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Biodegradation of Guanidinium Ion in Aerobic Soil Samples

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The manufacture of several munitions, polymeric resins, flame retardants, and pharmaceuticals utilizes guanidine salts as starting materials (Patterson, 1984). For example, as much as 40 ton/day of the military propellant nitroguanidine could be synthesized in a process involving the production of guanidine nitrate, followed by its dehydration in strong acid to form the munition (Small and Rosenblatt, 1974). Significant levels of guanidinium, the predominant ionic species of guanidine, have been detected in wastewaters from nitroguanidine pilot production operations, together with nitroguanidine, inorganic ions and a diversity of low-level organic by-products (Burrows, 1983; Burrows et al., 1984).

Introduction of guanidinium into the soil environment could result from the ponding of such wastes as well as from spills and cleanup activities associated with manufacturing operations. In soil, guanidinium has been reported to inhibit microbial ammonium nitrification and has been associated with toxic effects to at least one plant species (Lees and Quastel, 1947; Konishi and Imanishi, 1941).

Aerobically, microorganisms in a variety of environmental waters can degrade guanidinium slowly as a sole growth substrate and more rapidly in the presence of a carbon source, in which case its nitrogen is used for growth. In both cases, most of the metabolized guanidinium carbon is mineralized (Mitchell, 1987). Microbial degradation of the cation in soil under anaerobic conditions also has been reported and requires supplemental carbon for significant expression (Ebisuno and Takimoto 1981; Kaplan and Kaplan, 1985). In aerobically incubated soil, rapid guanidinium degradation thought to be a result of microbial action has been observed at a low concentration (0.28 mg/Kg) (Williams and MacGillivray, 1987). It is the purpose of this report to further define aerobic guanidinium biodegradation in soil.

MATERIALS AND METHODS

Following the removal of surface litter, field soil samples were collected from a depth of from 1 to 4 inches. Samples were main-

tained as described by Pramer and Bartha (1972) to preserve biological activity and were passed through a 2 mm screen prior to use. Although results reported below were obtained with local soil samples (Duffield silt loam, pH 6.4 to 7.1), samples of two other Maryland soils (Hagerstown silt loam, pH 7.1, and Collington loamy fine sand, pH 6.3) were also used in initial screening studies (Miller, 1967). None of the sites had a history of exposure to guanidinium.

The mineralization of guanidinium carbon was measured in the incubation and trapping system described by Marrinucci and Bartha (1979). Triplicate test flasks received 1 μCi carbon-14 guanidine chloride (Amersham, 22 mCi/mMole), sufficient distilled water to achieve 60% soil moisture holding capacity, and the equivalent of 20 g dry weight soil. Chemical additions including unlabeled guanidine chloride and the inhibitors mercuric chloride and sodium azide were made in conjunction with the moisture adjustment. The organic carbon sources, cellulose and glucose, were added as sterile dry solids (4% by weight) and were mixed to homogeneity with a sterile glass rod. Following incubation for the desired times at 20^{o}C, flasks were flushed with 10 volumes of laboratory air for 10 min and head space gases were collected in duplicate organic traps (11 mL Econofluor) and duplicate alkaline traps (10 mL Instagel plus 1 mL 0.5 N KOH). Trapped radioactivity was counted in a Beckman Model 3801 scintillation spectrophotometer, and quench corrected data were converted to disintegrations per minute (Beckman, 1985). The amount of guanidinium mineralized was calculated from percentages of the label mineralized in the replicate flasks multiplied by the initial concentration of guanidinium. Trapping efficiencies, measured by collecting known amounts of acidified carbon-14 bicarbonate, were routinely 90 to 95%. Significant label was not collected in the organic traps and only results for labeled carbon dioxide are reported below. The stability of the cation in sterile soil was demonstrated by extracting labeled guanidinium with 100 mL 0.5 N HCl from 20 g dry weight samples and analyzing thin layer chromatograms (Milks and Janes, 1956) of the extract with a System 200 Imaging Scanner (Bioscan, Inc.).

RESULTS AND DISCUSSION

Screening for guanidinium degradation was conducted with Duffield soil and a total of 45 nM guanidine chloride (0.22 mg/Kg soil). Levels of labeled guanidinium carbon converted to carbon dioxide are shown in Figure 1. More than 40% of the guanidinium carbon was mineralized by the first day of incubation in the soil, after which time mineralization continued at a decreasing rate until roughly 80% of the substrate's carbon was converted to carbon dioxide. In soil samples containing the metabolic inhibitors mercuric chloride or sodium azide (0.1%), mineralization was inhibited by 73 and 97.5% respectively; essentially no guanidinium carbon was mineralized in heat killed controls. Thus guanidinium can be degraded readily in the Duffield soil test system at low concentration, and its degradation results from biological action.

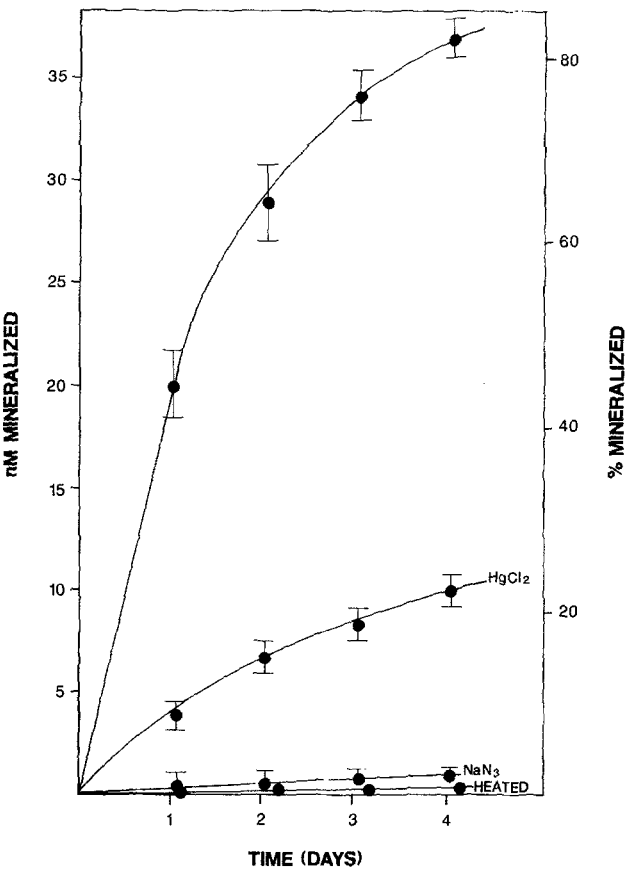

Figure 1. Mineralization of Guanidinium Carbon in Unamended, Poisoned, and Heat Sterilized Duffield Soil Samples.

Mineralization of guanidinium carbon could also be demonstrated in soils from both other screening sites listed in Methods. Results obtained from a second silt loam (Hagerstown) were essentially the same as those of Figure 1; however, mineralization of the cation's carbon was at least four times slower in the fine sandy loam sample (Collington) and did not exceed 50% after eight days incubation. Thus microflora from geographically distinct sites can biodegrade the cation, but its degradation is not necessarily as rapid or extensive from site to site.

Mineralization studies were also carried out over a wide range of guanidinium concentrations (0.22 to 400 mg/Kg soil) in Duffield soil. Results are shown in Figure 2 for guanidinium ("A") and for urea ("B") as a basis of comparison in identical soil samples. Although rates (24 hour) of guanidinium carbon mineralization increased with the concentration of the cation, increases were not in proportion and tended toward saturation of the biodegrading system. Unlike guanidinium, the rate of urea carbon mineraliza-

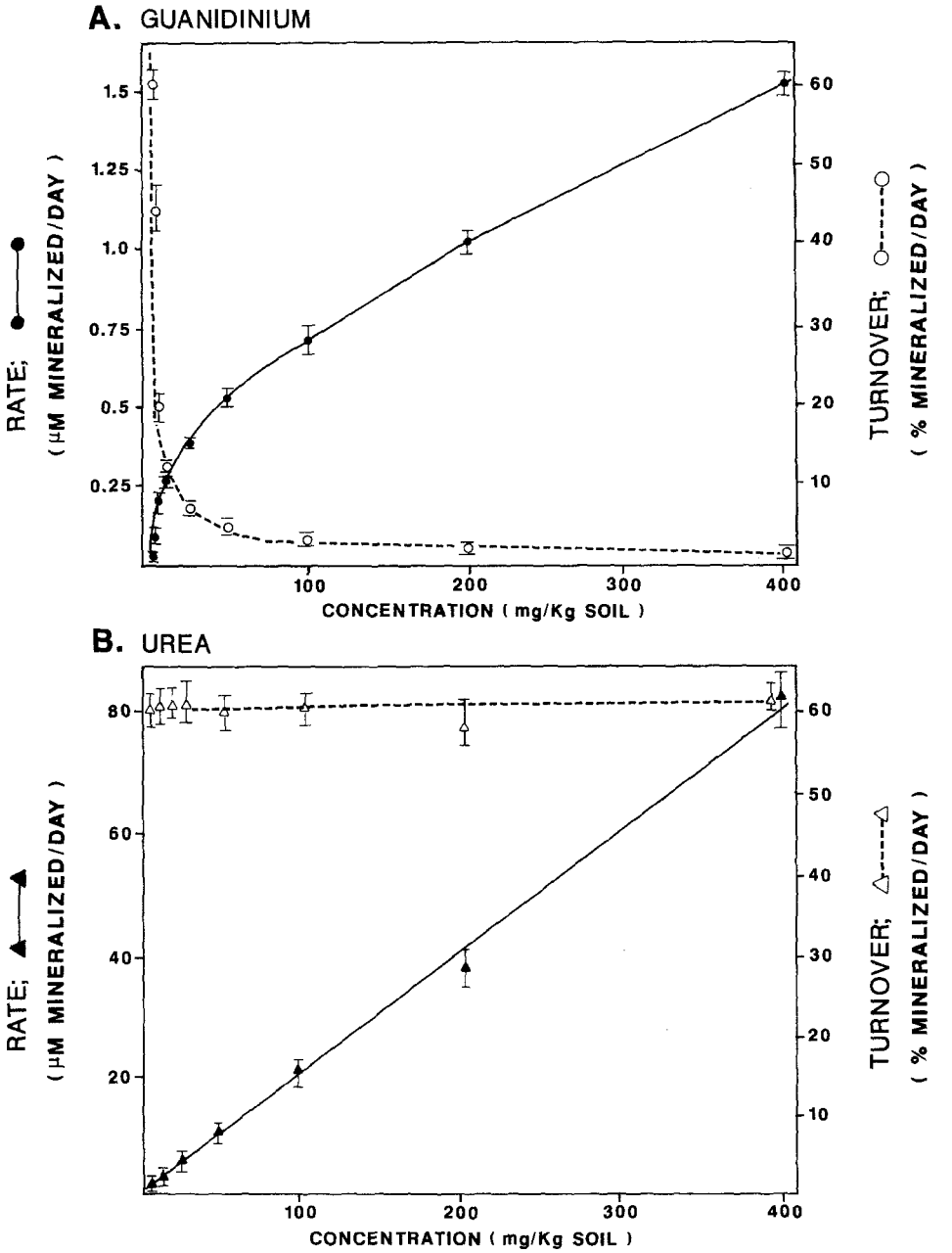

Figure 2. Mineralization of Guanidinium (A) and Urea (B) Carbon with Increasing Concentrations in Duffield Soil Samples.

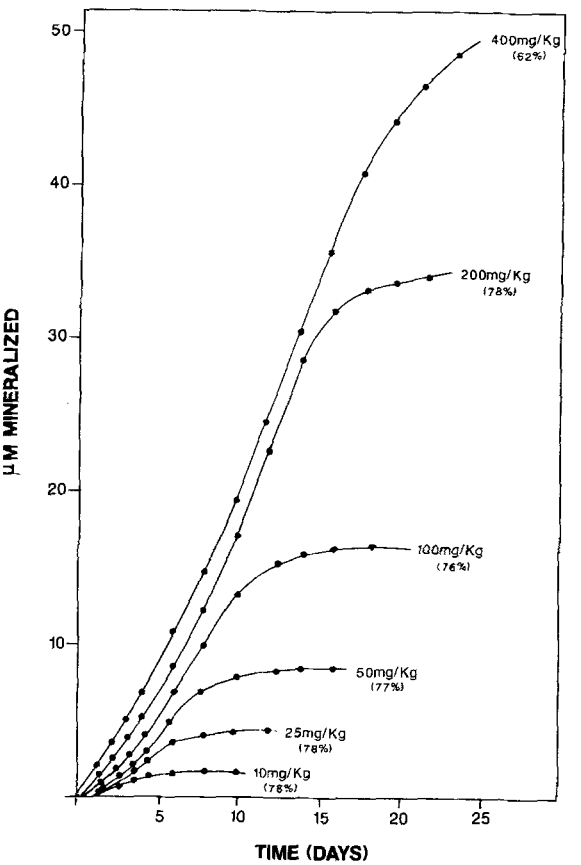

Figure 3. Mineralization of Guanidinium Carbon with Time at Several Concentrations in Duffield Soil. The final percentage of the input guanidinium carbon mineralized is in parentheses.

tion in the same soil samples increased linearly with the concentration and showed no tendency to saturate at the levels tested. At the highest concentration tested, urea carbon mineralization was more than 50 times that of guanidinium carbon. Thus, although carbon turnover (percentage mineralized per day) for both substances is nearly equal at very low concentrations, it decreases drastically with increasing concentrations of guanidinium but not of urea.

The mineralization of guanidinium carbon with extended incubation at several higher input concentrations is shown in Figure 3. In all cases tested, microbial activity ultimately resulted in the conversion of from 62 to 78% of the guanidinium carbon to carbon dioxide. Mineralization rates showed no tendency to decrease at any concentration until most of the cation was degraded, and the concave nature of the curves apparent at 25 mg/Kg and above indicates that rates could as much as double during continued exposure

of the microbial population to the cation. Thus, although at higher concentrations guanidinium turnover is relatively slow (Figure 2), soil microflora remain active long enough to mineralize most of the added guanidinium carbon upon prolonged incubation and also appear to adapt somewhat to facilitate increases in degradation rates.

Shown in Table 1 are the effects of two carbon sources and three major nitrogenous components in nitroguanidine wastestreams on guanidinium carbon mineralization. Both glucose and to a much lesser extent its natural polymer cellulose enhanced 24 hour mineralization rates: production of radiolabeled carbon dioxide was nearly four times the unamended control in the presence of glucose and was increased by twenty percent in the presence of cellulose. The wastewater components nitroguanidine, ammonium, and nitrate showed only a slight tendency to inhibit guanidinium mineralization whether alone at equimolar concentrations to guanidinium or in concert. Previous results have demonstrated that environmental microorganisms in surface water can mineralize guanidinium carbon slowly as a sole substrate and more rapidly in the presence of metabolizable carbon (Mitchell, 1987). While the relative contribution of such populations to the mineralization of the cation in soil can not be assessed in the present study, results with the added carbon sources suggest that the latter process can also be stimulated in soil.

Table 1. Effects of Carbon and Nitrogen Compounds on the Mineralization of Guanidinium Carbon in Duffield Soil.

Sample	nM Mineralized/24Hours (1σ)	%Control (1σ)
Control[1]	634 (4)	100 (<1)
Carbon added as[2]:		
Cellulose	776 (26)	122.4 (4)
Glucose	2481 (273)	391.4 (43)
Nitrogen added as[2]:		
Ammonium	579 (47)	91.3 (7)
Nitrate	596 (8)	94 (1.3)
Nitroguanidine	542 (22)	85.5 (3.5)
Composite	578 (44)	91.7 (7)

1. Triplicate flasks each contained 1 μCi and 20.9 μM guanidinium at a concentration of 100 mg/Kg soil.
2. Carbon and nitrogen compounds were 4% (w/w) and 20.9 μM respectively.

Guanidinium biodegradation in aerobic soil samples can be deceiving. Biodegradation of the cation can be rapid, extensive, and can approach levels of urea degradation at low concentrations. However, the fraction of input guanidinium degraded can also decrease significantly with increased loading and, by comparison at higher concentrations, be only a small percentage of the amount

decrease significantly with increased loading and, by comparison at higher concentrations, be only a small percentage of the amount of urea degraded. Even at a low concentration, the time required for guanidinium degradation can vary with the soil in question. Given sufficient time or perhaps a carbon supplement, aerobic soil microflora have the potential to degrade relatively large amounts of the cation. Thus, while microorganisms in aerobic zones of some soils contaminated with low levels of guanidinium could bring about its rapid removal, the time required for clearance of the cation in the same soils at higher levels, or at low levels in other soils, could be considerably longer.

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