Evaluation of RDX Biodegradation Using Compound-Specific Stable Isotope Analysis (CSIA) and Stable Isotope Probing (SIP)

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Background/Objectives. Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is commonly detected in groundwater at military facilities, including many operational training and testing ranges. Remedial alternatives for this and other munitions constituents include monitored natural attenuation (MNA) and enhanced bioremediation via carbon source amendment. However, one limitation for MNA or enhanced remediation of RDX is the inability to accurately determine whether the nitramine is biodegrading under field conditions, particularly in groundwater. The objective of this study was to quantify the microbial fractionation of C and N stable isotopes in RDX under differing geochemical conditions and by different pure cultures and to assess whether such isotope fractionation could be used to document RDX biodegradation in the field. In addition, stable isotope probing (SIP) with both ^{15}N-RDX and ^{13}C-RDX was used to determine which organisms were biodegrading RDX under different terminal electron accepting conditions in samples from an active range.

Approach/Activities. Gas-chromatography isotope-ratio mass spectrometry (GC-IRMS) methods were developed to quantify stable isotope ratios of both nitrogen (δ^{15}N) and carbon (δ^{13}C) in RDX. These methods were subsequently used to assess stable isotope enrichment during aerobic and anaerobic degradation of RDX by pure cultures that degrade the nitramine via different pathways. Stable isotope ratios of C and N also were analyzed in RDX extracted from groundwater samples from an active DoD range where a biobarrier was installed to enhance anaerobic degradation of RDX in groundwater, and SIP analysis was conducted with samples from the same aquifer that were incubated under differing geochemical conditions.

Results/Lessons Learned. Stable isotope fractionation of C and N in RDX by pure cultures differed appreciably based upon the dominant pathway. Among four aerobic RDX-degrading strains, fractionation of N but not C was observed. The mean ε^{15}N for the four strains was -2.4 ± 0.5 ‰, and the ε^{13}C was -0.3 ± 1.0 ‰ (not different from zero). A larger N fractionation (ε^{15}N between -6.8‰ and -13.2 ‰ for different strains) was observed for seven different pure cultures degrading RDX through various anaerobic pathways. This large N fractionation is consistent with various proposed mechanisms of initial enzymatic attack on the RDX molecule, most of which involve a N-N bond. Interestingly, fractionation of C in RDX under anaerobic conditions also was observed (ε^{13}C between -2.1‰ and -7.1‰ for different strains). In groundwater samples from an active testing range, the ε^{15}N in RDX was -7.8 ‰, and ε^{13}C was -2.7 ‰ downgradient of an emulsified oil biobarrier, which confirms RDX biodegradation, and is consistent with data from pure cultures. Degradation product analysis and SIP of large-scale mesocosms from the range site indicated that different organisms and pathways contributed to RDX degradation under differing electron accepting conditions. Overall, the results suggest that CSIA and SIP can be valuable approaches to document RDX biodegradation.