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Joint Photomicrobial Process for the Degradation of the Insensitive Munition \( N\)-Guanylurea-dinitramide (FOX-12)

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ABSTRACT: \( N\)-Guanylurea-dinitramide (FOX-12) is a very insensitive energetic material intended to be used in the composition of next-generation insensitive munitions. To help predict the environmental behavior and fate of FOX-12, we conducted a study to determine its photodegradability and biodegradability. When dissolved in water, FOX-12, a guanylurea-dinitramide salt, also named GUDN, dissociated instantly to produce the dinitramide moiety and guanylurea, as demonstrated by high-performance liquid chromatography (HPLC) analysis. When an aqueous solution of FOX-12 was subjected to photolysis using a solar-simulated photoreactor, we found a rapid removal of the dinitramide with concurrent formation of \( \text{N}_2\text{O}, \text{NO}_2\), and \( \text{NO}_3\). The second component, guanylurea, was photostable. However, when FOX-12 was incubated aerobically with the soil isolate \textit{Variovorax} strain VC1 and protected from light, the dinitramide component of FOX-12 was recalcitrant but guanylurea degraded effectively to ammonia, guanidine, and presumably \( \text{CO}_2\). When FOX-12 was incubated with strain VC1 in the presence of light, both components of FOX-12 degraded, giving similar products to those described above. We concluded that the new insensitive explosive FOX-12 can be effectively degraded by a joint photomicrobial process and, therefore, should not cause persistent contamination of surface waters.

INTRODUCTION

Traditionally, the defense industry has focused on the use of explosives, such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), 2,4,6-trinitrotoluene (TNT), nitrocyclulose (NC), and nitroglycerine (NG), but handling and use of these chemicals is often associated with a high risk of unwanted detonation. Efforts are now underway in North America, Europe, and Australia to develop insensitive materials to replace TNT, RDX, and HMX in the manufacturing of munition formulations. Examples of emerging insensitive explosives include dinitramide acid, \( \text{HN(NO}_2\text{)}_2\), and salts derived from the acid, such as ammonium dinitramide. Because of their importance, several theoretical and experimental studies have been carried out to determine their physicochemical properties, thermal stability, and applications. Recently, a Swedish team developed another nitramide salt, \( N\)-guanylurea-dinitramide (FOX-12), whose performance matches those of RDX and TNT but shows low sensitivity to impact and friction, thus allowing the chemical to be used as an ingredient in the composition of very insensitive high explosives and propellants and as a gas-generating material in automotive safety airbags.

On the basis of past experience on the environmental risks associated with the use of the traditional explosives, it is imperative to understand the environmental impacts associated with the manufacturing and use of the new generation of insensitive munitions. In general, little information is available on the transformation pathways of these emerging explosives, and no reports on the degradability of FOX-12 are currently available. A study by Mill et al. indicated that ammonium dinitramide can be rapidly photodegraded but is stable toward hydrolysis in water and recalcitrant to biotransformation in water and soil. To our knowledge, bacterial degradation of guanylurea has not yet been reported. However, we previously isolated a strain of \textit{Variovorax} that degraded nitroguanidine and its degradation product nitrourea, as well as other guanidine derivatives. \textit{Variovorax} species are ubiquitous in nature and degrade a diversity of pollutants, including the phenylurea herbicides linuron and diuron. Therefore, \textit{Variovorax} strain VC1 was selected in this study to assess biodegradation of FOX-12.

The present study thus aims at determining the photodegradation of FOX-12 under solar-simulated conditions and its biotransformation using the soil bacterial isolate \textit{Variovorax} strain VC1. Understanding the transformation pathways of FOX-12 will provide insight into the environmental fate of this new energetic material, which, in turn, should help site managers and environmental officers to manage their activities and training needs.

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Growth was monitored spectrophotometrically at 600 nm rotary shaker under visible light (desk lamp) or in the dark. Resting Cells of Strain VC1. Centrifuged, washed in distilled water, and resuspended at an OD

Controls consisted of strain VC1 in MSMG without FOX-12 and FOX-12 salt dissociates instantly to its two components, protonated \([\text{M} + \text{H}]^+\) and deprotonated \([\text{M} − \text{H}]^-\) molecular mass ions. The mass range was scanned from 40 to 500 Da.

**RESULTS AND DISCUSSION**

**Dissociation of FOX-12 in Water.** HPLC/ultraviolet (UV) analysis demonstrated that, in aqueous solution, the FOX-12 salt dissociates instantly to its two components, guanylurea and dinitramide. The HPLC chromatogram at 210 nm in Figure 1 clearly shows two peaks: one appearing at a retention time of 5.8 min identified as guanylurea by comparison to a reference standard material and another appearing at 9.1 min identified as dinitramide and confirmed by liquid chromatography–ultraviolet/mass spectrometry (LC–UV/MS) showing characteristic UV absorptions at 219 and 285 nm, and a deprotonated molecular mass ion \([\text{M} − \text{H}]^-\) at 106 Da.
Because the FOX-12 salt completely dissociates in water to guanylurea and dinitramide and each component is detected at a different retention time by HPLC, it was possible to look at the stability of each component separately. The following sections thus describe how the two components of FOX-12, guanylurea and dinitramide, behave in the presence of light and bacteria in aqueous solutions.

**Photodegradation of FOX-12.** When we subjected an aqueous solution of FOX-12 (253.4 μM) to light from a SolSim photoreactor simulating solar irradiation, more than 96% (243.9 ± 0.3 μM) of the initial dinitramide from FOX-12 was photodegraded over 30 min of photolysis. Guanylurea stayed intact, indicating that only the dinitramide part of the FOX-12 salt was photoactive (Figure 2). The UV absorption of the two moieties of FOX-12, i.e., guanylurea ($λ_{max}$ of 210 nm) and dinitramide ($λ_{max}$ of 219 and 285 nm) shown in Figure 1 clearly supports the photostability of guanylurea and the photodegradability of dinitramide using a solar simulator, whose emission spectrum and intensity match the standard solar AM1.5 (280–800 nm).

The disappearance of dinitramide was accompanied by the formation of NO$_2^-$ (209.9 ± 1.0 μM), NO$_3^-$ (146.9 ± 3.4 μM), and N$_2$O (51.2 ± 4.8 μM) (Figure 2), representing a nitrogen recovery of 63.6%. After 5 min of irradiation, the NO$_3^-$/NO$_2^-$ ratio was relatively high, reaching 4.4, which is in line with the ratio of 4 obtained by Mill et al.$^{13}$ during photolysis of ammonium dinitramide in water (pH 7). The NO$_3^-$/NO$_2^-$ ratio decreased to 0.37 after 7 days, indicating that NO$_2^-$ might have been photo-oxidized to NO$_3^-$.$^{19}$ In an earlier study involving photolysis of the nitramine RDX (NO$_2$–N–N–CH$_2$)$_3$, we found that NO$_2^-$ formed from the photolysis of N–NO can be photooxidized to NO$_3^-$.$^{20}$ Earlier, Mill et al.$^{13}$ investigated the hydrolytic stability of ammonium dinitramide in water and found the salt to be stable in the dark at 25 °C. However, the salt was rapidly photodegraded to NO$_2^-$, NO$_3^-$, and N$_2$O at wavelengths close to those naturally available from the sun (June sunlight), with $t_{1/2} = 3.2$ min and $Φ = 0.1$. They reported a similar N balance from photolysis of potassium–dinitramide (61%) and ammonium–dinitramide (59%) in water. Guanylurea in FOX-12 (and as a control of guanylurea alone) was photostable under the same conditions, with no loss encountered over 7 days; likewise, photostability of guanylurea after 120 min of irradiation was previously reported.$^{21}$ Although it is known that, during photolysis of dinitramide several reactive free radicals can be produced, including *NO, NO$_3^-$, and $OH.$* none of these radicals was able to degrade guanylurea. The potential participation of these radicals in other side reactions with dinitramide might have led to the formation of unidentified products that contributed to a non-stoichiometric N mass balance.

**Aerobic Biotransformation of FOX-12 with Variovorax Strain VC1.** We first determined that *Variovorax* strain VC1 grew in MSMG with FOX-12 as the sole N source. The cells reached an OD$_{600}$ of 0.68 ± 0.09 when grown with 424 μM FOX-12 and 20 mM glucose (Figure 3). No lag time was observed when acclimated VC1 cells (pre-grown on FOX-12) were used as inocula. Figure 3 also shows that strain VC1 degraded the guanylurea moiety of FOX-12 but did not attack dinitramide. This is in contrast to what we observed with light (Figure 2). Cometabolic biodegradation of dinitramide with glucose has been previously reported without mentioning any products;$^{13}$ mineralization of ammonium dinitramide to ammonia in digested sewage sludge under strict anaerobic conditions was also reported.$^{22}$ A *Bacillus* soil isolate from our lab$^{23}$ also degraded dinitramide cometabolically in rich medium (Luria–Bertani broth) at a rate of 3.9 μM h$^{-1}$ (data not shown). These experimental findings indicate that biodegradation of dinitramide is possible although not achieved by strain VC1. Degradation of guanylurea was accompanied by the formation of guanidine, which could not be quantified because of interfering substances in the culture supernatant.

To further assess biotransformation of the guanylurea moiety in FOX-12, resting cells of VC1 were incubated with either FOX-12 or pure guanylurea (from Sigma) as substrates (Figure 4). VC1 mostly transformed guanylurea from FOX-12 to guanidine and ammonia; dinitramide remained intact (Figure

![Figure 2](image-url)  
**Figure 2.** Effect of simulated sunlight irradiation on dinitramide (DNA-FOX12) and guanylurea (GUU-FOX12) moieties of FOX-12.

![Figure 3](image-url)  
**Figure 3.** Cells growth (OD$_{600}$) and guanylurea (GUU) degradation by *Variovorax* strain VC1 with FOX-12 as the sole N source. DNA-FOX12, dinitramide in FOX-12; GUU-FOX12, guanylurea in FOX-12.
The disappearance of guanylurea was accompanied by the concurrent formation of guanidine, followed by the appearance of ammonia that was only detected after 6.5 h of incubation (Figure 4a). After 26 h, 0.70 ± 0.11 mol of guanidine and 0.74 ± 0.02 mol of ammonia were produced for each mole of guanylurea degraded, corresponding to a N mass balance of 71%. Neither nitrite, nitrate, nor N₂O were observed, consistent with the fact that dinitramide was not degraded.

Biotransformation of pure guanylurea (from Sigma) by VC1 was faster than biotransformation of guanylurea (from FOX-12) (Figure 4b). Removal of guanylurea (from Sigma) was accompanied by the formation of guanidine and ammonia. After 26 h, for each disappearing mole of guanylurea, 0.56 ± 0.06 mol of guanidine and 0.94 ± 0.04 mol of ammonia were formed, representing a N mass balance of 65%. Seemingly, dinitramide had an inhibitory effect on the degradation of guanylurea. From Figure 4b, we also observed that, in contrast to Figure 4a, the formation of ammonia was concurrent with the formation of guanidine. The presence of dinitramide appeared to affect the formation of ammonia.

The missing N from both panels a and b of Figure 4 could be attributed to several products that we detected by LC–MS and that were tentatively identified as secondary product(s) of guanidine. One of the products showed a protonated molar mass ion [M + H]⁺ at m/z 112 Da, matching a molecular formula of C₉H₂N₄ that we tentatively identified as the heterocyclic compound formoguanine. It is also known that guanidine is a reactive molecule and condenses in the presence of an amine to form oligomers and polymers. All of these products taken together can lead to a reduction in the N mass balance. These products were not detected in the controls of VC1 cells in the absence of substrate.

**Joint Photomicrobial Transformation of FOX-12.** We combined bacteria and light to achieve degradation of both components of FOX-12. Strain VC1 was allowed to grow in MSMG supplemented with FOX-12 and exposed to room lighting. Under these conditions, guanylurea and dinitramide degraded simultaneously with almost similar rates, i.e., 15.9 and 16.9 μmol L⁻¹ h⁻¹, respectively (Figure 5). After 2 days, 1.74 ± 0.11 mol of nitrite ion was formed for each mole of dinitramide degraded. Part of the anion was later consumed by the microorganisms (Figure 5). Nitrous oxide was formed at 0.170 ± 0.002 mol per mole of dinitramide. A small amount of NO⁻ was also detected and supposed to originate from dinitramide degradation (data not shown).

Dinitramide degradation in the combined photomicrobial system was significantly decelerated in comparison to what was observed in the solar simulator (Figure 2). The kinetic retardation can be explained by the fact that, in contrast to photolysis in the solar simulator, the combined assay (Figure 5) was performed under suboptimal conditions using Erlenmeyer flasks exposed to a 13 W fluorescent tube and in the presence of bacterial cells that interfered during irradiation.

**Insights into the Photomicrobial Degradation Pathways of FOX-12.** Using time course studies and product distribution from the photodegradation (Figure 2), biotransformation (Figure 3 and panels a and b of Figure 4), and joint photodegradation–biotransformation experiments (Figure 5), we constructed a degradation pathway that can best describe FOX-12 photobiodestruction, as shown in Figure 6 (routes a, b, and c). The pathway consisted of a primary route that first lead to the complete dissociation of FOX-12 to its two acid/base components, dinitramide and guanylurea (route a in Figure 6). When the dissociated aqueous solution of FOX-12 was subjected to light, dinitramide degraded to NO₂⁻, NO₃⁻, and N₂O.
and N₂O, leaving guanylurea intact (route b in Figure 6). In this context, dinitramide would first undergo photodenitration to initially produce NO₂⁻ and the hydroxyl-nitramide, NH(OH)·NO₂. The latter being unstable should undergo spontaneous decomposition to give NO₂⁻, N₂O, and water.¹³ It is known that photolysis of NO₂⁻ can lead to NO₃⁻.¹⁹,²⁰

When another FOX-12 aqueous solution was incubated with the bacterium Variovorax strain VC1 without light, guanylurea degraded to guanidine, ammonia, and presumably CO₂ but, this time, leaving dinitramide intact (route c in Figure 6). We presume that the primary step in the biodegradation of guanylurea involved the loss of the cyanic acid, HCNₐ, a precursor of the carbamic acid, NH₂COOH, which, upon decomposition in water, would produce NH₃ and CO₂ (route c in Figure 6). The lag time in the ammonia production during guanylurea (from FOX-12) incubation with VC1 (Figure 4a) suggests that HCNₐ was formed prior to the formation of the unstable carbamic acid, NH₂COOH. Cyanic acid intermediate have been detected during hydrolytic decomposition of urea by urease.²⁷ As we mentioned earlier, the lag time in the ammonia production was not observed during incubation of pure guanylurea with VC1 (Figure 4b), suggesting that dinitramide might have stabilized the precursor intermediate, leading to the formation of ammonia.

Guanidine can then be transformed to ammonia and CO₂ (Figure 6). Many guanidine derivatives were shown to be mineralized by microorganisms in soil and water.²⁸,²⁹ Mineralization of another guanidine derivative, nitroguanidine, by the same strain was reported to give the same products NH₃ and CO₂.¹⁴

The present study demonstrates that, by combining photolytic and microbial processes, the new insensitive explosive FOX-12 can be effectively degraded to nontoxic products. This first assessment of the degradation of FOX-12 suggests that dinitramide would be broken down quite rapidly by sunlight in the upper layers of surface waters. In the absence of light in subsurface environments, dinitramide may persist significantly longer, particularly in oligotrophic ecosystems. Studies to date only revealed cometabolic degradation of dinitramide. Likewise, degradation of guanylurea by VC1 was achieved with glucose, and its remediation without organic carbon may be limiting in practice. On the other hand, guanylurea with its four amino groups serves as a valuable N source for microbial growth and eventually should be completely mineralized. Therefore, in addition to its insensitivity to impact and friction, FOX-12 is more environmentally friendly than traditional explosives.

Figure 6. Proposed photobiodegradation routes of FOX-12 in water (a) using light (b) and Variovorax strain VC1 (c). Compounds in parentheses were not detected.

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Notes

The authors declare no competing financial interest.

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