THE EFFECTS OF FERRIC IRON ON PHOTOCHEMICAL AND MICROBIAL DEGRADATION OF 2,4,6 -TRINITROTOLUENE

Sean Cook, Huey-Min Hwang, and Harriet Course
Environmental Science Program, Department of Biology, Jackson State University

INTRODUCTION

2,4,6 -Trinitrotoluene (TNT) has been used extensively as an explosive since 1902 (Higson 1992). Accidental release of TNT has contaminated groundwater and soil at numerous munitions manufacturing sites. It is estimated that TNT alone is produced in amounts close to 2 million pounds a year and threatens human life through the food chain (Hawari et al. 1999). The compound is mutagenic and toxic and has a tendency to persist in the environment. There have been many attempts to biodegrade TNT, but thus far the compound has been found to undergo biotransformation rather than degradation.

The reported pathways for biological reduction of TNT have been the same as for chemical reduction. The nitro groups are reduced to the corresponding amines via nitroso and hydroxylamino intermediates. Reduction of the second nitro group does not begin until the nitro group has converted to the amine. Most microorganisms studied catalyze the preferrential reduction of the 4-nitro group to yield 4-amino-2, 6 -dinitrotoluene (4ADNT) (Fiorella and Spain 1997).

"Pink water" produced during photolysis of aqueous solutions containing mainly photo-decomposed TNT and its photoproducts, is a complex mixture, which has been found to be more photosensitive than TNT. Some of these are; 4, 6- dintroanthranil, 2,4,6-trinitobenzyaldehyde (TNBAL), and 2,4,6-trinitrobenzyl alchohol (Burlinson et al. 1979). Ferric and ferrous iron, used by microorganisms for electron transport systems, has been shown to aid in the remediation of aromatic pollutants in water and soil in the form of Fenton's reagent, which is a combination of ferrous iron and hydrogen peroxide. Photochemically, it has already been demonstrated that dissolved Fe (III) species undergo sunlight photoredox reactions on a time scale of minutes in atmospheric water. Thus, during daytime, the dissolved iron in atmospheric water drops is rapidly cycling between Fe (III) and Fe (II), very probably on a time scale of minutes, due to rapid (photo)redox reactions occuring in the water drops (Faust 1986). It is probable that similar mechanisms could be contributing to the iron redox cycling in acidic surface waters and perhaps even at the higher pH values encountered in oceanic waters (Faust 1994).

In this study, a series of experiments were run to asses the rate of the transformation of TNT over time and the effectiveness of ferric chloride as a sensitizer to the combination of photochemical and biological transformation of TNT in samples of natural freshwater. This was done using HPLC analysis and radiotracing techniques.

MATERIALS AND METHODS

Water samples were collected from the Mississippi River near Vicksburg, MS and the Ross Barnett Reservoir in Ridgeland, MS. The pH of the water samples from both locations ranged from 7.0 to 7.5 and the temperature ranged from 25°C to 28°C, during spring and summer, and 13°C to 15°C, during the winter. TNT (analytical grade; Chem Service Co., Pennsylvania) was dissolved in methanol (HPLC grade; Fisher Scientific) and added to 50 mL of river water sample into 150-mL quartz flasks (GM Associates, Inc., Oakland, California) and incubated in triplicate. TNT degradation was measured with production of $^{14}CO_2$ (mineralization analysis) and detection of parent compound (transformation study with HPLC). Radiolabled TNT final concentration was 10- 14 μg/L (for mineralization study). In the transformation study using the HPLC final concentration was 10 mg/L. Later mineralization experiments included 10 mg/L of non- labeled TNT along with 10 μg/L of C^{14}-UL-TNT (s.a. 11.85 mCi/mol; purity 97.3; NEN Co., Boston, MA.) in the final solution. Ferric chloride was added at 0.1, 1, 10, and 100 μM in four different sample groups of TNT and river water. Light exposure samples were put in quartz flasks. The

flasks allowed 100% transmission of light at wavelength 300 nm. Dark exposure sample groups were placed in 150- mL glass bottles (Corning Co.) wrapped in aluminum foil. Killed control samples were autoclaved (for transformation study) at 250°F at 15 psi for 25 minutes. For mineralization experiment killed controls were autoclaved and poisoned with formaldehyde (0.4% final concentration; for assuring sterile conditions). All flasks and bottles were sealed with silicon stoppers. The quartz flasks and wrapped bottles were then suspended in an outdoor tub in a continuous running water bath. This maintained a constant temperature of 28 ± 2°C. During transformation studies, samples were filtered (0.45 µm; Schleicher and Schuell, Keene, New Hampshire) before being injected into a Waters 996 HPLC for analysis. The system was fitted with a reverse- phase Supelcosil LC- 8 column (Supelco Co., Bellefonte, Pennsylvania) and a mobile phase of 0.5 mL/min of HPLC grade methanol and 0.5-mL/min HPLC grade water. TNT wavelength detection was set at 228 nm. Rate of transformation of TNT over time was done at 15, 30, 45, and 60 min intervals.

For the purpose of mineralization study, the silicon stoppers of each sample had plastic center wells placed on the bottom containing filter paper soaked with 0.6 mL of 1N sodium hydroxide as an initial $^{14}CO_2$ trapping solution. Sample incubation times were at 1, 2, and 3 days (to allow for the mineralization of C^{14}-TNT). After each groups designated exposure times, the samples were brought in and 0.7 mL of 2N sulfuric acid was injected into the samples to stop the reaction. Injections were done through the stopper with a 1 cc-tuberculin syringe. After this was done a second group of 150- mL Corningware bottles were autoclaved. 3 mL of 2N sulfuric acid was added to the bottles, and the sodium hydroxide soaked filter paper placed in them. The bottles were then sealed with center wells containing filter paper soaked with 0.5 mL of β-phenylethylamine (double trapping technique), and allowed to sit overnight. The β-phenylehtylamine, saturated filter paper was then removed from the bottles and placed in plastic vials containing Ultima Gold Liquid Scintillation high flashpoint solution. The vials were then placed in a TRI-CARB 1600 TR Liquid Scintillation Analyzer (Packard, Conberra Co., Meriden, CT.) for counting.

RESULTS AND DISCUSSION

The mean percentage and standard deviation, from the initial mineralization of TNT study during the 1, 2, and 3 day period (14 µg/L of C^{14}-TNT in solution only), were calculated from the DPM1 reading of scintillation analysis. Rate of mineralization was highest in the live sample groups exposed to sunlight (light live sample group) at 2 and 3 days (63% and 73% mineralization to $^{14}CO_2$ respectively). The light exposure where the microorganisms were killed (light killed exposure group) also showed some significant mineralization but at a lower percentage (14% and 41% for 2 and 3 days respectively) (Table 1). The dark killed and dark live sample groups showed very little or no mineralization of TNT (Table 1). Mathematically microbial degradation accounted for 44% of the total TNT mineralized. The contribution of microbial activity was more significant (79%) in day 2. Even though microbial activity played a major role in the mineralization process, results of the dark live groups show that microbial activity alone cannot mineralize TNT without the initial transformation done by photolysis (Table 1).The experiment utilizing a spike of non-labeled TNT along with radio -labeled TNT (10 mg/L and 10 µg/L) showed less mineralization (light live 1.7% and 2.7% for 2 and 3 days of exposure) (Table 2). From these two sample groups, the same synergistic trend between photolysis and microbial activity was observed with the light live sample groups having the highest rate of mineralization (Table1). The next mineralization experiment included different concentrations of ferric chloride (0.1, 1, 10, & 100 µM) to discern whether it would act as a sensitizer to photochemical and biological degradation of TNT. Bacteria may respire using NO_3, SO_4, oxidized (ferric) iron [Fe(III)] or a variety of metals (such as arsenic and uranium) as an oxidant (Sulzberger and Laubscher 1995). Probably the most crucial inorganic property of iron, from the point of view of nutrition of living cells, is the profound insolubility of ferrous and ferric iron. The Fe(II) and Fe(III) salts of strong acid anions are soluble, but the phosphates, carbonates, and phytates, are insoluble (Neilands 1973). The presence of Fe (III) in natural waters should increase the rate of photomineralization of natural organic matter (Faust 1994).

Samples were suspended in water bath for a 3-day period. The light killed and light live sample groups, which contained TNT only, showed the most mineralization (at 7.4% and 19.2% respectively) (Table 3). The light killed sample group containing 0.1 μM and 1 μM ferric chloride had very similar readings to the light killed without ferric chloride with only slight differences in the standard deviation of each sample group. In the light live sample groups with 1 μM ferric chloride was the only group that was close to matching the rate of mineralization behind the light live without ferric chloride group (i. e. 18.3%). This indicates that ferric chloride at lower concentrations (eg. 0.1 μM) has little or no effect on the photolysis of TNT, while higher concentrations, (eg. 100 μM) may inhibit photolysis and thus inhibit biodegradation of TNT.

HPLC analysis of TNT was first conducted to show rate of transformation at different time periods (15, 30, 45, and 60 min). The dark sample groups were similar to the mineralization readings in that very little or no degradation occurred (Table 4). The marked difference comes while comparing the light with the light live sample groups. Light killed and light live at 60 min, had the highest rate of transformation with the light kill group being the highest at 72% and light live at 64.3% (Table 4). This was true for the other time periods as well. This may be due impart to microbial activity. Microorganisms, such as algae, may sequester TNT from instant transformation by photolysis (Hwang et al. 1985). This indicates that microbial transformation and degradation of TNT is a slower process than photochemeical transformation and degradation, and may take a period of days instead of just hours to totally degrade TNT. Ferric chloride in sample solutions (0.1, 1, 10, and 100 μM final concentration of four sample groups) were also analyzed using the HPLC. Disappearance of the parent compound was more significant in the 1 μM and the 10 μM light live sample groups, both groups showed 16% degradation (Table 5). This suggests the same trend as the mineralization analysis. Ferric chloride at very low concentrations have no real affect on the photochemical and biotransformation of TNT, while ferric chloride in high concentrations (100 μM) may inhibit photochemical and microbial transformation of TNT due to light attenuation by the color, crystalline structure, and acidity of the chemical. Another factor is that there was no significant change in pH after the samples were brought out of the sunlight (pH 7.0- 7.5). Level of pH of surface water samples may have to be lowered in order to see more significant reactions between Fe (III) and TNT while undergoing photolysis and microbial degradation. Fe (III) may be increasing microbial population, but it is interfering with the photochemical and microbial breakdown of TNT. Tests, such as spread plate analysis, will be run to get a glimpse at the effect ferric chloride has on microbial growth. In conclusion, Fe (III) in the form of ferric chloride at high concentrations has an inhibitory effect upon photochemical and microbial degradation of TNT.

ACKNOWLEDGEMENTS

This research was supported by: (1) Department of Energy #DE-AC03-76SF00098 (to LBL); subcontract # 6482515 to JSU; (2) NIH- MBRS S06GM08047 (to JSU); (3) Department of Energy DE- FG02-97ER62451 (to UGA); subcontract # RR100-239/4891914 to JSU; and (4) the Army HPCRC under the auspices of the Department of the Army, Army Research Laboratory. The content does not necessarily reflect the position/ policy/ endorsement of the government.

REFERENCES

Burlinson, N. E., Kaplan, L. A., Adams, C. E., Oct. 1979. "Photochemistry of TNT: Investigation of the "Pink Water" Problem," Technical report NOLTR 73- 173.

Faust, B. C. and Hoffmann, M. R. 1986. Photo induced reductive dissolution of α-Fe_2O_3 by bisulfite, Environ. Sci. and Technol. 20, 943, 1986.

Faust, B. C. 1994. A Review of the photochemical Redox Reactions of Iron(III) Species in Atmospheric, Oceanic, and Surface Waters: Influences on Geochemical cycles and Oxidant Formation. In Aquatic and Surface Photochemistry 1994, edited by G. R. Helz, R. G. Zepp, and D. G. Crosby; Lewis Publisher, Chelsea, MI

Fiorella, P. D. and Spain, J. C. 1997. Transformatiopn of 2,4,6- Trinitrotoluene by *Pseudomonas pseudoalcaligens* JS52. Appl. and Environ. Microbiol. 63 (5): 2007- 2015.

Hawari, J., et. al. 1999. Biotransformation of 2,4,6- Tinitrotoluene with *Phanerochaet chrysosporium* in Agitated Cultures at pH 4.5, Appl. and Environ. Microbiol., 65 (7): 2977-2986.

Higson, F. K., 1992, Microbial degradation of nitroaromatic compounds, Adv. Appl. Microbiol. 37: 1- 19. Microbiol., 37: 1- 19.

Hwang, H- M., R. E. Hodson, R. F. Lee. 1985. "Photochemical degradation of 1,4,5-trichloroaniline ina freshwater lake", Appl. and Environ. Microbiol., 50: 1177- 1180.

Neilands, J. B. 1973. Department of Biochemistry, University of California, Berkeley, California, "Chemistry of Iron in Biological Systems", Metal Ions in Biological Systems, 1973, edited by Sanat K. Dhar, vol. 40: 13- 41.

Sulzberger, B., Laubscher, H. U., Karametaxas, G. 1994. In Aquatic and Surface Photochemistry 1994, edited by Helz, G., Zepp, R., Crosby, D.; Lewis Publishers, Chelsea, MI.

Table 1. Mineralization of C^{14}- TNT #1.

TREATMENTS	% MINERALIZED
Dark killed Time 1 (3 days)	0.20 ± 0.01
Dark killed Time 2 (3 days)	0.40 ± 0.07
Dark live Time 1 (1 day)	0.10 ± 0.01
Dark live Time 2 (3 days)	0.60 ± 0.40
Light killed Time 1 (1 day)	0.10 ± 0.01
Light killed Time 2 (2 days)	13.5 ± 9.4
Light killed Time 3 (3 days)	41 ± 4
Light live Time 1 (1 day)	0.20 ± 0.04
Light live Time 2 (2 days)	63 ± 18
Light live Time 3 (3days)	73 ± 14

Table 2. Mineralization of C^{14}- TNT #2.

TREATMENT	% MINERALIZED
Dark killed Time (1 day)	0.3 ± 0.4
Dark killed Time (3 days)	0.20 ± 0.02
Dark live Time (1 day)	0.40 ± 0.06
Dark live Time (3 days)	0.20 ± 0.03
Light killed Time (1day)	0.5 ± 0.1
Light killed Time (2 days)	0.6 ± 0.1
Light killed Time (3 days)	1.4 ± 0.7
Light live Time (1 day)	1.5 ± 0.3
Light live Time (2 days)	1.7 ± 0.4
Light live Time (3 days)	2.7 ± 0.2

Table 3. Mineralization of C^{14}- TNT with ferric chloride added as a sensitizer.

TREATMENT	% MINERALIZED
Light killed w/o ferric chloride	7.4 ± 1.8
Light killed (0.1 μM)	7.3 ± 2.8
Light killed (1 μM)	7.1 ± 3.6
Light killed (10 μM)	6.4 ± 1.7
Light killed (100 μM)	5.0 ± 2.0
Light live w/o ferric chloride	19.2 ± 4.4
Light live (0.1 μM)	12.2 ± 1.4
Light live (1 μM)	18.3 ± 2.3
Light live (10 μM)	13.3 ± 3.1
Light live (100 μM)	11.0 ± 2.0

Table 4. HPLC analysis of the rate of transformation of TNT over time.

TREATMENTS	% TRANSFORMATION
Dark killed Time 1 (15 min)	3.3 ± 1.2
Dark killed Time 2 (30 min)	5.3 ± 4.1
Dark killed Time 3 (45 min)	3.4 ± 3.0
Dark killed Time 4 (1 hr)	4.2 ± 3.0
Dark live Time 1	4.2 ± 4.0
Dark live Time 2	5.6 ± 5.6
Dark live Time 3	4.6 ± 1.5
Dark live Time 4	4.0 ± 3.2
Light killed Time 1	22.2 ± 3.6
Light killed Time 2	35.5 ± 3.5
Light killed Time 3	52.0 ± 9.0
Light killed Time 4	72.0 ± 16.0
Light live Time 1	21.0 ± 4.0
Light live Time 2	23.0 ± 0.5
Light live Time 3	42.0± 0.9
Light live Time 4	64.3 ± 4.2

Table 5. HPLC analysis of TNT transformation with ferric chloride as a sensitizer.

TREATMENTS	% TRANSFORMATION
Light live w/o ferric chloride	10.7 ± 1.3
Light live (0.1 μM)	13.6 ± 6.5
Light live (1 μM)	16 ± 6
Light live (10 μM)	16 ± 4
Light live (100 μM)	14.5 ± 5
Light killed w/o ferric chloride	3 ± 0.6
Light killed (0.1 μM)	6.7 ± 6.5
Light killed (1 μM)	5.8 ± 3
Light killed (10 μM)	6.6 ± 2
Light killed (100 μM)	4.5 ± 0.3