Biodegradation **15:** 41–48, 2004.

Detection and decontamination of residual energetics from ordnance and explosives scrap

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Accepted 3 October 2003

Key words: anaerobic bioslurry, ion mobility spectrometer, ordnance and explosives, 2,4,6-trinitrotoluene (TNT)

Abstract

Extensive manufacturing of explosives in the last century has resulted in widespread contamination of soils and waters. Decommissioning and cleanup of these materials has also led to concerns about the explosive hazards associated with residual energetics still present on the surfaces of ordnance and explosives scrap. Typically, open burning or detonation is used to decontaminate ordinance and explosive scrap. Here the use of an anaerobic microbiological system applied as a bioslurry to decontaminate energetics from the surfaces of metal scrap is described. Decontamination of model metal scrap artificially contaminated with 2,4,6-trinitrotoluene and of decommissioned mortar rounds still containing explosives residue was examined. A portable ion mobility spectrometer was employed for the detection of residual explosives residues on the surfaces of the scrap. The mixed microbial populations of the bioslurries effectively decontaminated both the scrap and the mortar rounds. Use of the ion mobility spectrometer was an extremely sensitive field screening method for assessing decontamination and is a method by which minimally trained personnel can declare scrap clean with a high level of certainty.

Abbreviations: DAC – Defense Ammunition Center; DAD – diode array detector; HPLC – high performance liquid chromatography; IMS – ion mobility spectroscopy; OE – ordnance and explosives; ORP – oxidation-reduction potential; RDX – hexahydro-1,3,5-trinitro-1,3,5-triazine; TAT – 2,4,6-triaminotoluene; TNT – 2,4,6-trinitrotoluene; PETN – pentaerythritol tetranitrate; Tetryl: 2,4,6-trinitrophenyl-n-methylnitramine

Introduction

TNT (2,4,6-trinitrotoluene) has been a widespread, significant environmental contaminant since the extensive manufacture of ordnance and explosives for World War I and II (Kaplan & Kaplan 1982; Rieger & Knackmuss 1995; Wikström et al. 2000). Remediation of explosives compounds has often focused on TNT because it is the most prevalent and persistent of the nitroaromatic explosives in the environment (Bhadra et al. 1999). Numerous abiotic variables determine the fate of TNT in the environment (Elovitz & Weber 1999; Gordon & Hartley 1992; Hoffstetter et al. 1999; Weissmahr et al. 1999). Likewise, biotic transformations of TNT depend upon a variety of factors such as the availability of carbon and energy sources, types of organisms involved, and oxygen concentration within the contaminated site (Rieger & Knackmuss 1995; Shen et al. 2000). Biotic transformations may also, in turn, significantly influence abiotic processes like the binding of TNT metabolites to organic matter and soil particles (Achtnich et al. 1999, 2000; Achtnich & Lenke 2001; Lenke et al. 1998).

Numerous chemical and physical treatment technologies have been proposed to reduce or remove hazardous energetic organonitro compounds from the environment (Kaplan 1990). Microbiological treatment technologies have also been employed and include denitrification (Kaplan 1990), batch and continuous fermentation systems (Daun et al. 1998; Funk et al. 1995a, b; Lenke et al. 1998; Razo-Flores et al. 1997) and composting (Funk et al. 1995a; Isbister et

al. 1984; Tuomi et al. 1997; Williams et al. 1992). Microbiological treatment is often desirable, due to relatively low cost compared to chemical or physical treatment technologies and the innocuous nature of typical end products, i.e., CO_2 and H_2O or non-toxic polymers (Emery & Faessler 1997; Funk et al. 1995b; Lenke et al. 1998).

The majority of these treatment technologies have targeted contaminated soil or water; however, other materials contaminated with explosive residues also exist. Demilitarization programs have produced large amounts of ordnance and explosives (OE) scrap metal contaminated with residual energetic compounds. OE scrap is defined as ammunition, ammunition components, chemical or biological warfare material; the exact amounts of which are unknown because Department of Defense record controls no longer account for these materials (http://www.poh.usace.army.mil/waikoloa/EECA/Ch 12.pdf). Typically, when OE scrap can be safely moved to another site, a temporary open burn and open detonation site is established, or a burn-in-place procedure is initiated if the OE scrap cannot be safely moved (http://www.tourtelotcleanup.com). Residual explosives residues are then removed by incineration or chemical treatment. Ultimately, this scrap may be destined for recycling or entry into a waste stream if it is certified to be clear of residual energetics (http://www.cr.nps.gov/nagpra/graphics/bases.pdf). However, high levels of residual explosives on incinerated OE scrap may result in explosions, presenting obvious danger to operators as well as the potential for equipment and resource destruction. Increasing public concern and the Clean Air Act Amendments of 1990 also continue to severely restrict or eliminate the use of incineration and open burn and open detonation of energetic materials (Defense Ammunition Center 2000). The limited available public information on the decontamination of OE scrap contains only the descriptions of chemical and physical methods, while microbiological decontamination methods have not been attempted on OE scrap, although they may be safer and cheaper.

The goals of the present research were to demonstrate the effectiveness of bioremediation for cleaning OE scrap and develop and test a field portable method to determine the cleanliness of treated OE scrap materials. The US Army Corps of Engineers had set forth two stipulations for field portable detection methods. First, it was to be easily used by any member of a demilitarization team. Second, the method was to be sensitive enough that members of a demilitarization team could pronounce with statistical confidence that the OE scrap was clean to specific standards. Since this method would potentially be used to determine the cleanliness of the scrap before shipment for recycling, development and implementation of this method was critical to acceptance of bioremediation as an alternative to currently used methods.

Three field portable technologies are available for explosive residue detection. Ion mobility spectrometry (IMS) is a field portable explosives detection technology most commonly employed in airports to detect explosive residues on luggage. IMS is highly sensitive and can specifically detect more than one type of explosive compound at the same time. A drawback to this technology is the initial capital outlay needed to purchase a portable IMS unit (approximately $20,000). Two other field portable techniques, immunoassays (Crockett et al. 1996; Heiss et al. 1999; Higson 1992; Patra & Mitra 1979) and colorimetry (Crockett et al. 1996) were considered, although not used in this study primarily due to interferences experienced in soil systems.

Here we report research to first, determine if a commercially available, anaerobic biological reactor technology could be used to clean residual energetics from OE scrap and second, to develop and implement a field portable explosives residue detection technique that could be used by demilitarization personnel to determine the cleanliness of the OE scrap post-treatment.

Materials and methods

Establishment of test reactors

Small-scale bench reactors were used as models for field-scale anaerobic treatment systems. A mineral salts medium was employed for every experiment and contained the following per liter at a pH of 7.2: 13 g K_2HPO_4, 4 g KH_2PO_4, 0.1 g NH_4Cl, 2 ml $MgSO_4$ (1M), 6 g molasses (Grandma's unsulfured). The medium (2.5 L) was added to tall, 2.5-L Pyrex beakers with the tops open to the air. A soil inoculum of 0.2% (w/v) was added to the experimental beakers. Sterile controls contained the same mineral salts medium and molasses (which were autoclaved separately and then added together) but did not contain inoculum. The oxidation-reduction potential (ORP) of inoculated reactors was measured daily using a calibrated ORP

electrode (Microelectrodes, Inc., Bedford, NH 03110-6805). TNT was added at an appropriate concentration when measurements reached below −400 mv (∼24–96 h). ORP was measured throughout the experiment and found to remain below −400 mv during TNT degradation.

TNT transformation in test reactors

Six 2.5-L reactors were prepared with mineral salts medium. Three of these reactors were inoculated with 5 ml of an overnight culture from previously established reactors to minimize variability in the microbial communities during the various experiments. The remaining three reactors were left uninoculated and served as controls. TNT was added to all reactors at a final concentration of 125 ppm.

Degradation of TNT from the surfaces of model metal scrap and mortar rounds

TNT was added to small, fabricated metal scrap pieces to a final concentration of 20 mg TNT per metal piece. TNT was delivered to the metal in acetone solution and was evaporated onto the metal for 24 h at 55 °C. Ten metal pieces (1.25 cm^2 each) were then suspended with fishing line into each reactor, hanging from a dowel located across the top of the reactor.

Ten 2.5-L reactors were prepared with mineral salt medium. Half of the reactors were inoculated with 5 ml of an overnight culture, and the remaining five reactors were established as uninoculated controls. The metal pieces were randomly sampled from the reactors at various time points. Each metal piece was submerged in a capped test tube containing 5 ml high performance liquid chromatography (HPLC)-grade methanol and sonicated for 1.5 h. One ml of the methanol solution was then filtered into an HPLC vial and analyzed by HPLC. Liquid medium samples were taken directly from the middle of the reactors, and 1 ml from each was filtered into an HPLC vial for HPLC analysis.

Eighty-one-mm mortar rounds that were visibly contaminated with an unknown amount of Composition B (Comp B), a mixture of TNT and RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), were received from the Defense Ammunition Center (DAC, McAlester, OK). Two of the rounds were submerged in 20-L reactors containing mineral salts medium; one reactor was inoculated with 2.5 g of soil, and the other was established as an uninoculated, sterile control. The degradation of TNT in the medium was analyzed with HPLC and verified with gas chromatography-mass spectrometry (GC-MS).

HPLC analysis

Reverse phase HPLC was used to monitor the removal rate of TNT and the presence of TNT metabolites during the experiments. A Hewlett Packard Series II 1090 Liquid Chromatograph with a diode array detector (DAD) equipped with a Phenomenex Luna 3 μm 150 × 4.60 mm phenyl-hexyl column was used. The HPLC was operated in a gradient mode where initial fractions of 70% H_2O: 30% methanol was ramped to 100% methanol over 20 min. This level of 100% methanol was held for 15 min at a constant flow rate of 0.85 ml/min. The oven temperature was maintained at 40 °C. The DAD was set at 254 nm and 219 nm, both with reference peaks at 550 nm. The injection volume of the sample was 25 μl with a draw speed of 83.3 μl/min.

GC-MS analysis of TNT and its metabolites

Samples (25 ml) for GC-MS analysis were taken from reactors using sterile pipettes and placed into 50-ml Falcon tubes. An extraction with 15 ml CH_2Cl_2 was performed, and the organic layer was separated. The aqueous layer was acidified to pH 2 with 12N HCl and extracted with 15 ml CH_2Cl_2, again, separating out the organic layer. The resultant 30 ml organic fraction was then dried under N_2 flow, dissolved in 2 ml CH_2Cl_2, and filtered into a glass HPLC vial.

A HP 5890 Series II gas chromatograph equipped with a HP 6890 Series autoinjector and a Phenomenex ZB5 30 m capillary column (0.25 mm × 0.2 μm), coupled with a HP 5989A mass spectrometer was employed for analysis of metabolites. Injector temperature was set at 250 °C, and the initial oven temperature was set at 100 °C. The temperature ramp was set at 9 °C/min to 200 °C, then 5 °C/min to 300 °C. A HP quadrupole MS (5989A) controlled by HP MS Chemstation software (PC version) was used for MS and analysis under the following conditions: repeller, 7 V; emission, 300 V; electron energy, 70 eV. The source temperature was 250 °C, and the quadrupole temperature was 125 °C. The scan parameters were 30 to 350 or 30 to 750 m/z. Interpretation of the MS spectrum was aided by the Wiley and National Institute of Standards and Technology library of mass spectra stored in the Chemstation database (approximately 200,000 spectra). Three μl of sample were injected. Standard curves were generated for TNT

and the three predominant metabolites shown in Figure 2 and compared against the resultant peak areas calculated from each of the samples.

Determination of a field portable method to be employed during full-scale applications

A colorimetric assay was tested in our lab for use with metal scrap. Surfaces to be tested were wiped with ethanol-soaked, cotton-tipped swabs. The swabs were placed in 5 ml of 95% ethanol and used to agitate the solution. Approximately 0.5 mg of $NaBH_4$ was added to the ethanol solution, which was mixed and resulting colored solution was observed immediately. The time between the addition of $NaBH_4$ and observation was critical since the reaction colors faded with time.

A SABRE 2000 IMS unit (Barringer/Smiths Detection, Warren, NJ) was also examined as a possible explosives detection device for use in the field. Model metal pieces were contaminated with specific amounts of TNT using an acetone solution that was evaporated onto the metal for 24 h at 55 °C. The total surface of each metal piece was approximately 1.25 cm^2, and the contamination levels ranged from 5 ng to 5 μg per surface area. To detect samples of TNT from the surface of the metal pieces, a few drops of acetone were placed on IMS sampling swabs provided by the manufacturer, the metal surface was wiped three times, and the swab was placed inside the sampling port of the IMS. When explosive residues were detected, an alarm sounded and the signature peak and name of the compound was listed.

Results

Transformation of TNT in test reactors

TNT was added to each of the reactors, which were then sampled at various time points and monitored with GC-MS. TNT was found in notably lesser concentrations within 3 h of its introduction into established, inoculated reactors, as compared to control reactors where most of the TNT was still present (125 ppm) (Figure 1). Within 18 h, the TNT concentration was negligible (less than 3 ppm) in the inoculated reactors, but still within the 125 ppm range in controls (Figure 1).

Analysis of the degradation products of TNT was also performed using GC-MS (Figure 2). TNT was added to the medium at a final concentration of 125 ppm, which is above its limit of solubility in water

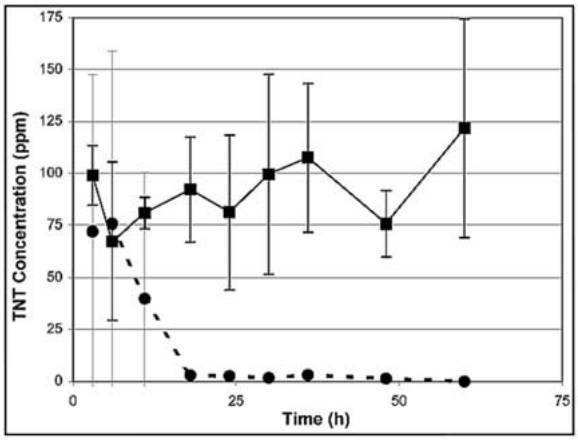

Figure 1. Analysis of TNT degradation from liquid medium containing 125 ppm TNT using GC-MS. Error bars represent ± one standard deviation of three replicates. Symbols: inoculated (dashed line), sterile control (solid line).

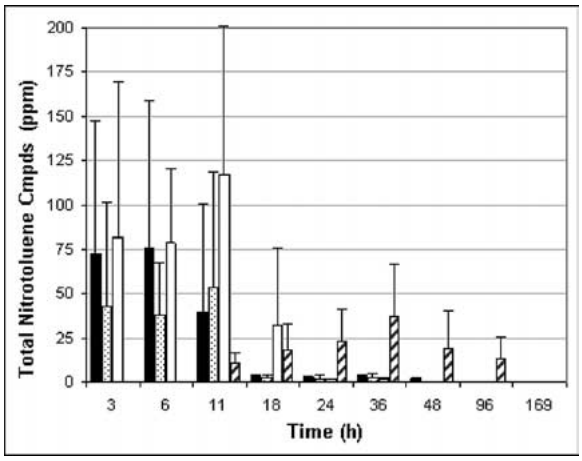

Figure 2. Analysis of TNT degradation and transformation from liquid containing 125 ppm TNT using GC-MS. Error bars represent ± one standard deviation of three replicates. Symbols: TNT (■), 2-amino-4,6-dinitrotoluene (⊡), 4-amino-2,6-dinitrotoluene (□), 2,4-diamino-6-nitrotoluene (◪).

(85-100 ppm), in order to provide for a time released effect of the TNT and allow for easier analysis of transformation products. Unfortunately, insoluble TNT in the medium caused interferences in the extraction process, ultimately altering the stoichiometric relationship between TNT and its transformation products. Consequently, mass balances were not performed. TNT was transformed within 3 h of its introduction into established, inoculated reactors. The typical reduction products of monoamino-dinitrotoluenes were formed first, followed by the formation of 2,4-diamino-6-nitrotoluene. Only mono- and diamino-nitrotoluene compounds were detected in the estab-

lished, inoculated reactors within 18 h (Figure 2). By 169 h, neither TNT nor the predominant transformation products were detectable. The potential intermediate 2,4,6-triaminotoluene (TAT) was never detected, although control studies using TAT standards in aqueous systems demonstrated that our extraction methods were appropriate for detecting TAT (data not shown). Degradation or transformation products were never detected in control reactors (data not shown).

Decontamination of model metal scrap contaminated with TNT

The data (Figure 3) display the general trend that TNT initially dissolved from the metal into the surrounding liquid medium. In control reactors the aqueous TNT concentration climbed steadily as it solubilized from the surfaces of the metal pieces over time, whereas in inoculated reactors once the TNT dissolved into the medium, it was degraded or transformed. TNT was detected at negligible levels from metal pieces (less than 0.5 mg) and the surrounding medium (less than 0.5 ppm) of inoculated reactors by 15 h yet was found at elevated concentrations in control medium (Figure 3). The 0 h metal extraction, which was contaminated with 20 mg TNT, yielded a reading of only 10 mg TNT, indicating that the limit of TNT solubility in methanol for our extraction conditions had been reached. We chose to disregard this limitation and proceeded, since dissolution into the liquid medium occurred at such a fast rate and values under 10 mg were previously shown to be dependable (data not shown).

Decontamination of 81-mm mortar rounds in test reactors

The 81-mm mortar rounds received from DAC were considered clean enough for incineration (DAC, pers. comm.), yet there was visible explosives residue on both the lips and on internal surfaces of the rounds (Figure 4). After introduction into bioreactors, mortar rounds were examined visibly every week to see if explosives residue was still evident on or in the rounds. TNT was not detected in the liquid medium of the inoculated reactor after 3 months (Table 1), at which time the explosives residue was visibly absent. Thus, the bench-scale system was successful at eliminating dissolved Comp B. The limiting step of degradation appeared to be the dissolution of the large masses of Comp B into the medium.

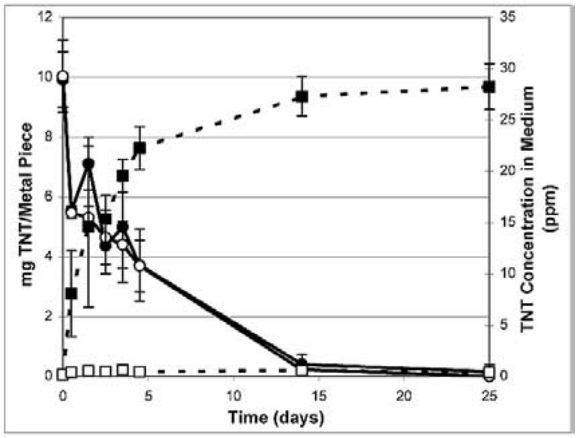

Figure 3. Analysis of TNT degradation from TNT-contaminated metal scrap (solid lines) and surrounding liquid medium (broken lines) using HPLC. Error bars represent ± one standard deviation of five replicates. Symbols: sterile control (●, ■), inoculated (○, □).

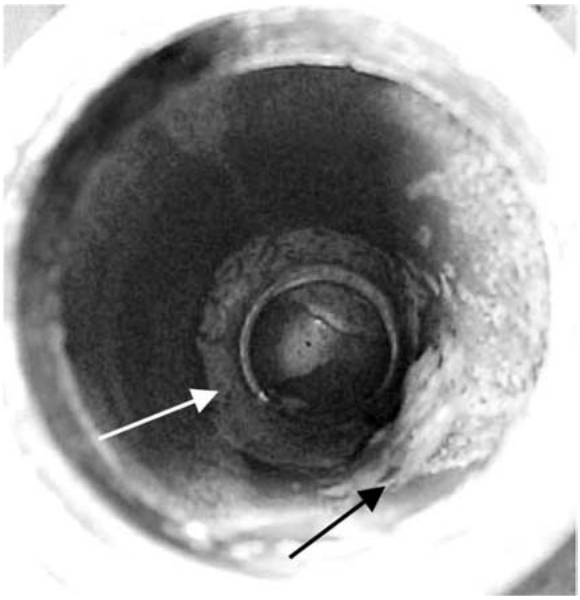

Figure 4. Top-down view of 81-mm mortar round before microbiological treatment. Arrows point to visible pieces of Comp B explosive residue still remaining after bulk cleaning on-site.

Table 1. Analysis of TNT degradation from reactor medium containing Comp B contaminated 81-mm mortar rounds using HPLC

	TNT concentration (ppm)	
Time (days)	Sterile Control	Inoculated
7	9.38	0.78
42	69.64	0
84	66.82	0

Examination of field portable explosives detection methods

The immunoassay method was not tested due to previously documented difficulty in using this assay in soil or mixed systems and because of its high cost (Heiss et al. 1999). The colorimetric method for TNT detection was extremely easy to perform and interpret in non-metal, control experiments. However, rust and particulate matter from even relatively clean metal scrap pieces caused interferences that made this method too inconsistent and the results unreliable (data not shown). Therefore, we chose to investigate the semi-quantitative, easy to use, and highly reliable technique of IMS. The sensitivity and utility of IMS in measuring TNT on metal surfaces were examined (Table 2). The IMS consistently (triplicate samples) detected and sounded an alarm for TNT from model metal scrap samples contaminated in the 250- to 5,000-ng range, although the instrument could be fine-tuned to detect and alarm at even lower concentrations. Under the conditions in the lab, we did observe peaks corresponding with the retention time of TNT when the 5-ng samples were swabbed, but the instrument was not able to identify the peak as TNT due to background interferences. In comparison, the lowest quantity detectable with extraction and subsequent HPLC analysis was approximately 100 μg/metal piece (data not shown), approximately 200 times greater than the level detected by IMS in these experiments.

A final laboratory experiment was conducted on one of the demilitarized 81-mm mortar rounds received from DAC. Samples swabbed on the inner and outer surfaces of the mortar round and analyzed by the IMS revealed the presence of TNT and RDX, which indicated that Comp B residue still remained on this round.

Use of IMS to test "clean" munitions at Jefferson Proving Ground (Madison, IN)

Pilot-scale tests were undertaken to determine the feasibility of using the portable IMS to detect the level of contamination on actual OE scrap at a military site. Most of the scrap found at Jefferson Proving Ground (Madison, IN) was assumed to be clean due to the nature of operations at the site. The scrap had been recovered from an impact area of the firing range and was stacked in a storage shed. Scrap metal pieces, primarily mortar rounds of various sizes, were randomly gathered and analyzed using the swab method discussed above on both the internal and external surfaces. Of the 137 samples analyzed, 14 scrap pieces were found to have some level of contamination with explosives residue (Table 3).

Table 2. IMS bench-scale analyses of TNT-contaminated scrap metal pieces. + = signature peak detected and/or alarm sounded; – = no peak detected or alarm sounded; +/– = inconsistent positive alarm

Sample (ng/metal piece)	TNT signature peak found	Alarm at TNT detection
5,000	+	+
2,500	+	+
1,000	+	+
500	+	
250	+	+/–
125	+	+/–
75	+	–
50	+	–
25	+	–
10	+	–
5	+	–
0	–	–

Table 3. IMS analyses of OE scrap metal positive for explosive residue(s). Lower detection limits established at the ppb level

Sample(s)	Signature peak found	Relative amount
Large mortar	TNT	Low
2 Large mortars	TNT	Trace
3 Large mortars	TNT	High
9" mortar	TNT	Trace
9" mortar	TNT	High
Random scrap	PETN	Trace
Random scrap	RDX	Trace
Random scrap	Tetryl	Trace
Large shell (30")	TNT	High
Large shell (30")	TNT/PETN	High/Trace
Large shell (30")	TNT/PETN	High/Trace

Discussion and Conclusions

This project aimed to develop an inexpensive, effective method to remove residual energetics from the surface of OE scrap as an alternative to incineration

or chemical treatment processes (Emmrich 1999; Li et al. 1997; Rho et al. 1998). We investigated use of a microbiological variation of a bioslurry process designed to support a combination of aerobic, and preferentially, anaerobic microbial communities. This process has been described in detail in an EPA Superfund Innovative Technology Evaluation (SITE) report, where it achieved 99% treatment efficiency after 9 months, based on a starting TNT concentration of 1,500 ppm (U.S. Environmental Protection Agency 1995). Another report has shown 99% treatment efficiency after 40 days when the TNT concentration was <500 ppm (Tuomi et al. 1997). Although the anaerobic process is a proven TNT remediation technology, its utility in remediating explosives residue from contaminated metal surfaces has not been previously examined.

Overall, the bench-scale anaerobic bioremediation system presented here was extremely successful at eliminating dissolved TNT from aqueous solutions during all experiments. The limiting step in the degradation of these compounds from metal surfaces appeared to be the dissolution of the solid compounds from the metal into the liquid phase. However, solubilization in the field may be increased by the use of detergents, active mixing, or sonication, which would increase the rate of TNT dissolution. Alternatively, a consortium might be grown as a biofilm on the surfaces of scrap.

Thus, the anaerobic system may be a viable alternative strategy to circumvent the substantial cost and hazards associated with physical or chemical treatment technologies such as incineration, which is currently used to decontaminate OE scrap metal. Our bench-scale studies using both model metal pieces and decommissioned mortar rounds demonstrated the ability of the anaerobic bioslurry process to transform explosives into less hazardous compounds. However, this process may have limitations with respect to OE scrap until the low dissolution rate problem can be addressed during the engineering of a full-scale system. Further, full-scale tests on decommissioned or exploded OE scrap are still needed to validate process effectiveness.

This project also aimed to find and develop an on-site, field portable method to detect explosives residues on OE scrap. On-site analytical methods are generally more cost efficient because shipping costs and safety concerns are not incurred, numerous samples may also be run, and the investigation can be directed to areas of concern without significant waiting periods (Crockett et al. 1996). While the use of immunoassay and colorimetric techniques does not seem reasonable for use in soil or mixed systems such as bioslurries, the use of a portable IMS in the field for detection of various explosives residue on OE scrap appears promising. Laboratory studies using metal scrap pieces contaminated with known concentrations of TNT established the lower limit of detection at 5 ng/cm^2. IMS field tests on random OE scrap suspected to be free of explosives residue were also successfully performed. The ease of use, sensitivity, reliability, and semiquantitative nature of the instrument makes this an incredibly appealing tool for the screening and detection of explosives residue on OE scrap in the field. However, more field studies are needed to fully validate this technique.

This research confirms the effectiveness of anaerobic bioslurries as a bioremediation technology for the cleaning and decommissioning of OE scrap. When coupled with the use of the IMS explosives monitoring and detection technology, it should be possible to successfully apply this remediation system in the field. Thus, additional study of this alternative technology is merited, preferably in the field using well-mixed bioreactors at full-scale.

Acknowledgements

The authors would like to thank Dr. Dave Atkinson from the Idaho National Engineering and Environmental Laboratory (INEEL), Jefferson Proving Ground, Madison, IN, and Nicole Gardner and Sheri Wardwell. We would also like to thank Cornelia L. Sawatzky for assistance in manuscript preparation. This work was funded by the U.S. Army Corps of Engineers, Cooperative Agreement DACA87-00-H-0023; Cooperative Agreement to Conduct a Study Testing the SABRE Process for Removing Residual Energetics from Ordnance and Explosives Scrap.

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