitrogen levels reported here are near regulatory limits for surface water release. Final effluent ammonia levels could have been lower with more strict ammonia additions as a nutrient supplement during the study. Ammonia is also produced by some bacteria during denitrification of nitrate and nitrite. Ammonia regulatory limits are normally higher than those for nitrite and nitrate. Even though nitrogen levels are near regulatory compliance, it is believed that effluents should be released to a municipal waste treatment facility rather than to surface waters. The aerobic phase of an activated sludge municipal system would further decrease ammonia levels.

Total nitrogen levels were lower in the Strategy 2 processing approach. It was believed throughout the study that these effluent nitrogen numbers could have been improved upon but are likely sufficient for release to a municipal waste stream.

6. SUMMARY

In this study, we evaluated the combined effect of neutralization, oxidation, and biotreatment to eliminate hazardous and closely regulated compounds during a proposed destruction process for three obsolete propellants. The specific application for this study was destruction of propellants with potential chemical agent contamination. The neutralization process employed was borrowed from that designed for neutralization of chemical agents

because agent destruction is a primary requirement prior to propellant destruction. Once agent destruction is confirmed, the process for propellant destruction becomes safer but not necessarily easy.

The neutralization of propellant produces a rich mixture of compounds, some of which are easily bio-degraded and others that are not. The analysis and identification of all the compounds was challenging leading to the application of more generic indicators of process monitoring that included benchtop assays for Chemical Oxygen Demand (COD), Microtox, and nitrogen. These proved reliable throughout the study and are routinely used for wastewater regulation and characterization.

The use of combined peroxone oxidation and biotreatment in this study was successful at eliminating hazardous chemicals and reducing nutrients to near regulatory levels, dependant on location and established limits. The two treatment strategies studied were successful at meeting treatment goals. Strategy 1, which employs initial biotreatment followed by peroxone oxidation, takes advantage of the low cost and low technical destruction capabilities of a microbial culture. This approach may reduce energy requirements and initial capitol equipment costs by allowing the use of a smaller oxidation system in a stand alone system design. An additional separation or filtration step to remove suspended solids may offset some of the cost savings.

Strategy 2 may be a simpler design that reduces material handling and given sufficient oxidative treatment, may lend itself to a more streamlined approach. Once a more detailed engineering study with specific propellants or other chemicals of interest are considered and site specific treatment requirements defined, Strategy 2 may allow release to an existing municipal waste water treatment system, thus saving on dedicated treatment system capitol costs. Municipal waste water treatments systems generally have an anoxic component aimed at nitrogen removal that if scaled appropriately will allow dual use for the propellant waste stream.

As of this writing, continuous flow peroxone treatment systems are being offered commercially, which may include additional oxidation schemes that may improve overall efficiency over our lab scale batch system. These systems can be engineered into dedicated biotreatment systems to allow seamless transition and operation of an integrated, site specific, treatment solution. This study targeted potentially contaminated propellant materials from demilitarization operations associated with the CWC. Similar treatment schemes should also be useful for treatment of other compounds of military interest often associated with weapons production, waste management at testing/training activities, range sustainability, pollution prevention, and waste minimization programs. This treatment process should be easily accepted by the public and special interest environmental groups, and thus be more easily sited and permitted.

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