# Rapid Degradation of Atrazine by *Rhodococcus* Sp. NI86/21 and by an Atrazine-Perfused Soil

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Rapid mineralization of the *s*-triazine ring carbons of atrazine in the absence of other substrates was accomplished by a mixed microbial consortia in soil that had been extensively perfused with a solution of the herbicide. Most (83%) of the labeled *s*-triazine ring carbons were mineralized to $^{14}CO_2$, and nearly all (93%) of the labeled carbon in the ethyl side chain of atrazine was also mineralized at a similar rate as the ring carbons to $^{14}CO_2$. No metabolites accumulated in the broth; however, several metabolites were detected in small amounts after 72 h of incubation. One of these metabolites was identified as hydroxyatrazine. The degradation of atrazine by *Rhodococcus* sp. NI86/21 was also studied, and two metabolites, deisopropylatrazine and deethylatrazine, were identified by mass spectrometry. The major metabolite, deisopropylatrazine, contained 69% of the radiolabel of the parent atrazine, and the minor metabolite, deethylatrazine, accounted for 25% of the parent atrazine.

**Keywords:** *Atrazine; mineralization; perfusion; N-dealkylation; Rhodococcus sp. NI86/21*

## INTRODUCTION

Atrazine is a widely used *s*-triazine herbicide for pre- and post-emergent weed control in crops such as maize and sorghum, as well as for weed control in irrigation channels for cotton production (Barret *et al.*, 1991). Concerns that atrazine may enter groundwater as well as leave phytotoxic residues in soil have prompted interest in obtaining a better understanding of its biodegradation and environmental fate. In Australia, a 1994 Draft Drinking Water Guideline for atrazine was set at 20 $\mu g\ L^{-1}$ (Cooper, 1994). Atrazine has been classified as a class C carcinogen by the U.S. EPA with a preventive action limit set at 0.35 $\mu g\ L^{-1}$ in water supplies (Meisner *et al.*, 1993). The formation of *N*-nitrosoatrazine by ingestion of atrazine in conjunction with nitrite and also by chemical formation of atrazine in the presence of nitrite at low pH has been demonstrated (Weisenburger *et al.*, 1987) and shown to cause up to 10 000 times the normal chromosome breakage in human lymphocytes (Meisner *et al.*, 1993). Environmental epidemiology studies linked the presence of nitrate and atrazine in groundwater supplies to an increased incidence of non-Hodgkin's lymphoma (Weisenburger, 1990).

A New South Wales Department of Water Resources survey (Preece and Whalley, 1993) of the northern rivers in New South Wales, Australia, has detected atrazine in river water at concentrations up to 15.9 $\mu g\ L^{-1}$. Atrazine was the most widely and frequently detected herbicide in the northwestern river systems during the study. Many reports indicate long half-lives of atrazine in soil. Using $^{14}C$-tracer studies, Wolf and Martin (1975) showed that only 18% of applied atrazine in soil was mineralized in 550 days. Atrazine was found to be extremely persistent in both clay and sandy loam soils at 15 °C, with half-lives of 105 and 166 weeks, respectively (Bowmer, 1991). *Rhodococcus* strains degrading EPTC have recently been found also to be capable of dealkylation of atrazine (Behki *et al.*, 1993). Axenic cultures of *Pseudomonas* (Behki and Khan, 1986; Khan and Behki, 1990) and *Nocardia* (Giardina *et al.*, 1980) were also reported to be capable of degrading atrazine, predominantly by dealkylation of the side chains.

Recently, mixed cultures in soil or soil bacterial extracts have resulted in complete degradation of the *s*-triazine ring of atrazine to $CO_2$ (Mandelbaum *et al.*, 1993). These microbes were shown to use the atrazine as a source of nitrogen. However, in that work, sucrose and citrate were provided as carbon sources. The *s*-triazines have been reported to be poor sources of energy (Ericson and Lee, 1989), and studies of *s*-triazine degradation have usually required supplements with carbon compounds (Wierinck and Verstraete, 1990; Cook and Huetter, 1984). The formation of chloride and nitrate has been demonstrated as a result of atrazine mineralization (Gschwind, 1992). During this process, atrazine concentration fell from 20 mg $L^{-1}$ to 10 $\mu g\ L^{-1}$ in liquid batch cultures with soil microbes within 3 days.

In the study described here, the rapid mineralization of the *s*-triazine ring of atrazine by an atrazine-perfused soil in the absence of other carbon substrates and the partial degradation of atrazine by a previously uncharacterized strain of *Rhodococcus* sp. NI86/21 are examined.

## MATERIALS AND METHODS

**Chemicals.** Uniformly ring-labeled [$^{14}C$]atrazine (1.98 MBq $mg^{-1}$) and [*ethyl*-$^{14}C$]atrazine labeled only in the side chain (0.277 MBq $mg^{-1}$) as well as analytical atrazine, deethylatrazine, and hydroxyatrazine were gifts from Ciba-Geigy AG, Switzerland. Technical grade atrazine was a gift of Ciba-Geigy Australia. Analytical grade EPTC was obtained from Rhone Poulenc Australia. All solvents used were of Mallinckrodt Nanograde or HPLC grade.

**Microorganisms.** *Rhodococcus* sp. NI86/21 was obtained from the National Collection of Agricultural and Industrial Micro-organisms, Department of Microbiology, University of Horticulture and Food Industry, Hungary. A Baxter Microscan (Pos MIC Panel Type 6) was used to determine antibiotic

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resistance in *Rhodococcus*. The microbe was maintained on media containing 10 $\mu$g mL$^{-1}$ kanamycin.

**Soil.** A slow draining gray cracking clay soil from the sullage pit of a cotton farm in Narrabri, northern New South Wales, was used in the experiments. Soil properties: pH, 8.2; EC, 0.014 S/m; OC, 0.7%; $CaCO_3$, <0.1%; sand, 17%; silt, 13%; clay, 69%; $Cl^-$, 39 mg/kg; CEC, 522 mmol/kg (Triantafilis and McBratney, 1993).

**Perfusion of Atrazine through Soil.** A 30 cm by 8 cm i.d. glass column was used to contain the soil for perfusion of a sterilized solution of 50 $\mu$g L$^{-1}$ atrazine. The column consisted of 5 cm of washed and sterilized sand at the base of the column held in place by fiber glass cloth. Soil from the sullage pit was mixed with about equal quantities of vermiculite. This mixture was placed over the sand to bring the column height to 25 cm. An aluminum-lined rubber stopper with an inlet for the atrazine solution and filtered air was inserted in the column. A 2 L Nalgene reservoir fitted with a tap to regulate flow was placed above the soil column. The aqueous atrazine solution (30 $\mu$g mL$^{-1}$) was passed through the column at laboratory temperature at about 100 mL/week. Fresh sterile atrazine solution was replaced every 15 weeks. The perfusion was allowed to run for 3 years before experiments on the soil began.

**Assaying the Degradation of Uniformly Ring-Labeled [$^{14}$C]Atrazine and Ethyl Side Chain Labeled [$^{14}$C]Atrazine.** Atrazine-perfused soil/vermiculite mix (20 g) was placed in an autoclaved 250 mL ground glass stoppered conical flask containing 20 mL of distilled water. Control flasks were then autoclaved. The concentration of both uniformly ring-labeled [$^{14}$C]atrazine (1.98 MBq mg$^{-1}$) and ethyl side chain labeled [$^{14}$C]atrazine (0.277 MBq mg$^{-1}$) was brought to 50 $\mu$g L$^{-1}$ using a stock solution of 1000 $\mu$g mL$^{-1}$ in methanol. A ground glass stopper with an attachment to suspend a 2 mL glass vial inside the system was used to seal the flask. All glassware was sterilized by autoclave prior to use. The vial contained 1 mL of autoclaved 2 M NaOH enabling the $^{14}CO_2$ to be collected. The efficient collection of $^{14}CO_2$ by NaOH has previously been demonstrated (Guerin, 1993; Oades and Jenkinson, 1979). Samples were taken aseptically at days 1, 2, 3, 5, 10, and 15, and the NaOH was replaced at each sampling time. The flasks were incubated on a shaker between 25 and 30 °C. A similar system was used for broth cultures of *Rhodococcus* sp. NI86/21. Basal minimal nutrient broth (20 mL) with yeast and glycerol (BMNGYE) as carbon source was used for growth of *Rhodococcus* (Tam *et al.*, 1987).

**Extraction of the Atrazine Residues into an Organic Phase from the Soil Slurry.** At each sampling, 2 g of soil slurry was removed and placed in a ground glass stoppered flask. Twenty milliliters of 10% methanol in chloroform was added and the flask shaken at room temperature for 1 h. The organic phase was filtered through anhydrous sodium sulfate and evaporated to near dryness under a stream of nitrogen. The sample was drawn into a 250 $\mu$L syringe, and small volumes of chloroform were added to dissolve remaining residues and bring the volume to 250 $\mu$L.

**Extraction of Bacterial Broth Cultures.** Two milliliters of broth culture was extracted using 10% methanol in chloroform in a Mixxor liquid/liquid extractor (Guerin *et al.*, 1992; Feng *et al.*, 1994).

**Determination of $^{14}CO_2$.** The 1 mL of 2 M NaOH used to collect the $^{14}CO_2$ in the sealed flask system was transferred to a 20 mL Packard glass scintillation vial; 10 mL of Hionic Fluor was added, and the samples were shaken and allowed to stand in the dark for 0.5 h before being counted in a United Technologies Packard Tri-carb 4000 Series scintillation counter.

**Estimation of [$^{14}$C]Atrazine and Degradation Products Using Thin Layer Chromatography.** A glass dome tipped syringe was used to load 10 $\mu$L of the chloroform extract onto a Kieselguhr Silica 60 aluminum-backed TLC plate (20 cm × 20 cm). The TLC plate was developed using 10% methanol in chloroform solvent for 2 h. The TLC plate was then allowed to air-dry briefly before being guillotined into 1 cm vertical grids for each sample applied. The 18 TLC segments were placed in 20 mL scintillation vials, 1 mL of 10% methanol in chloroform was added, and the vials were shaken prior to the addition of 10 mL of Packard 299 general purpose scintillant. The sample was again shaken and allowed to stand in the dark for 0.5 h before counting.

**Confirmation of Atrazine Metabolites.** Kieselguhr Silica $60_{F254}$ aluminum-backed TLC plates (20 cm × 20 cm) were used to confirm $R_f$ values of atrazine and its metabolites. Standards and metabolites appeared brown on a green background when viewed under UV light.

**Unlabeled Atrazine and EPTC Studies.** The degradation of unlabeled atrazine and EPTC was assayed for both the *Rhodococcus* sp. NI86/21 and the atrazine-perfused soil using BMNGYE broth. Broths were inoculated with about $10^7$ cells.

**Extraction of Unlabeled Atrazine and EPTC.** A 5 mL sample of the culture was extracted with a Mixxor liquid/liquid extractor using 25% acetone in hexane or diethyl ether as sovlent. Samples were then evaporated under reduced pressure and redissolved in hexane or methanol for GC analysis.

**Gas Chromatographic Analysis of Atrazine and Deethylatrazine.** A Hewlett-Packard 5890 Series II gas chromatograph equipped with a $^{63}$Ni electron capture detector was used to analyze the atrazine and deethylatrazine. A J&W Scientific 30 m by 0.32 mm i.d., 0.25 $\mu$m film thickness, fused silica Durabond DB-17 fused silica capillary column was fitted to the instrument. An autosampler injected 1 $\mu$L of sample in hexane, the injection port was purged at 1 min, and a temperature program (60 °C for 1 min−170 °C at 25 °C min$^{-1}$−260 °C at 5 °C min$^{-1}$, final time 5 min) was used. Helium was the carrier gas with a column head pressure of 24 kPa, giving a linear gas flow of 40 cm s$^{-1}$ at the midrun temperature, with nitrogen as the detector makeup gas (25 mL min$^{-1}$). The injector temperature was 240 °C, and detector temperature was 300 °C. Deethylatrazine eluted at around 14 min and atrazine at around 15 min, with a total chromatographic analysis time of 25 min. The chromatograms were recorded and integrated by a Hewlett-Packard Vectra computer with Chemstation software. The limit of detection of atrazine and deethylatrazine using the ECD was 50 pg of injected component.

**Identification of Atrazine, Deisopropylatrazine, and Deethylatrazine.** The identities of atrazine and its metabolites were confirmed with a Hewlett-Packard Model 5971 mass spectrometer interfaced with the gas chromatograph. A J&W Scientific DB-Wax fused silica capillary of similar dimensions to that previously described was fitted. The temperature of the mass spectrometer interface was set to 250 °C. Ions with a mass to charge ratio of between 50 and 400 were selected for detection.

**Gas Chromatographic Analysis of EPTC Using MSD Quantification.** A variation in the temperature program was used for EPTC analysis (70 °C for 1 min−140 °C at 6 °C min$^{-1}$−250 °C at 30 °C min$^{-1}$, final time 5 min). The mass spectrometer was in selected ion mode (SIM) for the analysis of EPTC, with 10 prominent ions selected. Ions with a mass to charge ratio of 27, 30, 43, 62, 86, 128, 160, and 189 were sought with a MS dwell time of 100 ms/ion. The resulting voltage after autotune was 1400 eV. The limit of detection for EPTC was 1 ng of EPTC injected.

## RESULTS

**Mineralization of Radiolabeled Atrazine by the Atrazine-Perfused Soil.** Almost complete mineralization of the three labeled carbons in uniformly ring-labeled [$^{14}$C]atrazine was achieved by the microbial population in the atrazine-perfused soil. Within 360 h, 83% of these carbons were mineralized to $^{14}CO_2$, as shown by Figure 1. No $^{14}CO_2$ was produced by the autoclaved control.

Thin layer chromatography of the extract from the incubation taken at 144 h revealed a minor metabolite with an $R_f$ of 0.39, accounting for 4% of the initial $^{14}$C label present. An analytical standard of hydroxyatrazine detected by quenching of the fluorescent indicator had the same $R_f$, indicating that the metabolite in the

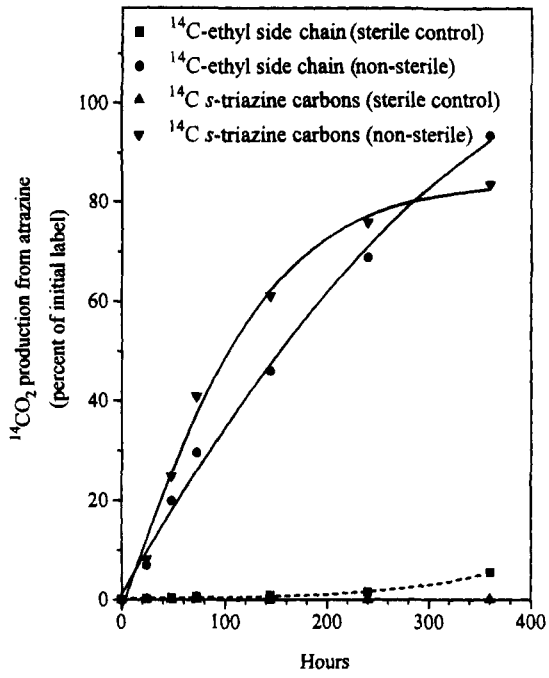



**Figure 1.** Percent conversion of uniformly ring-labeled [$^{14}$C]atrazine and [*ethyl*-$^{14}$C]atrazine to $^{14}CO_2$ by the atrazine perfused soil.

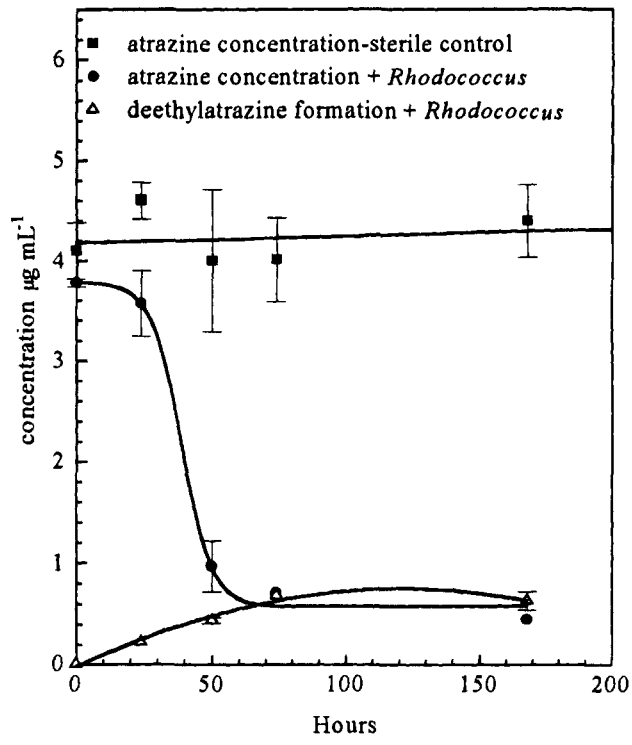
incubation with the perfused soil was hydroxyatrazine. No metabolites were detected in the autoclaved control. Only 17% of the parent atrazine ($R_f$ of 0.94) remained in the nonsterile treatment at 144 h, while 100% remained in the autoclaved control.

Experiments were also conducted with these soil cultures to determine the fate of the labeled carbon in the ethyl side chain of atrazine. As with the labeled ring carbons, the carbon of the labeled ethyl side chain was mineralized to $^{14}CO_2$ (Figure 1). Within 360 h, 93% of the total side chain label was mineralized to $^{14}CO_2$, and the rates of mineralization of the ethyl side chain carbon and of the ring carbons were similar. Less than 7% of the side chain label in the autoclaved control was found as $^{14}CO_2$.

Thin layer chromatography of the extract from the incubation with atrazine-perfused soil taken at 72 h showed a number of metabolites were present, but at low levels. The most prominent product had an $R_f$ of 0.61, accounting for 4.5% of the total initial side chain radiolabel. Others, with $R_f$ values of 0.28 and 0.44, each accounted for 2% of the $^{14}$C label.

The microbes responsible for the mineralization of both the $^{14}$C ring carbons as well as the ethyl $^{14}$C side chain into $^{14}CO_2$ were found to be firmly associated with the soil particulate matter. Samples from these broths as well as a sample of the solid soil were used as inocula in a BMNGYE broth containing 20 $\mu$g mL$^{-1}$ atrazine. Only the solid soil inoculum led to atrazine metabolism. Tests showed that the original soil not perfused with atrazine was unable to degrade atrazine during a 360 h sampling period in the same system.

**Degradation of Atrazine by *Rhodococcus* Sp. NI86/21.** *Gas Chromatographic Studies Using Unlabeled Atrazine.* *N*-Dealkylation of atrazine by *Rhodococcus* sp. NI86/21 was shown in BMNGYE nutrient broth. One of the *N*-dealkylated metabolites, deethylatrazine, was quantified in these studies. The other, deisopropylatrazine, could not be quantified in these studies using the hexane/acetone extraction procedure and GC analysis on the DB-17 column. Assays com-



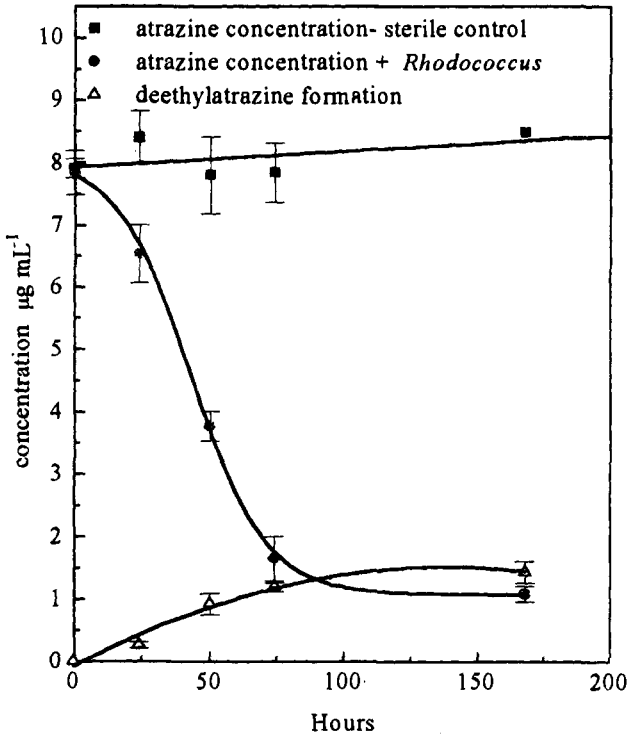




**Figure 2.** Metabolism of 4 and 8 $\mu$g mL$^{-1}$ atrazine by *Rhodococcus* sp. NI86/21 to deethylatrazine.

mencing with 4 and 8 $\mu$g mL$^{-1}$ of atrazine in the broth (Figure 2) were performed to determine the percentage formation of deethylatrazine from the parent compound atrazine. In the assay with 4 $\mu$g mL$^{-1}$ of atrazine, the reaction was complete at around 75 h after inoculation and the time taken to reach half the initial concentration of atrazine was 39 h. The assay with 8 $\mu$g mL$^{-1}$ of atrazine was completed at around 125 h, and the time to reach half the initial concentration was 43 h. The concentrations of atrazine used appear to have kinetically saturated the system, as the rates of degradation

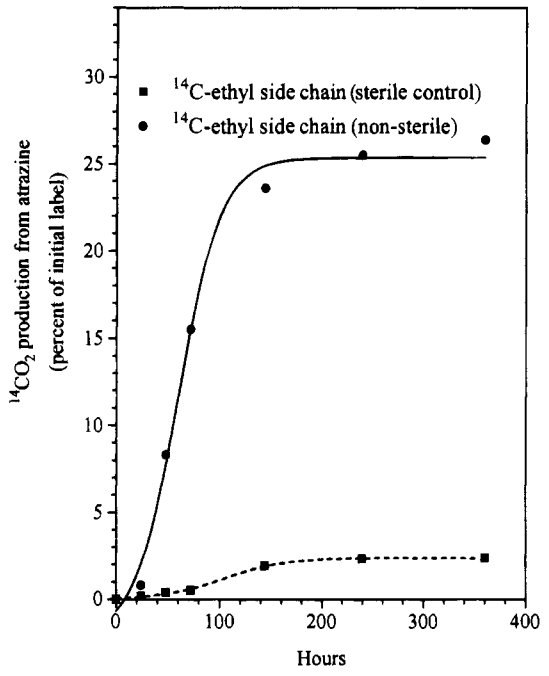



**Figure 3.** Percent conversion of [*ethyl*-$^{14}$C]atrazine to $^{14}CO_2$ by *Rhodococcus* sp. NI86/21.

were similar for each concentration. Between 3 and 4 $\mu$g mL$^{-1}$ of atrazine was degraded by the *Rhodococcus* within the first 50 h at both concentrations.

After an incubation of 168 h, around 12% of the atrazine remained unchanged when the starting concentration was 4 $\mu$g mL$^{-1}$, and 14% remained with 8 $\mu$g mL$^{-1}$. The assay with 4 $\mu$g mL$^{-1}$ atrazine showed that 17% of the parent compound was metabolized to deethylatrazine by 168 h, while 18% was converted to this product with 8 $\mu$g mL$^{-1}$ of atrazine.

*Studies Using Uniformly Ring-Labeled [$^{14}$C]Atrazine.* *Rhodococcus* sp. NI86/21 was unable to mineralize the *s*-triazine ring of atrazine, as no $^{14}CO_2$ was detected in the incubation with uniformly ring-labeled [$^{14}$C]atrazine. The thin layer chromatogram showed metabolites of atrazine having $R_f$ values of 0.72 and 0.83, deisopropylatrazine and deethylatrazine, respectively. The peak of radioactivity at $R_f$ 0.83 was identified with an analytical standard of deethylatrazine by quenching of fluorescent indicator. The deethylatrazine accounted for 25% of the initial radiolabel. The more prominent metabolite with an $R_f$ 0.72 was later shown by mass spectrometry to be deisopropylatrazine, accounting for 69% of the initial radiolabel. A similar TLC profile was found by Adams *et al.* (1990), who studied the metabolism of atrazine by rat hepatic microsomes. The degradation of atrazine by this strain of *Rhodococcus* therefore appears to be limited to the removal of the ethyl and isopropyl side chains, as only these two metabolites were detected by TLC.

*Studies Using Ethyl Side Chain Labeled [$^{14}$C]Atrazine.* Assays to determine the fate of the ethyl $^{14}$C label in incubations with *Rhodococcus* sp. NI86/21 were conducted. Figure 3 shows a time course of conversion of the side chain label into $^{14}CO_2$. By 72 h, 16% of the total label had been metabolized into $^{14}CO_2$, while a maximum of 25% conversion was reached at about 120 h. The removal of the $^{14}$C label on the ethyl side chain corresponded to the formation of the metabolite deethylatrazine. It can therefore be concluded that a maximum of 25% of the atrazine was metabolized by *Rhodococcus* sp. NI86/21 to deethylatrazine.

**Table 1. Principal Ions in the Mass Spectra of Atrazine and *N*-Dealkylated Metabolites of *Rhodococcus* Sp. NI86/21**

| peak retention time (min) | *m/z* | relative abundance % | structural assignment | compound |
|---|---|---|---|---|
| 19.87 | 217.05 | 27.1 | [M] + $^{37}$Cl | atrazine |
| | 215.10 | 64.5 | [M] | |
| | 202.00 | 40.0 | [M − $CH_3$] + $^{37}$Cl | |
| | 200.05 | 100.0 | [M − $CH_3$] | |
| | 173.00 | 29.5 | [M − $C_3H_7$]$^+$ | |
| 26.65 | 189.05 | 16.7 | [M] + $^{37}$Cl | deethyl- |
| | 187.05 | 37.6 | [M] | atrazine |
| | 174.00 | 28.9 | [M − $CH_3$] + $^{37}$Cl | |
| | 172.00 | 100.0 | [M − $CH_3$] | |
| | 145.05 | 25.9 | [M − $C_3H_7$]$^+$ | |
| 29.81 | 175.00 | 37.6 | [M] + $^{37}$Cl | deisopropyl- |
| | 173.00 | 100.0 | [M] | atrazine |
| | 160.00 | 20.2 | [M − $CH_3$] + $^{37}$Cl | |
| | 158.05 | 62.1 | [M − $CH_3$] | |
| | 145.05 | 80.9 | [M − $C_2H_5$]$^+$ | |

Deethylatrazine could not be detected as a radioactive spot at $R_f$ 0.83 on the thin layer chromatogram when the starting substrate was ethyl side chain labeled [$^{14}$C]atrazine, as the $^{14}$C label had been removed and $^{14}CO_2$ formed. The second metabolite with an $R_f$ of 0.72 accounted for 55% of the total initial label after 72 h and 69% after 144 h. This metabolite of atrazine was confirmed to be deisopropylatrazine by GC−mass spectrometry.

**Mass Spectral Identification of Unlabeled Metabolites.** Three gas chromatographic peaks were detected in extracts obtained after *Rhodococcus* sp. NI86/21 was incubated with atrazine at 20 $\mu$g mL$^{-1}$. Table 1 summarizes the prominent ions for these three peaks in the total ion chromatogram. The mass spectrum of atrazine, which eluted at 19.87 min, showed a base peak at *m/z* 215 and characteristic ions at *m/z* 217, 202, 200, and 173. Deethylatrazine eluted at 26.65 min and had a base peak of *m/z* 187. Prominent ions were at *m/z* 189, 174, 172, and 145. The compound eluting at 29.81 min had a base peak of *m/z* 173 and prominent ions of *m/z* 175, 160, 158, and 145. It can be concluded that the compound eluting at 29.81 min was deisopropylatrazine. Its theoretical molecular weight of 173 was found in the mass spectrum, and the ion at *m/z* 175 was attributed to the $^{37}$Cl isotope.

**Studies on Deethylatrazine Degradation.** Deethylatrazine has been shown above to be a metabolite of atrazine in incubations with *Rhodococcus* sp. NI86/21. It was demonstrated that this strain of *Rhodococcus* was unable to further metabolize deethylatrazine at a concentration of 5 $\mu$g mL$^{-1}$ when it was supplied to a BMNGYE broth. This supports the results in Figure 2 indicating that deethylatrazine is a terminal metabolite of atrazine in incubations with *Rhodococcus* sp. NI86/21.

**Studies on EPTC Degradation.** The ability of *Rhodococcus* sp. NI86/21 to degrade EPTC (Nagy *et al.*, 1987, 1991) was confirmed in these studies by GC−MS detection using SIM. The assay was performed in triplicate and showed the *Rhodococcus* removed 100% of the applied EPTC (20 $\mu$g mL$^{-1}$) in nutrient broth within 24 h. *N*-Dealkylation processes for thiocarbamate herbicides such as EPTC have been previously investigated, with the ability to degrade both EPTC and atrazine shown to be associated with expression of genes in a 50.5 MDa plasmid (Tam *et al.*, 1987).

## DISCUSSION

Rapid mineralization of the *s*-triazine ring of atrazine has been reported on only a few occasions (Mandelbaum *et al.*, 1993; Gschwind, 1992). The process of atrazine degradation by the mixed microbial community of the atrazine-perfused soil reported here was much more extensive than the limited *N*-dealkylation activity of *Rhodococcus* sp. NI86/21. Both processes, however, destroy the herbicidal activity of atrazine. The atrazine-perfused soil was capable of cleaving the *s*-triazine ring of atrazine, with 83% of the $^{14}C$ label from the ring being mineralized to $^{14}CO_2$ within 360 h. In the same period, 93% of the $^{14}C$ label in the ethyl side chain was mineralized into $^{14}CO_2$.

Mandelbaum *et al.* (1993) has also demonstrated rapid mineralization of the *s*-triazine ring of atrazine. Over 200 bacterial colonies were isolated from their soil and tested for atrazine degradation. None were found to individually degrade atrazine; however, when mixed together, the degrading ability was restored.

The atrazine-degrading microbial consortia in our studies were enriched *in situ* using a perfusion of atrazine solution through the soil. The soil initially had the potential to have microbes adapted for xenobiotic degradation as it was taken from the pit where storage drums and tanks containing pesticides were washed. This soil was not able to rapidly degrade atrazine prior to the perfusion with the herbicide. However, after the perfusion, it was capable of rapid atrazine mineralization. Early soil perfusion experiments have shown that the lag phase in 2,4-D degradation could be eliminated by continual perfusion of the herbicide through the soil (Audus, 1952). It is likely that the rapid mineralization of atrazine by the soil reported in our results after perfusion of the herbicide was due to the removal of the lag phase associated with its degradation; however, the possibility of microbial adaptation to degrade atrazine cannot be excluded.

The microbes in our perfused soil were restricted to the favorable microecological site of soil particles, as we demonstrated that it was essential to have the atrazine-perfused soil present to achieve degradation of the herbicide. The microbes responsible for atrazine mineralization were not free to dissociate into the liquid phase of the soil solution in our experiments. It is well understood that bacterial cells are rarely free in the liquid phase of the soil because most cells adhere to clay particles and humus (Alexander, 1977).

The results given here show that *Rhodococcus* sp. NI86/21 limited *N*-dealkylation of the atrazine to two metabolites, deisopropylatrazine and deethylatrazine. This strain of *Rhodococcus* forms deisopropylatrazine as its major metabolite, with a maximum of 69% of the atrazine being metabolized to deisopropylatrazine after 144 h, and deethylatrazine accounted for only 25% of the initial atrazine.

*Rhodococcus* strain TE1 (Behki *et al.*, 1993) also degraded atrazine to two metabolites, deethylatrazine and deisopropylatrazine. This TE1 strain predominantly metabolized atrazine to deethylatrazine, thus contrasting with *Rhodococcus* sp. NI86/21 in our studies. *Rhodococcus* strain B-30 (Behki and Khan, 1994) also produced deethylatrazine as its major metabolite, and a third metabolite, 2-chloro-4,6-diamino-*s*-triazine, was detected.

The role of *Rhodococcus* sp. NI86/21 as a plant biosafener against the herbicide EPTC is described in the patent by Nagy *et al.* (1987). The bacteria were applied as a suspension in physiological medium to the soil prior to sowing of crops such as maize and sorghum, which were susceptible to the herbicide, and protected them from the phytotoxic effects of the herbicide. This rhizosphere engineering (Nagy *et al.*, 1991), which has been successfully applied to the thiocarbamate herbicides, may also be useful for reducing the phytotoxic effects of atrazine in soil. In previous research, the application of microbes degrading 2,4-D to roots of canola and wheat has demonstrated that these rhizosphere-associated microbes were capable of degrading the 2,4-D and protecting the plant from the phytotoxic effects of the herbicide in a hydroponic system (Van Zwieten *et al.*, 1995). Trials in which *Rhodococcus* sp. NI86/21 is applied to the roots of seedlings are currently under investigation to determine the plant biosafening effects of this organism with atrazine residues.

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