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ECBC-TR-457

TREATMENT OF M1 AND M8 PROPELLANT HYDROLYSATES WITH IMMOBILIZED CELL BIOREACTORS

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14. ABSTRACT
Chemical agents in bulk form and chemical weapons in assembled rockets and mortars are scheduled to be destroyed in accordance with the Chemical Weapons Convention. Several technologies that include neutralization/biodegradation, supercritical water oxidation, and incineration have been selectively chosen to perform this task. Neutralization followed by biodegradation has been selected as the technology for the destruction of assembled chemical weapons by the Assembled Chemical Weapons Assessment (ACWA) program for mustard containing munitions at the Pueblo Chemical Depot (PCD), Pueblo, CO. As part of the overall destruction, the propellants and explosives from the assembled rounds must also be destroyed. While explosives integral to the assembled round will be processed simultaneously with the munition payload, final disposition of the propellant associated with the chemical round has not been determined. Parsons/Honeywell, the proposed technology provider for PCD, has proposed that the propellants be destroyed in a process similar to that used for hydrolyzed mustard agent: caustic neutralization followed by biodegradation. This laboratory study represents the initial attempt at destroying M1and M8 hydrolyzed propellants using the same immobilized cell bioreactors and culture techniques. System performance was considered inadequate at meeting destruction goals under the operating conditions employed.

15. SUBJECT TERMS

Immobilized Cell Bioreactor	MICROTOX (MTX)
Assembled Chemical Weapons Assessment (ACWA)	M1 Propellant
Propellant Hydrolysate	M8 Propellant

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PREFACE

The work described in this report was authorized under Project Nos. 778017 and 778117, Assembled Chemical Weapons Assessment (ACWA) Program. This work was started in September 2000 and completed in June 2001.

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1. INTRODUCTION

Under U.S. law and the terms of the Chemical Weapons Convention (CWC), the U.S. Army is required to destroy its stockpile of 30,000 T of chemical warfare agents by April 2007. While incineration has been the baseline method used for the demilitarization of these materials, public and political opposition have necessitated the evaluation of alternate technologies, such as biodegradation. Hot water hydrolysis, followed by biodegradation, has been shown to be an effective means of disposing of the blister agent, sulfur mustard (HD).

The ability of the immobilized cell bioreactors (ICBs) to deal with a mixture of hydrolyzed HD and tetrytol (tetrytl and TNT) was evaluated under the Assembled Chemical Weapons Assessment (ACWA) program at Aberdeen Proving Ground (APG) in Maryland. Successful laboratory testing and follow-on pilot scale testing eventually led to the selection of neutralization followed by biodegradation as the destruction method for assembled chemical projectiles stored at Pueblo Chemical Depot (PCD), Pueblo Co.[1,2] Destruction of HD containing assembled weapons has specific application to the PCD, which holds the major US stockpile of 4.2-in. Mortar rounds and 155-mm rockets.[3]

In addition to a proven ability to degrade HD and tetrytol, the stockpile destruction technology used at PCD may also need to address the destruction of the propellant in the chemical rounds, just like incineration or any other technology. Presently, there is believed to be approximately 78,000 lb. of M1 propellant and 60,000 lb. of M8 stored at PCD in assembled and unconfigured rounds.

This study illustrates a laboratory-scale examination of the ability of ICBs to degrade the hydrolysates of energetics M1 and M8 grown on HD/tetrytol. This scenario, proposed by Parsons/Honeywell (Parsons Infrastructure and Technology Group, Pasadena, CA) is for the disposal of propellants associated with the assembled chemical rounds stored at PCD. The treatment scheme planned for PCD also includes water recycling and waste minimization by recycling bioreactor effluent and the drying of biomass solids prior to land filling. Process performance and its suitability for propellant destruction will be measured by the elimination of the priority chemicals, the overall breakdown or product removal, and the characterization of waste and process streams within the total approach.

Two sets of ~ 600-mL ICBs in series were inoculated with sewage sludge and biomass from a large-scale ICB and fed a mixture of HD and tetrytol hydrolysates. After the cultures were established, the feed was switched to increasing concentrations of either M1 or M8 hydrolysates as a sole carbon source. The ICB effluents were tracked for numerous process monitoring analytes. Biofeed, effluents, and culture biomass samples were characterized for the designated chemicals. The ability of the 2 systems to make the changeover from HD/tetrytol to M1 or M8, and to detoxify and degrade the respective hydrolysates were compared and discussed.

2. METHODS

Both the M1 and M8 propellants are mixtures of compounds. The propellant materials were removed from 155-mm projectiles and shipped to the U.S. Army Edgewood Chemical Biological Center (ECBC) just prior to the hydrolysate production. The composition of each propellant prior to hydrolysis is listed in Table 1.

Table 1. Composition of M1 and M8 Propellants.

M1 Propellant Composition		**M8 Propellant Composition**	
Compound	%wt / wt	Compound	%wt / wt
Nitrocellulose	84.0	Nitrocellulose	52.15
Dinitrotoluene	9.0	Nitroglycerine	43.00
Dibutylphthalate	5.0	Diethylphthalate	3.00
Diphenylamine	1.0	Potassium Nitrate	1.25
Lead Carbonate	1.0	Ethyl Centralite	0.60

The M1 propellant, shown in Figure 1, was in the form of rod-shaped pellets about ¼ in. in length and 1/16 in. in diameter.

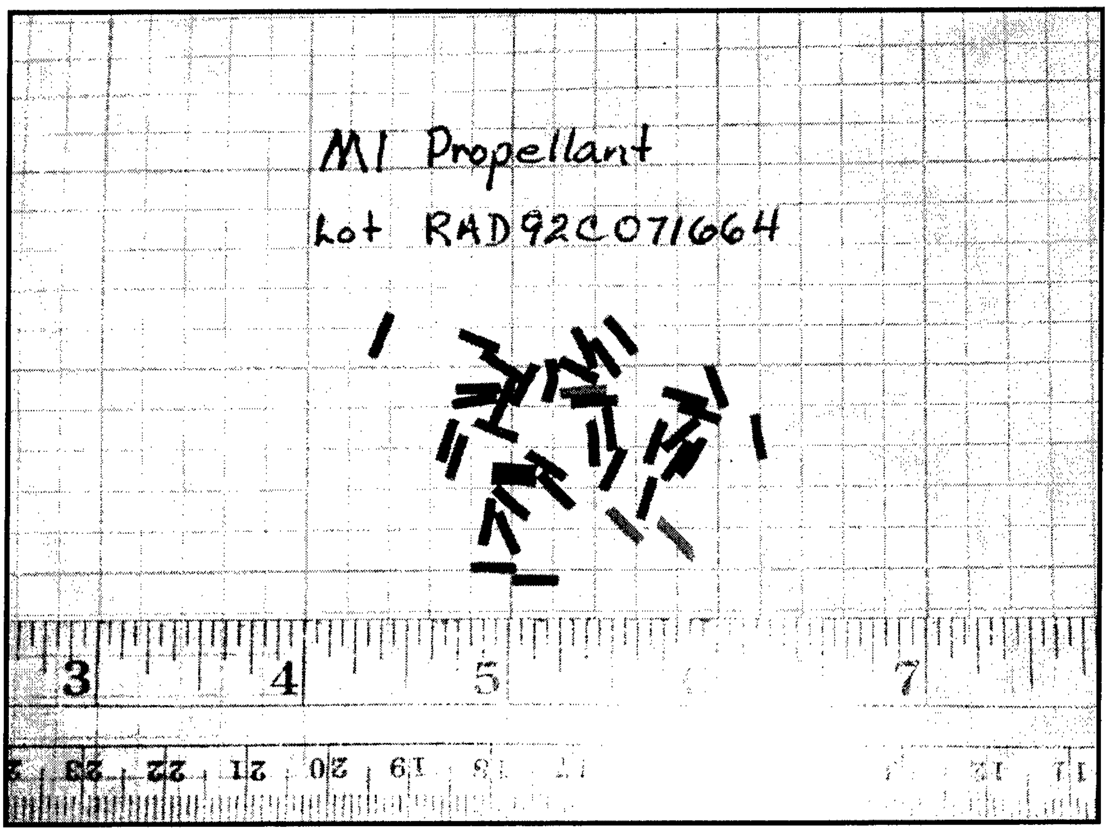

Figure 1. M1 Pellets prior to Hydrolysis.

The M8 propellant, shown in Figure 2, was produced in sheets that were cut to size and sewn together to obtain the required thickness. The solid sheets were prepared at 6.5% (wt / wt) propellant in sodium hydroxide solution. The propellant hydrolysates were formed by neutralizing in a 6% NaOH solution, heating and stirring in laboratory flasks over an 8-hr period. After cooling and coarse filtration, the hydrolysates were divided into 4-l batches and used as biofeed.

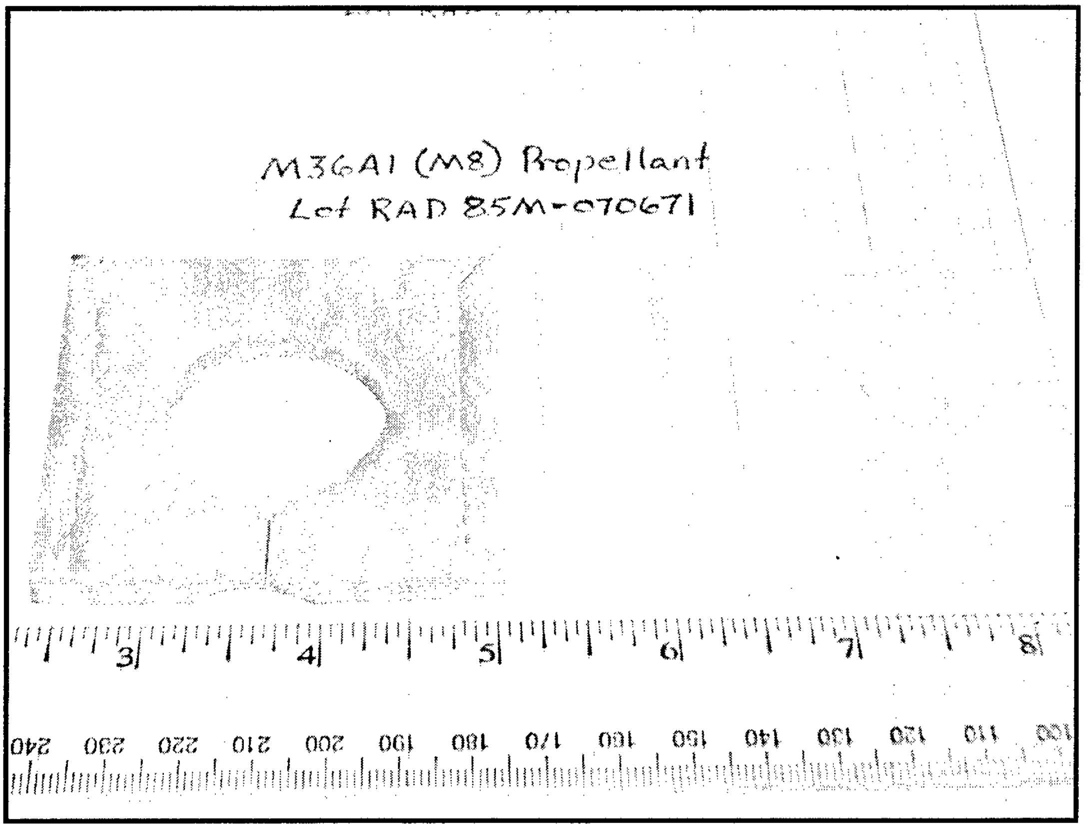

Figure 2. M8 Propellant prior to Hydrolysis.

Each hydrolysate sample was analyzed for chemicals. The results of these analyses are presented in the Appendixes.

Biofeed for the reactors was made in 4-l batches. The reactors were inoculated with a bioculture sample removed from the pilot scale reactor that was started several months prior to this study. In a full-scale process plant, the HD/tetrytol rounds would likely be processed prior to the propellant and near the completion of the HD/tetrytol campaign, the full-scale reactor would be switched over to processing the M1 and M8 hydrolysates. For the laboratory study design, the culture was grown on the HD/tetrytol feed and then switched over to M1 and M8. The concentration of hydrolyzed propellant in the feed was slowly increased while the HD/tetrytol concentration was eliminated. Fresh samples of activated sludge were also added to the culture to provide additional culture diversity during the feed change. The standard full-strength biofeed formula is listed in Table 2.

Table 2. Propellant Hydrolysate Feed Formulation.

Compound	Amount
Propellant Hydrolysate (M1 or M8)	800 mL
Potassium Phosphate Di-Basic	0.64 gm
Wolin Salts	20 mL
Distilled/Dionized Water	To Volume (4L)
Neutralize with HCl to pH 7.5	As Required for pH=7.5

The laboratory ICBs used for this study were glass cylinders of approximately 650-ml internal volume per reactor. Two glass cylinders were used to simulate a 2-celled bioreactor. The working volume of the reactor at start-up was approximately 1.2 l. In an ICB, the culture grows on an expanded foam media. Spacers mixed with the foam keep the culture from becoming plugged and allow air and the aqueous media to mix. The actual M8 ICB is shown in Figure 4. The expanded foam and spacer packing materials are shown in Figure 5. Under normal growth conditions, the working volume of the ICB decreases to approximately 600 mL for both cylinders combined. Air supply to the culture enters the ICB through a glass fret in the bottom and exits through a tube inserted into a butyl rubber stopper at the top of the ICB. Effluent leaves the ICB through an overflow.

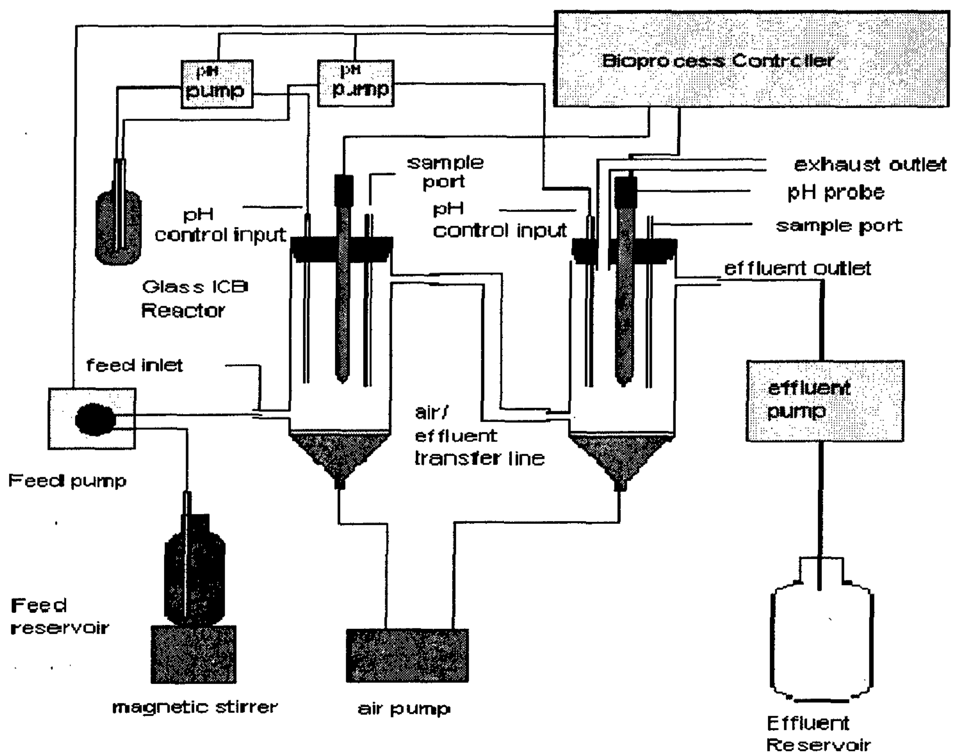

Figure 3. Sketch of Two ICBs in Series.

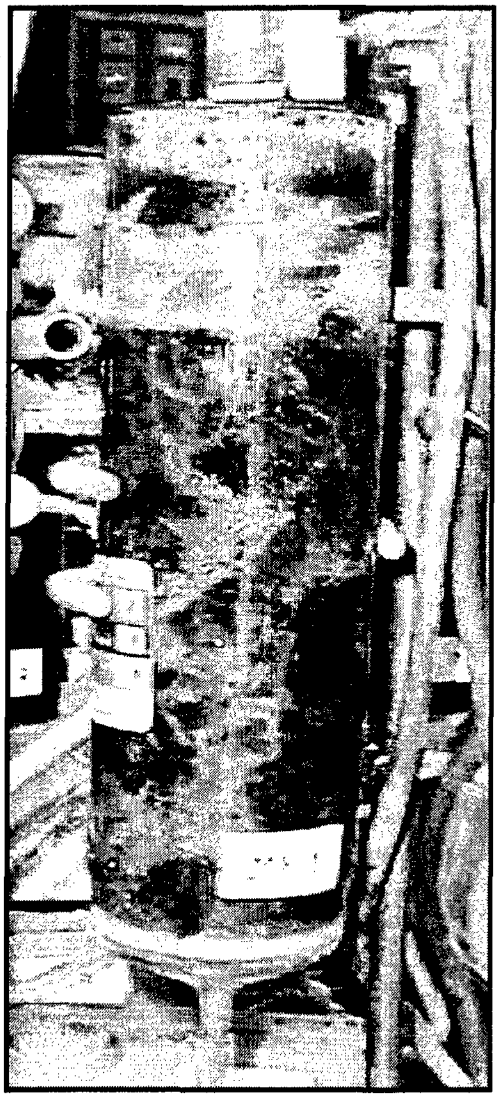

Figure 4. M8 Propellant ICB.

Figure 5. ICB Expanded Foam and Spacer Support Material.

The propellant hydrolysate bio-feed was pumped continuously into the ICB at 300 mL/day for a Hydrolyic Residence Time (HRT) of 5 days. Approximately 300 mL/min of air was supplied to each ICB by diaphragm pumps. The media pH was continuously monitored and controlled with acetic acid early in the testing to provide additional carbon and hydrochloric acid during the second half of the 80-day validation period.

Process monitoring samples were taken 3 times per week and analyzed for chemical oxygen demand (COD) nitrogen, ammonia, and phosphate. Samples for these analytes were analyzed using Hack analysis kits. Samples of the effluent were taken near the end of each feed batch and screened for aquatic toxicity using the MICROTOX (MTX) Assay.

Contract laboratories performed validation or steady state biofeed and effluent characterization analysis. Analytical samples were collected by ECBC scientists and sent to a sample coordinator for shipping and tracking. Arthur D. Little, Inc. (Cambridge, MA) compiled the analytical results in a consolidated database. Analytical results for biofeed and effluent characterization are discussed in either the results section or listed in the Appendixes. Analytical methods for test compounds are listed in Table 3.

Table 3. List of Steady-State-Methods and References for Compounds of Interest.

Compound	Method Reference	Method Source	Type
Energetics and Nitroglycerine	GC/ECD (CAD 42.1)	CHPPM[1]	GC/ECD
Mercury (M28 Mod)	7470 (ACWA-3503)	SW846[4]	CV
Metals	6010B	SW846	ICP
Nitrocellulose	SEC/FTIR	MRI[3]	GPC/FTIR
Nitrocellulose (m28)	SEC/FTIR (m28 Mod)	MRI	GCP/FTIR
Specific Gravity	Specific Gravity	US Army	Hygrometer
SVOC	8270C	SW846	GC/MS
SVOC (m28 Mod)	8270C (ACWA-3505)	SW846	GC/MS
TCLP (Metals)	1311/6010B	SW846	ICP
TCLP (SVOC)	1311/8270C	SW846	GC/MS
TCLP (VOC)	1311/8260B	SW846	GC/MS
TDS	160.1	MCAW[2]	Gravimetric
TOC	9060	SW846	Combustion
TOC (M28 Mod)	9060 (ACWA-3506)	SW846	Combustion
TOC (PIH Mod)	9060	SW846	Combustion
TSS	160.2	MCAW	Wet Chemistry
VOC	8260B	SW846	GC/MS
VOC (M28 Mod)	8260B (ACWA-3507)	US Army	GC/MS
VSS	160.4	MCAW	Gravimetric

[1]CHPPM-Center for Health Promotion and Preventive Medicine
[2]MCAW- Manual of Methods for Chemical Analysis of Water and Wastes
[3]MRI- Medical Research Institute
[4]US EPA, SW846, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods

3. RESULTS

3.1 Feed Schedule and Chemical Oxygen Demand (COD) Results.

The COD measurement is used as a near real-time measure of the utilization degree of degradable compounds by the bio-culture. With COD, analysis can be completed in just over 2 hr, which is useful in assessing the effectiveness of the cultures at degrading the propellant feed until more complete analysis is available. The COD does not indicate degradation or utilization of a single compound, although it is mostly associated with carbon compounds and, to lesser degree, nitrogen containing compounds. Generally, COD removal efficiencies of near 90% are considered nearly complete removal of biodegradable compounds. The remaining 10% may contain measurable culture waste products or process by-products that are not biodegradable. The COD of the effluents and the COD removal efficiency of each of the propellant reactors are presented in Figures 6 and 7.

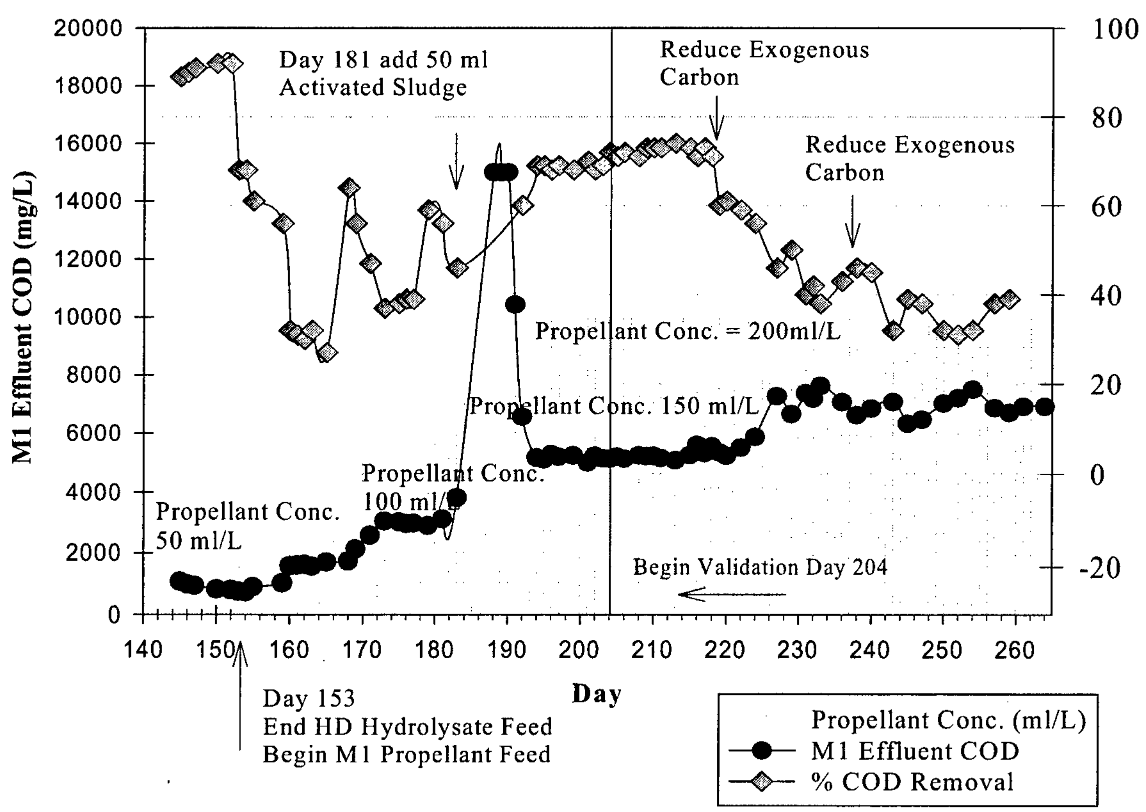

Figure 6. M1 Feed Schedule, Effluent COD, and COD Removal Efficiency.

Figure 6 represents the effluent COD results and feeding schedule for the M1 reactor. The culture was inoculated with bacteria from the HD/tetrytol pilot-scale. The reactor was seeded and the HD/tetrytol hydrolysate feed was started on Day 1. The culture was grown-upon the HD/tetrytol biofeed. Normally, acclimation and growth of a culture takes 20 to 40 days. The switch to propellant feed was targeted for around the 45th day. However, the receipt and hydrolysis of the propellants was repeatedly delayed. Figure 5 begins at day 140, just prior to the switch over and addition of the propellant feed.

On day 150, 3 days before changing to propellant feed, the COD removal efficiency of the reactors was at approximately 90%. The propellant feed was started on day 153 (Figure 6--vertical bars); COD removal efficiency decreased dramatically even though the feed load was greatly decreased. The culture COD removal efficiency improved, as the culture adapted even when feed loading was increased. On day 181, additional activated sludge from a local treatment plant was added to the culture. The spike in the effluent COD was a result of adding the carbon rich activated sludge. COD removal efficiency stabilized near day 190. Removal efficiency began dropping even though feed COD decreased as exogenous carbon, in the form of acetic acid, was gradually removed from the feed and pH control systems. Acetic acid was used to neutralize the high pH of the hydrolyzed propellant and to control pH within the reactor. Acetic acid for pH control and adjustments was replaced with hydrochloric acid (HCl) as it was the acid of choice for the full-scale operation.

The 80-day validation sampling of the effluent began on day 204. Validation sampling results contain more detailed analysis for the constituents of the ICB effluents and propellant hydrolysate including measures of volatile organic compounds (VOC), metals and mercury, Total Organic Carbon (TOC) energetics and nitroglycerine, total dissolved solids, total suspended solids and volatile suspended solids, and the Toxic Characteristic Leaching Procedure (TCLP).

Figure 7 represents the M8 effluent COD, COD removal efficiency, and feeding schedule. Like the M1 ICB in figure 3, the M8 ICB was grown on HD/tetrytol feed from an initial inoculum from the HD/tetrytol pilot-scale ICB. The change-over to the propellant feed and the incremental feeding schedule are similar to those of the M1 ICB. All changes in pH control, sludge addition, validation start date and exogenous carbon removal are the same. However, the M8 reactor received less exogenous carbon than the M1 reactor due to the lower acid requirement for neutralization to 7.5 pH of the M8 bio-feed. The M1 feed received 5.4 mL/L acetic acid while the M8 feed received only 2.5 mL/L acetic acid. After removal of exogenous carbon feed, COD removal effeciency decreased in both reactors. However, as shown in Table 4, effluent COD increased in ICB M1.

Table 4. Summary of M1 and M8 COD Results.

	M1-ICB			**M8-ICB**		
	COD	**(mg/L)**	**Removal**	**COD**	**(mg/L)**	**Removal**
	Feed	**Effluent**	**Effeciency**	**Feed**	**Effluent**	**Effeciency**
Average w/ Acetic Acid	13,650	5,614	59%	11,528	3,336	71%
Average w/o Acetic Acid	10,228	7,214	30%	8,165	2,915	64%
Average Over All	11,549	6,516	44%	9,426	3,103	67%

The effect of the change-over to propellant feed, sludge addition, and incremental increases in feed loading to each reactor had similar effects on COD removal efficiency. However, COD removal efficiency decreased more dramatically in the M1 reactor than the M8 reactor.

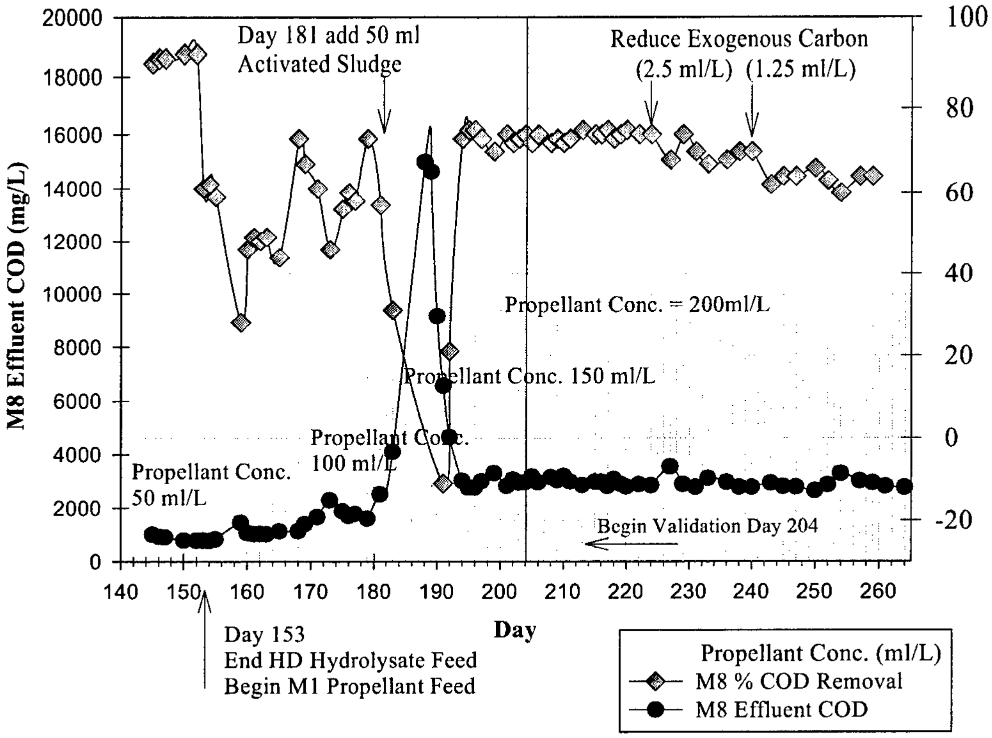

Figure 7. M8 Feed Schedule, Effluent COD, and COD Removal Efficiency.

3.2 Process Monitoring: Nitrogen Ammonia.

Nitrogen, a required nutrient in fermentor feed stocks, was not exogenously added to the biofeed. Normally, breakdown products from the hydrolysis of the propellants should supply the necessary nitrogen and the culture should breakdown these nitrogen containing compounds to extract the required nitrogen. In this study, nitrogen was measured as a laboratory process monitoring sample using the Hach[7] kit test-n-tube high range analysis for nitrogen-ammonia. The nitrogen values observed during the 80-day steady-state period were fairly stable and trendless. The summary statistics for these analyses are listed in Table 5.

Table 5. Summary Statistics for Ammonia-Nitrogen in Reactor Biofeed and Effluents.

	M1 Biofeed	M1 Effluent	M8 Biofeed	M8 Effluent
Mean	12.63	12.83	33.46	5.31
Standard Error	1.51	1.15	1.75	0.41
Median	12.55	12.10	35.20	5.60
Mode	14.00	8.00	35.20	2.10
Standard Deviation	6.05	7.19	6.98	2.50
Minimum	6.40	1.40	9.50	1.60
Maximum	32.10	30.20	39.30	12.70
Count	16.00	39.00	16.00	38.00

3.3 Process Monitoring: Phosphorus.

Phosphorus, a required nutrient for biological cultures, was not present in the hydrolized propellants in sufficient quantities to sustain the culture. Phosphorus was added to the biofeed in the form of Potassium Phosphate di-basic. Process monitoring for phosphorus was measured using the Hach Kit. The results were fairly stable and trendless. Summary statistics for the phosphorus results are listed in Table 6.

Table 6. Summary Statistics for Phosphorus Results in Reactor Biofeed and Effluents.

	M1 Biofeed	**M1 Effluent**	**M8 Biofeed**	**M8 Effluent**
Mean	152.63	171.19	130.69	143.51
Standard Error	6.59	8.18	12.38	8.60
Median	145.50	159.50	125.50	124.00
Mode	138.00	162.00	127.00	119.00
Standard Deviation	26.35	49.10	49.53	52.33
Minimum	133.00	116.00	35.10	104.00
Maximum	244.00	388.00	294.00	376.00
Count	16.00	36.00	16.00	37.00

3.4 MICROTOX (MTX) Analysis.

The MTX Bioassay exposes a bioluminescent marine bacterium (*Vibrio fischeri*) to a sample of unknown toxicity and measures the change in light output as the means of determining the effects on the organism. A reduction in light output is a direct indication of metabolic inhibition. The bacterium was cultured by Azur Environmental and shipped in lyophilized form. The bacterium (stored frozen) was re-hydrated immediately before testing. Each bioassay used less than 3 mL of sample and was performed in a temperature-controlled photometer. Due to interference caused by suspended particulate, the samples were centrifuged for 10 min at 500 Relative Centrifugal Force (RCF) and the supernatant decanted and used in testing. The samples were diluted with MTX Diluents and pH adjustments were done using 10% HCl as needed. The assays were performed in glass cuvettes in temperature-controlled wells of a photometer. The assay must have a minimum of 4 dilutions exhibiting a dose response for optimum accuracy in predicting toxicity. The addition of bacteria was referred to as time zero. Five minutes after time zero, the control cuvette was used to calibrate the photometer to 100% light output. The control and treatment cuvettes were returned to the incubator and measured again at 15 min. Data was analyzed with the MTX Test Protocol software to determine the EC_{50} (the effective concentration causing a 50% reduction in light output).

MICROTOX assays were performed on the propellant feed and ICB effluents during the study. Figure 8 represents the comparative toxicities of the propellant feed at each incremental propellant hydrolysate concentration. The MTX results for the HD feed are also included for comparison. The results indicated that the HD/tetrytol feed is less toxic at its design biofeed strength than the the M1 feed at its lowest biofeed concentration during the reactor switchover to propellant biofeed. The M1 at 50 mL/L, its lowest concentration, has nearly the same toxicicity as the the M8 feed at 200 mL/L. This may be attributed to the lead in the M1 propellants composition.

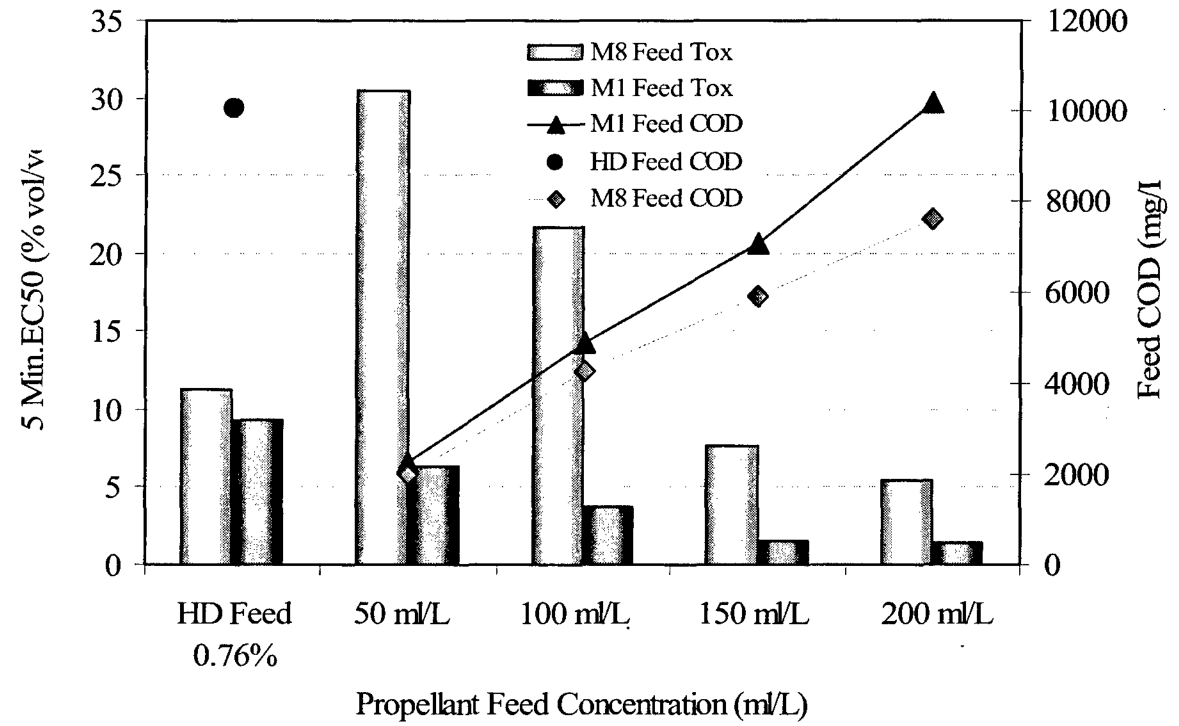

Figure 8. Chart of MTX and COD Results for ICB Propellant and HD Feeds.

Additional MTX Assay results are presented in Figure 9. A 5-min EC_{50} of >70 is considered non-hazardous. As shown, the effluent generated in each of the reactors while being fed the HD hydrolysate was quite low at MTX values greater than 80. The toxicity increased immediately after the switch to propellant hydrolysate feed. The M8 reactor recovered shortly after the addition of acetic acid to neutralize the feed. The M1 reactor did not do as well with the M1 propellant feed. At the target biofeed concentration of 200 mL/L hydrolysate, the M1 reactor effluent became quite toxic and did not recover by the end of the steady-state period. The decrease in carbon added through acetic acid seemed to have a negative effect on the M8 effluent toxicity, even though COD removal was still fair at >60% (Figure 5).

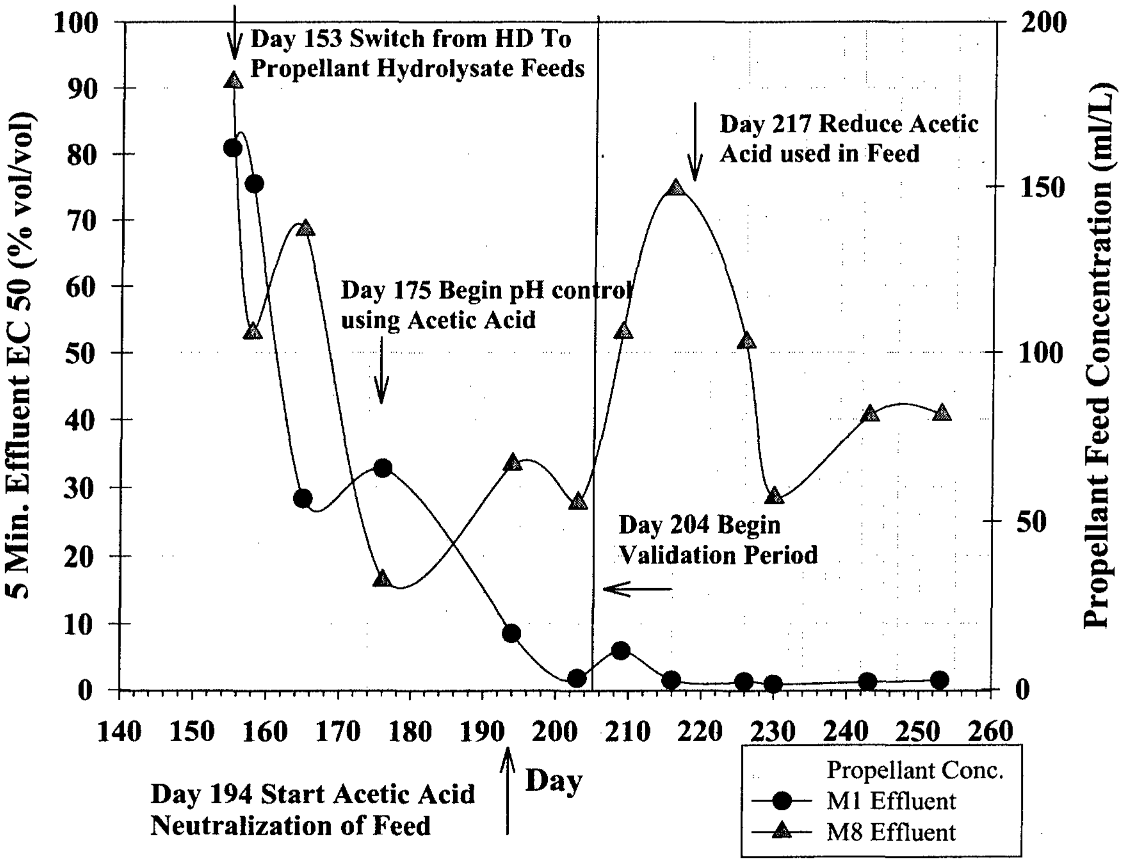

Figure 9. MTX Assay Results for M1 and M8 ICB Effluents.

3.5 Total Organic Carbon (TOC).

Analysis of the TOC, which is a good measure of the ability of the culture to metabolize carbon sources in the biofeed, was performed by off-site contracted labs. In this study, no carbon was added to the biofeed. All carbon sources for the culture were to be derived from carbon compounds in the propellant hydrolysate. The ability of the culture to degrade the various carbon containing compounds and utilize them for food is a direct measure of the success of the degradation process. Compounds that may be too recalcitrant or toxic will likely pass through the system. At times, some compounds that are degraded may release or produce by-products that are also toxins and can affect the overall health of the culture and the degradative process differently.

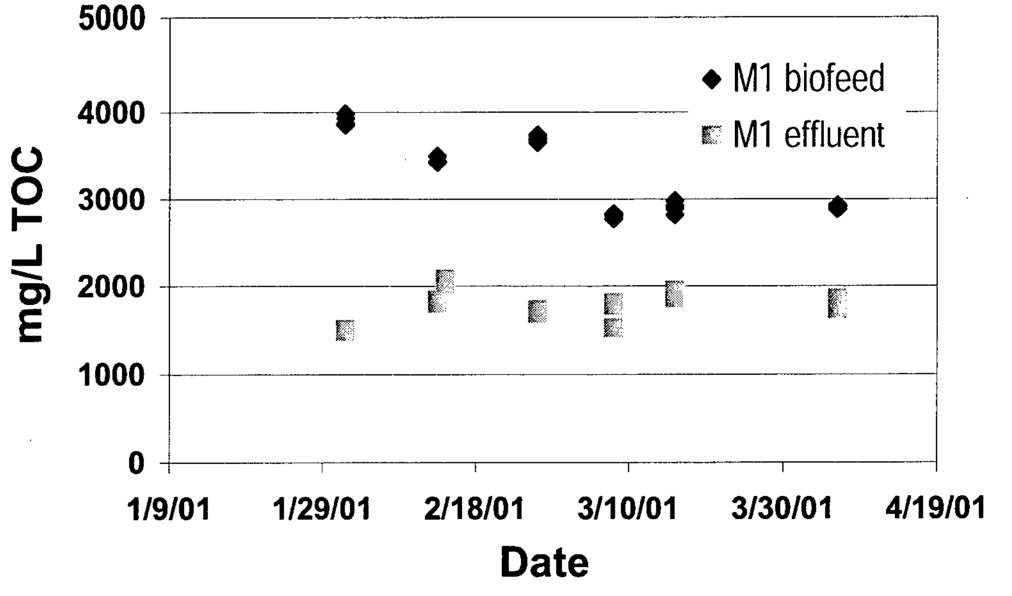

Figure 10. TOC Concentrations for the M1 Reactor during Steady-State Period.

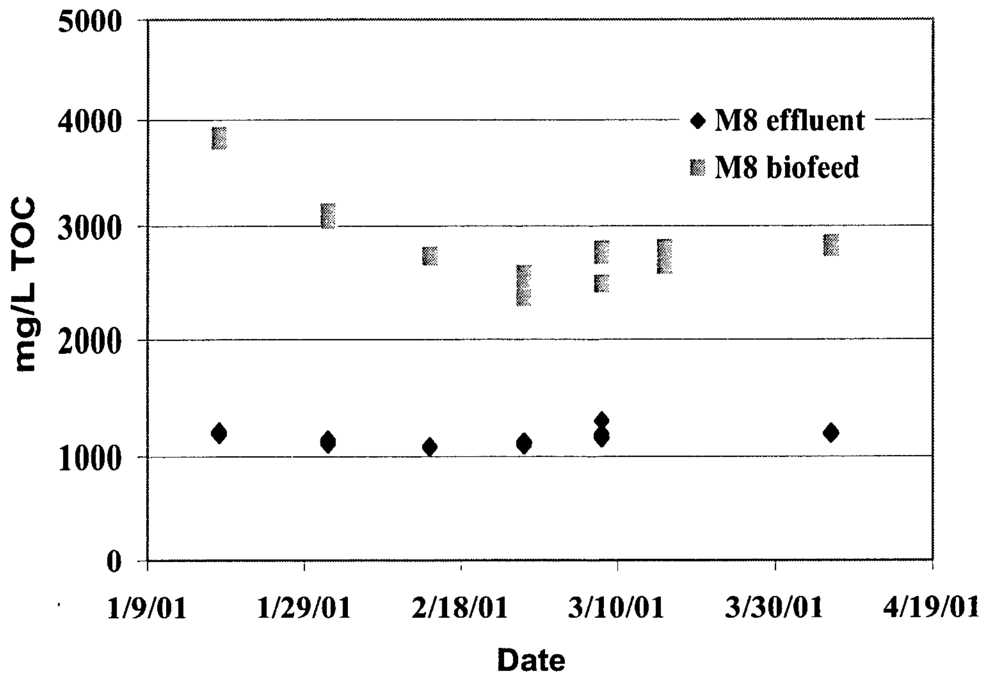

Figure 11. TOC Concentrations Chart of M8 Biofeed and Effluent.

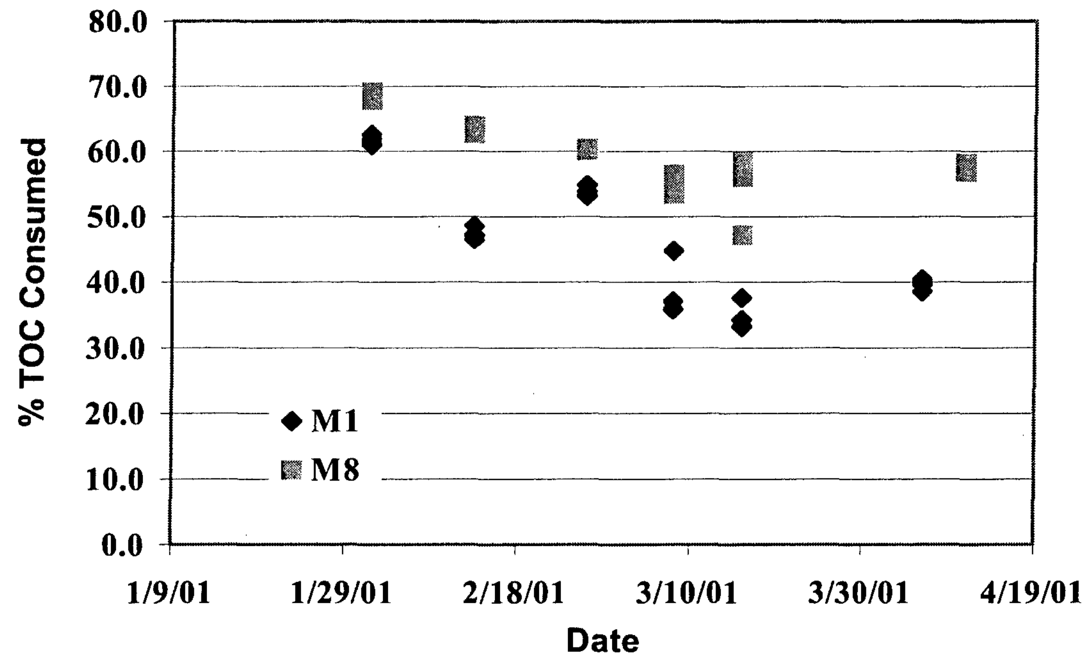

Figure 12. Chart of M1 and M8 TOC Removal Efficiencies.

3.6 Energetics.

The propellant hydrolysate, biofeed, and effluents were tested for energetic compounds and energetic breakdown products. All the compounds that were present in the hydrolysate can be catagorized as energetic breakdown products since they were originally part of an energetic mixture. The compounds produced during neutralization became progressively simpler the longer the hydrolysis procedure was continued. Most of the simpler compounds were listed as VOCs and SVOCs and many were never completely identified. Positive results for the compounds generally considered only slightly removed from the original energetic materials are listed in Table 7. Some compounds are also listed as VOCs and SVOCs.

Table 7. List of Energetics and Energetic Breakdown Products Detected.

Feed	Compound	Hydrolysate (µg/L)	Biofeed (µg/L)	Effluent (µg/L)
M1	1,2-Dichlorobenzene		12	7
M1	2,4,6-Trinitrotoluene	120		
M1	2,4-Dinitrophenol		1400	617
M1	2,4-Dinitrotoluene	8900	4900	1065
M1	2-Methylphenol		30	9
M1	2-Nitrophenol		145	54
M1	2-Nitrotoluene	26000		

Table 7. List of Energetics and Energetic Breakdown Products Detected (Continued).

Feed	Compound	Hydrolysate (µg/L)	Biofeed (µg/L)	Effluent (µg/L)
M1	3,4-Methylphenol			9
M1	3-Nitrotoluene	2700		
M1	4,6-Dinitro-2-methylphenol		2200	2550
M1	4-Chloro-3-methylphenol			8
M1	4-Methylphenol		30	10
M1	4-Nitrophenol		200	185
M1	4-Nitrotoluene	2600		
M1	Nitrobenzene	1000	407	172
M1	Nitrocellulose	5250	2210	250
M8	2,4-Dinitrophenol		270	
M8	2,4-Dinitrotoluene	200	2900	
M8	2-Methylphenol			1
M8	2-Nitrophenol	400	36	
M8	4,6-Dinitro-2-methylphenol		32	
M8	Nitrobenzene		51	
M8	Nitrocellulose	8930	1900	260

3.7 Nitrocellulose.

Nitrocellulose is one of the principle components of the M1 and M8 propellants. Nitrocellulose was degraded during the hydrolyzation process; however, low concentrations were still present in the hydrolysate. Though nitrocellulose can be biodegraded, its breakdown and utilization by the ICB culture is slow. Nitrocellulose analytical results for the hydrolysate, biofeed, and ICB effluents are listed in Table 7. Nitrocellulose content in the effluent was much lower than the feed, indicating a large portion had been utilized but not completely degraded.

3.8 Volatile Organic Chemicals (VOCs).

Propellant hydrolysate, biofeed, and effluents were analyzed for the VOCs listed in Table 8.

There were 821combined analyses for VOCs in the hydrolysate, biofeed, and effluents. The sheer number VOCs present made the analysis difficult due to the interfering peaks. Numerous compounds, not reported in the hydrolysate, were detected in the biofeed. The reported hydrolysate compounds may not have been complete due to problems in identifying or quantifying a detected compound. A complete listing of positive VOC analytical results, including qualified data, can be found in the Appendixes. A summary of the detected validated VOCs is shown in Table 9.

Table 8. Listing of VOC Compounds for ICB Propellant Hydrolysate, Biofeed, and Effluent.

VOC Compounds		
1,1,1-Trichloroethane	Bromofluorobenzene (surr)	Ethylbenzene
1,1,2,2-Tetrachloroethane	Bromoform	m,p-Xylene
1,1,2-Trichloroethane	Bromomethane	Methylene chloride
1,1-Dichloroethane	Carbon Disulfide	o-Xylene
1,1-Dichloroethene	Carbon Tetrachloride	Styrene
1,2-Dichloroethane	Chlorobenzene	Tetrachloroethene
1,2-Dichloropropane	Chloroethane	Toluene
2-Butanone	Chloroform	Toluene-d8 (surr)
2-Hexanone	Chloromethane	trans-1,2-Dichloroethene
4-Methyl-2-pentanone	cis-1,2-Dichloroethene	trans-1,3-Dichloropropene
Acetone	cis-1,3-Dichloropropene	Trichloroethene
Benzene	Dibromochloromethane	Vinyl chloride
Bromodichloromethane	Dibromofluoromethane	

Table 9. Positive Results Analysis for Total VOCs.

Location	Number Positive	Total VOCs	Compounds
M1 Hydrolysate	4	3700 µg/L	1-Butanol, acetone ether and toluene
M1 Biofeed	12	1402 µg/L	Mostly acetone, toluene, benzene and unidentified
M1 Effluent	8	467 µg/L	Mostly acetone, toluene and unidentified
M8 Hydrolysate	3	170 µg/L	Acetone, benzene and ethanol
M8 Biofeed	15	650 µg/L	Mostly Acetone, chloroform and unidentified
M8 Effluent	8	198 µg/L	Mostly Acetone

3.9 Semi-Volatile Organic Chemicals (SVOCs).

Propellant hydrolysates, biofeed, and effluents were tested for SVOCs. In all, there were 1330 analyses for SVOCs and 16 positive results for quantifiable SVOCs. The test results are listed in Table 10. Additional results, including qualified data, are presented in the Appendixes.

Table 10. Positive Results for SVOCs in ICB Prepared Biofeed and Effluent.

Feed	Sample Location	Sample Date	Compound	Result (µg/L)
M8	Prepared Biofeed	3/9/2001	Di-n-butylphthalate	25
M8	Prepared Biofeed	3/9/2001	N-Nitrosodiphenylamine	12
M8	Prepared Biofeed	4/11/2001	Naphthalene	40
M8	Prepared Biofeed	4/10/2001	Nitrobenzene	51
M1	Prepared Biofeed	3/9/2001	2,4-Dinitrotoluene	3800
M1	Prepared Biofeed	3/9/2001	Di-n-butylphthalate	11000
M1	Prepared Biofeed	3/9/2001	N-Nitrosodiphenylamine	3000
M1	Prepared Biofeed	3/9/2001	Nitrobenzene	700
M1	Prepared Biofeed	4/6/2001	2,4-Dinitrotoluene	6000
M1	Prepared Biofeed	4/6/2001	Di-n-butylphthalate	5500
M1	Prepared Biofeed	4/6/2001	N-Nitrosodiphenylamine	430
M1	Prepared Biofeed	4/6/2001	Nitrobenzene	470
M1	ICB Effluent	4/6/2001	2,4-Dinitrotoluene	1300
M1	ICB Effluent	4/6/2001	Nitrobenzene	140
M1	M1 Hydrolysate	4/6/2001	2,4-Dinitrotoluene	8900
M1	M1 Hydrolysate	4/6/2001	N-Nitrosodiphenylamine	18000

3.10 Solids Data.

Measurements of Total Suspended Solids, Total Dissolved Solids, and Volatile Suspended Solids were taken from the ICB hydrolysate, biofeed, effluent and composite biomass. Most of the solids for the samples were below detection limits. The positive results for hydrolysate, biofeed, effluent, and the composite biomass sample are listed in Table 11. Complete results of the solids analyses are available in the Appendixes.

Table 11. Solids Analysis Results for M1 and M8 Hydrolysate, Biofeed, Effluent, and Composite Biomass Samples.

Feed	Sample Location	TSS Result (mg/L)	VSS Result (mg/L)	TDS Result (mg/L)
M1	Hydrolysate	260	<100	103000
M1	Effluent	840	760	23867
M1	Composited Biomass	44000	40600	
M8	Hydrolysate	<100	<100	102000
M8	Effluent	194	180	23114
M8	Composited Biomass	43800	35400	

4. CONCLUSION

The intended purpose of this study was to evaluate the manner in which the hydrolyzed propellants removed from chemical rounds currently awaiting destruction at PCD should be handled. Neutralization followed by biodegradation was the treatment technology of choice. An additional concern was the possible destruction of the hydrolyzed propellants removed from the chemical rounds by the proposed treatment.

Parsons/Honeywell, the technology provider for PCD, proposed a processing campaign following the destruction of the mustard agents for the propellants associated with the chemical rounds. The test design was based on the premise that the ICBs would already contain the bacterial culture previously used for the mustard destruction process and thus be able to destroy the hydrolyzed M1 and M8 propellants. The ICBs were inoculated using activated sludge from a publicly owned treatment facility and grown on neutralized mustard for several months. The media was then changed over from the full-strength hydrolyzed mustard recipe to a hydrolyzed propellant base. At first, the propellant media was diluted and then increased to the proposed design strength and feed rate. An additional small amount of fresh activated sludge inoculum was added to the culture at the change over as may be the case at the full size facility.

The success of the treatment strategy was dependent on the ability of the inoculums to remove carbon and nitrogen compounds, and any energetics surviving the neutralization process. The ability of the inoculum to produce a non-toxic waste stream was also judged. During laboratory and pilot-scale testing, neutralized mustard agent as the base media carbon removal efficiency was generally measured around 90%. The 90% benchmark is regarded as a successful treatment process and is also a goal for the treatment of the propellant based media. The destruction of carbon based materials and other chemicals of concern were measured in the lab using a colorimetric assay that measures effluent COD. The COD includes chemically oxidizable materials that are carbon and non-carbon based, a good indicator of biotreatment performance, but should be used in concert with other measurement parameters.

4.1 Chemical Oxygen Demand (COD).

Prior to the switch from the neutralized mustard based media, COD removal efficiency was greater than 90%, but it quickly decreased after the switch to propellant based media (Figures 6 and 7). The COD removal efficiency varied widely during the ramp-up of the propellant concentration. During this phase, pH control of the culture also switched from single directional control using a base, sodium hydroxide to single direction control using an acid. Acetic acid was initially used to adjust the pH of the prepared biofeed and control pH within the ICB. Biofeed preparation protocol was changed to specify hydrochloric acid for pH adjustment and later hydrochloric acid was used to control ICB pH as well.

At each of the 2 changes, the decrease in exogenously added carbon coincided with a decrease in the COD removal efficiency of the culture and increased the effluent COD. By the end of the validation period, COD removal efficiency in the M1 reactor was approximately 40% and 60 to 65% for the M8 reactor. Relative to previous studies with the neutralized mustard based media, these were poor removal efficiencies. The higher COD removal efficiency observed during the period with exogenously added carbon indicates a level of co-metabolism of the propellant based media in the presence of the carbon containing acetic acid.

4.2 Organic Carbon.

The removal of organic carbon was also a measure of bioreactor performance. Total Organic Carbon measurement during the study indicates a decrease in removal efficiency after the switch to propellant based media and removal of exogenous carbon in both reactor systems. Total Organic Carbon removal by the end of the validation period was near 40% in the M1 reactor and 55% in the M8 reactor (Figures 10-12), indicating poor performance in each, although the M8 performed slightly better.

4.3 MICROTOX (MTX) Toxicity.

The relative toxicity of the prepared biofeed and effluent was measured using the MTX assay, which indicated that the effluent from the mustard based biofeed was nontoxic prior to the switch to the propellant based biofeed (Figure 8). MICROTOX also indicated that the propellant biofeeds once ramped up to design feed were more toxic than the neutralized mustard based feed (Figure 8). Figure 9 shows that the propellant bioreactors effluent toxicity increased throughout the study. At the end of the validation period, the M1 EC_{50} was less than 5% and the M8 EC_{50} near 35%, indicating an effluent from each reactor system that is fairly toxic to microorganisms using the MTX assay. According to MTX, toxic media and effluent have not been shown to be easily biodegraded.

4.4 Energetic Compounds.

Of the energetic compounds in the original propellant formulation, only nitrocellulose was present in the bioreactor effluents. Most of the primary energetics was removed during the neutralization process. Nitrocellulose, the principal energetic and base material, was present in the low concentrations of the hydrolysate and feed, and decreased in concentration across each bioreactor, but was present in low concentration in each effluent. Most of the organic containing compounds were breakdown products of the original energetic mixtures. Many of the compounds were identified as VOCs and SVOCs, with a large portion of the total being unidentified by the methods used.

4.5 Semi-Volatile Organic Compounds (SVOCs).

The quantitation of SVOCs was difficult in the process streams due to the high number of compounds and the difficulty in resolving and identifying many individual peaks. The majority of the SVOCs were low molecular weight alcohols and alkenes, which were not individually identified. Since identification was difficult, calibration and quantitation was impossible. Many of the values reported were estimates based on levels of similar compounds. The ICB culture was unable to remove many of the SVOCs to non detection levels. Perhaps an increase in the length of the hydrolyzation process could have made these compounds easier to biodegrade.

4.6 Volatile Organic Compounds (VOCs).

Twelve VOCs were detected in the ICB process streams. Many compounds, not identified specifically in the hydrolysate, were present in the biofeed. The largest quantifiable contributor in the effluent was acetone, which was the only quantifiable VOC identified in the M8 effluent.

The M1 effluent contained 6 VOCs, with acetone being the largest contributor. Data integration was also difficult as it was with the SVOCs. Other than acetone and toluene, all other concentrations reported are estimates.

4.7 Regulated Metals.

Analysis for metals in the M8 process streams revealed that mercury was detected at µg/L levels in the effluent and biomass. The hydrolysate contained barium, cadmium, chromium, and zinc at sub-mg/L levels. Barium, cadmium, chromium, lead, nickel and zinc were detected in the feed effluent and biomass at µg/L to mg/L levels. The biomass also contained antimony. The only metals detected in the TCLP analysis of the brine solids were mercury at 1.1 µg/L and barium at 0.11 mg/L. The level of the zinc in the biomass, 4.36 mg/L, exceeded the universal treatment standard (UTS) of 2.61 mg/L. Otherwise detected metals were below UTS.

The M1 process streams contained mercury in the biofeed, effluent and biomass as µg/L levels. Lead, which is a reported component of M1 propellant, was not detected in the hydrolysate feed or effluent. Lead was detected in the biomass at 1,230 µg/L, which is greater than the Resource Conservation Recovery Act (RCRA) UTS of 0.69 mg/L. Total constituent analysis of the brine solids detected lead at 3.4 mg/kg, but lead was not detected in the brine solids with the TCLP analysis. The only metal detected in the TCPL analysis of the brine solids was barium at 0.11 mg. Antimony, barium, chromium, nickel, and zinc were detected in various process streams at low levels. With the exception of lead in the biomass, detected metals were below UTS.

4.8 Solids.

Water reuse becomes an increasingly important requirement when facilities are located in arid environments and ultimate disposal of reactor effluents can be costly. Though not the primary objective of this study, data was collected to support engineering designs for solids and water reuse. Handling and reuse of effluent can depend greatly on the level of solids in the ICB effluent. Solids, removed and collected from effluent streams, must be disposed of according to local hazardous waste requirements if the biomass is determined to be a hazardous waste. A summary of solids collected from the ICBs at the end of this study is included in the Appendixes.

One method of proposed water reuse involves the collection and cleaning of effluents using an evaporator/crystallizer. This process is similar to distillation in that is produces a purified effluent and leaves behind a concentrated waste. In the case of bioeffluent from a caustically hydrolyzed food source, the concentrated waste becomes high in salt, so here it is referred to as brine. Suspended solids are also part of the concentrated brine. The brine may be further processed through a filter pressing process that further removes water to minimize the weight and volume of material that may be disposed of as a hazardous waste. A summary of the analysis of the concentrated brine and brine solids are included in the Appendixes.

5. SUMMARY

Test results show that stand-alone processing of the hydrolyzed propellants, using a culture grown on hydrolyzed sulfur mustard (HD), results in poor reactor performance and destruction of the hydrolyzed propellant components. Poor performance is noted in the areas of organic carbon removal, and the removal of chemical oxygen demand and specific chemicals of concern, including acetone, nitrobenzene, 2,4-Dinotrotoluene, and nitrocellulose. The hydrolyzed propellants produce a biofeed and effluent that is relatively toxic to microorganisms as indicated using the MICROTOX (MTX) Assay. The operating protocols for Pueblo Chemical Depot (PCD) plan for at least partial reuse of ICB effluent as process water. The prevalence of undigested Volatile Organic Chemicals (VOCs) and Semi-Volatile Organic compounds (SVOCs) in the bioculture effluent will complicate chrystallizer operation and increase off-gassing compounds that would need to be destroyed by a catalytic oxidizing system or collected on a filter cartridge for later disposal. While the approach would probably work, the goal is still to remove as many compounds biologically as possible before resorting to destruction or collection as off-gasses.

As designed, the test did not perform well as a stand-alone approach. The removal of a majority of the chemicals and apparent increased performance with exogenously supplied carbon indicate the potential for co-processing with the hydrolyzed mustard campaign. The high nitrogen content of the propellant hydrolysates could partially augment immobilized cell bioreactor (ICB) culture nitrogen requirements during hydrolyzed HD destruction. Removal of more difficult chemicals and nitrogen compounds may be aided by the addition of an anoxic cycle or chamber within the ICB process.

If a stand-alone process is required, an inoculum, selected and enriched specifically for the propellant biofeed, may perform better than the left over culture from the hydrolyzed mustard campaign. Additionally, a tertiary oxidative treatment of the propellant prior to or as an intermediate treatment to biotreatment may decrease propellant feed toxicity and the level of recalcitrant compounds to improve the overall performance of the ICB culture.

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APPENDIX A

M1 and M8 HYDROLYSATE CHARACTERIZATION

Positive Results for Chemicals of Concern.

Sample Date	Method Name	Compound Name	M1 Result	M8 Result	Units
4/6/2001	Metals (M28 Mod)	Aluminum	2400	1260	µg/L
4/6/2001	Metals (M28 Mod)	Barium	45.9	50.7	µg/L
4/6/2001	Metals (M28 Mod)	Calcium	16300	16200	µg/L
4/6/2001	Metals (M28 Mod)	Cadmium		69.5	µg/L
4/6/2001	Metals (M28 Mod)	Chromium	91.9	27	µg/L
4/6/2001	Metals (M28 Mod)	Copper	113	656	µg/L
4/6/2001	Metals (M28 Mod)	Iron	6740	937	µg/L
4/6/2001	Metals (M28 Mod)	Magnesium	10500	4960	µg/L
4/6/2001	Metals (M28 Mod)	Manganese	39.5	36.3	µg/L
4/6/2001	Metals (M28 Mod)	Nickel	131		µg/L
4/6/2001	Metals (M28 Mod)	Potassium	15900	448000	µg/L
4/6/2001	Metals (M28 Mod)	Zinc	147	127	µg/L
4/6/2001	Nitrocellulose (M28)	Nitrocellulose	5.25	8.93	mg/L
4/6/2001	Energetics	2,4,6-Trinitrotoluene	120		µg/L
4/6/2001	Energetics	2-Nitrotoluene	26000		µg/L
4/6/2001	Energetics	3-Nitrotoluene	2700		µg/L
4/6/2001	Energetics	4-Nitrotoluene	2600		µg/L
4/6/2001	SVOC (M28 Mod)	2,4-Dinitrotoluene	8900	2900	µg/L
	SVOC	2,4-Dinitrophenol		200	µg/L
	SVOC	2-Nitrophenol		400	µg/L
	SVOC	Nitrobenzene	1000		µg/L
	SVOC	Di-n-butylphthalate	2000		µg/L
4/6/2001	SVOC (M28 Mod)	N-Nitrosodiphenylamine	18000		µg/L
	SVOC	Unknowns	11200	90127	µg/L

APPENDIX A Table (Continued)

Sample Date	Method Name	Compound Name	M1 Result	M8 Result	Units
4/6/2001	TOC (M28 Mod)	TOC	13000	14700	mg/L
4/6/2001	VOC (M28-Mod)	1-Butanol	1100		µg/L
	VOC	Acetone	2000	1000	µg/L
	VOC	Benzene		50	µg/L
4/6/2001	VOC (M28 Mod)	Ether	170		µg/L
4/6/2001	VOC (M28 Mod)	Ethanol		170	µg/L
4/6/2001	VOC (M28 Mod)	Toluene	430		µg/L
4/6/2001	Specific Gravity	Specific Gravity	1.05	1.04	g/mL
4/6/2001	TDS	Total Disolved Solids	103000	102000	mg/L
4/6/2001	TSS	Total Suspended Solids	260		mg/L

APPENDIX B

POSITIVE RESULTS FOR SVOCS IN THE M1 HYDROLYSATE, BIOFEED, AND EFFLUENT

The large number of SVOC compounds detected made the complete identification and quantification of many of the compounds challenging. Previous discussions included only those SVOCs that were clearly identifiable and quantifiable. Many of the identifiable analytes and unknown concentrations are estimated. Because of the sheer number and quantity of SVOCs that fall into this category, it would be inappropriate to completely ignore this data.

Feed	**Compound**	**Hydrolysate (µg/L)**	**Prepared Biofeed (µg/L)**	**ICB Effluent (µg/L)**
M1	1,2-Dichlorobenzene		12	7
M1	2,4-Dinitrophenol		1400	617
M1	2,4-Dinitrotoluene	8900	4900	1065
M1	2-Methylphenol		30	9
M1	2-Nitrophenol		145	54
M1	3,4-Methylphenol			9
M1	4,6-Dinitro-2-methylphenol		2200	2550
M1	4-Chloro-3-methylphenol			8
M1	4-Methylphenol		30	10
M1	4-Nitrophenol		200	185
M1	Benzamine, 2-nitro-N-(2-nitrophenol)		1900	
M1	Benzene, 1-methyl-2-nitro-		9950	
M1	Benzoic Acid		1050	380
M1	Benzyl Alcohol		10	10
M1	bis(2-Ethylhexyl)phthalate			180
M1	Di-n-butylphthalate	2000	6500	
M1	Naphthalene			4
M1	Nitrobenzene	1000	407	172
M1	N-Nitrosodiphenylamine	18000	10500	
M1	Phenol		20	
M1	Unknowns	11200	5880	8390
M1	Unknown alcohols		1370	3290
M1	Unknown alkenes		1400	915
M1	Unknown organic acid		630	540
M1	Unknown Substituted Benzene		5600	

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APPENDIX C

POSITIVE RESULTS FOR SVOCS IN THE M8 HYDROLYSATE, BIOFEED, AND EFFLUENT

Due to the large number of SVOC compounds detected, it became extremely challenging to completely identify and quantify many of the compounds. Previous discussions of the SVOCs included only those that were clearly identifiable and quantifiable. Many of the identifiable analytes and unknowns concentrations are estimated. Because of the sheer number and quantity of SVOCs that fall into this category, it would be inappropriate to completely ignore this data which should be suspect due to its qualitative nature.

Feed	Compound	Hydrolysate (µg/L)	Prepared Biofeed (µg/L)	ICB Effluent (µg/L)
M8	2,4-Dinitrophenol	200	270	
M8	2-Methylphenol			1
M8	2-Nitrophenol	400	36	
M8	4,6-Dinitro-2-methylphenol		32	
M8	Benzenamine, ethyl- isomer		320	33
M8	Benzoic Acid		101	3
M8	Benzyl Alcohol			2
M8	bis(2-Ethylhexyl)phthalate		7	
M8	Diethylphthalate		3	
M8	Di-n-butylphthalate		25	
M8	Ethylbenzamine Isomer			648
M8	Nitrobenzene		51	
M8	N-Nitrosodiphenylamine		13	8.5
M8	Phenol		11	3
M8	Unknown	39160	20993	39886
M8	Unknown alcohols	12000	590	
M8	Unknown Alkanes	38967		
M8	Unknown alkenes		4550	
M8	UNKNOWN AMINE			37
M8	Unknown organic acids		2290	
M8	Unknown Substituted Benzene			150

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APPENDIX D

POSITIVE RESULTS FOR VOCS IN ALL M1 AND M8 PROCESS STREAMS WITHOUT QUALIFIERS

These data met all the requirements for reportable, quantifiable analytes.

Feed	Compound	Hydrolysate (µg/L)	Prepared Biofeed (µg/L)	ICB Effluent (µg/L)
M1	1-Butanol	1100		
M1	Acetone		380	143
M1	Benzene		14	3.8
M1	Bromodichloromethane		5.5	
M1	Bromomethane			3.6
M1	Chlorobenzene		7	5
M1	Chloroform		28	
M1	Chloromethane		7.7	14
M1	Ether	170		
M1	Toluene	430	415	26
M8	Acetone		290	103
M8	Benzene		29	
M8	Bromodichloromethane		7.45	
M8	Chlorobenzene		2.65	
M8	Chloroethane		3.4	
M8	Chloroform		29.5	
M8	Chloromethane		15	
M8	Dibromochloromethane		1.12	
M8	Ethanol	170		
M8	Toluene		3	

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APPENDIX E

POSITIVE RESULTS FOR VOCS IN ALL PROCESS STREAMS INCLUDING QUALIFIED DATA.

The values are estimates because of poor calibration, interference, or poor spike recovery.

Feed	Compound	Hydrolysate (μg/L)	Prepared Biofeed (μg/L)	ICB Effluent (μg/L)
M1	1,2-Dichloroethane		2	
M1	1-Butanol	1100	185	
M1	Acetone	2000	420	139
M1	Benzene		14	2.3
M1	Benzene, 1-methyl-2-nitro-		175	
M1	Bromodichloromethane		5.25	
M1	Bromomethane		3	6.55
M1	Chlorobenzene		6.9	5.8
M1	Chloroform		28	
M1	Chloromethane		7.7	19
M1	Ether	170		
M1	Toluene	430	415	48.3
M1	Unknown		140.5	189
M1	Unknown Alkene			58
M8	1,2-Dichloroethane		2.5	
M8	2-Butanone		26.5	
M8	Acetone	1000	420	190.7
M8	Benzene	50	29	0.58
M8	Bromodichloromethane		5	
M8	Bromomethane		6.2	0.3
M8	Carbon Disulfide		0.35	
M8	Chlorobenzene		2.65	0.09
M8	Chloroethane		3.4	
M8	Chloroform		29.5	
M8	Chloromethane		15	1.25
M8	Dibromochloromethane		1.12	
M8	Ethanol	170		
M8	Toluene		3	0.41
M8	Unknown		38.47	3.1
M8	Unknown Alkene		58	2.1

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APPENDIX F

M8 AND M1 ICB BIOFEED AND EFFLUENT TOC RESULTS

Quantified Data of Average of 4 replicates.

Feed Source	Sample Date	Method	Biofeed Result	Effluent Result	Units
M8 Hydrolysate	2/2/2001	TOC (PIH) Mod)	3875	1205	mg/L
M8 Hydrolysate	2/13/2001	TOC (PIH))Mod)	3080	1125	mg/L
M8 Hydrolysate	2/26/2001	TOC (PIH) Mod)	2727.5	1085	mg/L
M8 Hydrolysate	3/9/2001	TOC	2510	1117.5	mg/L
M8 Hydrolysate	3/9/2001	TOC (PIH) Mod)	2505	1115	mg/L
M8 Hydrolysate	3/16/2001	TOC	3275	1212.5	mg/L
M8 Hydrolysate	3/16/2001	TOC (PIH Mod)	2695		mg/L
M8 Hydrolysate	3/30/2001	TOC	2602.5	1195	mg/L
M8 Hydrolysate	3/30/2001	TOC (PIH) Mod)	2737.5		mg/L
M8 Hydrolysate	4/10/2001	TOC	2800	1102.5	mg/L
M8 Hydrolysate	4/10/2001	TOC (PIH) Mod)	2825	1197.5	mg/L
M1 Hydrolysate	2/2/2001	TOC (PIH) Mod)	3910	1497.5	mg/L
M1 Hydrolysate	2/13/2001	TOC (PIH) Mod)	3452.5	1817.5	mg/L
M1 Hydrolysate	2/13/2001	TOC (PIH Mod)		2050	mg/L
M1 Hydrolysate	2/26/2001	TOC (PIH) Mod)	3697.5	1710	mg/L
M1 Hydrolysate	3/9/2001	TOC	3117.5	1730	mg/L
M1 Hydrolysate	3/9/2001	TOC (PIH) Mod)	2807.5		mg/L
M1 Hydrolysate	3/16/2001	TOC	2700	2317.5	mg/L
M1 Hydrolysate	3/16/2001	TOC (PIH) Mod)	2902.5	1897.5	mg/L
M1 Hydrolysate	4/6/2001	TOC	3132.5	2062.5	mg/L
M1 Hydrolysate	4/6/2001	TOC (PIH) Mod)	2910	1757.5	mg/L
M1 Hydrolysate	4/6/2001	TOC (PIH) Mod)		2247.5	mg/L
M1 Hydrolysate	4/6/2001	TOC (PIH) Mod)		1825	mg/L

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APPENDIX G

SUMMARY OF M1 AND M8 BIOFEED AND EFFLUENT METALS

Average Over Validation Period.

Feed Source	Sample Date	Compound	Biofeed Result (µg/L)	Effluent Result (µg/L)
M1	Validation Avg.	Aluminum	685	656
M1	Validation Avg.	Barium	51	70
M1	Validation Avg.	Calcium	11600	12320
M1	Validation Avg.	Cobalt	130	122
M1	Validation Avg.	Copper	125	139
M1	Validation Avg.	Iron	708	898
M1	Validation Avg.	Magnesium	8735	9756
M1	Validation Avg.	Manganese	880	852
M1	Validation Avg.	Phosphorus	35500	40338
M1	Validation Avg.	Potassium	90000	115486
M1	Validation Avg.	Sodium	6930000	7485000
M1	Validation Avg.	Zinc	203	721
M8	Validation Avg.	Aluminum	316	380
M8	Validation Avg.	Barium	55	49
M8	Validation Avg	Cadmium	13	
M8	Validation Avg.	Calcium	11025	12180
M8	Validation Avg	Chromium	6	
M8	Validation Avg.	Cobalt	119	132
M8	Validation Avg.	Copper	190	180
M8	Validation Avg.	Iron	552	885
M8	Validation Avg.	Magnesium	8068	9454
M8	Validation Avg.	Manganese	872	865
M8	Validation Avg.	Molybdenum	26	35
M8	Validation Avg.	Phosphorus	34440	37486
M8	Validation Avg.	Potassium	122000	133500
M8	Validation Avg.	Sodium	6250000	6697143
M8	Validation Avg.	Zinc	181	448

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APPENDIX H

TABLES FOR ENERGETICS ANALYSIS

Table H-1. Positive Results of Energetics Analysis in Hydrolysate, Biofeed and Effluent

Sample Date	Feed Type	Sample Location	Compound	Result	Units
2/26/2001	M8 Hydrolysate	Prepared Biofeed	2,4-Dinitrotoluene	2900	µg/L
4/6/2001	M1 Hydrolysate	M1 Hydrolysate	2,4,6-Trinitrotoluene	120	µg/L
4/6/2001	M1 Hydrolysate	M1 Hydrolysate	2,4-Dinitrotoluene	8800	µg/L
4/6/2001	M1 Hydrolysate	M1 Hydrolysate	2-NT	26000	µg/L
4/6/2001	M1 Hydrolysate	M1 Hydrolysate	3-NT	2700	µg/L
4/6/2001	M1 Hydrolysate	M1 Hydrolysate	4-NT	2600	µg/L

* Energetics may also be reported in SVOCs and VOCs.

Table H-2. Analyzed but Undetected Compounds.

1,3,5-Trinitrobenzene
1,3-Dinitrobenzene
2,6-Dinitrotoluene
2-Amino-4,6-dinitrotoluene
4-Amino-2, 6-dinitrotoluene
HMX
Nitrobenzene
RDX
Tetryl

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APPENDIX I

M1 and M8 COMPOSITE BIOMASS CHARACTERIZATION

Positive Results for Chemicals of Concern.
Quantified data from the Composite Culture Biomass collected at the end of the study 04/25/2001.

Sample Location	Sample Series ID	Method Name	Compound	M8 Result	M1 Result	Units
Biomass	XXAX	Elemental Analysis	%C	30.4	34.2	%
Biomass	XXAX	Elemental Analysis	%H	4.3	3.9	%
Biomass	XXAX	Elemental Analysis	%N	7.8	10.2	%
Biomass	XXAX	Elemental Analysis	%O	20	18.6	%
Biomass	XXAX	Elemental Analysis	%S	0.235	0.197	%
Biomass	XXAX	Metals	Mercury	1.16	1.24	µg/L
Biomass	XXAX	Metals	Aluminum	3510	10100	µg/L
Biomass	XXAX	Metals	Barium	604	537	µg/L
Biomass	XXAX	Metals	Cadmium	485		µg/L
Biomass	XXAX	Metals	Calcium	48100	64900	µg/L
Biomass	XXAX	Metals	Chromium	159		µg/L
Biomass	XXAX	Metals	Cobalt	66.2	125	µg/L
Biomass	XXAX	Metals	Copper	3120	1700	µg/L
Biomass	XXAX	Metals	Iron	18800	36000	µg/L
Biomass	XXAX	Metals	Lead	605	1230	µg/L
Biomass	XXAX	Metals	Magnesium	30000	53300	µg/L
Biomass	XXAX	Metals	Manganese	4920	15400	µg/L
Biomass	XXAX	Metals	Molybdenum	50.8	50.3	µg/L
Biomass	XXAX	Metals	Nickel	239	381	µg/L
Biomass	XXAX	Metals	Phosphorus	370000	242000	µg/L
Biomass	XXAX	Metals	Potassium	285000	114000	µg/L
Biomass	XXAX	Metals	Sodium	7500000	6890000	µg/L
Biomass	XXAX	Metals	Zinc	4360	6530	µg/L
Biomass	XXAX	TSS	TSS	43800	44000	µg/L
Biomass	XXAX	VSS	VSS	35400	40600	µg/L

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APPENDIX J

CHARACTERIZATION OF EVAPORATED BRINE SAMPLE

Quantified Data From Biomass Solids Removed from the Reactor at the End of the Study Period 4/25/2001

Feed Name	Sample Name	Sample ID	Method Name	Compound	Result	Data Units
M8	Evaporated Brine Solid	PIP04MEXXBX	(pH)	pH	9.04	pH units
M8	Evaporated Brine Solid	PIP04MEXXBX	Metals	Barium	1.77	mg/kg
M8	Evaporated Brine Solid	PIP04MEXXBX	Metals	Calcium	532	mg/kg
M8	Evaporated Brine Solid	PIP04MEXXBX	Metals	Cobalt	4.05	mg/kg
M8	Evaporated Brine Solid	PIP04MEXXBX	Metals	Copper	6.49	mg/kg
M8	Evaporated Brine Solid	PIP04MEXXBX	Metals	Magnesium	333	mg/kg
M8	Evaporated Brine Solid	PIP04MEXXBX	Metals	Manganese	27.8	mg/kg
M8	Evaporated Brine Solid	PIP04MEXXBX	Metals	Phosphorus	1460	mg/kg
M8	Evaporated Brine Solid	PIP04MEXXBX	Metals	Potassium	5030	mg/kg
M8	Evaporated Brine Solid	PIP04MEXXBX	Metals	Sodium	247000	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Anions	Chloride	239000	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Anions	Fluoride	282	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Anions	Nitrate-N	12200	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Anions	Nitrate-N	15800	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Anions	o-Phosphate-P	920	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Anions	Sulfate	2510	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	(pH)	pH	9.12	pH units
M1	Evaporated Brine Solid	PIP04MOXXBX	Metals	Barium	2.51	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Metals	Calcium	558	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Metals	Cobalt	4.21	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Metals	Copper	5.49	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Metals	Magnesium	359	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Metals	Manganese	29.1	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Metals	Phosphorus	1340	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Metals	Potassium	3420	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Metals	Sodium	216000	mg/kg
M1	Evaporated Brine Solid	PIP04MOXXBX	Metals	Zinc	24.7	mg/kg

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APPENDIX K

TABLES FOR SOLIDS ANALYSIS

Table K-1. TCLP Positive Results for Evaporated M1 And M8 Brine Solids.

Feed	Sample Location	Sample Date	Method	Media	Compound	Result	Units	Qualifier
M8	Evaporated Brine Solid	5/16/2001	TCLP (Metals)	Solid	Barium	0.11	mg/L	J
M8	Evaporated Brine Solid	5/16/2001	TCLP (Metals)	Solid	Mercury	0.0011	mg/L	J
M1	Evaporated Brine Solid	5/16/2001	TCLP (Metals)	Solid	Barium	0.11	mg/L	J

J: The analyte was positively identified but the quantitative result reported is an estimated value due to data quality issue(s).

Table K-2. Compounds Analyzed for, but Not Found

Test	Compound	Test	Compound
TCLP (Metals)	Arsenic	TCLP (SVOC)	Nitrobenzene
TCLP (Metals)	Cadmium	TCLP (SVOC)	Pentachlorophenol
TCLP (Metals)	Chromium	TCLP (SVOC)	Pyridine
TCLP (Metals)	Lead	TCLP (VOC)	1,1-Dichloroethene
TCLP (Metals)	Selenium	TCLP (VOC)	1,2-Dichloroethane
TCLP (Metals)	Silver	TCLP (VOC)	1,4-Dichlorobenzene
TCLP (SVOC)	2,4,5-Trichlorophenol	TCLP (VOC)	2-Butanone
TCLP (SVOC)	2,4,6-Trichlorophenol	TCLP (VOC)	Benzene
TCLP (SVOC)	2,4-Dinitrotoluene	TCLP (VOC)	Carbon Tetrachloride
TCLP (SVOC)	2-Methylphenol	TCLP (VOC)	Chlorobenzene
TCLP (SVOC)	4-Methylphenol	TCLP (VOC)	Chloroform
TCLP (SVOC)	Hexachlorobenzene	TCLP (VOC)	Tetrachloroethene
TCLP (SVOC)	Hexachlorobutadiene	TCLP (VOC)	Trichloroethene
TCLP (SVOC)	Hexachloroethane	TCLP (VOC)	Vinyl chloride

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APPENDIX L

MERCURY ANALYSIS TABLE

Positive Results for Mercury in Process Streams.

Feed	Sample Location	Sample Date	Method	Media	Units	Result	Qualifier
M1	Prepared Biofeed	2/13/2001	Mercury-liquid	Liquid	µg/L	1.1	J
M1	ICB Effluent	2/13/2001	Mercury-liquid	Liquid	µg/L	3.77	
M1	ICB Effluent	2/14/2001	Mercury-liquid	Liquid	µg/L	1.8	J
M1	Evaporated Brine Solid	5/16/2001	Mercury-solid	Solid	mg/kg	0.031	J
M1	Composited Biomass	4/25/2001	Mercury-liquid	Slurry	µg/L	1.24	
M8	ICB Effluent	2/14/2001	Mercury-liquid	Liquid	µg/L	2.3	J
M8	Composited Biomass	4/25/2001	Mercury-liquid	Slurry	µg/L	1.16	
M8	Evaporated Brine Solid	5/16/2001	TCLP (Metals)	Solid	µg/L	0.0011	J

J: indicates the analyte was positively identified but the quantitative result reported is an estimate due to data quality issue(s).

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APPENDIX M

TOTAL DISSOLVED SOLIDS (TDS), TOTAL SUSPENDED SOLIDS (TSS), AND VOLATILE SUSPENDED SOLIDS (VSS) DATA

Feed	Sample Location	Sample ID	Sample Date	Measurement	Result (mg/L)	Qualifier
M8	ICB Effluent	PIP02ME1A0X	2/1/2001	TSS	34	J
M8	ICB Effluent	PIP02ME1B0X	2/13/2001	TSS	194	
M8	ICB Effluent	PIP02ME1C0X	2/14/2001	TSS	170	J
M8	ICB Effluent	PIP02ME1E0X	3/8/2001	TSS	100	U
M8	ICB Effluent	PIP02ME1G0X	3/29/2001	TSS	140	J
M1	ICB Effluent	PIP02MO1A0X	2/1/2001	TSS	840	
M1	ICB Effluent	PIP02MO1B0X	2/13/2001	TSS	96	J
M1	ICB Effluent	PIP02MO1C0X	2/14/2001	TSS	243	J
M1	ICB Effluent	PIP02MO1E0X	3/8/2001	TSS	100	U
M1	ICB Effluent	PIP02MO1G0X	4/6/2001	TSS	100	U
M8	Composite Biomass	PIP03MEXXAX	4/25/2001	TSS	43800	
M1	Composite Biomass	PIP03MOXXAX	4/25/2001	TSS	44000	
M1	M1 Hydrolysate	PIP05MOXXCX	4/6/2001	TSS	260	
M8	M8 Hydrolysate	PIP06MEXXCX	4/6/2001	TSS	100	U
M8	ICB Effluent	PIP02ME1A0X	2/1/2001	TDS	20700	
M8	ICB Effluent	PIP02ME1B0X	2/13/2001	TDS	21300	
M8	ICB Effluent	PIP02ME1C0X	2/14/2001	TDS	23000	J
M8	ICB Effluent	PIP02ME1E0X	3/8/2001	TDS	24000	
M8	ICB Effluent	PIP02ME1G0X	3/29/2001	TDS	22400	J
M1	ICB Effluent	PIP02MO1A0X	2/1/2001	TDS	2200	J
M1	ICB Effluent	PIP02MO1B0X	2/13/2001	TDS	22500	
M1	ICB Effluent	PIP02MO1C0X	2/14/2001	TDS	25600	J
M1	ICB Effluent	PIP02MO1E0X	3/8/2001	TDS	25400	
M1	ICB Effluent	PIP02MO1G0X	4/6/2001	TDS	23700	
M1	M1 Hydrolysate	PIP05MOXXCX	4/6/2001	TDS	103000	
M8	M8 Hydrolysate	PIP06MEXXCX	4/6/2001	TDS	102000	
M8	ICB Effluent	PIP02ME1A0X	2/1/2001	VSS	24	J
M8	ICB Effluent	PIP02ME1B0X	2/13/2001	VSS	180	
M8	ICB Effluent	PIP02ME1C0X	2/14/2001	VSS	158	J
M8	ICB Effluent	PIP02ME1E0X	3/8/2001	VSS	100	U
M8	ICB Effluent	PIP02ME1G0X	3/29/2001	VSS	112	J
M1	ICB Effluent	PIP02MO1A0X	2/1/2001	VSS	760	
M1	ICB Effluent	PIP02MO1B0X	2/13/2001	VSS	70	J
M1	ICB Effluent	PIP02MO1C0X	2/14/2001	VSS	200	J
M1	ICB Effluent	PIP02MO1E0X	3/8/2001	VSS	100	U
M1	ICB Effluent	PIP02MO1G0X	4/6/2001	VSS	100	U
M8	Composite Biomass	PIP03MEXXAX	4/25/2001	VSS	35400	
M1	Composite Biomass	PIP03MOXXAX	4/25/2001	VSS	40600	
M1	M1 Hydrolysate	PIP05MOXXCX	4/6/2001	VSS	100	U
M8	M8 Hydrolysate	PIP06MEXXCX	4/6/2001	VSS	100	U