

## Biodegradation of the Emerging Insensitive Munitions Compound 3-Nitro-1,2,4-Triazol-5-One (NTO) by Soil Microorganisms

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**Background/Objectives.** Insensitive munitions compounds, such as 5-nitro-1,2,4-triazol-3-one (NTO), have been used by the army as replacements for conventional explosives. Unlike the traditional explosives, NTO detonation requires higher activation energy, avoiding the occurrence of accidental explosions. As the use of NTO becomes widespread, there is more concern about the toxicity of this compound once it reaches the soil and water bodies. Since NTO is very soluble in water and does not adsorb onto soil particles, it is very mobile in the environment. The objective of this research is to investigate approaches to promote NTO mineralization by soil microorganisms.

**Approach/Activities.** In this study, the transformation of NTO in soil was investigated in batch bioassays containing soil as the microbial inoculum. Under anaerobic conditions and using pyruvate as electron donor, NTO was reduced to 3-amino-1,2,4-triazol-5-one (ATO). In order to investigate ATO biodegradability, ATO-degrading enrichment cultures were derived from an initial culture containing soil, mineral medium and ATO. After multiple transfers (10% volume/volume), ATO mineralization was investigated in closed bottles experiments through measurements of CO<sub>2</sub> and N<sub>2</sub> in the headspace and inorganic nitrogen ions (ammonium, nitrate and nitrite) in the liquid phase. The results indicate extensive mineralization of ATO, since nearly all of ATO-carbon and ATO-nitrogen were recovered as inorganic species (CO<sub>2</sub>, N<sub>2</sub> and NH<sub>4</sub><sup>+</sup>). Bacteria suspected of being responsible for ATO mineralization were identified in a clone library study using a universal bacterial primer set.

**Results/Lessons Learned.** The results obtained in this research suggest that the first step of NTO degradation pathway is its reduction to ATO. ATO can then become completely biodegraded by an enrichment culture releasing inorganic compounds as the degradation products, which are safer for the environment. This study indicates that bioremediation is a potential alternative for cleaning up sites contaminated with NTO.