

BIODEGRADATION OF HYDROLYZED MUSTARD FROM AN ACWA PROJECTILE WASHOUT STUDY

Mark A. Guelta and Laurie Fazekas-Carey
US Army Edgewood Chemical and Biological Center
APG MD 21010

ABSTRACT

In 1996, public laws 104-208, 105-261, and 106-79 established and expanded the Assembled Chemical Weapons Assessment (ACWA) Program. To address public concerns over safe destruction of the U.S. chemical weapon stockpile; the ACWA program was tasked to identify two or more viable alternatives technologies to the “baseline” destruction method of incineration. Neutralization followed by biodegradation was one technology to be successfully demonstrated in a pilot facility at the Edgewood Chemical and Biological Center (ECBC) APG, MD. A successful Engineering Design Study (EDS) followed the demonstration and the Neutralization/Biodegradation process was subsequently approved for destruction of assembled chemical weapons stored at the Pueblo Chemical Depot. During the laboratory and pilot-scale studies hydrolyzed mustard taken from ton storage containers and tetrytol from storage was used to simulate the agent and explosive fills of the M60 chemical round. Presently, rocket cutting and washout engineering studies continue at PCD in preparation for eventual destruction of the chemical rounds. Concern has risen over the possible effect undissolved heel material may have on the biodegradability of the hydrolyzed payloads. This follow-on laboratory study uses mustard agent and tetrytol removed during rocket cutting and washout testing on actual chemical rounds stored at PCD.

INTRODUCTION

Prior Demonstration¹ (Demo) 1 and Engineering Design Study (EDS) testing conducted by PMACWA validated biological treatment of a mixture of HD and tetrytol hydrolysates using the Honeywell Immobilized Cell Bioreactor (ICB). The HD hydrolysate used in the previous tests was made from neat agent obtained from ton containers. Because the Pueblo Chemical Depot² (PCD) stockpile consists of assembled munitions that contain both liquid agent and solid material (heel), the hydrolysate used in prior ICB testing was not fully representative of hydrolysate that will be produced at Pueblo. Therefore, ICB testing using hydrolysate prepared with liquid agent and heel from actual munitions was planned and executed and is the subject of this report.

Parsons/Honeywell³ has conducted EDS testing on a projectile washout system (PWS) on actual 4.2-inch HD mortars from the stockpile at Deseret Chemical Activity, Utah. These are the same type of mortars that make-up a large portion of the Pueblo stockpiles. Results of the PWS testing indicate that these munitions contain a significant amount of heel in the agent cavity. On average 16% of the HD in the test munitions had solidified. In the PWS testing, the heel was washed out of the munitions and combined with liquid agent drained from the munitions. The combined streams were then neutralized and the resulting HD hydrolysate was used in this laboratory-scale ICB study.

Report Documentation Page

Form Approved
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE 01 JUL 2003	2. REPORT TYPE N/A	3. DATES COVERED -			
4. TITLE AND SUBTITLE Biodegradation Of Hydrolyzed Mustard From An ACWA Projectile Washout Study		5a. CONTRACT NUMBER			
		5b. GRANT NUMBER			
		5c. PROGRAM ELEMENT NUMBER			
6. AUTHOR(S)		5d. PROJECT NUMBER			
		5e. TASK NUMBER			
		5f. WORK UNIT NUMBER			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) US Army Edgewood Chemical and Biological Center APG MD 21010		8. PERFORMING ORGANIZATION REPORT NUMBER			
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S)			
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)			
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES See also ADM001523.					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 8	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

The specific objectives of this test were:

- Confirm the ability of the laboratory-scale ICBs to effectively treat the PWS-generated HD hydrolysate at the hydraulic residence time (HRT) that is representative of full-scale design.
- Assess the impact of suspended solids in HD hydrolysate on ICB performance.
- Confirm the ability of the laboratory-scale ICBs to eliminate thiodiglycol (TDG) in the HD hydrolysate.
- Characterize ICB effluents.

Due to the limited space of this manuscript we have concentrated on the reactor performance and the effect, if any, of the hydrolyzed heel material on the reactor performance and operation.

MATERIALS AND METHODS

The HD hydrolysate used for this test was produced from the water hydrolysis of drained agent and heel from 4.2-inch HD mortars as part of the PWS study. It was produced at a nominal HD loading of 3.8 wt%.

Tetrytol hydrolysate was prepared at ECBC for this test by caustic hydrolysis of tetrytol at a nominal tetrytol loading of 6.67% (wt/vol). Methods for producing HD agent and tetrytol hydrolysates have been previously described¹.

TEST SETUP

Two ICBs were used for this test; one received feed with unfiltered HD hydrolysate and the other received feed with filtered HD hydrolysate. Each ICB consisted of two glass columns, or cells A and B. The working volume of each cell was 630 ml (nominal) before biomass loading. The two cells of each ICB were operated in series to closely resemble the configuration of the previously demonstrated multi-celled 1000-gallon pilot-scale ICB. Three Applikon Bioprocess Controllers⁴ (biocontrollers) monitored and controlled the automated portions of the ICB operation.

FEED SCHEDULE

The ICB feed was pumped from a 1-L reservoir into the first ICB in the series. The feed pump operated at a fixed speed. An automated timer controlled feed to the reactor by turning the pump on at specific intervals. To maintain a 5-day hydraulic residence time (HRT) (252 ml/day) the pump was set to operate 0.82 percent of the time using masterflex size 16 tubing.

pH CONTROL

The pH was controlled in only one direction (upward) since the breakdown and consumption of TDG, the primary organic constituent of HD hydrolysate produces an acid. The pH was controlled by addition of a 0.9M sodium bicarbonate solution.

EFFLUENT REMOVAL

An effluent pump, also operating on timed intervals, pumped effluent from Cell B to the effluent reservoir. Effluent was allowed to accumulate in the reservoir between sampling events. After each effluent sampling event the effluent was placed in a composite sample reservoir until needed.

A diaphragm pump supplied air through a glass frit in the bottom of Cell A. Air and effluent from Cell A overflowed into Cell B through a large tygon tube. Air was exhausted from Cell B into the facility hood system.

The required 20-liters of HD hydrolysate was received in a single container. Before preparation of the feed the 20-L container was shaken vigorously for 5 minutes to suspend the hydrolysate undissolved solids. A portion of the HD hydrolysate was removed immediately after shaking and used directly in the preparation of the unfiltered feed (ICB 1). For the filtered feed (ICB 2), HD hydrolysate was poured into a 1-L glass bottle and the solids were allowed to settle for 48 hours. The supernatant was poured into 500-mL bottles and centrifuged at 9500 rpm for 15 minutes. The clarified hydrolysate was then used to make the filtered feed. The unfiltered and filtered ICB feeds were prepared in 4-L batches. The biofeed recipe is listed in table 1.

Table 1. HD Hydrolysate Reactor Biofeed Recipe

Item	Quantity
HD hydrolysate (3.8 wt%)	300 mls
Tetrytol hydrolysate (6.67 wt/vol%)	14 mls
NH ₄ Cl	0.825 gms
Mono potassium phosphate (KPO ₃)	0.15 gms
Sulfur-free Wolin Salts	10 mls
Tap water to volume	~3676 mls
Final volume	1000 mls

Sampling of the ICB feeds, contents, and effluents occurred in two stages. The first stage was the ramp-up period in which only in-house process monitoring parameters were measured. In-house measurements included bench-top analysis for chemical characteristics using a Hach⁵ kit. Standard methods⁶ for wastewater analysis were used for feed and effluent solids measurement. In-house process monitoring included the following analyses:

- COD, Hach method 8000, Reactor digestion method
- Ammonia (NH₃), Hach method 10030, Salicylate method(NH₃-N)
- Phosphate (PO₄), Hach method 8178 (orthophosphate) amino acid method
- Total suspended solids (TSS), Method 2540 D
- Volatile suspended solids (VSS), Method 2540 E
- Total dissolved solids (TDS), Method 2540 C

Steady state sampling occurred after the ramp-up period was completed. The steady state period started when the ICB feed reached the test design strength of 300 ml HD hydrolysate per liter of feed at a 5-day HRT. Steady state sampling included the process monitoring analyses mentioned above as well as additional feed and effluent characterization analyses. The Analytical Chemistry Team (ACT) using HPLC analyzed TDG concentration during the steady state period in-house.

The bacterial inoculum for the test was activated sludge obtained from the Back River Wastewater Treatment Facility⁷. Each cell of the reactor was given 35 ml of the concentrated sludge. The cells were filled to ½ capacity with tap water prior to starting addition of the biofeed.

RESULTS

CHEMICAL OXYGEN DEMAND

COD is a measure of the chemically oxidizable compounds in an aqueous sample. COD was one of the major process parameters used to measure the overall system effectiveness in treating the combined HD/tetrytol hydrolysates. The major sources of COD in the feed are TDG and other organic hydrolysis products. Because COD analysis is inexpensive, has a quick turn-around time, and it can be done as a process monitoring sample, it was used as a primary indicator of the biomass health and performance throughout the test. TDG analysis of steady state samples was also performed and the results are presented later in this report.

After the initial batch operating period, the initial feed strength in continuous mode was 1/8th the design strength. The feed strength was adjusted as the biomass grew and became acclimated to the feed, as indicated by COD removal. The COD concentration was routinely measured in the feed, in Cell A, and in the effluent of each ICB. The COD removal efficiency was calculated as follows:

$$\text{COD}_{\text{REMOVAL EFF, \%}} = [(\text{COD}_{\text{input, mg/Day}} - \text{COD}_{\text{output, mg/Day}}) / \text{COD}_{\text{input, mg/Day}}] * 100$$

During the ramp-up phase, the strength of the ICB feed was adjusted by diluting the full-strength feed with tap water. The ICB biofeed COD during ramp-up and COD consumption for both the unfiltered and filtered feed ICBs are represented in figure 1.

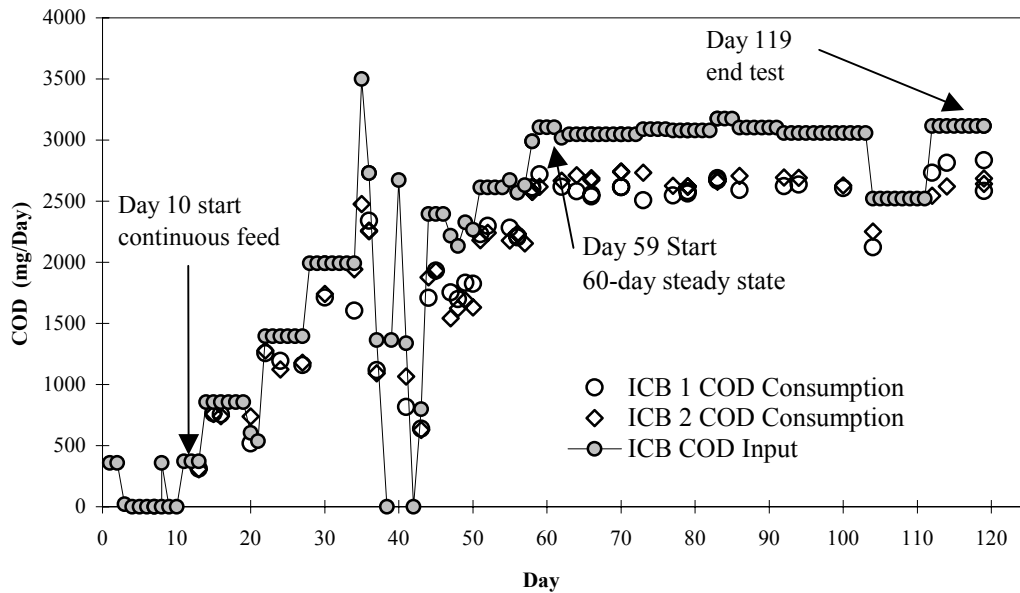


Figure 1. Ramp-up of the COD input and COD consumption in the ICB's during the study.

The quantities of COD removed, or consumed, in the ICBs per unit volume of ICB were also calculated. COD consumption is calculated as:

$$\text{COD consumption} = \text{COD}_{\text{input (mg/day)}} - \text{COD}_{\text{output (mg/day)}}$$

The values calculated from the outfall for ICBs 1 and 2 are presented in table 2. No difference in consumption between the unfiltered and filtered feed is apparent. During the ramp-up period consumption dropped on days 38 and 40 when the feed was stopped due to elevated COD in one cell of ICB 1. These results are useful for comparison to previous studies using reactors of different size and type. Normalized data for comparison are presented later.

Table 2. Summary Values Of ICBs Feed And Effluent COD And COD Removal Efficiency

Parameter	ICB1 Feed COD (mg/L)	ICB 1 Effluent COD (mg/L)	COD (% rem.)	ICB 2 Feed COD (mg/L)	ICB 2 Effluent COD (mg/L)	COD (% rem.)
Mean	12027	1758	85.51	12207	1763	85.8
Min	10000	1110	80.89	10480	1520	83.4
Max	12600	2310	90.82	12710	2080	87.9
Std-D	883	301	2.49	608	144	1.3

THIODIGLYCOL

Thiodiglycol is the principle organic compound in the HD hydrolysate. Once the ICB biomasses reached steady state the ICB biofeeds were sampled twice per 4-L feed batch. Field duplicates and effluent composite samples were also taken during the steady state period. The effluent from each ICB was sampled for TDG four times per feed batch. A summary of all TDG results is presented in Table 3. These data include results from field duplicates and effluent composite samples.

Table 3. Summary of TDG results from Steady State Operation

Sample Location	Mean (mg/L)	Min (mg/L)	Max. (mg/L)	Std-D (mg/L)	Number of Observations	Number BDL
ICB 1 unfiltered (HD hydrolysate)						
Feed	5592	4205	7614	659	12	---
Effluent	56.8	BDL	193	61.0	22	9
ICB 2 filtered (HD hydrolysate)						
Feed	5416	4108	6373	585	12	---
Effluent	37.5	BDL	202	54.7	24	11

BDL = below detection limit (1.0 mg/L).

BDL results were treated as zero in the statistical summary.

IMPACT OF HD HYDROLYSATE SOLIDS

The biofeeds of the two ICBs differed in the amount of solids that were in the HD hydrolysate from hydrolysis of the HD heel material. The feed in ICB 1 contained a representative quantity of heel from

the actual HD hydrolysate. The hydrolysate used to make ICB 2 biofeed was settled prior to mixing the biofeed. The TSS values measured in the biofeed and effluents for the two ICBs during the steady state period are summarized in table 4. Results of combined TSS data from the 60-day steady state operation period were analyzed using the student t-test to determine any statistical differences between input and output solids concentrations from the ICBs. The student t-test indicates a significant difference between the TSS values measured in the biofeed between ICBs 1 & 2, at the 95% confidence level. TSS values were significantly higher in ICB 1 biofeed than in ICB 2 biofeed.

Table 4. Summaries Of ICB Feed And Effluent TSS Values

	ICB 1 Feed TSS (mg/L)	ICB 1 Effluent TSS (mg/L)	ICB 2 Feed TSS (mg/L)	ICB 2 Effluent TSS (mg/L)
Mean	409	1062	272	620
Min	208	134	59	158
Max	700	3734	681	1563
Std-D	166	1046	205	366
count	11	34	11	34

Statistical analysis indicated there was no significant difference in the TSS of the two reactor effluents at the 95 % confidence level. Any difference in the biofeed TSS attributable to the hydrolyzed heel material was not apparent in the TSS of the reactor effluents. Perhaps this difference is masked by the increase in biomaterial in the effluent. If this is the case we cannot prove it from this test. There is no net effect observed by the addition of hydrolyzed heel material on the ICB effluent total suspended solids. There is no significant statistical difference in effluent TSS between ICB 1 and 2.

THIODIGLYCOL DESTRUCTION

The ECBC Analytical Chemistry Team (ACT) measured TDG in reactors biofeed and effluent during the 60-day steady state period. The TDG concentrations reported during the steady state operations were compared using the student t-test. Data used included only data points from the scheduled sampling events. Field duplicated and composite samples are not included in this analysis.

Results of the student t-test of the biofeeds from the 60-day steady state period using ACT reported TDG concentrations indicate no significant statistical difference between TDG in the two ICB biofeeds at the 95% confidence level.

Results of the student t-test comparing ICB 1 and 2 effluent TDG concentrations indicate no significant statistical difference between the two effluent streams. There is no difference observed in the ICBs ability to remove TDG whether heel material is added to the biofeed or not. There is therefore no effect on TDG degradation from inclusion of hydrolyzed heel material in the ICB biofeed in this study.

Analysis of the ICB effluents detected TDG on several sampling events during the study. Even though there was TDG in the ICB effluents, the ICBs performed quite well when fed hydrolyzed mustard from actual chemical rounds. When compared to previous studies using the pilot-scale 1000-gallon ICB, the lab scale systems consumption was higher on a per liter working volume basis. A brief comparison of the COD and TDG consumption between pilot and lab scale ICBs is presented in table 5. COD and TDG

values are normalized to reflect TDG input/output per day per liter of reactor volume. Even though there is some TDG breakthrough in the ICB-PWS, the mean detected TDG level over the duration of the study is low. The calculated TDG consumption rate for the ICB-PWS compared favorably to Demo I, and EDS testing. Operational optimization and reactor configuration may be able to eliminate effluent TDG in a scaled-up ICB. TDG breakthrough may also be eliminated by decreasing the loading in the biofeed or increasing the hydraulic residence time.

Table 5. Comparison Of COD And TDG Consumption Between The Laboratory And Pilot Scale ICBs

Test ICB	COD Input (mg/Day/L)	COD Output (mg/Day/L)	COD Removal efficiency	TDG Input (mg/Day/L)	TDG Consumption (mg/Day/L)
PWS ICB 1 (Unfiltered HD hydrolysate)	2374.8	348.9	85.5	1141	1128
PWS ICB 2 (Filtered HD hydrolysate)	2411.0	349.8	85.6	1092	1084
Demo I ICB	1297.6	115.5	91.1	612	612
EDS ICB	2266.0	216.6	90.4	1069	1069

CONCLUSION

From section 1 of this report, the specific objectives of this test were:

- Confirm the ability of the laboratory-scale ICBs to effectively treat the PWS-generated HD hydrolysate at the hydraulic residence time (HRT) that is representative of full-scale design.
- Assess the impact of suspended solids in HD hydrolysate on ICB performance.
- Confirm the ability of the laboratory-scale ICBs to eliminate thiodiglycol (TDG) in the HD hydrolysate.
- Characterize ICB effluents.

The ICBs were able to treat the HD hydrolysate generated from the projectile washout study. Even though some TDG was detected in the reactor effluents, the reactors were able to treat the HD hydrolysate at unit loadings higher than in Demo 1 and EDS pilot-scale testing.

The hydrolyzed heel (solids) material present in the HD hydrolysate was removed from the biofeed from ICB 2. Heel material was left in the biofeed to reactor 1 at the same concentration as in the HD hydrolysate. Statistical analysis of the TDG detected in the effluent streams from the two reactors indicates no significant difference in performance from the addition of the hydrolyzed heel material.

While TDG was detected in the effluents from both reactors at various times, the overall average TDG removal efficiencies were still greater than 89 percent. ICB TDG consumption rates were comparable to EDS testing using HD hydrolysate from ton containers, which is considered a cleaner, and more easily degradable food source for the ICB culture. Regular elimination of TDG to non-detect levels should be attainable through adjustment of feed loading and reactor design and operational optimization in a larger bioreactor system.

The ICB biofeed and effluents underwent extensive testing for chemicals of concern. Many of the chemical compounds of interest were below detectable limits. These data from effluent characterization

and analysis for chemicals of interest will be available in a technical report in printing or from PMACWA⁸.

ACKNOWLEDGEMENTS

The authors wish to thank PMACWA for funding this study and Mr. Joe Mashinski, PM ACWA for his considerations during the study. We also thank Mr. Jim Earley and Mrs. Janet Scanlon for their assistance during the study.

REFERENCES

1. Guelta, M.A, Chester, N.A., Lupton, F.S., Koch, M., Fry, I.J., Kim, M.H., *Biodegradation of HD and Tetrytol Hydrolysates in a Pilot Scale Immobilized Cell Bioreactor*, ECBC-TR-192, U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, July 2001
2. Pueblo Chemical Depot, Pueblo Colorado
3. Parsons/Honeywell, 50 East Algonquin Rd, Box 5016, Des Plaines, Illinois, 60017-5016
4. Applikon Biotechnology, P.O. Box 4014, Clinton, N.J., 08809-4014
5. Hach Co. P.O. Box 389, Loveland Colorado, 80539-0389
6. *Standard Methods for the Examination of Water and Wastewater*, APHA, 18th Ed. American Public Health Assoc., Washington, D.C. 1992
7. Back River Waste Water Treatment Plant, Eastern Avenue, Baltimore MD.
8. PMACWA, Aberdeen Proving Ground, MD 21010