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Microbial Degradation of Penoxsulam in Flooded Rice Field Soils

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The degradation of penoxsulam {2-(2,2-difluoroethoxy)-*N*-5,8-dimethoxy[1,2,4]triazolo[1,5-C]pyrimidin-2-yl-6-(trifluoromethyl)benzene-sulfonamide} was studied in flasks simulating flooded rice field conditions using four representative rice field soils from the Sacramento Valley. Degradation half-lives ($t_{1/2}$ values) ranged between 2 and 13 days. Increased degradation rates were observed in flask systems with steeper redox gradients between the flooded soil layer and the overlaying water. Two transient metabolites were identified that were temporarily formed in amounts exceeding 5% of the total initial mass of penoxsulam. The results of high-performance liquid chromatography/^{14}C radiodetection studies indicate that the degradation of the triazolopyrimidine system and its substituents is the main pathway of microbial transformation processes. Microbial activity, as measured by dehydrogenase activity, was not affected by penoxsulam concentrations corresponding to the proposed maximum annual use rate of 40 g active ingredient/ha.

KEYWORDS: Anaerobic transformation; paddy incubation conditions; environmental fate; Granite; triazolopyrimidine sulfonamide; acetolactate synthase inhibitor; metabolic activity

INTRODUCTION

Penxosulam (trade name Granite) is a triazolopyrimidine sulfonamide herbicide intended for postemergence control of annual grasses, sedges, and broadleaf weeds in rice culture (*1*). It acts as an acetolactate synthase (ALS) inhibitor and targets the biosynthesis of branch-chained amino acids, a metabolic pathway found in plants, fungi, and microorganisms (*1*, *2*).

Knowledge of pesticide degradation processes and possible degradation products is needed to predict residual levels after field applications and to assess potential ecological risks associated with exposure. In flooded fields, anaerobic degradation processes are expected to dominate the microbial transformation of pesticides (*3*, *4*).

Currently, environmental fate information on penoxsulam is obtained from studies submitted to the Environmental Protection Agency required for registration, and there is little information available in the open literature. Penoxsulam is a water soluble pesticide that is hydrolytically stable, nonvolatile, and sorbs only weakly to nonacidic soils and moderately to acidic soils (*1*, *5*). Therefore, degradation rates and water-holding periods are expected to control dissipation rates of penoxsulam in the rice field environment. In a previous study, field dissipation rates in flooded rice fields were determined with first-order half-lives of 3−7 days, indicating that penoxsulam is nonpersistent in aquatic systems (*1*). However, potential degradation pathways, transformation products, and the ecotoxicity of penoxsulam have not yet been fully characterized. Hence, there may be ecological risks to plants and microbial communities (*6*). In a second study, we determined photochemical transformation pathways. No persistent photoproducts were found (*7*). To our knowledge, there are no previously published studies on the microbial transformation of penoxsulam.

This study describes the microbial degradation of penoxsulam in flasks simulating flooded rice field conditions. The objectives were to (i) determine transformation rates of penoxsulam in representative flooded soils from the Sacramento Valley and (ii) characterize the occurrence and rates of formation and decline of degradation products to which plants and soil organisms may be exposed.

Table 1. Selected Properties of the Rice Field Soils

oil series	organic carbon (%)	pH	sand (%)	silt (%)	clay (%)
Sacramento	1.3	6.5	26	34	40
San Joaquin	0.5	5.3	37	43	20
Stockton	0.7	4.6	17	33	50
Willows	1.1	6.8	15	48	37

MATERIALS AND METHODS

Soils. Representative soils and water were collected from four flooded rice fields in the Sacramento Valley, located on commercial farms. The Sacramento soil series was from the Schiedel Ranch, the Willows soil series was from the Maxwell-Dennis Ranch (both located in Colusa County near Maxwell, CA), the San Joaquin soil series was from the Matthews Ranch (Yuba County, near Mello, CA), and the

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Table 2. Summary of Redox Potential (Eh) and pH Measurements in Flasks by Experiment

soil series	system	*n* samples	pH	Eh (mV) water	Eh (mV) soil	Eh (mV) water−soil
Sacramento	14 days acclimated, bottles	10	6.9 ± 0.3	−47 ± 61	−145 ± 11	98 ± 67
San Joaquin	14 days acclimated, bottles	10	6.7 ± 0.3	86 ± 131	−155 ± 35	241 ± 125
Stockton	14 days acclimated, bottles	10	6.3 ± 0.2	−101 ± 35	−182 ± 12	82 ± 42
Willows	14 days acclimated, bottles	10	6.5 ± 0.2	0 ± 66	−123 ± 36	123 ± 87
Willows	3 months acclimated, bottles	10	8.1 ± 0.2	94 ± 51	−190 ± 49	237 ± 81
Willows	14 months acclimated, tubes, C^{14} experiments	10	6.7 ± 0.3	107 ± 140	−217 ± 39	324 ± 134

Table 3. Microbial Degradation Rates and Half-Lives of Penoxsulam in Flooded Rice Soils

soil series	$k \times 10^{-3}$ ($days^{-1}$)	$t_{1/2}$ (days)	r^2	significant differences[a]
Sacramento	−134.1	5.2	0.966	A
San Joaquin	−54	12.8	0.697	B
Stockton	−97.4	7.1	0.871	C
Willows	−59.6	11.6	0.703	D
Willows, 3 months stored	−370.3	1.9	0.986	A, B, C, D
Willows, XDE-638-Ph-UL-^{14}C	−116.8	5.9	0.514	
Willows, XDE-638-TP-^{14}C	−273.5	2.5	0.853	

[a] Same letter = significant difference at 99% confidence level.

Stockton soil series was from the Thompson Ranch (Glenn County, near Butte City, CA). The collected soils were taxonomically classified as follows: The Sacramento soil series is a fine, montmorillonitic, noncalcareous, thermic Cumulic Haplaquoll (*11*); the San Joaquin soil series is a fine, mixed, thermic Abruptic Durixeralf (*12*); and the Stockton and Willows soil series are fine, montmorillonitic, thermic Typic Pelloxererts (*12*). Paddy water was directly placed into 4 L amber glass solvent jugs. At each location, about 1 kg of water-saturated soil was collected in 1 gallon ziplock bags that were sealed underwater. Water and soil samples were transported in ice-filled coolers and kept at 5 °C in the cold storage room of the Department of Environmental Toxicology at UC Davis before experimental use. Soil physicochemical properties of the soils were determined by the Division of Agriculture and Natural Resources Analytical Laboratory (DANR Lab) at UC Davis and are summarized in **Table 1**. The soil composition (sand/silt/clay) was determined by the hydrometer method (*8*), the soil pH was measured from a saturated soil paste (*9*), and the soil organic carbon contents were measured by the Walkley−Black method (*10*).

Chemicals. Analytical grade samples of penoxsulam [XDE-638, 98.8% active ingredient (a.i.)], XDE-638-TP-^{14}C (28.1 mCi/mol, 97.1% a.i.), XDE-638-Ph-UL-^{14}C (30 mCi/mol, 98% a.i.), and ^{13}C-XDE-638 and analytical grade reference samples for nine transformation products (92−99% purity) were provided by Dow Agrochemicals LLC. Cloransulam-methyl was obtained from ChemService (West Chester, PA). All solvents were high-performance liquid chromatography (HPLC) grade.

Microbial Transformation of Nonlabeled Penoxsulam in Flasks Simulating Flooded Rice Field Conditions. *Experimental Design.* Water-saturated soils were placed into 60 mL Qorpak amber wide-mouth bottles (All-Pak Inc., Bridgeville, PA) with a spatula to yield a soil layer of approximately 2.5 cm depth that was then flooded to a depth of 1−5 cm with paddy water to yield soil:water ratios of 1.6 ± 0.5. In experiment 1, freshly collected soils (stored <21 days) were used and the flasks were acclimated for 14 days prior to the start of the experiment. In experiment 2, the soil was stored for 6 months before use and experimental flasks were acclimated for 3 months (and periodically replenished with stored paddy water to replace evaporation losses). At the start of each experiment, 6 μg of penoxsulam (2 μg/mL in HPLC grade water) was applied to each flask, corresponding to the recommended maximum annual application rate of 40 g active ingredient/ha. The flasks were closed with PUF plugs and incubated at 25 °C in the darkness. Experiment 1 was run in duplicate while experiment 2 had triplicate flasks, which were removed at periodic intervals for sampling.

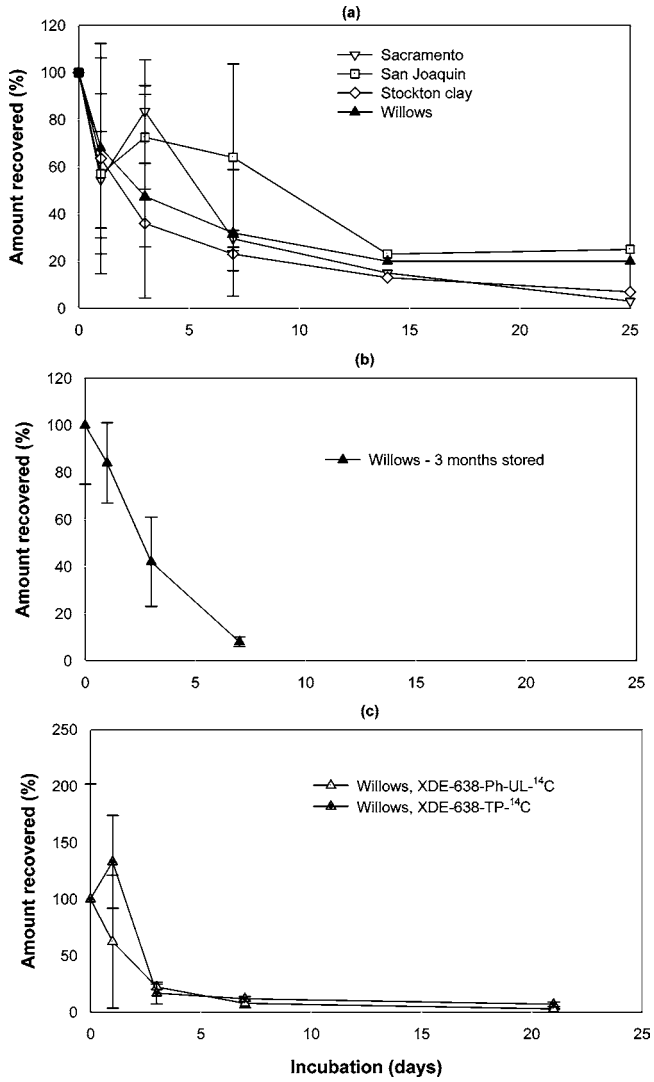

Figure 1. Degradation of penoxsulam incubated in simulated flooded conditions with (**a**) fresh soils (Sacramento, San Joaquin, Stockton, and Willows soil series), (**b**) Willows soil series stored for 3 months, and (**c**) fresh Willows soil series in C^{14} radioisotope studies with a modified experimental system (see Methods).

Relevance of Abiotic Transformations. The chemical stability of penoxsulam in sterile conditions was established in previous studies (*5*).

Extraction and Analysis. To determine the concentrations of penoxsulam and its transformation products in the water phase, samples of 1.0 mL were withdrawn, spiked with cloransulam-methyl as a surrogate recovery standard (to monitor recovery rates during extraction), and extracted with solid phase extraction (SPE) cartridges. The extracted analytes were eluted with 1 mL of acetonitrile:methanol:acetic acid (50:50:0.1 %). An additional 1.0 mL aliquot was filtered and directly injected to quantify transformation products that were not extractable by SPE. All analytes were quantified with a liquid chromatography/

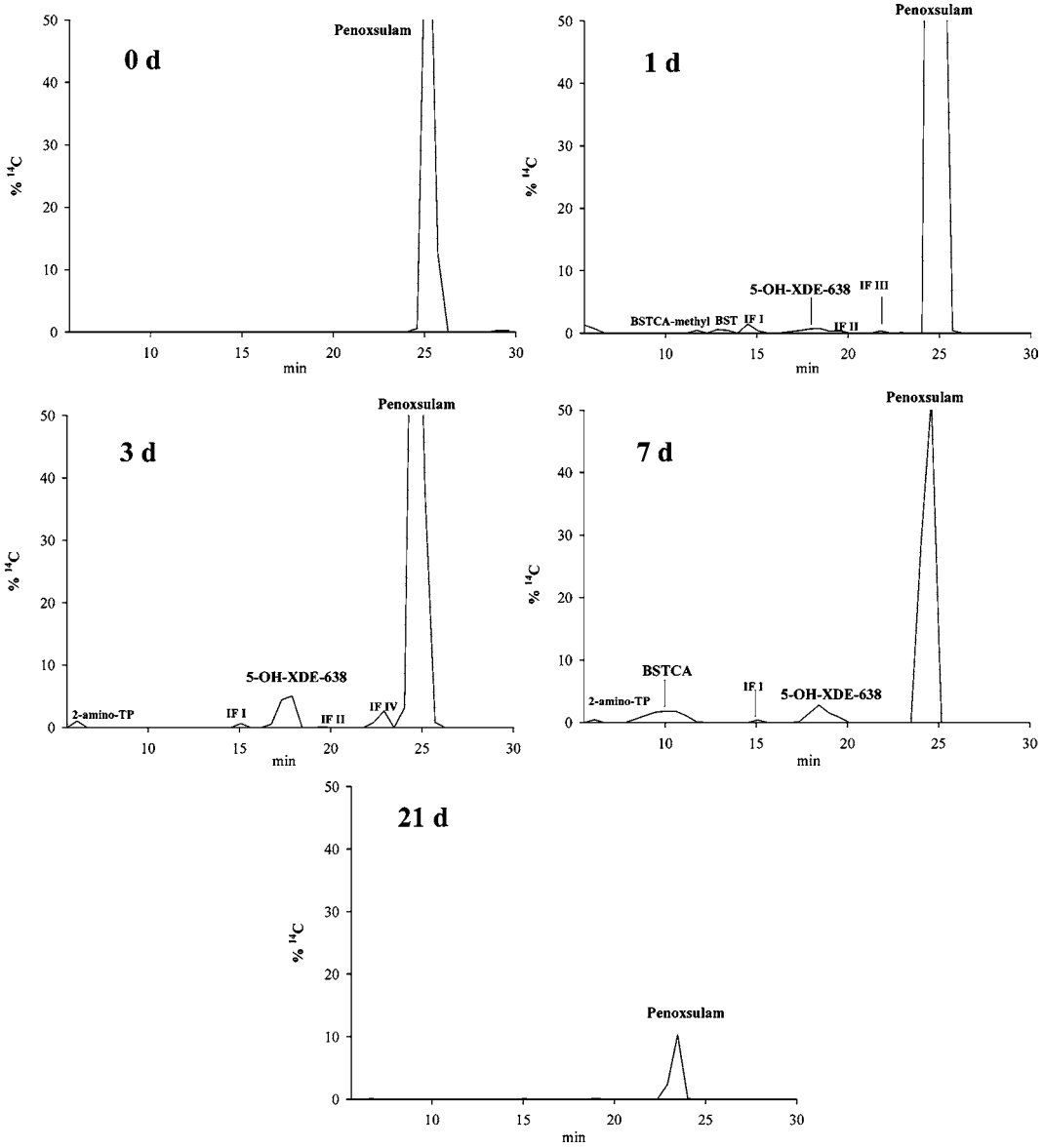

Figure 2. ^{14}C HPLC profile of radiolabeled penoxsulam (XDE-638-TP-^{14}C) and its biodegradation products in the water phase of glass tubes after 0, 1, 3, 7, and 21 days of incubation in the dark. Full compound names are given in **Figure 3**. IF I–IV, unknown fractions of intermediate polarity.

tandem mass spectrometry system (LC-MS/MS) that combined an Agilent model 1100 LC system (Palo Alto, CA) equipped with a 50 mm × 4.6 mm Luna 3 μm silica column (Phenomenex, Torrance, CA) and an API 2000 MS/MS (MDS Sciex, South San Francisco, CA) fitted with an electrospray interface. LC-MS/MS methods and analytical conditions are described in detail in ref *7*. Quantification of penoxsulam and degradation products was performed using external standard calibration curves (range, 0.33−30000 ng/mL) and ^{13}C-penoxsulam as an internal standard.

To quantify soil concentrations, approximately 5 g of wet soil was weighed and extracted for 1 h each with 25 and 15 mL of acetonitrile on a mechanical shaker. The extract was decanted into a graduated cylinder, and solvent lost during the extraction procedure was replaced to yield a total of 40 mL. The extract was then transferred to a rotoevaporator and reduced to less than 1 μL. One mL 0.1 N hydrochloric acid was added, and samples were then run through a SPE step before quantitation by LC-MS/MS analysis. After extraction, the soils were oven-dried at 90 °C and the soil dry weight was determined.

Radioisotope Transformation Studies. *Determination of Degradation Products.* Water-saturated Willows series soil was transferred to 200 mm × 25 mm glass test tubes, which created a soil layer of approximately 5 cm depth. The soil layer was covered to a depth of 5 cm paddy water to yield soil:water ratios of 1.2 ± 0.4. Prior to the addition of radiolabeled penoxsulam (either 4.5 μCi XDE-638-TP-^{14}C/unit or 4.7 μCi XDE-638-Ph-UL-^{14}C/unit), the flasks were acclimated for 14 days. At the start of the experiment, radiolabeled penoxsulam was added and rubber stoppers were placed in the flask that had funnels inserted in them. The funnels were layered with activated coal, dimethyl sulfoxide (DMSO)-treated glass wool, soda lime, and finally a layer of activated carbon. In periodic intervals, duplicate flasks were removed for analysis.

Determination of Organic Volatile Compounds. At each sampling interval, the DMSO-treated glass wool was removed from the funnel topping the glass tubes, transferred to a glass Petri dish, and sonicated for 15 min with 4 mL of dichloromethane. One milliliter of the extract was transferred to an 8 mL scintillation vial, mixed with 7 mL of Ultima Gold scintillation cocktail (PerkinElmer, Wellesley, MA), and capped for analysis by liquid scintillation counting (LSC) with a TRI-CARB Liquid Scintillation Analyzer model 2000CA (Packard, Downers Grove, IL).

Extraction and HPLC/^{14}C Radiodetection. The water phase from a sampled flask was decanted, and an aliquot of 1.0 mL was withdrawn. One aliquot was directly analyzed by LSC. The other sample was separated by HPLC using the conditions described by Jabusch and Tjeerdema (*7*). The HPLC eluate was collected with a fraction collector in 0.53 min intervals and analyzed by LSC. Analytes in the fractions

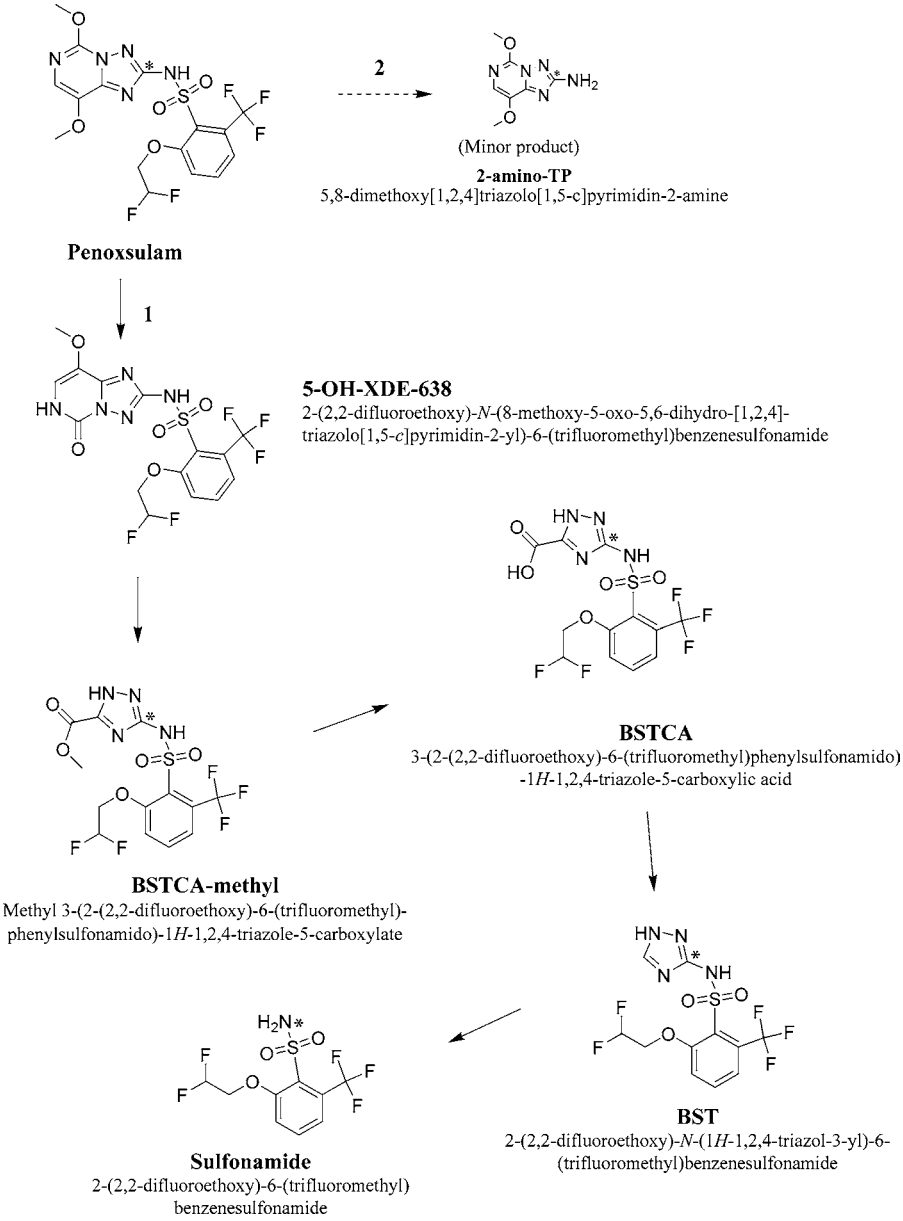

Figure 3. Suggested transformation pathway of penoxsulam in flooded rice soils. * = position of ^{14}C atom in XDE-638-TP-^{14}C radioisotope and its products.

were identified by retention time and matching with a nonlabeled standard mixture that was analyzed using the same HPLC conditions.

For HPLC/^{14}C analysis of soils, a spatula was used to mix the water-saturated soil and transfer a sample of approximately 1 g to a 7 mL Teflon-lined screwcap vial. Analytes were extracted with 4 mL of acetonitrile on a mechanical shaker. One 1 mL aliquot of the extract was passed through a 0.2 μm Acrodisk for HPLC/^{14}C analysis, and another 1 mL was directly transferred to a scintillation vial for determination of total ^{14}C by LSC.

Dehydrogenase Activity. The dehydrogenase activity was measured to assess the microbial metabolic activity in the flask systems and whether it was affected by penoxsulam, following the method described by Casida and Pamatmat and Bhagwat in refs *13* and *14*.

Redox Potential (Eh) and pH. The Eh was determined in both the water (Eh_w) and the water-saturated soil (Eh_s) of each sampled flask at the time of collection. During the acclimation phase, the redox potential was determined daily in a set of triplicate flasks that was designated for this purpose. For the measurements, the electrode was either suspended in the water phase or entirely immersed in the water-saturated soil. The pH was determined in the water phase of each sampled flask and daily in the water phase of the monitor flasks during the acclimation phase. To avoid cross-contamination in experiments with radiolabeled penoxsulam, the measurements were conducted in monitor flasks that were maintained at the same conditions but without ^{14}C added.

Statistics. The Tukey−Kramer honestly significant difference test was employed for statistical comparisons of redox potential and dehydrogenase activity. Student's *t*-test was used for comparing degradation rates.

RESULTS AND DISCUSSION

Flasks. Table 2 provides a summary of average redox potential and pH values for each experiment. Although there was considerable variability in Eh, and particularly in Eh_w, between individual samples, there were no apparent temporal trends within the experiments. There were also no differences between samples with penoxsulam and controls. Average Eh_s values ranged between −123 and −217, which is within the range of values observed in flooded rice fields (*15*).

Degradation Rates. Degradation rate constants were obtained by fitting penoxsulam degradation data from all experiments to the first-order kinetic equation

$$\ln (C/C_0) = -kt \quad (1)$$

where C_0 is the initial concentration of penoxsulam (μg/g), C is its concentration (μg/g) after time t (days), and k is the degradation rate constant. The r^2 values obtained for the correlations ranged between 0.514 and 0.986 (**Table 3**). The corresponding half-lives $t_{1/2}$ were calculated as $t_{1/2} = \ln 2/k = 0.693/k$. The calculated $t_{1/2}$ values ranged between 1.9 and 12.8 days (**Table 3**), indicating that penoxsulam is readily transformed by microorganisms in flooded rice fields.

Although there were differences in the degradation rates between certain soils in experiment 1, they were not as marked as those observed in a single soil (Willows), which was submitted to different incubation conditions (**Figure 1** and **Table 3**). The $t_{1/2}$ of penoxsulam decreased from 11.6 days in freshly collected Willows series soils to 1.9 days in soils stored for 6 months and then acclimated in the flasks for another 3 months. This increase in the degradation rate is associated with an increase in the average Eh_s from -123 to -193 mV and an increase in the pH from 6.5 to 8.2 (**Table 2**). Increases in pH in sediments are common in reductive conditions and due to the anoxic production of ammonium by microorganisms (*16*).

The observation that the degradation rate constant was higher in stored than in fresh soil is expected if degradation is anaerobically mediated. It is possible that the periodic replenishing of field water during the long acclimation time supplied the necessary electron acceptors and substrates to allow the establishment of an acclimated anaerobic microbial community.

Volatile Products. There was no significant production of volatile degradation products. Volatile products were only detected in two samples in the ^{14}C trials, in amounts <0.01% of the total initial mass applied.

Transformation Pathway and Products. The formation of transformation products was determined in experiment 1 (conventional analytes) by LC-MS/MS and in experiment 3 (radioisotopes) by HPLC/^{14}C radiodetection. **Figure 2** shows as an example the HPLC profile of samples collected from the water phase of glass tubes incubated with XDE-638-TP-^{14}C. The chromatographic profiles obtained from the soil phase and from the experiment using the XDE-638-Ph-UL-^{14}C penoxsulam are similar to those in **Figure 2**. In general, the greatest percentage of transformation products was found on day 1 and declined during the remainder of the experiment. BSTCA-methyl and BST (see **Figure 3** for full names) were the most abundant transformation products and accounted for an average of 10.2 and 5.7% of the total percent mass of ^{14}C derived from XDE-638-TP-^{14}C (water and soil phase combined) on day 1. In the experiment using 638-Ph-UL-^{14}C, BSTCA-methyl was not detected, and BST accounted for only 1.1% of the total ^{14}C on day 1. BSTCA-methyl and BST were also the only two metabolites detected by LC-MS/MS in experiment 1. Half-lives in this experiment were slower (**Table 2**), and the greatest average concentrations of BST and BSTCA-methyl were detected on days 3 and 7, with averages of 4.4 and 14.4% of the total mass.

Figure 3 provides a suggested pathway and information on transitional products of microbial degradation in flooded rice fields. Roberts et al. (*1*) note that biological degradation proceeds via the triazolopyrimidine system and its substituents.

Dehydrogenase Activity. Dehydrogenase activity is a marker of soil microbial activity and has been recommended as an indicator of soil health (*17*, *18*). The measurements were highly variable and revealed differences in the general microbial activity of different soil but not between controls and samples treated with penoxsulam (**Figure 4**).

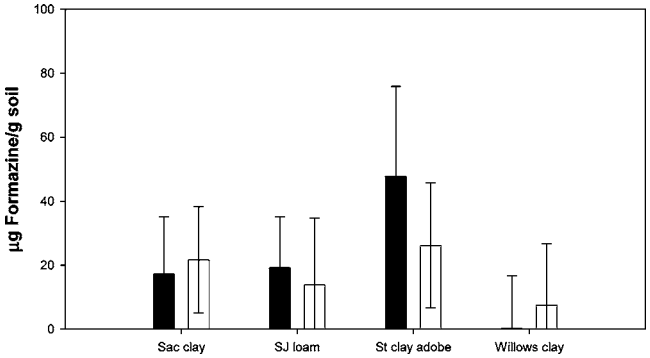

Figure 4. Effect of penoxsulam on dehydrogenase activity in flooded soils.

Conclusions. The study indicates that the anaerobic microbial transformation of penoxsulam in flooded soils is rather rapid and can be of equal significance for its overall fate as photodegradation (*7*).

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