Treatment of Hydrolysates of the High Explosives Hexahydro-1,3,5-Trinitro-1,3,5-Triazine and Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine Using Biological Denitrification

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ABSTRACT: Alkaline hydrolysis byproducts of the high explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), consisting of acetate, formate, formaldehyde, and nitrite, were treated in a denitrifying (anoxic) packed-bed upflow reactor. Additional nitrite or nitrate was added to match the carbon oxidation requirement. In a 2-year study, more than 90% removal of the organic compounds and nitrite were observed in a reactor with a 3-hour retention time. Removal was quantified by measuring actual compound concentrations and total organic carbon. The stoichiometry of the experimental results closely matched the predicted stoichiometry. Formaldehyde and acetate were preferentially removed over formate. The system removed N (nitrite):C (acetate):C (formaldehyde):C (formate) in a relative ratio of 1:0.07:0.36:0.50, respectively. The volumetric removal rate was as high as 170 mg/L of nitrite-nitrogen per day with existing carbon sources. The observed cell yield (mass basis) of nitrite reduction with acetate/formate was 0.21 mg cells/mg total organic carbon or 0.16 mg cells/mg chemical oxygen demand at 20 °C. This culture was also capable of biodegrading RDX and HMX when using nitrate as an electron acceptor. *Water Environ. Res.*, **71**, 148 (1999).

KEYWORDS: hydrolysate (hydrolysis byproducts), biological denitrification, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), acetate, formate, formaldehyde, nitrate, nitrite.

Introduction

Explosives have been manufactured in the U.S. and other countries for many decades. Among all the high explosives (HEs) that are manufactured hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and 2,4,6-trinitrotoluene (TNT) are the most common. The explosive RDX is classified as a possible human carcinogen (class C) by the U.S. Environmental Protection Agency (U.S. EPA) and has various effects on mammals, fish, and protozoa (McLellan et al., 1988a). The explosive HMX is produced with RDX and typically is found together with RDX as an environmental contaminant. It has adverse effects on the central nervous system in mammals, but at higher concentrations than RDX, and has been classified as a class D carcinogen by U.S. EPA (McLellan et al., 1988b). Both RDX and HMX are more energetic than TNT and have been used in both conventional and nuclear weapons.

The end of the Cold War created a worldwide surplus of both conventional and nuclear weapons. The U.S. and other countries are destroying large quantities of weapons, which creates the need for safe and reliable disposal technologies for energetic materials and wastewater associated with their processing. Bulk HEs were previously destroyed using open burning or detonation that can produce various unwanted toxic products, such as cyanic acid. Open detonation is not 100% efficient and explosives may be released to the environment with explosion byproducts.

In the past, wastewater containing RDX and HMX was often disposed in lagoons. This practice has sometimes resulted in soil and groundwater contamination. Therefore, environmental transformations of RDX and HMX and remediation methods have been extensively researched. Biodegradation of RDX and HMX to partially known byproducts under anaerobic or anoxic conditions have been investigated by McCormick et al. (1981) and in our laboratory (Alatriste-Mondragon, 1996; Chiou et al., 1997; and Hesselmann and Stenstrom, 1992). In spite of previous research, biological treatment of HE-containing wastewater is seldom practiced, and activated carbon adsorption is often used at munitions plants as well as groundwater remediation (Wujcik et al., 1992). Granular activated carbon (GAC) adsorption is effective in removing HE from contaminated water but produces HE-laden carbon. The spent carbon is a hazardous waste and, if allowed to dry, may become explosive.

Alkaline hydrolysis is a simple and effective process to convert RDX to smaller, less-harmful compounds such as acetate, formate, and nitrite (Spontarelli et al., 1993). The process is suitable for destroying bulk quantities; however, for dilute wastewater with relatively low RDX concentrations (RDX solubility is approximately 40 mg/L at 25 °C), this process is costly and ineffective. The cost of raising the pH and later neutralizing large volumes of water is prohibitive.

To overcome these costs, Heilmann et al. (1994) proposed a combined process that uses activated carbon adsorption and alkaline hydrolysis regeneration of the spent carbon. Adsorption concentrates the HE, which reduces the volume of material being treated to a manageable size. The combined process has all the advantages of carbon adsorption, and the waste carbon can be regenerated and reused. Furthermore, the regeneration process is relatively simple and may be performed without removing the

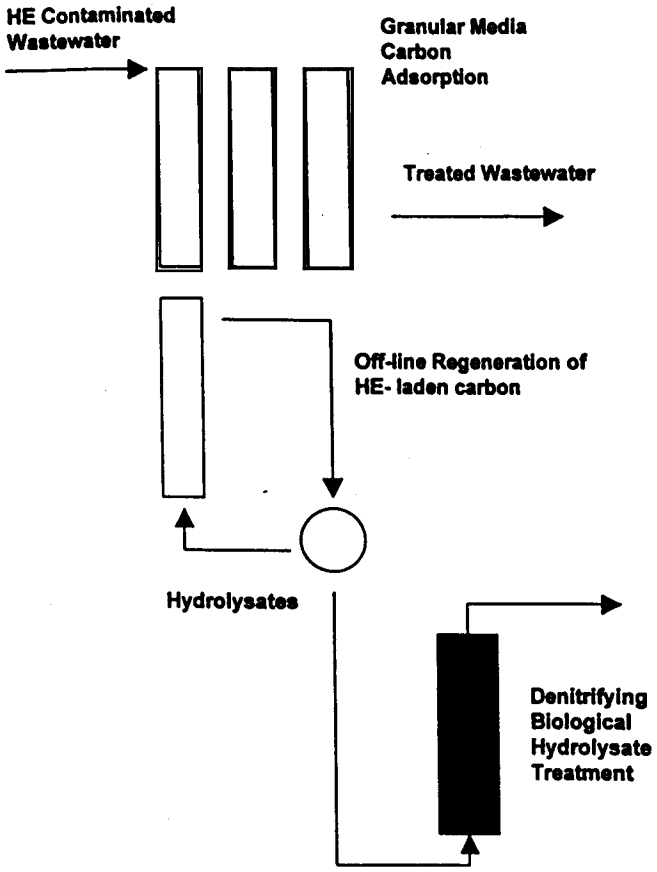

Figure 1—Treatment scheme for water and wastewater contaminated with high explosives (Heilmann et al., 1994).

carbon from the contactor. Figure 1 shows the proposed process. The HE-contaminated wastewater is first passed through granular activated carbon columns that dramatically reduce the volume of material to be treated. The laden carbon is treated with an alkaline solution that effectively regenerates it. Heilmann et al. (1996) found that alkaline hydrolysis of RDX yields 1.6 M nitrite (NO_2^-), 1.5 M formate ($HCOO^-$), 0.1 M acetate (CH_3COO^-), 1.1 M formaldehyde (HCHO), 0.9 M ammonia (NH_3), 1.1 M nitrous oxide (N_2O), and 0.34 M nitrogen gas (N_2) per 1 M of RDX hydrolyzed. Hydrolysis of adsorbed RDX proceeds at a slower rate than hydrolysis of dissolved RDX but produces the same byproducts. The explosive HMX hydrolyzes at slower rates than RDX but produces the same byproducts with slightly different stoichiometry.

Activated carbon adsorption with carbon regeneration using alkaline hydrolysis seems be an efficient and safe way to treat RDX- or HMX-containing wastewater; however, hydrolysates can contain high concentrations of acetate, formaldehyde, formate, ammonia, and nitrite. These hydrolysates cannot directly be released to the environment and require further treatment. The organic compounds will exert an oxygen demand on the receiving water and byproducts can have negative health consequences. Furthermore, nitrite probably could not be released because of its potential to contaminate groundwater. Nitrite can be oxidized to nitrate and both are important groundwater contaminants.

For these reasons it is necessary to develop a process to treat hydrolysates. An ideal process would remove both nitrite and organic compounds. Organic compounds are simple and quite amenable to biological degradation. The nitrite can be used as an electron acceptor. For these reasons, it was decided to demonstrate the effectiveness of a denitrifying treatment process. A synthetic wastewater identical in composition to RDX hydrolysates was used; it was not possible to use actual hydrolysate because safety in the laboratory (limited to several grams of explosives) would have been compromised with the required mass of explosives.

This paper describes the results of a 2-year investigation to demonstrate effective hydrolysate treatment. The process was developed in a stepwise fashion by gradually increasing the number of organic compounds and the mass of electron acceptor. Formate and acetate were first studied, and formaldehyde was added after obtaining stable conditions.

Methodology

Continuous Flow Experiment. Figure 2 shows the treatment scheme for water contaminated with hydrolysates of RDX. A continuous flow reactor constructed from a 200-mm acrylic column with an internal diameter of 25 mm, resulting in an empty bed volume of 98.17 mL, was used. The inlet and outlet to the reactor were equipped with stainless-steel screens to retain the packing material. This column was packed with 42 g of 2-mm diameter silicon tubing (size 13) cut to lengths of 1 to 2 mm. The column was sealed with Plexiglas end caps. Novel packing was used because it was difficult to find packing small enough from materials other than glass.

This column was inoculated with the mixed denitrifying cultures described later by injecting 1 mL of mixed culture into the lower quarter of the reactor, which had been prefilled with feed solution. Feed flow was initiated 2 weeks after inoculation to allow for microbial growth and attachment to packing material. The column was operated at room temperature (20 to 25 °C) in an upflow mode and fed by a cartridge pump. A single 2-L feed flask was used to supply the nutrients spiked with hydrolysates. Feed solution was mixed in this flask every other day. Influent was pumped to the reactor using a multicartridge peristaltic pump using Tygon tubing. A rubber stopper was used to prevent the transfer of oxygen to the feed. Nitrogen gas was used to purge the feed flask to further reduce the entry of oxygen to the reactor. The flask tubing was flushed with a dilute acid solution every other day to prevent growth of bacteria outside the reactor. The feed rate was 0.3 mL/min, except during the kinetics experiment. The influent and

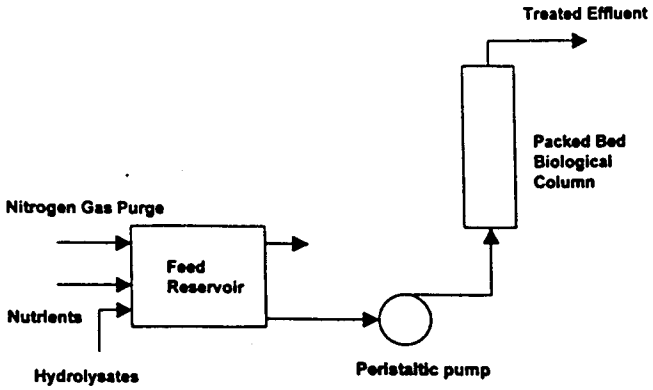

Figure 2—Schematic diagram of experimental set up.

Table 1—Composition of the culture medium used for continuous flow experiment.

Component	Concentration, mg/L
K_2HPO_4	5 000
$NaH_2PO_4 \cdot H_2O$	2 875
NH_4Cl	200
$MgCl_2 \cdot H_2O$	100
$CaCl_2 \cdot 2H_2O$	40
Na_2SO_3	19.7
$FeCl_3$	3.9
$MnCl_2$	0.95
$ZnCl_2$	0.66
$CoCl_2 \cdot 6H_2O$	0.58
$CuCl_2 \cdot 2H_2O$	0.30
$Na_2Mo_4 \cdot 2H_2O$	0.46
$Na_2B_4O_7 \cdot 10H_2O$	0.2

effluent ions and RDX and HMX concentrations were measured weekly. The influent sample was taken directly from the feed and, as the solution exited the reactor, the effluent was collected using a sampling tube located outside the reactor.

Denitrifying Cultures. The initial culture was started using anaerobic digester sludge from the Hyperion Wastewater Treatment Plant in El Segundo, California. All samples were diluted with oxygen-free phosphate buffer, filtered, and incubated for 3 weeks in a minimal medium with ethanol, potassium nitrate, and phosphate buffer. This culture was originally grown on ethanol and nitrate and was used to transform RDX and HMX to nonexplosive byproducts (Wilkie, 1994). The inoculum from this culture was used to start degrading the hydrolysates of RDX. Ethanol and nitrate for the degradation of RDX were removed gradually and replaced with nitrite (electron acceptor) and formate, acetate, and formaldehyde (carbon sources). All constituents of the liquid growth medium except the carbon sources and electron acceptors are listed in Table 1. Phosphate buffer was provided to maintain a pH between 7 and 7.5 and also as a nutrient.

Ion Chromatography—Formate, Nitrite, and Nitrate. Formate, nitrite, and nitrate (NO_3^-) were analyzed using a Dionex Ion Chromatograph (basic chromatography module CMB-2, gradient pump GPM-1; Dionex, Sunnyvale, California) with suppressed conductivity detection (conductivity detector CDM-1). An Ion Pac AS9-SC analytical column (4-mm i.d.) (Dionex) was used with a subsequent suppressor column. The mobile phase consisted of 0.75 mM sodium bicarbonate ($NaHCO_3$) and 2 mM sodium carbonate (Na_2CO_3) dissolved in deionized water from a Milli-Q water system (Millipore Co., Bedford, Massachusetts). The eluant flow rate was set to 2 mL/min. Samples were manually injected to a 50-μL sample loop.

Peaks were detected at retention times between 1.4 and 1.5 minutes for $HCOO^-$, 2.0 and 2.1 minutes for NO_2^-, and 2.8 and 2.9 minutes for NO_3^-, respectively. The peak area was a linear function of the concentration between 0.66 and 6.13 mg $HCOO^-$/L, between 0.77 and 6.13 mg NO_2^-/L, and 0.5 to 10.0 mg NO_3^-/L, respectively. For the external calibration, at least three datapoints were gathered for each standard concentration. The mean was then used for the calibration curve. All samples were filtered through sterile Acrodisc-13 0.2-μm syringe-microfilters (Gelman Sciences, Ann Arbor, Michigan) before injection.

High-Performance Liquid Chromatography—Formaldehyde. A modification of the technique reported by Kuwata et al. (1979) was used. The reaction solution for formaldehyde was prepared by dissolving 0.5 g of 2,4-dinitrophenylhydrazine (DNPH) in 500 mL of 2 N hydrochloric acid and purified by shaking with 5 mL of chloroform. Formaldehyde in the sample (0.4 mL) was first allowed to react with DNPH in acidic solution (4 mL) to form 2,4-dinitrophenylhydrazone. The solid-phase extraction (SPE) method (Richard and Junc, 1986) was used and the eluant was analyzed by high-performance liquid chromatography (HPLC).

The HPLC analysis was performed with a Hewlett Packard 1500 Series instrument (Avondale, Pennsylvania) equipped with a variable wavelength detector and autosampler. An Adsorbosphere C-18 10-μm, reversed-phase column (Alltech, Deerfield, Illinois) with prefilter element and guard column (C-18, 5-μm, Alltech) was used with a mobile phase consisting of 50% acetonitrile and 50% water (volume %) at a flow rate of 1.5 mL/min. A UV detector (254 nm) was used to detect the dinitrophenylhydrazone peak at a retention time of 5.9 minutes. The peak area was a linear function of the concentration between 0.5 and 20 mg/L of formaldehyde. Standards were prepared for 0.50, 1.00, 2.50, 5.00, 10.00, and 20.00 mg formaldehyde/L. For the external calibration, at least three datapoints were collected for each standard concentration. The mean was then used for calibration. All samples were also filtered through sterile Acrodisc-13, 0.2-μm, syringe-microfilters before injection.

Gas Chromatography—Acetate. Acetate ion analysis was performed on a Hewlett Packard model 5890 gas chromatograph. A glass column with 15% SP-1200/1% phosphoric acid on 100/200 mesh Chromosorb (Supelco, Bellefonte, Pennsylvania) and a guard column (Supelco) were used to resolve the compounds. Oven temperature and injection temperature was 115 °C; detector temperature was 200 °C. The retention time for acetate with this method was approximately 1.65 minutes. Standard curves were obtained from 2.93 to 29.25 mg C/L of acetate. The detection limit was approximately 3 mg C/L of acetate.

Solid-Phase Extraction for Hexahydro-1,3,5-Trinitro-1,3,5-Triazine and Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine. Richard and Junc's (1986) SPE method was used to concentrate liquid samples containing explosives for analysis. Varian Bond Elut (Harbor City, California) C-8 SPE cartridges were used and recoveries were typically greater than 90% when analyzing 5- to 10-mL samples. First, 10 mL of HPLC-grade methanol was passed through a 5-cc SPE cartridge under vacuum that provided a flow rate of approximately 5 mL/min. This was equivalent to approximately 25 mm Hg vacuum pressure. The sorbent bed was then washed with 5 mL of HPLC-grade water. Before the sorbent bed dried, a liquid sample that contained explosives was passed through the cartridge while the vacuum was still drawing liquid. When all of the sample had passed through the cartridge, 4 mL of HPLC-grade water was used to rinse the cartridge and remove interference. The SPE cartridge was then dried under full vacuum (~600 mm Hg) for 20 minutes (a maximum of 0.4 mg of explosive was retained on the column using this procedure). Next, 1 mL of HPLC-grade acetonitrile was used for elution. The eluant was then collected in a vial and analyzed using HPLC. Even though HEs can be concentrated up to ten times in the SPE column, the maximum concentration of HE is at most 50 mg/L; therefore, there is no explosive hazard associated with drying of the SPE tubes containing the explosives.

High-Performance Liquid Chromatography Analysis. The RDX and HMX were measured with the SPE method, followed by HPLC. The analysis was performed with the same HPLC as the one used in formaldehyde analysis. The mobile phase consisted of 40% water, 30% methanol, and 30% acetonitrile (volume %) at a flow rate of 1 mL/min. The sample injection volume and detection wavelength was set at 20 μL and 236 nm, respectively. Separation through a 10-μm, Adsorbosphere, C-18 reversed-phase column and corresponding 5-μm guard column (Alltech) at 25 °C resulted in retention times of 4.1 minutes (RDX) and 3.6 minutes (HMX), respectively.

Total Organic Carbon. A Beckman Model 915B total organic carbon (TOC) analyzer (Fullerton, California) was used following procedures described in *Standard Methods* (APHA et al., 1992). Samples were clarified by centrifugation, filtered through membrane filters, and acidified to pH 2 using phosphoric acid to liberate inorganic carbon dioxide. Organic carbon was determined by injecting 1 mL of the acidified sample.

Results and Discussions

Stoichiometry. Denitrification classically is considered to be a heterotrophic process by microorganisms that require a reduced organic substrate for respiration and cell synthesis (Gayle et al., 1989, and Knowles, 1982). Nitrate (NO_3^-) typically is used as the terminal electron acceptor and it is sequentially reduced to NO_2^- and finally to N_2 (Payne, 1973). Heterotrophic denitrifying bacteria can use a wide variety of organic compounds, such as methanol, ethanol, glucose, and acetate as well as the myriad compounds found in municipal wastewater.

The RDX hydrolysates contain formate, formaldehyde, and nitrite that are carbon sources and electron acceptors. However, only a few studies exist where nitrite is used as the electron acceptor (Kamath et al., 1991, and U.S. EPA, 1975). A possible reason is that high concentrations of nitrite are seldom found in municipal wastewater, and nitrifying activated-sludge plants typically completely convert ammonia to nitrate. Nitrite is reduced to nitrogen gas in a fashion similar to nitrate in accordance with the following half equation:

$$NO_2^- + 4\,H^+ + 3\,e^- \rightarrow 0.5\,N_2 + 2\,H_2O \quad (1)$$

The reduced organic carbon compounds present in the hydrolysates (formate, formaldehyde, and acetate) are oxidized according to the following half reactions:

$$HCOO^- \rightarrow CO_2 + 2\,e^- + H^+ \quad (2)$$

$$HCHO + H_2O \rightarrow CO_2 + 4\,e^- + 4\,H^+ \quad (3)$$

$$CH_3COO^- + 2\,H_2O \rightarrow 2\,CO_2 + 8\,e^- + 7\,H^+ \quad (4)$$

The combined equations for bacterial oxidation, ignoring cell synthesis can be written as follows:

$$NO_2^- + 1.5\,HCOO^- + 2.5\,H^+ \rightarrow 0.5\,N_2 + 1.5\,CO_2 + 2\,H_2O \quad (5)$$

$$NO_2^- + 0.375\,CH_3COO^- + 1.375\,H^+ \rightarrow 0.5\,N_2 + 0.75\,CO_2 + 1.25\,H_2O \quad (6)$$

$$NO_2^- + 0.75\,HCHO + H^+ \rightarrow 0.5\,N_2 + 0.75\,CO_2 + 1.25\,H_2O \quad (7)$$

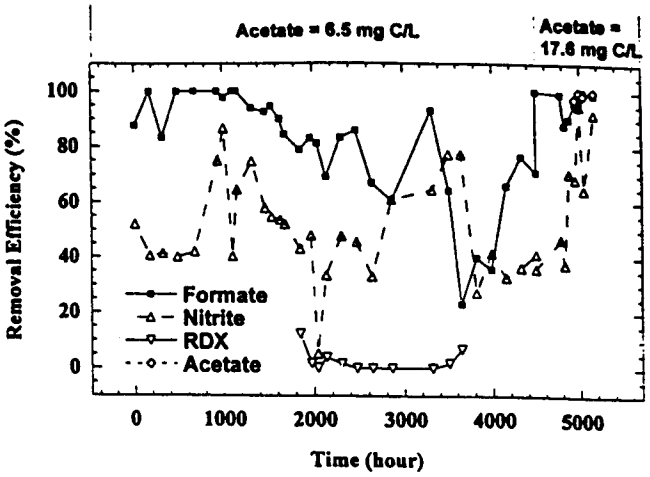

Figure 3—Removal efficiencies of hydrolysates and RDX: RDX was introduced at 1 800 hours, and HMX has similar removal as RDX. Acetate concentration was increased at 4 600 hours to get high removal of nitrite.

As indicated earlier, Heilmann et al. (1996) found that RDX hydrolysis yields 1.6 M NO_2^-, 1.5 M $HCOO^-$, 0.1 M CH_3COO^-, 1.1 M HCHO, 0.9 M NH_3, 1.1 M N_2O, and 0.34 M N_2 per M of RDX hydrolyzed. Equation 8 shows this relationship:

$$1\ RDX\ (C_3H_6N_6O_6) \rightarrow 1.6\ NO_2^-, + 1.5\ HCOO^- + 0.1\ CH_3COO^- + 1.1\ HCHO + 0.9\ NH_3 + 1.1\ N_2O \uparrow + 0.34\ N_2 \uparrow \quad (8)$$

In the following section, we describe the stoichiometry and experiments for denitrifying hydrolysates.

Denitrification of Nitrite Using Acetate and Formate. For simplicity, the system was first started with acetate, formate, and nitrite. First, the stoichiometric relationship (equation 9) was constructed by adding equation 5 to 0.27 (0.1/0.375) times equation 6:

$$1.6\,NO_2^- + 1.5\,HCOO^- + 0.1\,CH_3COO^- + 2.9\,H^+ \rightarrow 0.33\,NO_2^- + 0.63\,N_2 + 1.7\,CO_2 + 2.33\,H_2O \quad (9)$$

According to this stoichiometry, the amount of nitrite exceeds the amount required for complete oxidation of the organics. Therefore, acetate and formate should be oxidized completely; excess nitrite is present and only 80% can be removed if cell synthesis is neglected. However, some organic matter is always converted to cell material, which will require less electron acceptor and will further reduce the nitrite removal efficiency.

The biological column was operated for 3 months to demonstrate this conversion and to show that the system could effectively use nitrite as an electron acceptor at the concentrations anticipated. Figure 3 shows removal efficiencies of nitrite and formate. At the time of this experiment, we were not monitoring acetate removal. Until approximately 1 800 hours in this figure, formate removal averaged 95%, but nitrite removal averaged only 55.4%, which we attributed to cell synthesis.

The partial removal of nitrite can be used to approximate the cell yield, which typically cannot be measured in packed-bed reactors because of the difficulty in weighing the biomass that is attached to the column packing. Only 55.4% or 0.89 out of 1.6 moles of nitrite were reduced in the acetate/formate system. (For simplicity, we base our discussion on the coefficients from equation 9, as

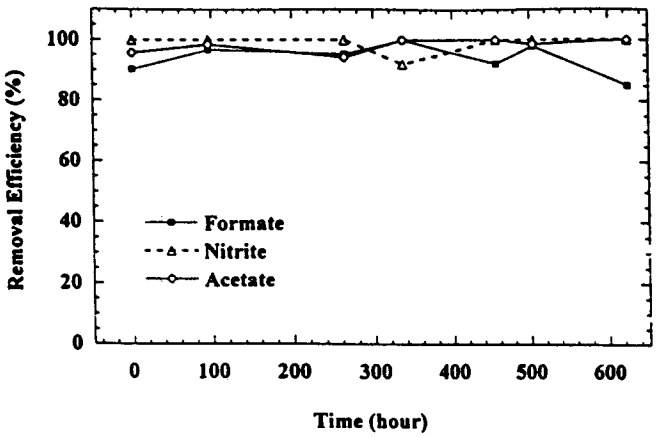

Figure 4—Removal efficiency of each hydrolysate after adding formaldehyde: formaldehyde was analyzed later in this experiment and completely removed.

opposed to the actual molar concentrations.) The amount of substrate used for cell synthesis can be estimated by balancing the amount of nitrite reduced with formate (acetate is neglected because of its low concentration and to simplify calculations). Equation 5 can be multiplied by 0.89 to obtain this result, which shows that 1.33 moles of formate can be reduced. If we subtract this amount from equation 9, we see that 0.17 out of 1.5 moles of formate are not reduced and, therefore, must be used for cell synthesis. Stoichiometrically, this amount of formate could produce a maximum of 0.034 moles of cells ($C_5H_7NO_2$). Therefore, the observed cell yield (mass basis) using this ratio (0.034/1.5) is 0.057 mg cells/mg formate, 0.21 mg cells/mg TOC, 0.16 mg cells/mg chemical oxygen demand (COD), or 0.31 mg cells/mg nitrite–nitrogen (NO_2^-–N). These results compare to an observed cell yield of 0.18 mg volatile suspended solids (VSS)/mg COD for nitrate reduction with methanol in an anoxic slurry reactor at 20 °C (Stensel et al., 1973) and a theoretical yield of 0.4 to 0.9 mg VSS/mg nitrate–nitrogen (NO_3^-–N) for typical denitrification processes (Metcalf & Eddy, Inc., 1991).

We next added acetate to balance the carbon source and electron acceptor to maximize nitrite removal. (A short experiment to assess the culture's ability to biodegrade RDX was performed in the intervening period and is reported later in the paper.) The stoichiometric relationship that best describes the reaction for this balanced condition using equation 5 and 6 is shown in equation 10.

$$1.6\ NO_2^- + 1.5\ HCOO^- + 0.1\ CH_3COO^- + \underline{0.13\ CH_3COO^-} + 3.33\ H^+ \rightarrow 0.8\ N_2 + 1.95\ CO_2 + 2.75\ H_2O \quad (10)$$

Using this stoichiometry, acetate concentration was increased (shown by underline in equation 10) from 6.53 mg C/L to 17.6 mg C/L (the theoretical amount is 14.5 mg C/L, but more acetate was added for cell synthesis) at approximately 4 600 hours in Figure 3. As shown in this figure, nitrite removal increased from 40 to more than 80%, suggesting that it is possible to balance the carbon sources and the electron acceptor. Acetate was completely removed while maintaining more than 90% formate removal from gas chromatography analysis.

Formaldehyde Addition. After developing stable conditions with acetate and formate, we added formaldehyde, which simulates the total hydrolysate system. The first step was to make a mass-balance equation, including formaldehyde. However, if the same hydrolysate mole ratio (equation 8) is used, nitrite becomes limiting and it is not possible to predict carbon oxidation. Therefore, additional nitrite was added to allow all carbon sources to be oxidized. The stoichiometric balance for treating hydrolysates, including additional nitrite, can be written as follows using equations 5 through 7:

$$\underline{(1.13\ NO_2^-)} + 1.6\ NO_2^- + 1.5\ HCOO^- + 0.1\ CH_3COO^- + 1.1\ HCHO + 4.33\ H^+ \rightarrow 1.37\ N_2 + 2.8\ CO_2 + 4.17\ H_2O \quad (11)$$

Equation 11 shows that insufficient nitrite is present and 1.13 moles (underlined) must be added to complete the carbon oxidation. Cell growth will reduce the required electron acceptor (nitrite); therefore, we initiated our experiment with 10% less nitrite.

Figure 4 shows the percentage removal of each hydrolysate in the bioreactor with this ratio. During one month, we obtained stable removals of more than 90% of all hydrolysates. This result was confirmed by checking TOC. As also shown in Figure 5, inlet total organic carbon is approximately 100 mg/L and effluent is approximately 10 mg/L. Table 2 also shows the agreement between calculated TOC and measured TOC. In this table, calculated TOC indicates the sum of all carbon sources. All data show that denitrification performed well in accordance with the mass-balance equation.

Degradation Kinetics of Carbon Sources. We anticipate that, in a commercial facility, upset conditions occasionally may allow breakthrough of one or more substrates. Therefore, it is desirable to know which carbon sources are preferentially degraded. To evaluate the reactor's preference among the three different carbon substrates, degradation efficiency was measured along the length of the reactor for each carbon source. Samples were collected with the reactor operating at 0.6 mL/min (twice the normal flow rate was used to stress the reactor) and an inlet feed concentration ratio of 197.4 mg of NO_2^-–N, 97.9 mg $HCOO^-$–C, 71.8 mg of HCHO–C, and 13.1 mg of CH_3COO^-–C per 1 L. The samples were taken over time from the inlet (x = 0 cm), the outlet (x = 20 cm), and from five ports along the reactor's length.

The degradation for the carbon sources is shown in Figure 6.

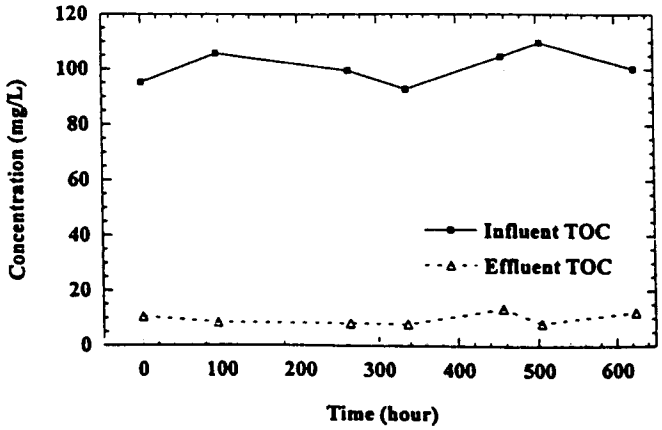

Figure 5—Comparison of influent and effluent total organic carbon.

Table 2—Comparison between calculated and measured total organic carbon.*

	Formate	Formaldehyde	Acetate	Total organic carbon	
				Calculated	Measured
Influent	49.9	35.6	6.5	92.1	100.2
Effluent	7.5	0.3	0.0	7.8	12.3

* Compounds are expressed in units of mg organic carbon/L.

The results indicate that acetate and formaldehyde are preferentially degraded over formate. Acetate and formaldehyde degradation rates are approximately equal.

Influent Concentration Effect. We next investigated the treatment capability of the biological reactor at increased influent concentrations. The concentration of each hydrolysate was increased by two and four times its original concentration (original composition is 98.7 mg/L of NO_2^-–N, 49.0 mg/L of $HCOO^-$–C, 35.9 mg/L of HCHO–C, and 6.53 mg/L of CH_3COO^-–C). Region A in Figure 7 corresponds to the original concentration. Region B corresponds to double the influent concentration. The removal of formate, the compound that is degraded last among organic hydrolysates, initially decreased but recovered after approximately 1 000 hours; the effluent pH was approximately 7.5. Region C corresponds to a quadrupling of the original concentration. At this loading, removal efficiency of each hydrolysate dropped to 30% or less and did not recover, even after 2 months. Reactor effluent pH increased to between 8 and 8.5. Therefore, the concentration of hydrolysates was decreased back to twice the original concentration, and the reactor efficiency recovered to more than 90%, as shown in region D in Figure 7.

The result suggests that this reactor can treat hydrolysates up to twice the original concentration. The alkalinity produced during conversion of nitrite to nitrogen gas and the oxidation of organic acids was sufficient to raise the pH and inhibit the reactor (the optimum pH range for denitrification is 7 to 7.5; U.S. EPA, 1975). Equation 11 predicts that 4.33 moles of hydrochloric acid (shown in bold) are needed to balance the alkalinity produced from the biological oxidation of the hydrolysates of 1 mole of RDX.

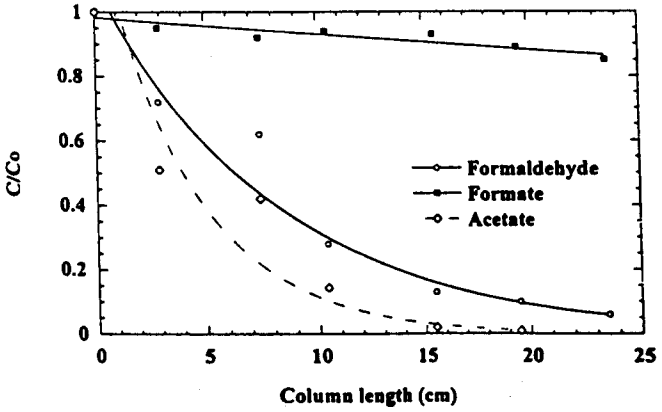

Figure 6—Degradation profile of carbon sources along the column length: nitrite removal is 78%, flow rate is 0.6 mL/min, and reactor inlet is located at 0 cm.

Changing Electron Acceptor. In a full-scale installation, it may be more desirable to use nitrate as opposed to nitrite as the additional electron acceptor for balancing the carbon sources. Nitrate is found more commonly in wastewater treatment and is less toxic. Region E in Figure 7 shows the results when using nitrate instead of nitrite. At 3 600 hours, the additional nitrite was replaced with nitrate and was effectively used as an electron acceptor without any adaptation period. The culture's ability to use nitrate as an electron acceptor has implications for full-scale sys-

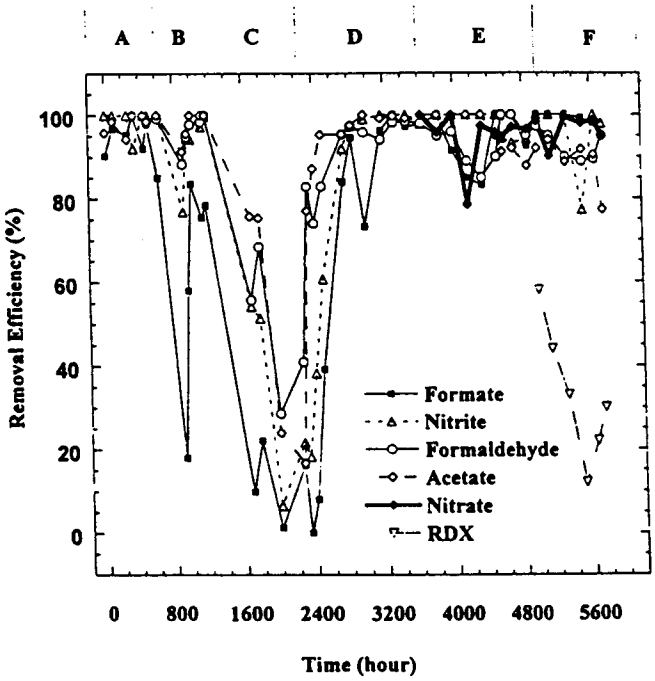

Figure 7—The change of hydrolysates and RDX removal efficiencies under various conditions: (a) original concentration system is acetate—6.53 mg C, formaldehyde—35.9 mg C, formate—49.0 mg C, and nitrite—98.7 mg N/L; (b) two times original concentration system; (c) four times original concentration system; (d) back to two times original concentration system; (e) keeping two times original concentrations system, nitrate (51.8 mg NO_3^-–N/L) as an additional nitrogen source was added instead of nitrite (75.7 mg NO_2^-–N/L); and (f) keeping two times original concentration of hydrolysates system and nitrate, trace amounts of RDX/HMX (5 mg RDX and 3 mg HMX/L) were added in the system containing nitrate as the additional nitrogen source.

tems. The hydrolysates contain ammonia, which is untreated in the anoxic system. An aerobic, biological process could be used after the anoxic process. This process could be operated to nitrify the ammonia and remove any untreated organic compounds. The produced nitrate could be used as the additional electron acceptor for anoxic process if it were recycled back to the anoxic column influent. The recycle might also help with pH control.

Biodegradation of Explosives. The ability to degrade high explosives is a desirable property for the hydrolysate treatment. If so, it could degrade trace amounts of RDX or HMX that inadvertently escaped alkaline hydrolysis. The inoculum used to start this culture (Wilkie, 1994) was also able to transform RDX and HMX to non-explosive byproducts using the same reactor system, but with nitrate as the electron acceptor and ethanol as the cosubstrate. Chiou et al. (1997) showed mineralization of ^{14}C RDX and ^{14}C HMX using the parent culture, with ^{14}C recovery (as $^{14}CO_2$) as high as 40%. Hesselmann and Stenstrom (1992) observed RDX biodegradation using acetate as a cosubstrate, with nitrate as the electron acceptor.

To test for RDX and HMX biodegrading ability, low concentrations of RDX and HMX were added to the influent. The first experiments were performed when nitrite was being used as an electron acceptor. Initially, 5 mg/L of RDX and 3 mg/L of HMX were added. No biodegradation of RDX and HMX were observed, and the culture's ability to oxidize the organic compounds was severely affected (at 1 800 hours in Figure 3). The concentrations of both RDX and HMX were decreased to 1 mg/L after 45 days (approximately 3 000 hours in Figure 3). However, gradual decline in organic carbon removal efficiency continued and no explosive transformation was observed. The results suggest that RDX and HMX cannot be degraded by the culture under these conditions, and RDX and HMX can inhibit carbon oxidation. The culture quickly recovered its ability for treating hydrolysates after the explosives were removed from the system (at 3 700 hours in Figure 3).

A second set of experiments was performed after adding nitrate as the electron acceptor. The results are shown in region F in Figure 7. Approximately 30% of the RDX was biodegraded and there was no effect on carbon oxidation. The culture retained its ability to biodegrade RDX even after long periods when it was absent from the feed.

Conclusions

The application of biological denitrification for treating synthetic hydrolysates of RDX was successfully demonstrated. The results show that postdenitrification of HE hydrolysates is feasible. Specifically, it can be concluded that the nitrite produced during alkaline hydrolysis can be used as the electron acceptor in the biological oxidation of acetate, formate, and formaldehyde produced in the hydrolysis.

Experimental results closely match calculated stoichiometric conversions. The removal efficiency of each byproduct and TOC was more than 90%. The observed cell yield (mass basis) with acetate/formate oxidation and nitrite reduction was 0.21 mg cells/mg TOC or 0.16 mg cells/mg COD.

The volumetric removal rate was as much as 170 mg/L NO_2^-–N per day with existing carbon sources, and acetate and formaldehyde were preferentially degraded over formate. In a full-scale system, some form of pH control will likely be required. Also, the culture was able to biodegrade RDX when using nitrate as an electron acceptor.

The study shows that the biological denitrification for treating hydrolysates of RDX is a feasible process in spite of the complexity of the system. One contribution of this work is demonstrating successfully the denitrification of nitrite using various low-molecular-weight compounds. The results of this research may be applicable to other industrial wastewaters containing nitrite or nitrate and multiple carbon sources. Pilot-scale research of this process is underway at the Pantex Plant in Amarillo, Texas. Research is also underway to treat hydrolysates from TNT.

Acknowledgments

Credits. This research was supported by Lawrence Livermore National Laboratory and Mason and Hanger, Inc., which operates the Pantex Plant in Amarillo, Texas. The authors are grateful for assistance and suggestions of Felipe Alatriste-Mondragon and Ed Ruth.

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Submitted for publication September 29, 1997; revised manuscript submitted May 26, 1998; accepted for publication September 15, 1998.

The deadline to submit Discussions of this paper is June 15, 1999.

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