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Mineralization of the *s*-Triazine Ring of Atrazine by Stable Bacterial Mixed Cultures

RAPHI T. MANDELBAUM,[1,2] LAWRENCE P. WACKETT,[1]* AND DEBORAH L. ALLAN[2]

Department of Biochemistry and Institute for Advanced Studies in Biological Process Technology[1] and Soil Science Department,[2] University of Minnesota, St. Paul, Minnesota 55108

Received 20 November 1992/Accepted 9 March 1993

Enrichment cultures containing atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) at a concentration of 100 ppm (0.46 mM) as a sole nitrogen source were obtained from soils exposed to repeated spills of atrazine, alachlor, and metolachlor. Bacterial growth occurred concomitantly with formation of metabolites from atrazine and subsequent biosynthesis of protein. When ring-labeled [^{14}C]atrazine was used, 80% or more of the *s*-triazine ring carbon atoms were liberated as $^{14}CO_2$. Hydroxyatrazine may be an intermediate in the atrazine mineralization pathway. More than 200 pure cultures isolated from the enrichment cultures failed to utilize atrazine as a nitrogen source. Mixing pure cultures restored atrazine-mineralizing activity. Repeated transfer of the mixed cultures led to increased rates of atrazine metabolism. The rate of atrazine degradation, even at the elevated concentrations used, far exceeded the rates previously reported in soils, waters, and mixed and pure cultures of bacteria.

Atrazine is the most widely used *s*-triazine herbicide in the United States (5). It has widespread use in the control of broad-leaf weeds in corn, sorghum, and certain other crops. Atrazine is moderately persistent in the environment (11), and it has a water solubility of 33 mg liter^{-1} (at 27°C) and a reported half-life in soils ranging from 4 to 57 weeks (3, 7). Spillage of this compound at herbicide-loading sites and subsequent runoff can cause crop damage and groundwater contamination. As a result, atrazine is detected in groundwater and soils in concentrations exceeding the maximum contaminant level of 3 μg liter^{-1} that took effect in 1992 (26). In Kansas, atrazine has been detected in well water at several locations at levels of up to 7.4 μg liter^{-1} (19). Point source spills of atrazine have resulted in levels as high as 25 μg liter^{-1} in some wells in Minnesota (10).

Herbicides containing an *s*-triazine ring are relatively persistent in the environment. This has stimulated investigations into the biodegradation of these compounds, which have had mixed success (11). In one report, 33 mixed bacterial cultures were examined, and all failed to degrade atrazine (14). Enrichment cultures from silty loam soil failed to mineralize atrazine to CO_2 (12). Generally, less heavily substituted and nonchlorinated *s*-triazines are more biodegradable than atrazine (8). For example, two soil fungi degrade cyanuric acid, but not atrazine, to CO_2 (29). Bacteria capable of utilizing *s*-triazine compounds as sole nitrogen sources have been isolated by enrichment culturing. However, *Pseudomonas* and *Klebsiella* strains which degraded various *s*-triazine ring compounds were not capable of metabolizing atrazine (8, 9). Several microorganisms that can degrade atrazine have been isolated, but in most cases only N-dealkylation of the atrazine side chains occurred, and complete metabolism of the *s*-triazine ring was not demonstrated. *Pseudomonas* strains reportedly N-dealkylate atrazine and use the side chain carbons for growth (2).

Kaufman and Blake (18) studied several soil fungi that were able to degrade atrazine by N-dealkylation, as evidenced by $^{14}CO_2$ evolution from ^{14}C-labeled ethyl or isopropyl atrazine. McMahon et al. (23) reported that the ethyl side chain of atrazine but not the *s*-triazine ring was degraded by microbial processes in alluvial-aquifer sediments. Most recently, very slow liberation of $^{14}CO_2$ from the atrazine ring was observed in soil bioreactors (24). Less than 10% of uniformly ring-labeled [^{14}C]atrazine was converted to $^{14}CO_2$ in 125 days.

The objective of this research was to enrich for microorganisms capable of mineralization of high concentrations of atrazine. Previous studies in which atrazine was used as the sole N source failed to yield bacterial cultures that effectively mineralize atrazine. In contrast to other studies, we used citrate as a carbon source and succeeded in obtaining stable mixed bacterial enrichment cultures that liberated $^{14}CO_2$ from the *s*-triazine ring of atrazine. The rates of atrazine degradation observed were significantly faster than those previously reported for atrazine side chain or *s*-triazine ring metabolism.

MATERIALS AND METHODS

Sampling sites. Soil samples were collected from three Minnesota sites which were formerly operated as agricultural chemical dealerships and are being considered for bioremediation. Two sites in Little Falls, Minn., and one site in Albany, Minn., are referred to below as the LFA, LFB, and ALB sites, respectively. The native soils at the LFA site are Hubbard and Nymore sands, and at the LFB site the native soils are loamy sands. The native soils at the ALB site are Cordoba loam and unclassified ponded histosols (15).

Twelve cores (1.9 cm [inner diameter] by 30 cm deep) were collected at random locations at each site and stored at 4°C until they were used. Some grassy weeds were also collected from the boundaries of the LFA site for isolation of potential atrazine-degrading microorganisms from the rhizoplane. Physical and chemical data are presented in Table 1.

Enrichment cultures. Selective enrichment cultures were used to obtain mixed bacterial populations that were capable of growth on atrazine as a nitrogen source. Atrazine, metolachlor, and alachlor concentrations in soil samples were analyzed as described by Koskinen et al. (20). For samples

* Corresponding author.

TABLE 1. Physical and chemical properties of the spill site soils

Sampling site	Soil type[a]	Particle size distribution			pH in water	pH in 0.1 N $CaCl_2$	Total N content ($\mu g\ g^{-1}$)	Olson P content ($\mu g\ g^{-1}$)	Total organic matter content (%)	Total organic carbon content (%)
		% Sand	% Silt	% Clay						
LFA	Loamy sand	85	7	8	5.04	4.88	1,240	135	1.5	0.90
LFB	Loamy coarse sand	87	7	6	7.04	7.18	500	95	0.8	0.44
ALB	Loam	44	42	14	5.48	4.88	1,280	500	2.3	1.65

[a] U.S. Department of Agriculture soil type.

that contained one or more herbicides, 10 g of soil was suspended three times in 30 ml of 0.1 M phosphate buffer (pH 7.3) and centrifuged at 7,000 × *g* for 10 min at 4°C, and the supernatants were discarded to reduce the quantity of extraneous nitrogen source(s).

To obtain enrichment cultures of weed rhizoplane microorganisms, loose soil particles were removed by hand, and the roots were submerged for 10 min in tap water, rinsed for 1 min under slowly running tap water, and air dried. Roots were cut into 5-cm segments, and 10 segments were shaken in a 250-ml Erlenmeyer flask with 10 g of glass beads (diameter, 3 mm) and 50 ml of 0.1 M phosphate buffer on a reciprocal shaker for 2 h. Enrichment cultures were prepared as described below by using 5 ml of the buffer as inoculum.

A 5-g wet soil pellet or 5 ml of a rhizoplane suspension was inoculated into 20 ml of atrazine medium containing (per liter of deionized water) 1.6 g of K_2HPO_4, 0.4 g of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of NaCl, 0.02 g of $CaCl_2$, 1 g of sucrose, 1 g of sodium citrate, 2.5 ml of atrazine stock solution, 20 ml of a salt stock solution, and 20 ml of a vitamin stock solution. The salt stock solution contained (per liter of deionized water) 2.5 g of EDTA, 11.1 g of $ZnSO_4$, 5.0 g of $FeSO_4$, 1.54 g of $MnSO_4 \cdot H_2O$, 0.4 g of $CuSO_4 \cdot 5H_2O$, 0.25 g of $Co(NO_3)_2 \cdot 6H_2O$, 0.18 g of $Na_2B_4O_7 \cdot 10H_2O$, and 5.0 ml of concentrated H_2SO_4 to retard precipitation of salts. The vitamin stock solution contained (per liter of deionized water) 5 mg of thiamine-HCl, 2 mg of biotin, 2 mg of folic acid, 10 mg of nicotinamide, and 10 mg of pyridoxine-HCl. The atrazine stock solution was prepared in methanol (20 mg/ml) and was vigorously shaken for several hours prior to incorporation into the medium. The salt and vitamin stock solutions were filter sterilized and kept at 4°C. The atrazine stock solution was stored unfiltered at room temperature in the dark. Cycloheximide (50 mg $liter^{-1}$) was added to media used for isolation of bacteria, and the pH was adjusted to 7.3. The media used for isolation of fungi were acidified to pH 5.5 with a concentrated HCl solution, but were not amended with antibiotics. Cultures were incubated without shaking at 30°C in the dark to preclude photolysis reactions. All enrichment cultures were subcultured into a homologous medium at 2-week intervals. From a 2-week-old culture, 0.5 ml was transferred to 20 ml of freshly prepared atrazine medium. After cultures were subcultured four times under conditions of nitrogen limitation, atrazine degradation was quantified by high-pressure liquid chromatography (HPLC) as described below. Positive enrichment cultures were harvested by centrifugation, resuspended in buffer, and either frozen at −20°C or amended with 20% glycerol (by volume) and stored at −70°C.

Each culture received a designation that consisted of the abbreviation for the site where the initial inoculum was collected, the sample number within the site, and a letter; A indicated that a culture was grown in an acidified medium for the first four growth cycles, N indicated that a culture was grown in a neutral medium throughout the enrichment period, and R indicated that a culture was isolated from the grassy weed rhizoplane.

Atrazine mineralization by growing cultures. Uniformly ring-labeled [^{14}C]atrazine was added to 100 ppm of unlabeled atrazine to yield a concentration of 1.1×10^{-3} μCi/ml. Portions (20 ml) of medium (pH 7.3) were aseptically transferred to 250-ml biometric Erlenmeyer flasks. The flasks were inoculated and sealed after being flushed for a few seconds under a stream of oxygen (99.6% pure) to ensure aerobic conditions and to reduce the amount of N_2 in the flask atmosphere. At periodic intervals, the flasks were opened, and the amounts of $^{14}CO_2$ evolved and trapped in a 2 N NaOH solution were determined with a scintillation counter. An aliquot of the atrazine medium was also analyzed for residual radioactivity. Prior to resealing and further incubation, the NaOH in each trap was replaced with a fresh solution, and each flask was flushed with oxygen again as described above.

Determination of microbial growth. Cell growth was measured by determining A_{600} with a Beckman model DU-70 spectrophotometer (Beckman Instruments, Fullerton, Calif.). The protein contents of the cultures were determined after cell lysis in 0.1 N NaOH at 80°C for 1 h. A bicinchoninic acid protein assay kit (Pierce Chemical Co., Rockford, Ill.) with a bovine serum albumin standard was used.

Assessment of nitrogen fixation activity in the enrichment cultures. The acetylene reduction assay was conducted as described by Krieg (21). The presence of nitrogen-fixing bacteria was also tested by using a *nif* gene probe and the hybridization procedure of Holden et al. (16). The probe was obtained from Michael Sadowsky (Department of Soil Science, University of Minnesota).

Analytical methods. The total N content in soils was analyzed by using the procedure of Bremner and Mulvaney (4). Soil phosphorus content was determined by the procedure of Olson et al. (25). Soil total organic carbon content was measured with a total organic carbon analyzer (Leco Corp., St. Joseph, Mich.); total organic matter content was measured by determining the loss of weight from a dry soil sample after pyrolysis. The ammonia content in the growth media was determined with an Ammonia 31 enzymatic kit (Sigma Chemical Co., St. Louis, Mo.). Radiorespirometry was performed in 250-ml biometric Erlenmeyer flasks containing 20 ml of medium supplied with uniformly ring-labeled [^{14}C]atrazine. The flasks were equipped with a CO_2 trap containing 2 ml of 2 N NaOH. Radioactivity levels in samples and CO_2 traps were determined with a Beckman model LS 6800 scintillation counter (Beckman Instruments, Irvine, Calif.) with channels set at 0 to 670 and 250 to 670 for background and sample readings, respectively. To determine the formation of nonvolatile atrazine metabolites in the growth medium, equal volumes of ethyl acetate and growth

medium were vigorously mixed, and the radioactivity in the aqueous phase was measured. Typically, more than 98% of the atrazine in noninoculated control treatments partitioned into the organic phase. Thin-layer chromatography was performed on silica gel thin-layer chromatography precoated plates with a 3-cm preadsorbant spotting layer (J. T. Baker, Inc., Phillipsburg, N.J.) by using chloroform-methanol-water-acetic acid (70:25:4:2, vol/vol). HPLC measurements were done with a system (Spectra Physics, San Jose, Calif.) consisting of a model 8800 pumping system equipped with a model 7125 valve-and-loop injector (Rheodyne, Cotati, Calif.) fitted with a 20- or 100-μl loop and a model UVIS-204 detector (Linear Instruments, Inc., Reno, Nev.). A_{220} was recorded. Routine analysis of residual atrazine levels in enrichment cultures was performed by using a Spheri-5 C_{18} RP column (length, 100 mm; internal diameter, 4.6 mm; Alltech Associates, Inc., Deerfield, Ill.). A reverse-phase isocratic HPLC mobile phase was adopted from the work of Wenheng et al. (28) and contained acetonitrile–aqueous 0.1 M H_3PO_4 and 20 mM *n*-heptanesulfonic acid (50:50, vol/vol). The eluant pH was adjusted to 2.8. The flow rate was adjusted to 1.0 ml/min, and chromatography was conducted at room temperature. To determine atrazine metabolites, a C_8 column (length, 300 mm; internal diameter, 4.6 mm; Phase Separation, Inc., Norwalk, Conn.) was used. The procedure suggested by Vermeulen et al. (27) was used, except that the isocratic mobile phase contained methanol and aqueous 50 mM ammonium acetate (50:50, vol/vol). The flow rate was 1 ml/min with the column at room temperature. The chromatograms were displayed on a Chromjet recording integrator (Spectra Physics). Authentic standards were chromatographed to aid in the identification of metabolites.

Chemicals. Atrazine (99.6% pure) was purchased from Chem Service, West Chester, Pa. Uniformly ring-labeled [^{14}C]atrazine (7.8 mCi/mmol; 99.6% radiochemically pure) was purchased from Sigma. Authentic samples of simazine, atrazine, desisopropylatrazine, desethylatrazine, hydroxyatrazine, hydroxydesisopropylatrazine, and hydroxydidesalkylatrazine were gifts from Ciba Geigy Corp., Greensboro, N.C. Individual 100-ppm stock solutions of authentic atrazine and metabolite standards were prepared in methanol–aqueous 0.1 N H_3PO_4 and stored at 4°C. All other chemicals used were of reagent grade or better.

RESULTS

Enrichment of microorganisms by using atrazine as the sole nitrogen source. Successful enrichment cultures at pH 5.5 and 7.3 prepared with atrazine at a concentration of 100 ppm as the sole nitrogen source were obtained from all three herbicide spill sites (Table 2). In the first three transfers, atrazine utilization was indicated by significant growth in 31 of 38 cultures. The 31 cultures were transferred for the fourth time, and 2 weeks later they were extracted and analyzed for atrazine disappearance by HPLC; 20 cultures had less than 50% of the atrazine remaining (Table 2).

Atrazine disappearance was determined to be the result of microbial metabolism. Control media which were not inoculated did not exhibit significant atrazine disappearance (<10%). In studies performed with [^{14}C]atrazine, less than 10% of the radioactivity was associated with the biomass, indicating that atrazine depletion was not a result of binding to cells. Additional experiments performed with [^{14}C]atrazine (see below) confirmed that atrazine was metabolized to nonvolatile metabolites and $^{14}CO_2$.

Utilization of atrazine as a nitrogen source was indicated by several lines of evidence. First, atrazine consumption occurred concomitantly with growth in nitrogen-limited medium (Fig. 1). Second, media lacking atrazine failed to support growth. Some cultures were screened for nitrogen-fixing activity by using the acetylene reduction assay and were found to be negative. Concomitantly, a *nif* gene probe failed to detect *nif* genes, while a positive control, *Rhizobium* sp., gave positive hybridization. Finally, the addition of ammonium nitrate to cultures supported growth but suppressed atrazine degradation (data not shown). Taken together, these data demonstrated that atrazine was consumed as a nitrogen source by cultures from all three soil sampling sites.

TABLE 2. Atrazine contents at spill site sampling locations and atrazine degradation in the corresponding enrichment cultures

Soil sample	Atrazine content in soil sample used as inoculum (ppm)	Concn of atrazine left in enrichment medium (ppm)[a]	
		pH 5.5	pH 7.3[b]
LFA1	12.90	ND[c]	ND
LFA2	5.57	44	50
LFA3	0.50	38	ND
LFA4	0.30	7	3
LFA5	0.30	12	8
LFA6	0.27	6	3
LFA7	0.07	7	3
LFB1	0.87	50	20
LFB2	0.71	54	9
LFB3	0.24	12	ND
LFB4	<0.01	55	99
LFB5	<0.01	58	20
LFB6	<0.01	18	4
ALB1	5.80	88	8
ALB2	4.76	65	10
ALB3	1.03	ND	100
ALB4	0.75	ND	ND
ALB5	0.67	70	11
ALB6	0.53	75	10

[a] The atrazine concentration was measured in the fourth-subculture growth medium after 14 days by HPLC. The starting concentration was 100 ppm for each culture.

[b] The medium was amended with cycloheximide.

[c] ND, not determined (cultures were abandoned prior to the fourth subculture because of slow or no growth in medium containing atrazine as the sole N source; these preparations could be considered to have 100 ppm remaining).

Table 2 shows that atrazine-degrading organisms were obtained from multiple locations at each site. Successful enrichment cultures were obtained at both pH 5.5 and pH 7.3. Generally, the degree of degradation appeared to be more related to the site than to the pH of the enrichment culture. Only at the LFA site did soils with lower atrazine contents give rise to bacterial enrichment cultures with high levels of atrazine-degrading activity. Since this site had a history of herbicide input, low atrazine levels might reflect higher levels of in situ atrazine degradation.

Plating of bacteria on atrazine-containing solid medium revealed the presence of 10 or more different colony types in most of the cultures. Random selection of more than 200 strains purified from the mixed cultures failed to yield a single organism capable of degrading atrazine in liquid culture. However, inoculation of liquid media with mixtures of colonies from plates resulted in cell growth (observed as turbidity) and atrazine mineralization. This suggested that

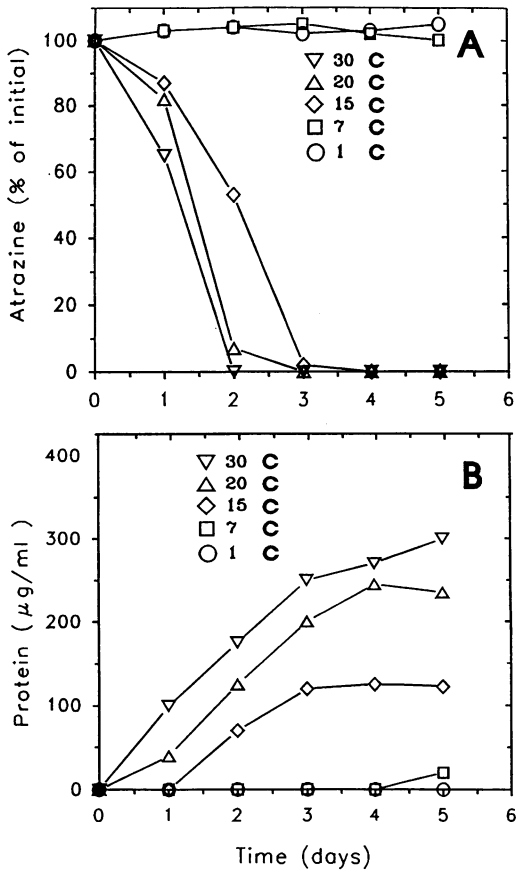

FIG. 1. Effect of temperature on atrazine degradation (A) and protein formation (B) by culture LFB5A during the 10th growth cycle.

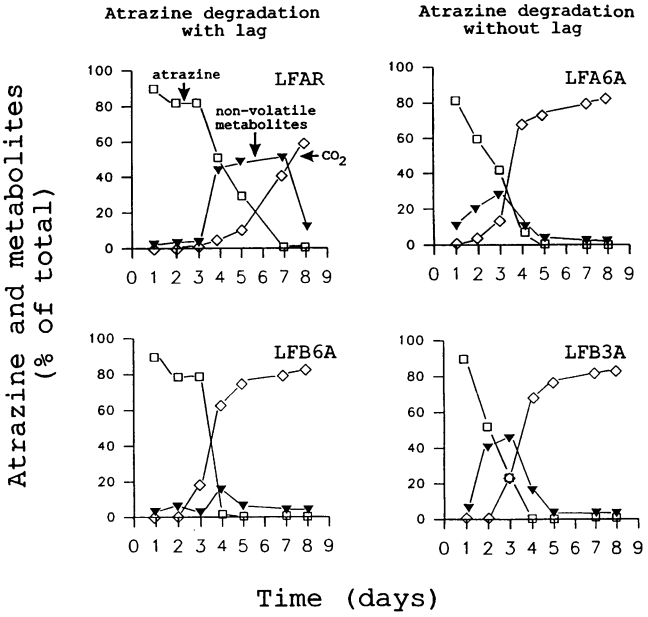

FIG. 2. Degradation of 100 ppm of [^{14}C]atrazine in growth media during the sixth growth cycle. Symbols: □, atrazine content as measured by HPLC; ▼, content of nonvolatile ^{14}C-labeled metabolites minus atrazine content as measured by HPLC; ◇, amount of $^{14}CO_2$ evolved. The culture designations are given on the figure (see Materials and Methods).

the metabolism of atrazine might require the combined metabolic activities of more than one organism. These mixed cultures were stable after repeated transfers and could be frozen and thawed. Additional studies were conducted to characterize atrazine degradation by these stable consortia.

Characteristics of stable atrazine-degrading consortia. Cultures that degraded more than 50 ppm of atrazine in the fifth growth cycle were selected for further study. Ring-labeled [^{14}C]atrazine was used to determine the fate of atrazine in the growth medium. Atrazine concentrations were determined by HPLC, and the concentrations of nonvolatile metabolites were determined by measuring radioactivity in the aqueous phase after extraction with ethyl acetate. Clear differences among the cultures were detected. Although 100 ppm of atrazine was consumed by all cultures within 7 days, the kinetic courses of metabolism differed (Fig. 2). First, while cultures LFAR and LFB6A exhibited a distinct lag period in atrazine degradation, cultures LFA6A and LFB3A degraded atrazine steadily over the first 3 days. In the latter cultures there was an immediate increase in the concentrations of nonvolatile ^{14}C-labeled metabolites. The concentrations of the metabolites decreased with time, and $^{14}CO_2$ was evolved. Following the lag, culture LFAR also exhibited transient formation of nonvolatile intermediates. In contrast, there was little accumulation of intermediates in LFB6A. In all cases, 70 to 85% of the initial atrazine could be accounted for, mostly as $^{14}CO_2$, by the end of the experiment.

The effects of temperature and pH on growth and atrazine degradation were examined with culture LFB5A during the 10th growth cycle. Atrazine degradation was negligible at or below 7°C, but significant metabolism occurred at temperatures of 15°C and above (Fig. 1A). Atrazine degradation (Fig. 1A) occurred concomitantly with an increase in protein content (Fig. 1B), although lower temperatures led to less biomass when stationary phase was reached. At 30°C, atrazine degradation was not markedly altered over the range from pH 5.5 to 8.5 (data not shown).

Effect of repeated subculturing in medium containing atrazine as the sole nitrogen source. Continuous subculturing of the enriched microorganisms on medium containing atrazine as the sole source of nitrogen resulted in an increase in degradation rates. This was illustrated with the eighth subcultures by monitoring the disappearance of ethyl acetate-soluble atrazine from culture media. In the control without cells, 97% of the added [^{14}C]atrazine partitioned into the organic phase, and this value remained constant at both 24 and 48 h (Table 3). In the acclimated cultures, significant amounts (up to 35%) of the [^{14}C]atrazine had been transformed to water-soluble metabolites by 24 h. The total amounts of ^{14}C in the organic and aqueous phases were similar to the control values, indicating that insignificant atrazine mineralization had occurred. After 48 h, most of the radioactivity was no longer in the organic or aqueous phases and in an independent experiment was determined to be in $^{14}CO_2$.

Figure 3 shows the differences in degradation patterns between the 6th and 12th growth cycles 24 h after inoculation. By the sixth subculture, almost no atrazine degradation occurred by the end of day 1, as indicated by similar partition patterns in the aqueous and organic phases of the noninoculated control and the inoculated media. However,

TABLE 3. Transient formation of water-soluble metabolites during the degradation of 460 nmol of atrazine per ml by eighth-cycle subcultures[a]

Culture	^{14}C partitioning (nmol/ml of culture)			
	24 h after inoculation		48 h after inoculation	
	Organic phase	Aqueous phase	Organic phase	Aqueous phase
Control	445	15	442	14
LFA5A	303	106	8	20
LFA7A	293	162	10	15
LFA6A	290	157	4	17
LFAR	343	103	13	10
LFB3A	327	101	10	10
LFB6A	405	50	10	50
ALB2N	359	60	15	55

[a] A 2-ml portion of culture medium containing [^{14}C]atrazine was extracted with 2 ml of ethyl acetate, and the aqueous and organic phases were analyzed by scintillation counting.

after another six subculturing cycles, a significant amount of degradation had occurred in most cultures 24 h after inoculation. Cultures LFB5A and LFB6A were particularly active; only 30 and 41%, respectively, were recovered in the organic phase. The rest of the ^{14}C label occurred as water-soluble metabolites (up to 30%) or as $^{14}CO_2$. The mass balance of radioactivity indicated a level of recovery of ≥90%. Complete mineralization was observed by the end of day 2 of the 12th subculture cycle in all of the cultures except ALB2N (data not shown).

Chromatography of nonvolatile metabolites. The culture that degraded atrazine most rapidly was analyzed by isocratic reverse-phase HPLC in an attempt to identify nonvolatile metabolites of atrazine degradation. A zero-time control (Fig. 4A) contained the starting materials, atrazine and simazine (2-chloro-4,6-diethylamino-1,3,5-triazine); the latter was a minor contaminant of the atrazine preparation (less than 0.5%). After 24 h, 75% of the atrazine and 85% of the simazine were degraded. Four new peaks were observed, and radioactivity from ring-labeled atrazine was associated with all four. Peaks I and II migrated close to the elution volume of the column and may have contained multiple polar materials. Peak III, at 4.8 min, had the same retention time as authentic desisopropylatrazine. The largest peak (peak IV), at 7.5 min, coeluted with authentic hydroxyatrazine. UV spectroscopy of material recovered from peak IV revealed an absorption maximum at 240 nm identical to that of hydroxyatrazine. Rechromatography of peak IV by thin-layer chromatography on silica gel plates gave an R_f value similar to that of synthetic hydroxyatrazine.

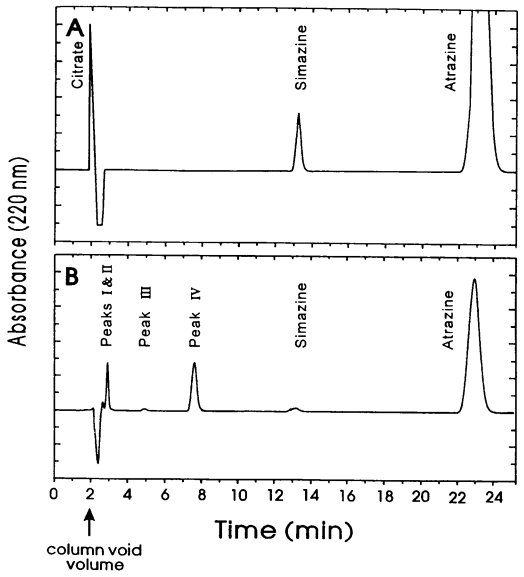

FIG. 4. HPLC chromatograms of the culture medium immediately after inoculation with culture LFB5A (A) and 24 h later (B). A 1-ml portion of culture was centrifuged at 15,000 × *g* for 3 min, and 100 μl of the supernatant was injected into the HPLC apparatus.

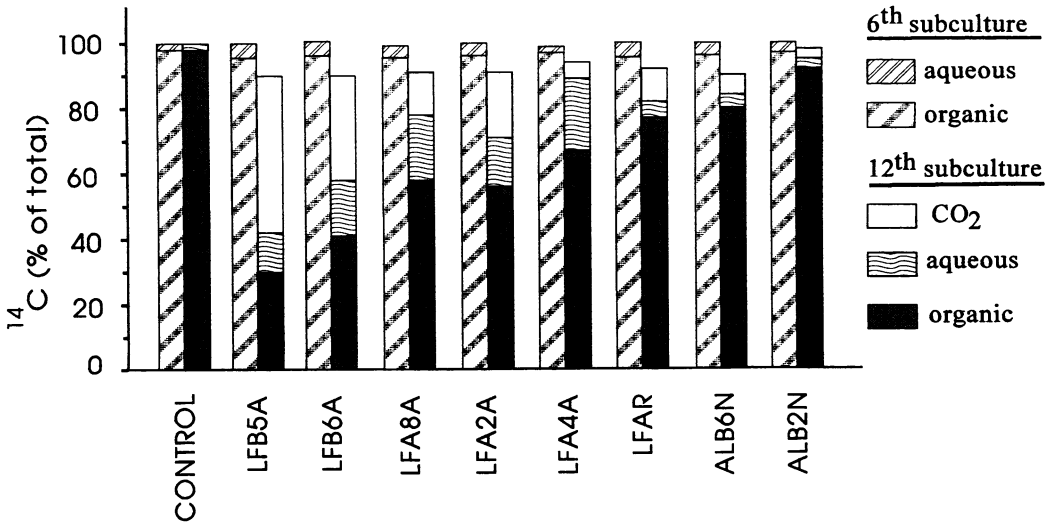

FIG. 3. Mass balance of atrazine and atrazine degradation products compared for the 6th and 12th growth cycles, each after 24 h of incubation. For each pair, the 6th subculture is on the left, and the 12th subculture is on the right. Explanations for culture names are given in Materials and Methods.

TABLE 4. Half-lives of atrazine disappearance under different conditions in this and previous studies

Prepn	pH	Temp (°C)	Half-life (days)	Reference
Soil	4.8–6.5	22	53–113	5
Soil	5.6–6.6	Field conditions	37–168	11
Soil-water slurries	4.5		65–113	1
Filtered, sterilized water	5–9	25	64–200	5
Bacterium from industrial wastes[a]			>7	14
Pseudomonas spp.	6.8–7.0	28	>35	2
Aspergillus fumigatus			24	15
Enrichment culture			>42	10
Early enrichment cultures	7.3	25	4–8	This study
Late enrichment cultures	7.3	25	0.5–2	This study

[a] Only 40% degradation was detected after 7 days of incubation. No further degradation was reported.

DISCUSSION

Previous studies have shown that atrazine is biodegraded by soils (1, 6, 13), mixed cultures (10), and pure cultures of microorganisms (2, 17, 18). Atrazine ring cleavage is rarely reported, and even side chain cleavage is typically very slow. This is reflected in a recent effort to overcome the recalcitrance of atrazine by using sequential ozonolysis and bioremediation (22).

In this study, rapid and extensive conversion (≥80%) of the ^{14}C-labeled *s*-triazine ring carbon atoms to $^{14}CO_2$ was demonstrated. The enrichment cultures differed from cultures in previous studies in that atrazine was used as the sole nitrogen source. This method was previously used successfully to obtain various *s*-triazine-degrading bacteria but yielded no strains capable of growth on atrazine (9). The use of citrate and sucrose as mixed carbon sources may have contributed to the success of the enrichment cultures in these studies.

The cultures described here exhibit the most rapid rates of biological atrazine degradation, as well as degradation at elevated atrazine concentrations (Table 4). Soils often exhibit half-lives of atrazine decrease on the order of months. Pure or enriched cultures typically give half-lives on a time scale of weeks. This is observed even with bacteria which use atrazine as a sole carbon source (2). The enrichment cultures in this study yielded half-lives of 4 to 8 days by the sixth subculture. After 12 subcultures, the half-life for degradation of 100 ppm of atrazine was 0.5 to 2 days. In most of the experiments described above we used atrazine at a concentration of 100 ppm, a concentration typical at a spill site but unlike the concentrations used in the field. Indeed, we have observed atrazine degradation on solid media with atrazine concentrations as high as 1,000 ppm (data not shown). This, coupled with the stability of the consortia described above, highlights the potential of the consortia for degrading atrazine under herbicide spill conditions.

Transient metabolites accumulated in most cultures, as demonstrated by the detection of nonvolatile water-soluble materials. HPLC analysis of media revealed the presence of multiple nonvolatile compounds resolvable by reverse-phase chromatography. The presence of hydroxyatrazine as an apparent intermediate was somewhat surprising. Previous studies have indicated that side chain N-dealkylation reactions are typical first steps in *s*-triazine metabolism and stressed that the presence of both alkyl groups on atrazine may be inhibitory for bacterial dechlorination (2, 11). Further studies are needed to establish the role of hydroxyatrazine in the metabolism of atrazine by these cultures. Also, research is under way to establish how the metabolism of atrazine by different microorganisms combines to liberate $^{14}CO_2$ from ^{14}C-labeled *s*-triazine ring carbon atoms.

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