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Chemical and Biological Degradation of Insensitive Munitions (IM) Mediated by Fe(III)-Reducing Microorganisms

Kevin T. Finneran (ktf@clemson.edu), Kameryn McGee, and Jolanta Niedzwiecka (Clemson University, Clemson, SC, USA)
Kayleigh Ann Millerick (Texas Tech, Lubbock, TX, USA)

Background/Objectives. The insensitive munitions (IM) 2,4-dinitroanisole (DNAN) and nitroguanidine (NQ) were investigated to determine the capacity for biodegradation using mixed chemical-biological reactions mediated by Fe(III)-reducing microorganisms. The objective of the work was to characterize how the rate and extent of IM degradation was infleunced by microbially-mediated secondary chemical reactions with biogenic Fe(II) and reduced electron shuttling molecules.

Approach/Activities. The data were generated using a series of laboratory batch incubations in the presence and absence of active microbial biomass. The chemical reductants were tested across a range of pH values from 6 to 9, and included ferrous iron or reduced electron transfer molecules such as anthrahydroquinone disulfonate. The cells used in the experiments included *Geobacter metallireducens*, *Shewanella oneidensis*, *Clostridium geopurificans*, and *Rhodobacter sphaeroides*.

Results/Lessons Learned. Both DNAN and NQ were rapidly degraded by ferrous iron at pH 7, 8, and 9, with rates increasing as the pH increased. The extracellular electron shuttle anthrahydroquinone-2,6-dilsulfonate (AH2QDS) degraded DNAN, but not NQ. DNAN degraded in less than one hour with AH2QDS and ferrous iron at pH 8 and pH 9; more than 90% of the initial 100 micromolar DNAN was degraded in several minutes, suggesting that this pathway will be exceptionally effective at DNAN removal depending on pH. Rates were slower at pH 7, but DNAN degraded in less than 36 hours. The primary products of DNAN degradation by Fe(II) was 2-methoxy-5-nitroaniline (MENA), recovered at approximately 60-75% of initial DNAN carbon. 2,4-diaminoansiole (DAAN) was also detected, but at a lower final percentage.

NQ degradation was much slower, with complete degradation taking 30 days at pH 8 and pH 9. NQ degradation at pH 7 was negligible. Unlike DNAN, NQ degradation required solid surfaces (primarily as aggregated $Fe(OH)_2(s)$) for reduction – soluble ferrous iron alone did not reduce NQ. NQ reduction products have not yet been identified, but work continues to characterize the NQ degradation pathway. The ferrous iron ligand, trihydroxybutyric acid (THBA), increased DNAN and NQ reduction at pH 7 and 8, respectively. THBA amendment may be a reasonable alternative for ex situ reactions, where it cannot other metals.
The Fe(III)-reducing bacterium *Geobacter metallireducens* also reduced DNAN; however, the products MENA and DAAN were not detected. The cells reduced the compound directly, and via electron shuttling reactions with iron and extracellular quinone/hydroquinone couples. Finally, very recent data indicate the DNAN is degraded by the phototroph *Rhodobacter sphaeroides*, suggesting sun energy will promote IM degradation.