# Atrazine biodegradation potential in a created wetland

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## Introduction

Atrazine is widely used in cornfields in the American Midwest as a pre-emergence broadleaf herbicide (Park et al., 2003). Its persistence in soils and sediments varies widely (Best and Weber, 1974; Seiler et al., 1992; Radosevich et al., 1997). Although atrazine can be transformed abiotically in small amounts (Burkhard and Guth, 1981), the primary mode of attenuation is microbial biodegradation (Kaufman and Kearney, 1970).

Past studies have shown atrazine mineralization capabilities in wetlands receiving agricultural runoff, such as the Olentangy River Wetland Research Park (ORWRP) (Anderson et al., 2002). There is, as of yet, an incomplete understanding of the factors affecting the activity and distribution of atrazine-degrading microorganisms in wetland environments. The purpose of this study was to determine the aerobic and anaerobic atrazine mineralization potential of the ORWRP as a means of assessing the wetland site for sampling for microbial isolation and identification.

## Methods

### Sampling

Surface water was collected from the Olentangy river using a core sampler. The samples were stored in glass jars, each representing a composite of three sub-samples. Sediment samples were taken from the inlet, middle section, and outlet from experimental wetland 1 at the ORWRP (Figure 1). The top 3- inch section of sediment was retrieved from a core sampler into a plastic sample bag at each sampling location.

### Atrazine Mineralization

River water was concentrated via centrifugation 60-fold (13,739.1 x g, 10 minutes). The concentrated water sample was dispensed in 1 ml aliquots and sediment samples in 5 g wet weight aliquots into 50 mL serum bottles. Duplicate aerobic biometers for each sample were constructed either with or without the addition of glucose (11.1 mM). Each biometer received 0.1 µCi (0.064 µM) of [U-ring-$^{14}$C]-atrazine. For trapping carbon dioxide, each biometer had a 2-ml vial suspended from a rubber septum by copper wire. The vial contained 1 ml of 0.5 M KOH as an alkaline trap. Biometers were closed with crimp-sealed septa.

Duplicate anaerobic biometers for each sample were constructed with no terminal electron acceptor (sterile double distilled $H_2O$) or with one of the following electron acceptors: sulfate as $Na_2SO_4$ (15 µM per biometer), nitrate as $KNO_3$ (25 µM), Fe(III) as ferrihydrite (125 µM). Each anaerobic biometer contained 0.1 Ci (0.064 µM) of [U-ring-$^{14}$C]-atrazine and an alkaline trap. The headspace of these biometers was flushed with oxygen-free nitrogen gas prior to sealing the rubber septa with crimp tops.

At 3-5 day intervals, the alkaline traps were removed and replaced with fresh KOH. The radioactivity in the alkaline trapping solution was measured by liquid scintillation counting using Scintiverse BD (Fisher Scientific, Fair Lawn, NJ) as scintillant. Atrazine mineralization was calculated as the percentage of the total amount of added [U-ring-$^{14}$C]-atrazine that was mineralized to $^{14}CO_2$.

## Results and Discussion

Atrazine mineralization in Olentangy River water samples under aerobic conditions is shown in Figure 2. Approximately 25% of the [U-ring-$^{14}$C]-atrazine was mineralized as $^{14}CO_2$ in 70 days (Figure 2). All but ~2% of the mineralization took place within the first 23 days of incubation, suggesting the microbial populations responsible for the activity were entering death phase at that point. Sediment samples from ORWRP experimental wetland 1, which is fed by the Olentangy River, showed


Figure 1. Diagram of experimental wetland 1. I = inflow; O = outflow; black squares mark approximate locations of the three sediment sampling sites.

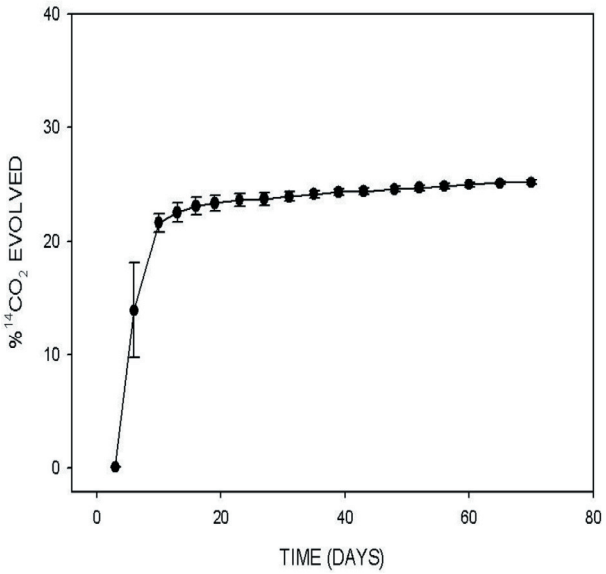


Figure 2. Aerobic biodegradation of [U-ring-14C]-atrazine in duplicate biometers containing 60-fold concentrated Olentangy River water (without glucose amendment). Biodegradation was expressed as the cumulative amount of $^{14}CO_2$ evolved as a percentage of the initial amount of radiolabeled atrazine added. The average of duplicate biometers is shown. Standard deviations only shown if larger than data points.

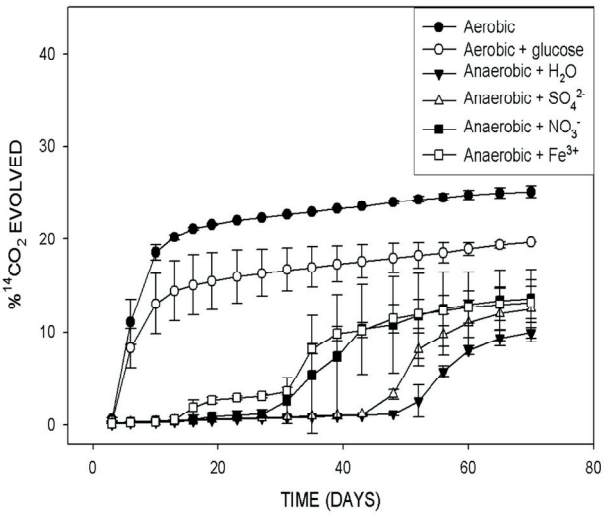


Figure 3. Aerobic and anaerobic biodegradation of [U-ring-$^{14}CO_2$]-atrazine containing ORWRP sediment. Duplicate biometers were constructed for each media condition. Biodegradation was expressed as the cumulative amount of $^{14}CO_2$ evolved as a percentage of the initial amount of radiolabeled atrazine added. The average of duplicate biometers is shown. Standard deviations only shown if larger than data points.

considerable variation in the extent of mineralization. This activity varied with the carbon and energy sources (aerobic biometers) and electron acceptor conditions (anaerobic biometers) (Figure 3). The smaller percentage of atrazine mineralization observed in glucose amended aerobic biometers as opposed to aerobic biometers without glucose amendment probably results from microbial utilization of atrazine as a carbon and energy source, not solely as a nitrogen source. Overall, anaerobic biometers with no added terminal electron acceptors (allowing fermentation as the only energy producing metabolic pathway) mineralized atrazine more slowly and to a smaller percentage of the total atrazine added than anaerobic biometers that included added electron acceptors (allowing for fermentation and anaerobic respiration), which in turn mineralized atrazine more slowly and to a lesser extent than aerobic biometers (allowing for fermentation, anaerobic and aerobic respiration).

## Conclusions

This brief survey revealed that both aerobic and anaerobic microorganisms exist that are capable of atrazine mineralization in the wetland system. Our studies in 2005 in the ORWRP experimental wetland 1 will use an in-situ entrapment technique in an attempt to enrich for atrazine-degrading microbes from the water column and sediments.

## References

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