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COMMUNICATIONS

Effect of Two Electron Acceptors on Atrazine Mineralization Rates in Soil

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Introduction

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] and its metabolites, deethylatrazine and deisopropylatrazine, are the most frequently detected pesticides in surface waters of the midwestern United States (*1*) and in Iowa groundwater (*2*). Atrazine is also one of the most heavily used pesticides for corn in the corn belt states (*3*). At the present time, Iowa and other states are considering increased labeling restrictions on atrazine or outright bans due to persistence of the compound in surface water and groundwater in excess of its maximum contaminant level allowable of 3 ppb.

In the upper soil profile, atrazine is biotransformed aerobically to deethylatrazine and deisopropylatrazine, but in deeper soil and groundwater, the role of an alternate electron acceptor for biotransformation becomes important as oxygen gets depleted. Nitrate is usually the alternate electron acceptor under agricultural fields in the corn belt states. Atrazine and other *s*-triazine biodegradation research in different media and environmental conditions have been extensively reviewed (*4–6*). Jessee et al. (*7*) found that a facultative anaerobic bacterium could degrade about half of 75 mg/L atrazine in 1 week under anaerobic conditions. Goswami and Green (*8*), however, did not detect mineralization of atrazine in anaerobic systems, but mineralization of atrazine in aerobic soils has been widely reported in the research literature (*9*). Behki and Khan (*10*) isolated three species of *Pseudomonas* from agricultural soil with a 14-year history of atrazine treatment and found that these species could utilize atrazine as a sole source of carbon. Dealkylation of both the side chains was performed with preferential utilization of the isopropyl side chain. Cook and Hutter (*11*) isolated three strains of *Pseudomonas* and two strains of *Klebsiella pneumoniae* that were able to use *s*-triazines as sole and limiting sources of nitrogen for growth. Giardina et al. (*12*) isolated a soil bacterium, a *Norcardia* strain from a soil enrichment culture, that could utilize atrazine as the sole source of carbon and nitrogen to form dealkylated metabolites and deaminated metabolites. They found that dealkylation preceded deamination in this species and that the final product formed was 2-chloro-4-amino-*s*-triazine.

However, no studies have been reported in the literature on atrazine mineralization under denitrifying conditions in soil systems. This research, therefore, compared the mineralization rates of ^{14}C-labeled atrazine in agricultural soil under oxygenated conditions and denitrifying conditions, to ascertain whether movement of atrazine to anoxic zones in the soil profile will affect biotransformation rates and subsequently its persistence.

Experimental Procedures

Soil used for the experiments was taken from the top 150 cm of the ground surface, from an experimental plot just adjacent to Lily Lake, Amana, IA. The soil is classified as of the Nodeway–Ely series and is of silt–loam texture (organic matter 2.2%, pH 6.3, and CEC 23.5 mequiv/100 g of soil). The soil was air-dried, pulverized, and passed through a 2-mm sieve. The soil was then homogenized, and 500-g portions of the whole soil were added to reactors. Three types of reactors were used for the batch studies. Wide-mouth 1000- and 500-mL Erlenmeyer Pyrex flasks were used for aerobic and anoxic bioreactor studies, respectively, while 50-mL serum bottles were used for microcosm reactor studies. For the microcosm reactors, 30 g of soil was added to each and sealed with gas-tight rubber caps. For all the reactors simulating anoxic conditions, the fresh soil was added directly to the reactors without any preparation. The bioreactors were then sealed with neoprene stoppers with two glass tubing inserts and were sealed air-tight with paraffin wax. Controls were prepared using glass beads and sterilized soil as media. The soil for the sterile controls was first autoclaved in trays for 1 h at 250 °F and 14 psi. The autoclaved soil was then transferred to the flasks, stoppered, and autoclaved again for 15 min at 250 °F and 14 psi. All reactors were prepared in replicates.

Uniform ^{14}C ring labeled atrazine (specific activity 19.4 μCi/mg) or ^{14}C isopropyl side chain labeled atrazine (specific activity 4.9 μCi/mg), obtained from Ciba-Geigy (Greensboro, NC) was added to the soil at a concentration of 0.37 ppm (μg/g of soil). Chemical purity was 95% and >99%, and radiochemical purity was 98.9% and 98.4%, for the ring labeled and isopropyl side chain labeled atrazine, respectively. Deionized water was added (deoxygenated for anoxic soils) after atrazine application to bring the soil to 60% field capacity (water-holding capacity) for aerobic soils and 100% field capacity for all anoxic soils. Nitrate at a soil water concentration of 10 mg/L NO_3^- N (0.16 mM), was added to the anoxic reactors only, to simulate anoxic conditions with nitrate as the electron acceptor. The anoxic bioreactors were supplemented with 10 mg/L NO_3^- N (0.16 mM) on days 30, 67, and 109, and the anoxic microcosm reactors on days 36, 68, and 92 to make up for nitrate depletion. The headspaces of the reactors were filled with N_2/O_2 or N_2 gas mixture for aerobic and anoxic incubations, respectively. The bioreactors were individually wrapped with aluminum foil to shield off light, and the microcosm reactors were placed in boxes shielded from light. The temperature in the laboratory was in the range of 20–25 °C.

At 10–14-day intervals, the air in the reactors was removed by a vacuum pump from one outlet and fresh gas was passed into the reactors from the other. The gases evacuated were trapped in CO_2 traps consisting of glass tubes and 0.2 N NaOH (prepared with CO_2-free deionized

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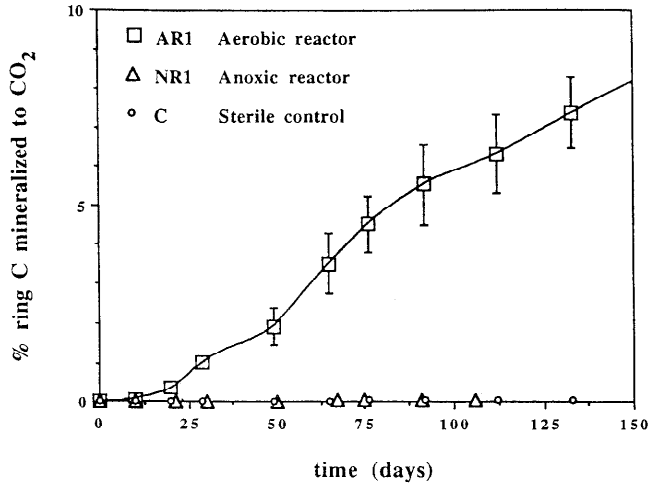

Figure 1. Mineralization of ^{14}C ring labeled atrazine in bioreactors maintained under two different electron acceptor conditions: atrazine applied at 0.37 ppm to Nodeway–Ely soil; soil at 60% field capacity for aerobic reactors and 100% for anoxic reactors; nitrate applied at 10 mg/L as N (soil water concentration) to anoxic reactors. Data points are average results of triplicate reactors. Error bars show standard errors for the data.

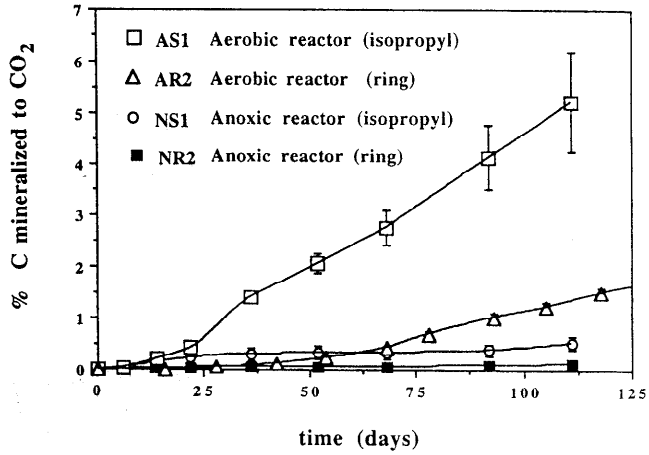

Figure 2. Mineralization of ^{14}C isopropyl side chain (AS1, NS1) and ring labeled atrazine (AR2, NR2) in microcosm reactors maintained under two electron acceptor conditions: atrazine applied at 0.37 ppm to Nodeway–Ely soil; soil at 60% field capacity for aerobic reactors (AS1, AR2) and 100% for anoxic reactors (NS1, NR2); nitrate applied at 10 mg/L as N (soil water concentration) to anoxic reactors. Data points are average results of six replicate reactors. Error bars show standard errors for the data.

water). The fresh gas was passed through a flask of CO_2-free deionized water to moisten the gas before it refilled the reactors. The samples from the traps were then pipeted into 20-mL polypropylene scintillation vials, a scintillation cocktail, Scintiverse E (Fisher Chemicals), was added at twice the volume of the sample, and the vials were counted using a Beckman Model LS 6000IC liquid scintillation counter. Counting efficiency for this sample/cocktail mixture was over 90%. Samples were counted until a fractional error of ±0.5% was obtained at the 95% confidence level, subject to a maximum allowable counting time of 15 min.

Results and Discussion

Figure 1 shows the importance of oxygen as an electron acceptor in atrazine ring mineralization. Bioflask reactors NR1 and AR1 have the same ^{14}C ring labeled atrazine soil concentration, except that NR1 had a nitrogen atmosphere in its headspace and nitrate was added initially to simulate an anoxic environment, with nitrate as the electron acceptor; AR1 had an aerobic headspace. Reactor set C, with sterilized soil maintained under aerobic conditions, was used as a control reactor. The anoxic reactors NR1 showed negligible mineralization even after 100 days of incubation. The soil used was a topsoil and may have had a low population of denitrifying microorganisms. Another experiment performed under the same denitrifying conditions, but with a saturated soil from 2-m depth, showed the same low mineralization amounts as the NR1 bioreactor. The first-order rate constant for atrazine ring carbon mineralization was more than 140 times slower under denitrifying conditions in the bioflasks. The aerobic mineralization rate of the atrazine ring was $(52 \pm 5.3) \times 10^{-5}$/day (half-life 3.6 ± 0.4 years) compared to $(0.37 \pm 0.05) \times 10^{-5}$/day (half-life over 500 years) under denitrifying conditions.

A similar experiment was performed with microcosm reactors with both ^{14}C isopropyl side chain and ring labeled atrazine. Figure 2 shows the results of this experiment. Reactors NR2 and AR2 had ^{14}C ring labeled atrazine and were maintained under denitrifying and aerobic conditions, respectively. Reactors NS1 and AS1 had ^{14}C isopropyl side chain labeled atrazine in the soil instead and had the same electron acceptor conditions as NR2 and AR2, respectively. Both the ring carbons and the isopropyl side chain mineralized much slower under anoxic conditions compared to aerobic conditions. The first-order rate for atrazine isopropyl side chain carbon mineralization was 10 times slower under denitrifying conditions. The aerobic mineralization rate of the isopropyl side chain was $(62 \pm 8.6) \times 10^{-5}$/day (half-life 3.0 ± 0.5 years) compared to $(5.9 \pm 1.2) \times 10^{-5}$/day (half-life 32 ± 0.8 years) under denitrifying conditions.

The mineralization rate of the isopropyl side chain was only slightly faster than mineralization of the atrazine ring. Skipper and Volk (*13*) showed that mineralization of the ethyl side chain was ~8 times faster than the isopropyl side chain. Dealkylation of the ethyl side chain and formation of deethylatrazine is fast (Figure 3), but dealkylation of the isopropyl side chain of atrazine and deethylatrazine is slow and may explain why deethylatrazine persists and is found frequently in unsaturated soils under agricultural fields (*14*).

Figure 4 shows the effect of oxygen concentration in microcosm reactors on mineralization of ^{14}C isopropyl side chain labeled atrazine. Reactor sets AS2, AS1, AS3, and NS1 had initial (after each sampling) oxygen concentrations (in the headspace of the reactors) of 100%, 40%, 20%, and 0%, respectively. The results demonstrated an oxygen limitation in the reactors with 0% and 20% oxygen concentrations in the headspace. The reactors were not aerated continuously because the reactors were meant to simulate deep unsaturated soil environments, so oxygen levels may have been decreased in the reactor set AS3 (20% oxygen) between sampling, causing an oxygen limitation. Reactor sets AS2 and AS1 did not have this oxygen limitation problem and showed higher rates of mineralization than the AS3 and NS1 reactor sets.

It can be estimated that atrazine applied at 2 kg/ha to a Nodeway–Ely soil as used here will be mineralized to 1 kg/ha in ~4 years (half-life) in the unsaturated zone, while under denitrifying conditions the half-life can be estimated as over 100 years. The half-life of atrazine disappearance cited in the research literature varies from 20 to 100 days in soil (*15*), which would indicate a relatively rapid disappearance. On the contrary, detections are frequent in

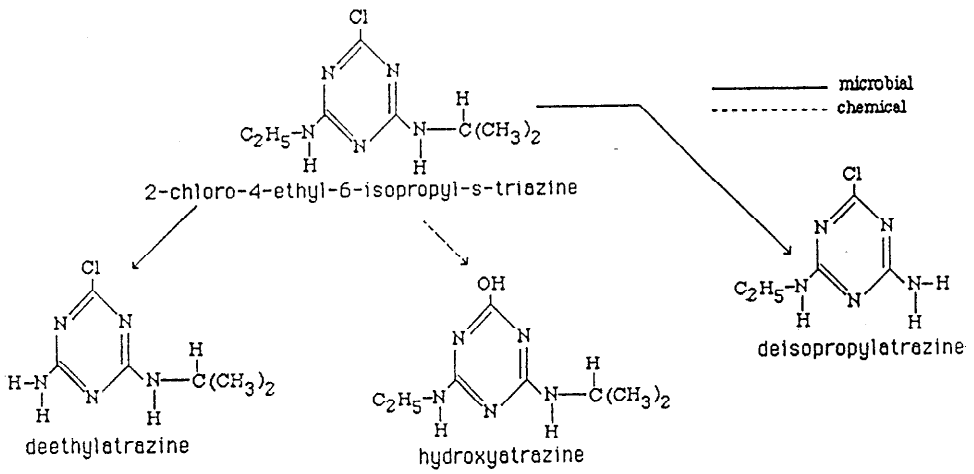

Figure 3. Transformation pathways of atrazine to its primary metabolites.

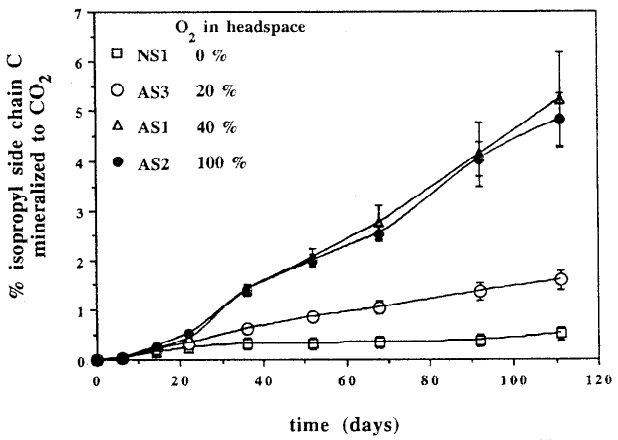

Figure 4. Effect of different oxygen contents in microcosm reactors on mineralization of isopropyl side chain labeled atrazine applied at 0.37 ppm to Nodeway–Ely soil: soil at 60% field capacity for aerobic reactors (AS1, AS2, AS3) and 100% for anoxic reactors (NS1); nitrate applied at 10 mg/L as N(soil water concentration) to anoxic reactors (NS1). Data points are average results of six replicate reactors. Error bars show standard errors for the data.

groundwater, especially at well depths less than 30 m (*2*).

Summary

Atrazine ring and its isopropyl side chain were mineralized much more slowly under denitrifying conditions than under aerobic conditions. Oxygen limitation at deeper soil depths will retard atrazine transformation and mineralization as the soil environment becomes more anoxic. The implication is that if atrazine or its metabolites are transported into the deeper soil and groundwater where denitrifying conditions may prevail, mineralization and transformation of atrazine and its metabolites will be greatly reduced. The slow biotransformation pathway for atrazine and deethylatrazine is dealkylation of the isopropyl side chain, and this in combination with denitrifying conditions may account for the frequent detection of atrazine and some of its metabolites, especially deethylatrazine, in groundwater (*2*, *16*). Also at deeper soil depths, microbial activity will be much lower with lower organic carbon, thus retarding the biotransformation processes even more. The slow dealkylation of the isopropyl side chain of atrazine and its even slower mineralization under anoxic conditions will have implications for bioremediation of sites contaminated with atrazine.

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Registry No. O_2, 7782-44-7; atrazine, 1912-24-9.

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