

Mineralization of Soil-Aged Isoproturon and Isoproturon Metabolites by *Sphingomonas* sp. Strain SRS2

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ABSTRACT

The aim of the study was to determine the effect of aging of the herbicide isoproturon and its metabolites monodesmethyl-isoproturon and 4-isopropyl-aniline in agricultural soil on their availability to the degrading bacterium *Sphingomonas* sp. strain SRS2. The ^{14}C -ring-labeled isoproturon, monodesmethyl-isoproturon, and 4-isopropyl-aniline were added to sterilized soil and stored for 1, 49, 71, or 131 d before inoculation with strain SRS2. The availability of the compounds was estimated from the initial mineralization and the amount of $^{14}\text{CO}_2$ recovered after 120 d of incubation. Aging in soil for 131 d reduced the initial mineralization of isoproturon and monodesmethyl-isoproturon and, in the case of isoproturon, also reduced the recovery of $^{14}\text{CO}_2$. Initial mineralization and recovery of $^{14}\text{CO}_2$ from aged 4-isopropyl-aniline were slightly reduced, but less $^{14}\text{CO}_2$ was generally produced than with isoproturon or monodesmethyl-isoproturon. Thus, recovery of $^{14}\text{CO}_2$ from ^{14}C -isoproturon and ^{14}C -monodesmethyl-isoproturon was 50.7 to 64.4% of the initially added ^{14}C , while recovery from ^{14}C -4-isopropyl-aniline was only 11.7 to 17.0%. Sorption measurements revealed similar Freundlich constants (K_f) for isoproturon and monodesmethyl-isoproturon, whereas K_f for 4-isopropyl-aniline was more than fivefold greater. The findings imply that in soil, partial degradation of isoproturon to 4-isopropyl-aniline may lead to reduced mineralization of the herbicide due to sorption of the aniline moiety.

THE PROCESSES WHEREBY ORGANIC CONTAMINANTS in soil become increasingly recalcitrant with time are collectively termed aging. Aging in soil reduces the bioavailability of organic contaminants, thereby reducing or preventing biological degradation and hence the natural attenuation rate in contaminated soil. This topic was recently reviewed by Alexander (2000). Sorption of organic compounds to soil is often initiated by a short phase of fast sorption followed by a slow and less reversible sorption phase, the net result of which is a fraction resistant to desorption and biodegradation (Pignatello and Xing, 1996). Pignatello and Huang (1991) found that residues of aged atrazine and metolachlor represented the most strongly sorbed fraction, which suggests that they are less susceptible to biodegradation than younger residues. Similar results were obtained by comparing desorption and biodegradation of 1,2-dibromoethane aged in soil for up to 19 yr with freshly added compound (Steinberg et al., 1987).

The phenylurea herbicide isoproturon [3-(4-isopropylphenyl)-1,1-dimethylurea] (IPU) is used for pre- and postemergence control of annual grasses and broad-leaved weeds in spring and winter cereals. It is widely used across Europe, and has been detected in ground

water in concentrations exceeding $0.1 \mu\text{g L}^{-1}$ (Spliid and Køppen, 1998), which is the European Union limit value for drinking water (European Commission, 1980). Degradation of IPU in soil is mainly microbial (Fournier et al., 1975), though photochemical (Kulshrestha and Mukerjee, 1986) and chemical transformation (Sørensen and Aamand, 2001) have been reported. Possible degradation products of IPU in soil include monodesmethyl-isoproturon [3-(4-isopropylphenyl)-1-methylurea] (MDIPU), didesmethyl-isoproturon [3-(4-isopropylphenyl)-urea] (DDIPU), 4-isopropyl-aniline (4IA), and several hydroxylated compounds (Mudd et al., 1983; Lehr et al., 1996a). Partial degradation of IPU by several soil fungi and some bacteria has been reported by Berger (1998), and accumulation of 4IA from degradation of IPU has recently been reported to be mediated by various bacteria isolated from agricultural soils (Tixier et al., 2002; Turnbull et al., 2001). Partial degradation of IPU is a potential problem as it may leave metabolites in the environment that are more toxic than the parent compound (Tixier et al., 2002; Remde and Traunsperger, 1994). Studies using ^{14}C -ring-labeled IPU have shown mineralization to $^{14}\text{CO}_2$ in soil at low to moderate rates, with 13 to 23% of the added ^{14}C recovered as $^{14}\text{CO}_2$ after 55 to 67 d at 22°C (Lehr et al., 1996b; Reuter et al., 1999). Considerable spatial heterogeneity has been observed in soils with respect to both the rate and kinetics of IPU degradation (Bending et al., 2001), however. A *Sphingomonas* sp. (designated strain SRS2) that has recently been isolated from an agricultural soil is able to degrade IPU to CO_2 and biomass via initial *N*-demethylation to MDIPU, further *N*-demethylation to DDIPU, cleavage of the urea-moiety to 4IA, and mineralization (Sørensen et al., 2001).

In soils where mineralization or degradation of IPU is slow, residence time of IPU and its metabolites can be expected to be long. Partial degradation may lead to accumulation of metabolites, either serving as end products or as intermediates for further degradation. Aging of IPU and its metabolites would consequently delay mineralization of the substances and hence their removal from the soil.

The aim of this study was to investigate the effect of aging of IPU and the two metabolites MDIPU and 4IA in agricultural soil on their mineralization by *Sphingomonas* sp. strain SRS2. In addition, sorption of the three compounds to the soil was studied by determining the distribution of ^{14}C derived from aged ^{14}C -labeled IPU, MDIPU, and 4IA into the water-extractable, NaOH-extractable, and nonextractable fractions. Standard

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Abbreviations: 4IA, 4-isopropyl-aniline; IPU, isoproturon [3-(4-isopropylphenyl)-1,1-dimethylurea]; MDIPU, monodesmethyl-isoproturon [3-(4-isopropylphenyl)-1-methylurea].

sorption and desorption measurements were also performed. This study is the first to describe the effect of aging on the biodegradation of a phenylurea herbicide and its major metabolites in agricultural soil.

MATERIALS AND METHODS

Soil Sampling and Treatment

Soil from the plow layer (0–30 cm) was collected near Flad-erne Creek, Karup in the western part of Denmark. This soil has previously been studied by Larsen et al. (2000) and Vinther et al. (2001), revealing a low potential for mineralization of IPU. The soil moisture content was measured gravimetrically by oven-drying for 24 h at 105°C. Total organic carbon was determined on dry samples by carbon combustion (CS-200, IR-212; LECO Corporation, St. Joseph, MI) after removal of carbonates using 6% sulfurous acid. The structure of the soil was determined by size fractionation of dried samples using sieves followed by gravimetric measurement of each fraction. Soil with a natural moisture content was sieved (2 mm), radiation-sterilized (29 kGy), and stored at 4°C until needed. The sterility of the soil was verified by plating on agar plates (R2A; Remel, Lenexa, KS). Three platings were made from each of three different portions of the sterilized soil.

Chemicals

The ^{14}C -IPU (4.43 MBq mg^{-1} , radiochemical purity $\geq 99\%$) was purchased from Amersham Life Science (Buckinghamshire, UK), ^{14}C -MDIPU (4.42 MBq mg^{-1} , radiochemical purity $\geq 99\%$) from Institute of Isotopes Co. (Budapest, Hungary), and ^{14}C -4IA (5.72 MBq mg^{-1} , radiochemical purity $> 98\%$) from International Isotope (Munich, Germany). All ^{14}C -labeled chemicals were uniformly ring-labeled. Unlabeled compounds were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and were all of analytical grade. The chemical structure of each of the compounds is shown in Fig. 1.

Cell Culture

Sphingomonas sp. SRS2 (Sørensen et al., 2001), deposited at the Institut Pasteur Collection under the number CIP107349, was grown in 250-mL Erlenmeyer flasks containing 100 mL of a R2A-based broth (Sørensen et al., 2001). The flasks were incubated at 20°C on a platform shaker at 150 rpm, and the cells harvested in the late exponential growth phase by centrifugation ($3500 \times g$, 20°C, 10 min). The supernatant was then removed and the cells washed twice and resuspended in a mineral salt medium supplied with 100 mg L^{-1} of casamino acids (MS-CA) (Sørensen et al., 2001). Cell density of strain SRS2 in MS-CA was estimated using the correlation between optical density at 600 nm and cell counts on R2A.

Aging and Mineralization

All glassware and liquids were autoclaved before use (121°C, 20 min) and preparation of samples and standards was performed in a laminar flow bench. All experiments were set up in triplicate. Ten-gram (dry weight) sterile soil samples and unlabeled and ^{14}C -labeled IPU, MDIPU, or 4IA were added to 100-mL glass flasks fitted with airtight glass stoppers to a final concentration of 5.0 mg kg^{-1} soil (dry weight) and approximately $1400 \text{ Bq flask}^{-1}$ (IPU and MDIPU) or $150 \text{ Bq flask}^{-1}$ (4IA). Isoproturon, MDIPU, or 4IA in acetone was added to a portion of the soil in each flask and the flasks were left open for one hour to allow the acetone to evaporate before the soil was thoroughly mixed with a sterile spatula. Milli-Q

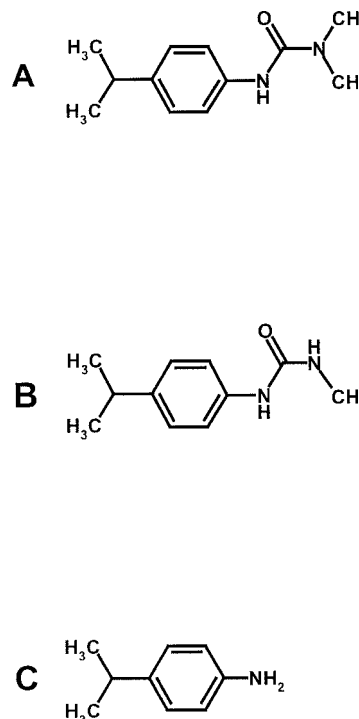


Fig. 1. Molecular structure of (A) isoproturon (IPU), (B) monodesmethyl-isoproturon (MDIPU), and (C) 4-isopropyl-aniline (4IA).

water (Millipore, Bedford, MA) was added to a final content of 2.5 mL per flask, resulting in an aqueous chemical concentration of 20 mg L^{-1} of each compound. A small test tube containing 2 mL 0.5 M NaOH was placed in each flask to trap $^{14}\text{CO}_2$ produced during mineralization of the ^{14}C -labeled compound. Flasks were stored in the dark at 20°C for 1, 49, 71 or 131 d before inoculation. Three additional flasks with each compound were prepared for control of sterility after four months by plating on R2A. No colonies were detected on the plates following two weeks of incubation at 20°C. Three flasks were included as pH controls during the experiment. For all compounds the soil pH was 5.0.

The flasks were then inoculated with strain SRS2 to a density of $8.0 \pm 2.3 \times 10^7$ cells per flask and incubated in the dark at 20°C. At frequent intervals during incubation the NaOH in the alkali trap was replaced in a laminar flow bench and flasks were left open for 5 min to replenish oxygen. The NaOH was mixed with 10 mL OptiPhase HiSafe scintillation liquid (Wallac, Turku, Finland), left for at least 10 h in the dark, and counted for 10 min on a Wallac 1409 liquid scintillation counter. Flasks with uninoculated soil that contained compounds aged for 131 d were included as abiotic controls. In these flasks, less than 1% of the initially added ^{14}C was trapped in the alkali traps during a 120-d period. Cumulated activity was corrected for quenching and background activity. The initial mineralization was defined as $^{14}\text{CO}_2$ produced after 8 d, while recovery was defined as accumulated $^{14}\text{CO}_2$ produced at the termination of the incubation after 120 d.

The data were statistically analyzed using one-way analysis of variance (ANOVA). Multiple comparisons of significant differences were made using a Tukey test (Fowler and Cohen, 1992, p. 184–185). With $^{14}\text{CO}_2$ recovery from ^{14}C -MDIPU aged for different periods the variances were dissimilar and the ANOVA test could not be applied. A Kruskal–Wallis test was therefore used instead (Campbell, 1990, p. 74, 199–200, 220).

Water-Extractable, Sodium Hydroxide-Extractable, and Nonextractable Carbon-14

In addition to the flasks used for measuring mineralization, a set of identically aged but uninoculated flasks was prepared and used to determine the distribution of ^{14}C following aging. Three fractions were estimated sequentially: (i) water-extractable ^{14}C , (ii) NaOH-extractable ^{14}C , and (iii) nonextractable ^{14}C .

The water-extractable fraction of ^{14}C was determined by adding 10 mL of Milli-Q water to each flask before placing the flasks on an orbital shaker at 150 rpm at 20°C for two hours. The slurries were then transferred to centrifuge tubes by washing with 10 mL Milli-Q water and centrifuging at $2150 \times g$ (20°C). The ^{14}C in the supernatant was measured by liquid scintillation counting. To assess abiotic degradation during aging, high performance liquid chromatography (HPLC) analysis of the water-extractable fraction was conducted as described by Juhler et al. (2001). The NaOH-extractable fraction was determined by replacing the supernatant with 10 mL of NaOH (0.5 M) and repeating the shaking and centrifugation procedure. For determination of the nonextractable ^{14}C , the soil was air-dried and triplicate subsamples of 0.25 g were combusted under conditions of excess O_2 in a Packard Model 507 oxidizer (Packard Instrument Company, Meriden, CT) with automatic capture of the $^{14}\text{CO}_2$ produced. Data were statistical analyzed as described above.

Sorption and Desorption Measurements

Before adding IPU, MDIPU, or 4IA, the soils for the sorption measurements were equilibrated with 0.01 M CaCl_2 . For the IPU and MDIPU sorption measurements, 5 g sterile soil (dry weight) and 4 mL 0.01 M CaCl_2 was mixed in glass test tubes with Teflon-lined screw caps for 24 h in a vertical rotator in the dark at 20°C. For the measurement of 4IA sorption, 1 g of soil and 9 mL CaCl_2 solution were used. The IPU, MDIPU, or 4IA were then added as 1-mL aliquots of CaCl_2 (0.01 M) solutions containing both ^{14}C -labeled and unlabeled compound. This resulted in initial concentrations of 0.5, 1.0, 5.0, 10.0, or 25.0 mg L^{-1} and 833 Bq tube^{-1} . To prepare the CaCl_2 solutions, IPU, or MDIPU from stock solutions in acetone were added to glass flasks and the solvent was evaporated before addition of CaCl_2 . Unlabeled 4IA and ^{14}C -labeled 4IA was added directly into the CaCl_2 solution from stock solutions in acetone and ethanol, respectively. Following addition of IPU, MDIPU, or 4IA, the soil- CaCl_2 suspensions were rotated for 96 h and centrifuged ($1483 \times g$, 20°C, 30 min) before determination of ^{14}C in the supernatants. The experiment was performed in triplicate. Sorption was calculated as a decrease in the aqueous concentration of ^{14}C relative to controls without soil.

To determine desorption, the supernatant was replaced with fresh CaCl_2 and the samples rotated for 24 h and centrifuged as described above. Aliquots (250–500 μL) of the supernatant were removed and the ^{14}C was determined. The volume of solution removed was replaced with CaCl_2 and the samples rotated again. Desorption was measured in this manner on a further three occasions each separated by 24 h of incubation. Desorption was calculated as an increase in concentration of ^{14}C in the aqueous phase taking dilution into account. For all sorption and desorption experiments, pH was 5.3 to 5.4.

RESULTS

Soil Characterization

The site is located on the Weichselian outwash plains and the soil has previously been classified in a study by

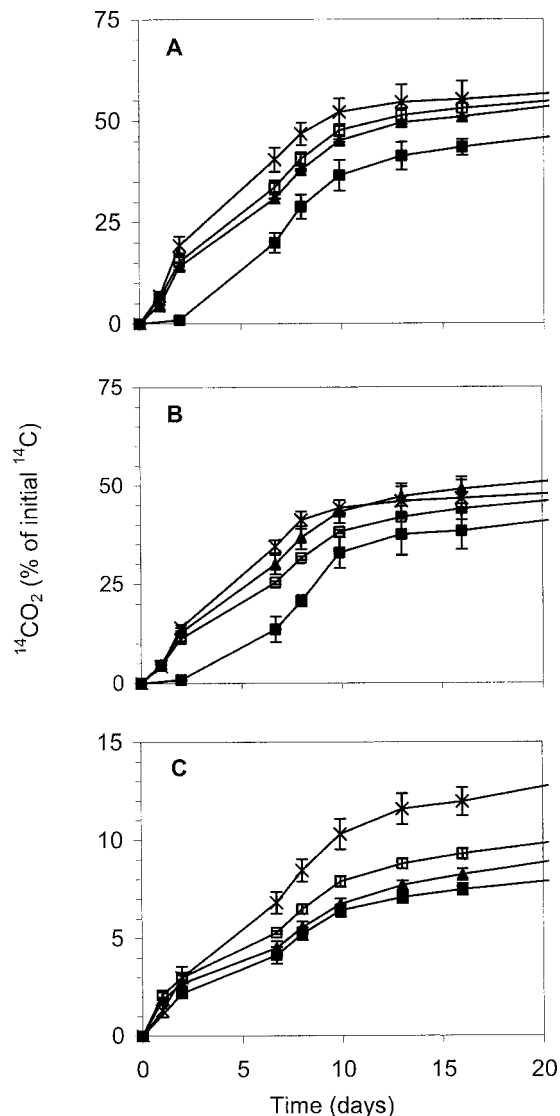


Fig. 2. Initial mineralization of (A) ^{14}C -isoproturon (IPU), (B) ^{14}C -monodesmethyl-isoproturon (MDIPU), and (C) ^{14}C -4-isopropyl-aniline (4IA) aged for 1 (crosses), 49 (closed triangles), 71 (open squares), or 131 (closed squares) d and inoculated with *Sporangium* sp. strain SRS2. Note that the ordinate scales differ. The values are the mean of two or three replicates. The vertical bars indicate ± 1 SD.

Vinther et al. (2001) as a Humic Podzol. The grain size distribution of the soil (in weight percent) is 10.3% silt and clay (<0.063 mm), 15.4% fine sand (0.063–0.20 mm), 60.9% medium sand (0.20–0.60 mm), 12.7% coarse sand (0.60–2.00 mm), and 0.6% gravel (>2.00 mm). The total organic carbon content was 1.6%.

Mineralization of Aged Isoproturon, Monodesmethyl-Isoproturon, and 4-Isopropyl-Aniline

Initial mineralization of IPU was slowest when it had been aged in soil for 131 d before inoculation (Tukey, $p < 0.05$) (Fig. 2A). Thus, only 28.8% of the added ^{14}C -IPU was mineralized to $^{14}\text{CO}_2$ after 8 d of incubation as compared with 37.9 to 46.9% when aged for shorter

Table 1. Mineralization of soil-aged ^{14}C -isoproturon (IPU), ^{14}C -monodesmethyl-isoproturon (MDIPU), and ^{14}C -4-isopropyl-aniline (4IA) following inoculation with *Sphingomonas* sp. SRS2 and incubation for 120 d.†

Compound	Mineralization to $^{14}\text{CO}_2$ after four different aging periods				
	1 d	49 d	71 d	131 d	Controls‡
	% of added ^{14}C				
IPU	64.1 ± 3.7	62.9 ± 0.8	64.4 ± 2.0	55.8 ± 3.7	0.1 ± 0.0
MDIPU	53.3 ± 9.7	57.7 ± 1.3	53.4 ± 1.0	50.7 ± 2.4	0.1 ± 0.0
4IA	16.9 ± 0.5	12.9 ± 0.5	14.6 ± 0.4	11.7 ± 1.2	0.5 ± 0.0

† Values are the mean of three replicates ± 1 SD.

‡ Mineralization of freshly added compound in controls without inoculation following 120 d.

periods. By the end of the experiment on Day 120, recovery of added ^{14}C as $^{14}\text{CO}_2$ was also significantly lower in soil containing IPU aged for 131 d (55.8%) than when aged for shorter periods (62.9–64.4%) (Table 1). Similarly, less MDIPU was initially mineralized when aged for 131 d than with shorter aging periods (Tukey, $p < 0.05$) (Fig. 2B). After 8 d, only 20.7% of the added ^{14}C -MDIPU was mineralized to $^{14}\text{CO}_2$ in soil containing MDIPU aged for 131 d, compared with 31.5 to 41.2% when aged for shorter periods. For all aging periods, mineralization of ^{14}C -MDIPU to $^{14}\text{CO}_2$ after 120 d was 50.7 to 57.7%, with a tendency for slightly less mineralization following aging for 131 d (Table 1). A significantly lower mineralization after aging for 131 d compared with aging for only 1 d was observed with 4IA, both on the initial mineralization (Fig. 2C), and on the recovery of $^{14}\text{CO}_2$ at the end of the experiment (Tukey, $p < 0.05$) (Table 1). In general, the level of 4IA mineralization was substantially lower than that of IPU and MDIPU (Fig. 2). The initial mineralization of ^{14}C -4IA aged in soil for 1 d was 8.5%, compared with 5.2 to 6.5% for longer aging periods. Likewise, the $^{14}\text{CO}_2$ production after 120 d was significantly higher from ^{14}C -4IA applied the day before inoculation (17.0% of added ^{14}C) than when aged for longer periods (11.7–14.6%).

Water-Extractable, Sodium Hydroxide-Extractable, and Nonextractable Carbon-14

Aging also influenced the distribution of ^{14}C in uninoculated soil containing ^{14}C -IPU (Fig. 3A), with less water-extractable ^{14}C (27.1% of initially added ^{14}C) and more NaOH-extractable ^{14}C (36.4%) in soil containing ^{14}C -IPU aged for 131 d than when aged for 1 d (40.1 and 26.3%) (Tukey, $p < 0.05$). No significant differences between the aging periods were observed in the nonextractable fractions of ^{14}C . In soil containing aged ^{14}C -MDIPU, a similar pattern was obtained (Fig. 3B). The water-extractable fraction was largest in soil containing ^{14}C -MDIPU aged for 1 d (38.5% of initially added ^{14}C) compared with longer aging periods (20.5–24.4%) (Tukey, $p < 0.05$). Moreover, the NaOH-extractable ^{14}C fraction was larger in soil containing ^{14}C -MDIPU aged for 131 d (31.8%) than when aged for 1 d (25.2%) (Tukey, $p < 0.05$). No differences in the nonextractable fractions of ^{14}C were observed. Slightly more ^{14}C from ^{14}C -4IA was water-extractable after 1 d in the soil

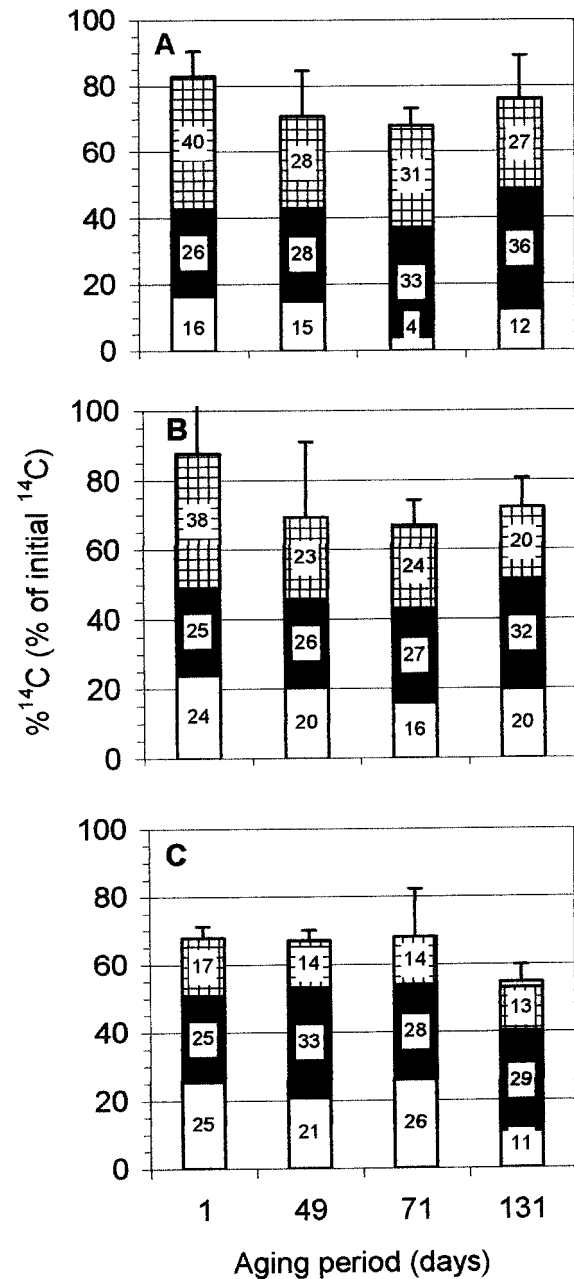


Fig. 3. Distribution of ^{14}C derived from aged (A) ^{14}C -isoproturon (IPU), (B) ^{14}C -monodesmethyl-isoproturon (MDIPU), and (C) ^{14}C -4-isopropyl-aniline (4IA) in uninoculated soils, showing water-extractable ^{14}C (checkered), NaOH-extractable ^{14}C (black), or nonextractable ^{14}C (white). The numbers on columns indicate the percentage of initially added ^{14}C . The values are the mean of three replicates except for soil with IPU aged for 131 d, which is the mean of two replicates. The vertical bars indicate ± 1 SD of the total recovery of ^{14}C .

(17.1% of initially added ^{14}C) than when aged for longer periods (12.8–14.4%) (Tukey, $p < 0.05$), but no significant differences were measured in the NaOH-extractable and the nonextractable fractions of ^{14}C (Fig. 3C). High performance liquid chromatography analysis of the water-extractable fractions showed no abiotic degradation of either IPU, MDIPU, or 4IA during the aging periods. No attempts were made to elucidate the nature

Table 2. Freundlich parameters for sorption and desorption of isotroturon (IPU), monodesmethyl-isotroturon (MDIPU), and 4-isopropyl-aniline (4IA) in soil. Sorption was determined after 96 h, desorption was determined after 96 h for IPU and MDIPU and after 24 h for 4IA. Values were calculated using triplicate samples and five concentrations of each compound.†

Compound	Sorption			Desorption		
	K_f	n	r^2	$K_{f,des}$	n	r^2
	$\mu\text{g}^{(1-1/n)} \text{ mL}^{1/n} \text{ g}^{-1}$			$\mu\text{g}^{(1-1/n)} \text{ mL}^{1/n} \text{ g}^{-1}$		
IPU	3.84	0.93	0.998	5.39	0.92	0.999
MDIPU	3.73	0.91	0.989	5.03	0.88	0.994
4IA	21.16	0.62	0.999	35.46	0.63	0.999

† K_f , Freundlich constant; n , slope of the isotherms; r^2 , correlation coefficient; $K_{f,des}$, Freundlich constant for desorption.

of the ^{14}C -labeled compounds associated with the NaOH- or nonextractable ^{14}C fractions.

Mass balances for ^{14}C were calculated based on mineralization to $^{14}\text{CO}_2$, water-extractable ^{14}C , NaOH-extractable ^{14}C , and nonextractable ^{14}C . The standard deviations between the means of triplicate flasks with uninoculated soils from each of the fractions ranged from 0.2 to 5.0% for the water-extractable ^{14}C , 1.2 to 5.5% for the NaOH-extractable ^{14}C , and 2.9 to 21.2% for the nonextractable ^{14}C . In inoculated soils the standard deviations ranged from 0.5 to 10.6% for the water-extractable ^{14}C , 0.8 to 17.5% for the NaOH-extractable ^{14}C , and 0.5 to 14.1% for the nonextractable ^{14}C . The overall recovery of ^{14}C was $70.9 \pm 12.4\%$ of the initially added ^{14}C with the uninoculated soils and $92.6 \pm 10.9\%$ with the inoculated soils.

Sorption and Desorption Measurements

Sorption and desorption isotherms were calculated using the Freundlich equation:

$$C_s = K_f C_w^n$$

where C_s is the concentration of the compound sorbed to the soil and C_w is the concentration in solution. The Freundlich constant K_f represent the degree of sorption and n is a measure of the nonlinearity of the sorption isotherms. The terms K_f , n , and the corresponding coefficients of determination (r^2) (Table 2) were determined from the linear form of the Freundlich equation:

$$\log C_s = \log K_f + n \log C_w$$

The term C_s was calculated as the difference between added ^{14}C and ^{14}C in solution. The K_f for sorption of 4IA was more than five times higher than those for IPU and MDIPU. The $K_{f,des}$ for desorption was also high, thus indicating that the majority of 4IA was still sorbed to the soil after the desorption process. The slopes of the Freundlich equations (n) for both sorption and desorption were close to 1 for IPU and MDIPU, with the correlation between the sorption or desorption of these compounds and the equilibrium concentrations thus being almost linear. In contrast, the lower value of n for 4IA indicates a decrease in the availability of sorption sites with increasing solution concentration (Schwarzenbach et al., 1993). Seven to ten percent of the sorbed ^{14}C -IPU and ^{14}C -MDIPU was desorbed within the first 24 h, after which desorption continued slowly to as much

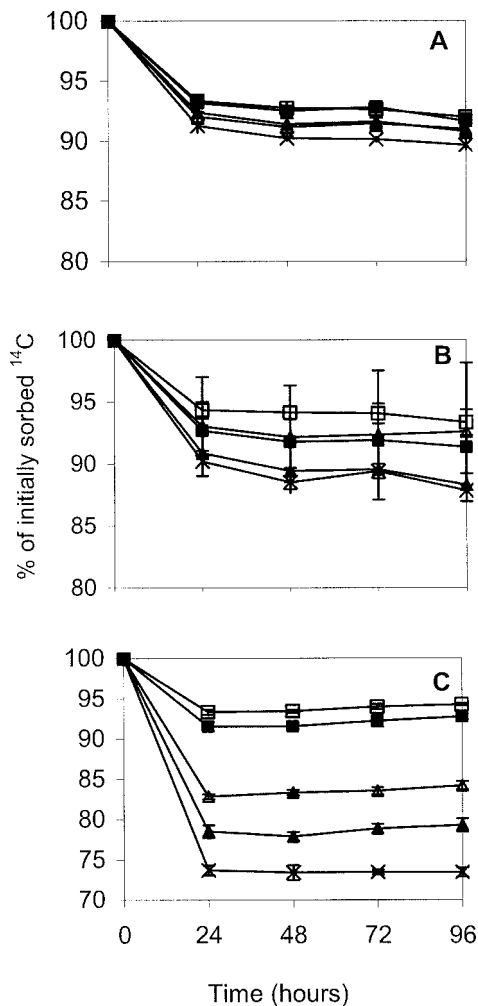


Fig. 4. Desorption of (A) ^{14}C -isotroturon (IPU), (B) ^{14}C -monodesmethyl-isotroturon (MDIPU), and (C) ^{14}C -4-isopropyl-aniline (4IA). Each figure shows the sorbed ^{14}C in percent of initially sorbed ^{14}C versus time for initial concentrations of 0.5 (crosses), 1.0 (closed triangles), 5.0 (open triangles), 10.0 (closed squares), and 25.0 mg L^{-1} (open squares). The values are the mean of two or three replicates. The vertical bars indicate ± 1 SD.

as 12% after 96 h (Fig. 4A,B). As equilibrium was not attained within the duration of the experiment, desorption at 96 h was chosen for calculation of the desorption parameters in Table 2. With 4IA, in contrast, desorption was completed within 24 h (Fig. 4C).

DISCUSSION

Aging of IPU, MDIPU, and 4IA in an agricultural soil resulted in reduced initial mineralization by *Sphingomonas* sp. SRS2. This suggests that aging reduced the availability of the compounds to strain SRS2, thereby rendering them more resistant to biodegradation. Furthermore, less 4IA was generally mineralized to $^{14}\text{CO}_2$ than with IPU and MDIPU, thus indicating that even after 1 d of contact with soil, a larger fraction of 4IA was unavailable to strain SRS2. Since strain SRS2 mineralizes ^{14}C -4IA to $^{14}\text{CO}_2$ as efficiently as ^{14}C -IPU and ^{14}C -MDIPU in a mineral salt medium without soil (Sørensen et al., 2001), this suggests that interactions

between 4IA and the soil were responsible for the low level of mineralization. Moreover, as only a minor reduction in the mineralization of IPU and MDIPU was observed after 120 d, the aging processes seem to be partly reversible. With 4IA, in contrast, the lower mineralization of aged than more recently added compound and the generally low level of mineralization compared with IPU and MDIPU was also evident after 120 d.

Although the processes behind aging of organic compounds in soil are not fully understood, suggested mechanisms include entrapment into micropores in soil aggregates due to slow diffusion (Steinberg et al., 1987), diffusion into soil organic matter (Brusseau et al., 1991), and chemical binding to soil constituents (Reuter et al., 1999). Biodegradation rates of aged compounds may thus be limited by diffusion rates from the micropores, partitioning out of the organic matter, or desorption from surfaces (Alexander and Scow, 1989). In addition, reactions that irreversibly transform the parent compound (e.g., chemical oxidation or reduction, photolysis, covalent binding to soil constituents, and polymerization) will reduce the amount of compound that is bioavailable.

Sorption measurements revealed similar behavior of IPU and MDIPU. In contrast, the behavior of 4IA was significantly different, with K_f being more than fivefold greater. Isoproturon is not considered to be sorbed to soil to a large extent compared with other herbicides (Singh et al., 2001). Distribution coefficients (K_d values) for IPU in silty clay loam and clay soils range from 2.7 to 4.4 L kg⁻¹ (Beck and Jones, 2001; Johnson et al., 1998; Worrall et al., 1996). In contrast, high 4IA sorption to soil has been reported by Bollag et al. (1978), who found that 52% of the ¹⁴C from added ¹⁴C-isopropyl-labeled 4IA was unextractable after 24 h despite extensive extraction.

Sorption of IPU in soil is related to the natural organic carbon content (Fouqué-Brouard and Fournier, 1996; Singh et al., 2001), and the mechanism is suggested to be analogous to partitioning of a compound between solvents (Worrall et al., 1997). Reduced bacterial mineralization of aged compounds in soil has also been observed for the herbicide atrazine (Kristensen et al., 2001; Radosevich et al., 1997) and for phenanthrene (Hatzinger and Alexander, 1995). With phenanthrene it was shown that the aging effect is more likely to be due to slow diffusion into micropores or partitioning into natural organic matter than to adsorption to surfaces (Hatzinger and Alexander, 1995). Like the phenylurea herbicides, atrazine and phenanthrene are nonpolar organic compounds and sorption in wet or saturated soils is thus expected to be controlled by the natural organic matter content (Chiou, 1989). The polar water molecules are believed to displace the organic contaminants at the mineral surfaces such that absorption of the contaminants into the macromolecular structure of natural soil organic matter becomes the dominating sorption mechanism in soils where the organic matter content exceeds approximately 0.2% (Schwarzenbach et al., 1993). Sorption by this mechanism exhibits linear sorption isotherms because no saturation of specific ad-

sorption sites occurs (Schwarzenbach et al., 1993). Moreover, the process is reversible because the intermolecular attractions between the nonpolar contaminant and the natural organic matter are relatively weak (Schwarzenbach et al., 1993). Based on the similar chemical structure between IPU and MDIPU together with the nearly identical behavior of these compounds observed in our experiments, it is likely that the mode of sorption of MDIPU is close to that of IPU. This view is supported by the virtually linear sorption isotherms obtained for both IPU and MDIPU in our soil. The reversible nature of the sorption of IPU and MDIPU is also apparent from our observation that desorption of IPU and MDIPU did not reach an equilibrium within the duration of our desorption experiment.

In contrast, desorption of 4IA was completed within 24 h. Weber et al. (2001) described sorption of aniline to a sediment with a high organic carbon content (3.3%) as consisting of an initial phase of 20 to 24 h with binding by both reversible cation exchange and irreversible covalent binding. With longer reaction periods, irreversible covalent binding became dominant. Li and Lee (1999) also found that reversible sorption of aniline to surface soils reached equilibrium within 1 d, whereas removal of aniline from the aqueous phase by irreversible processes proceeded for much longer time. Reuter et al. (1999) and Scheunert and Reuter (2000) demonstrated covalent binding of 4IA to synthetic soil organic matter. These findings suggest that a fraction of the 4IA irreversibly binds to organic matter in our soil, thus rendering it unavailable for degradation by strain SRS2.

With the uninoculated soils containing aged ¹⁴C-IPU and ¹⁴C-MDIPU, the ¹⁴C content of the aqueous extracts was low while that of the NaOH extracts was high. It has been suggested that aqueous compounds are available for microorganisms (Ogram et al., 1985), while NaOH-extractable compounds—which are thought to represent ¹⁴C associated with the alkali soluble humic and fulvic acids (McCall et al., 1981)—are probably less available. However, more ¹⁴C-IPU and ¹⁴C-MDIPU was mineralized to ¹⁴CO₂ in soil inoculated with strain SRS2 than was extracted with water in uninoculated soil at the time of inoculation, thus indicating that strain SRS2 was able to utilize more than just the water-extractable fraction. Fu et al. (1994) found that substantially more ¹⁴C-ring-labeled styrene was mineralized by the indigenous microorganisms in a loam soil and aquifer sediment than was desorbed under equilibrium conditions. However, when desorption was measured under nonequilibrium conditions by replacing the aqueous phase at regular intervals, much more ¹⁴C was desorbed, thus indicating that the correlation between the bioavailable and the water-extractable fractions may not be simple.

Previous studies have revealed that the availability of biphenyls and polycyclic aromatic hydrocarbons to degrading bacteria in soil may be strain specific, where some bacteria appear to access the sorbed fraction and others only the aqueous-phase fraction (e.g., Feng et al., 2000; Tang et al., 1998). The availability of IPU and its metabolites may also be strain specific, though the use of strain SRS2 to assess bioavailability is relevant

as recent results based on extraction and sequencing of 16S rRNA genes directly from IPU-treated soil strongly indicate that strain SRS2 had a role in in situ degradation within a British agricultural field (Bending et al., 2003). Additionally, strain SRS2 is so far the only characterized bacterium degrading IPU and the metabolites MDIPU and 4IA, making comparison with other degrading strains impossible at present.

CONCLUSIONS

This study demonstrates that aging of IPU and MDIPU in agricultural soil reduced the bioavailability to strain SRS2, although this effect was less significant after 120 d of incubation. Sorption of IPU and MDIPU to agricultural soils may increase their residence time and thereby time for degradation to occur. Our results shows that aging probably does not affect degradation when the residence time is long. With 4IA the availability to strain SRS2 after aging for just 1 d in the soil was markedly reduced compared with that of IPU and MDIPU. Sorption and desorption measurements together with the distribution of ^{14}C originating from ^{14}C -IPU, ^{14}C -MDIPU, and ^{14}C -4IA into the water-extractable, NaOH-extractable, and nonextractable fractions support the suggestion that different sorption mechanisms govern the bioavailability and hence the mineralization of IPU, MDIPU, and 4IA. Partial degradation of IPU to 4IA in the soil environment may significantly reduce mineralization of the herbicide molecule due to sorption of the aniline metabolite to soil. This renders the 4IA unavailable for biodegradation, which may reduce the natural attenuation rate of IPU in agricultural soils with respect to mineralization of the phenyl structure to CO_2 .

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