Bioremediation and Sustainable Environmental Technologies—2017. Fourth International Symposium on Bioremediation and Sustainable Environmental Technologies (Miami, FL; May 2017). ©2017 Battelle Memorial Institute, Columbus, OH, www.battelle.org/biosymp. A. Barton and S. Rosansky (Chairs)

Transformation Products of the Insensitive Munitions Explosive 2,4-Dinitroanisole in Biotic Systems

Craig L. Just (craig-just@uiowa.edu) and Hunter W. Schroer (hunter-schroer@uiowa.edu)
(University of Iowa, Iowa City, IA, USA)

Background/Objectives. The environmental fate of 2,4-dinitroanisole (DNAN), a component of new insensitive munitions explosives (IMX), is an emerging global issue and the new IMX formulations are replacing older explosives at a rapid pace. Emerging studies have described a few DNAN transformation products, soil binding behavior, and ecotoxicology, but not enough is known about the toxicity, transport and potential for remediation of DNAN in the environment. DNAN and other nitroaromatics have been shown to cause harm to human health and ecosystems, leading to concern over the widespread use of DNAN and its potential to contaminate soil and groundwater. Full-scale remediation will be necessary for military testing sites and ammunitions manufacturing plants as more countries adopt the new IMX formulations and the compounds are inevitably released. Therefore, to characterize ecosystem risk and inform future remediation efforts, knowledge regarding degradation of DNAN within natural systems is needed. We evaluated the potential for phytoremediation of DNAN contaminated sites by identifying transformation products of DNAN in willow trees and by a bacterial and a fungal species isolated from willow tissues.

Approach/Activities. To our knowledge, we have undertaken the first comprehensive study of DNAN transformation in a plant system. Metabolic pathways within whole trees were examined using isotope-labeled DNAN, liquid chromatography tandem mass spectrometry (LC/MS/MS), high resolution mass spectrometry (HRMS) and liquid scintillation counting. We determined the bulk partitioning of DNAN to roots, stems and leaves resulting from ^{14}C-labeled DNAN studies in willow trees grown in hydroponic media. Furthermore, we examined the metabolic transformation of DNAN using ^{13}C-DNAN and ^{15}N-DNAN stable-isotope analysis. Suspected DNAN metabolites were identified and confirmed with LC/MS/MS and HRMS. A *Rhizobium* sp. isolated from willow tree tissues degraded 5 mg L^{-1} of DNAN within 24 hours in liquid media with added carbon and nitrogen. The use of DNAN, ^{13}C-DNAN and ^{15}N-DNAN enabled the identification of 11 previously unknown *Rhizobium*-derived metabolites. A fungal *Penicillium* sp., also isolated from willow trees, degraded DNAN in solution within 14 days. Stable-isotope labeled DNAN and an untargeted metabolomics approach revealed 13 novel transformation products produced by *Penicillium*.

Results/Lessons Learned. Metabolites of DNAN such as 2-amino-4-nitroanisole and 2,4-dinitrophenol were confirmed in willow, as well as larger conjugated metabolites. *Rhizobium* reduced, sulfated and acylated DNAN in solution. Further mass spectral analysis provided evidence for various combinations of amino and hydroxylamino DNAN metabolites. Nitro-reduction was observed at the *para* position of DNAN to produce 4-amino-2-nitroanisole. *Penicillium* also reduced DNAN at the *para* position and further produced DNAN metabolites resulting from demethylation, acetylation, hydroxylation, malonylation and sulfation. Incubations with intermediate metabolites such as 2-amino-4-nitroanisole and 4-amino-2-nitroanisole as the primary substrates confirmed putative metabolite isomerism and pathways. No ring-cleavage products were observed, consistent with other reports that mineralization of DNAN is an uncommon metabolic outcome. Observed metabolites of DNAN in willow trees were consistent with expected metabolic pathways towards DNAN sequestration. Therefore, phytoremediation represents a potential remediation strategy requiring further study. Bio-augmentation to enhance phytoremediation was deemed impractical with the organisms studied.