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Dissipation of the Herbicide [^{14}C]Dimethenamid under Anaerobic Conditions in Flooded Soil Microcosms

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The objective of this research was to investigate the dissipation of the herbicide dimethenamid under anaerobic redox conditions that may develop in the soil environment. Soil−water biometers were prepared with a saturated soil and made anaerobic by either glucose pretreatment (according to the Environmental Protection Agency registration study for anaerobic fate) or N_2 sparging. Treatments included glucose pretreatment, $NO_3^- + SO_4^{2-}$ amendment, unamended, and autoclaved. Volatile, aqueous, extractable, and bound (unextractable) ^{14}C-residues were quantified and characterized. The redox potential decreased over time, and evidence of denitrifying, iron-reducing, sulfate-reducing, and methanogenic conditions was observed, depending on the amendments. Anaerobic degradation of ^{14}C-dimethenamid occurred in all treatments, and the time observed for 50% disappearance (DT_{50}) was 13−14 days for nonautoclaved treatments. ^{14}C-metabolites accumulated to up to 20% of applied ^{14}C. At least two major metabolites were observed in nonautoclaved treatments, whereas only one was observed in autoclaved microcosms. More than 50% of the applied ^{14}C was eventually incorporated into soil-bound residue.

KEYWORDS: Anaerobic aquatic metabolism; anaerobic herbicide degradation; chloroacetamide; dimethenamid; flooded soil; SAN 582 H

INTRODUCTION

Dimethenamid (2-chloro-*N*-(2,4-dimethyl-3-thienyl)-*N*-(2-methoxy-1-methylethyl)acetamide) is a chloroacetamide herbicide used for preemergence or early postemergence grass control in corn and soybean (*1*). This herbicide is water soluble (1174 mg L^{-1}, 25 °C), moderately sorptive (K_{OC}, 155 for nonaged residue, in soil with 0.5−4.9% OC and 12−36% clay), and is not particularly vulnerable to photodecomposition or volatilization (*1*). The aerobic half-life varies from 20 days to 5−6 weeks in the field and 38 days in vitro (*1*). Although dimethenamid has been registered since 1992, information concerning biodegradability and dissipation mechanisms is limited. A biodegradation study by Müller and Buser (*2*) demonstrated that degradation of dimethenamid and other acetamide stereoisomers in aerobic garden soil and sewage sludge was stereo- and enantioselective. The first-order half-lives of dimethenamid in aerobic soil and anaerobic sewage sludge were 7.8 and 29.6 h, respectively. Microbiological and abiotic degradation were not specifically addressed. The *Herbicide Handbook* (*1*) reports that in soil, the herbicide is transformed to dimethenamid oxalamide (2-[(2,4-dimethyl-3-thienyl)(2-methoxy-1-methylethyl)amino]-2-oxoacetic acid; **Figure 1**), which is comparable to the oxanilic acid (OXA) metabolites described for alachlor, acetochlor, and metolachlor, but the study that produced this information has not been published. OXA metabolites of alachlor and metolachlor have been detected in groundwater and surface water, but their formation under anaerobic conditions has not been documented.

Dimethenamid may be transported in the environment through leaching or runoff. In a field study that monitored dissipation of several chloroacetamides in the 0−8 cm fraction of the soil surface, the time observed for the disappearance of 50% applied dimethenamid (DT_{50}) ranged from 4 to 11 days (*3*). The authors suggested that the dissipation of the herbicides studied may have reflected transport from the soil surface with heavy rains. Detection of this and other chloroacetamide herbicides in surface water (*2*) and groundwater (*4*) supports the likelihood of offsite transport.

Anaerobic metabolism in soil and sediments is a potential mechanism of xenobiotic dissipation in the environment, but the influence of anaerobic microbial processes on herbicide persistence in soil has received very little research attention. The North Central states represent a major herbicide use area

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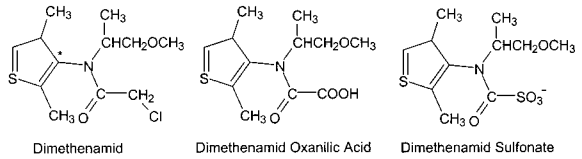

Figure 1. Chemical structures of dimethenamid, dimethenamid oxalamide, and dimethenamid sulfonate. The asterisk denotes the position of the radiolabel in dimethenamid.

with soils that are predisposed to flooding due to seasonal rainfall and poor drainage. Much of the land in crop production in this area exists in the Midwest flood plain and is frequently inundated during the season of herbicide application. In particularly wet corn fields, it sometimes becomes obvious that the crop will not recover from the flooding after the field dries out, and recropping the field with soybean may be considered. This, however, raises the question of how much herbicide applied to the corn crop (which may not be safe for soybean) remains in the soil following the flooding. In 1997, chloroacetamide herbicides were applied to 69% of the cropland in Illinois and the other North Central states. Dimethenamid represented over 2.1 million kg (8%) of the application. The importance of herbicide use and soil flooding indicates the necessity of characterizing herbicide degradation in saturated soils. Runoff can transport herbicides to drainage ditches and surface waters, where sediments are anaerobic.

Predicting herbicide fate in anaerobic environments requires a knowledge of the existing anaerobic redox conditions as well as herbicide susceptibility to degradation under the various redox conditions, particularly since the mechanisms of biodegradation differ depending on which anaerobic population is active (*5*−*13*). In general, information concerning anaerobic degradation of chloroacetamide herbicides is limited (*14*). This family of herbicides includes, in order of most to least applied in 1997, metolachlor, acetochlor, dimethenamid, alachlor, and propachlor as well as the newly registered BAY FOE 5043 (flufenacet). Of these, alachlor seems to be the only representative for which anaerobic degradation in soil or sediment has been reported (*14*, *15*). Of particular concern is the absence of literature on anaerobic soil dissipation of dimethenamid.

The objective of the current research is to characterize dimethenamid dissipation and metabolite formation under conditions that represent naturally occurring anaerobic redox regimes in the environment including denitrifying, Fe(III)-reducing, sulfate-reducing, and methanogenic conditions. The study employed four different systems in anaerobic fate determination: (i) aerobic preparation and incubation with glucose (to achieve anaerobic conditions) prior to dimethenamid addition (Environmental Protection Agency Protocol N-162-3, Anaerobic Aquatic Metabolism, used in herbicide registration in accordance with U.S. EPA, *16*); (ii) anaerobically prepared with N_2 sparging instead of glucose amendment; (iii) anaerobically prepared with N_2 sparging as well as nitrate and sulfate amendment above the concentrations present in the basal salts medium but without the addition of glucose; and (iv) an autoclaved version of treatment iii. The study was designed to represent herbicide-treated fields that undergo soil flooding and anaerobiosis.

MATERIALS AND METHODS

Chemicals. Both unlabeled dimethenamid (purity, 99.7% by high-performance liquid radiochromatography, HPLC) and [3-^{14}C]thienyl-dimethenamid (specific activity 1.87×10^6 Bq μmol^{-1}) were donated by Sandoz Agro., Inc. (Des Plaines, IL, presently BASF Corp., Greensboro, NC). A radiolabel purity of 94% was determined by thin-layer radiochromatography (TLC) and barium chloride precipitation. A barium chloride treatment to remove bicarbonate in a sterile solution of ^{14}C-dimethenamid resulted in precipitation of 6% of ^{14}C, thus accounting for the impurity. Two analytical metabolite standards were additionally provided by BASF Corp. (Greensboro, NC): dimethenamid oxalamide (HPLC purity, 99.5%) and the sodium salt of dimethenamid sulfonate (2-[(2,4-dimethyl-3-thienyl)(2-methoxy-1-methylethyl)amino]-2-oxoethansulfonate; HPLC purity, 97.5%). The structures of dimethenamid and these metabolites are illustrated in **Figure 1**. The metabolites are structurally similar to the previously described OXA and ethansulfonic acid metabolites for herbicides acetochlor, alachlor, and metolachlor (*17*).

Soil. A saturated Sawmill silty clay loam, classified as a mixed mesic Cumulic Haplaquoll, (Sangamon River, University of Illinois Allerton Park, Piatt County) was used in this study. The ≤2 mm fraction contained 21% sand, 50% silt, 29% clay, and 4% organic matter and was a composite of three stream bank (upper 6 cm, saturated) and three stream bed samples and, when mixed 1:1 with streamwater (dissolved O_2, 4 mg L^{-1}), had a pH of 7.3 and a redox potential (Eh) of +27 mV (hydrogen scale) at sampling.

Media. The anaerobic mineral salts and trace metals medium was a modification of the previously described BN medium (*18*) in which benzoate and nitrate were omitted and only 0.05 g L^{-1} $(NH_4)_2SO_4$ was used. Dissolved oxygen was removed, and the medium was made anaerobic and sterile as detailed by Crawford et al. (*19*) in accordance with accepted techniques (*20*−*22*). The medium had a background sulfate concentration of 2.5 mM due to the elemental salts. The medium prepared in this manner was determined to be O_2 free using gas chromatographic analysis (GC, *21*).

Experimental Design. Serum bottle biometers were employed in this study. In preparation of each biometer, 10 g (7.7 g air-dry) of saturated flood plain sediment and 10 mL of anaerobic medium ± nitrate or sulfate were added to an 80 mL serum bottle. Microcosms prepared according to the EPA protocol (glucose pretreatment) were amended with glucose-containing medium (9 mg D-glucose biometer^{-1}) and incubated prior to dimethenamid addition to allow the development of anaerobic conditions as a function of oxygen and glucose consumption. The EPA protocol recommends a 30 day pretreatment or a dissolved O_2 concentration of <2 mg L^{-1} at experiment initiation. In the present study, glucose depletion was confirmed after 8 days with an *o*-toluidine-based colorimetric glucose assay (Sigma Chemical Co., St. Louis, MO), and the anaerobic nature of the microcosms was characterized. Measurement of Eh (75 mV) and dissolved oxygen (0 mg L^{-1}) indicated that the glucose-treated microcosms were anaerobic by EPA standards; thus, the experiment was initiated at this time.

The other microcosm treatments were made anaerobic by sparging with N_2 after adding soil. They were then capped and transferred to an anaerobic chamber, where they were opened and anaerobic basal salts medium was added.

After all microcosms were anaerobic, the experiment was initiated by adding ^{14}C-dimethenamid and nitrate + sulfate, where applicable, to biometers. To apply the herbicide, 9710 Bq of [^{14}C]dimethenamid (specific activity, 1.65×10^4 Bq μmol^{-1}, diluted with unlabeled dimethenamid) in 15 μL of methanol was added to each biometer to achieve a final concentration of 16 mg kg soil^{-1}. This concentration corresponds to the recommended application rate (on an area basis for the soil used) of 1.63 kg ha^{-1} (*1*, *23*). A CO_2 collection trap containing 2 M KOH (1 mL) was attached to a 2.5 cm thick butyl rubber stopper and was suspended into the microcosm with stainless steel wire. After the herbicide was added, the microcosm was immediately stoppered and crimp-sealed. All biometers were incubated in the dark at 25 ± 3 °C. The treatments included 27 replicates each of glucose-pretreated, unamended, nitrate + sulfate-amended (5 mM each), and nitrate + sulfate-amended (5 mM each) autoclaved control (autoclaved 3 times at 1 day intervals, 90 min each time at 121 °C prior to amendment with the herbicide and terminal electron acceptors). The dimethenamid solution had been filter-sterilized (0.2 μm). The absence of oxygen in the biometer headspace was confirmed with GC analysis (*21*).

Sampling. Microcosms from each treatment were destructively sampled and processed after 6 and 12 h and 1, 2, 4, 8, 16, 32, and 142 days.

Headspace Analyses. *Analysis of Methane.* Both [^{14}C] and unlabeled methane were quantitatively determined at 142 DAT with a GC combustion technique (*7*). The total methane analysis of headspace samples (0.5 mL) was conducted with GC including a Carbosieve SII column, isothermal conditions of 150 °C, and He carrier (30.0 mL min^{-1}). Effluent gas was directed through a quartz tube held at 800 °C and containing CuO pellets for carbonaceous oxidation to CO_2. The oxidized samples were manually fraction-collected in 3 mL of 0.5 M NaOH, and ^{14}C in the sample was quantified with liquid scintillation spectrometry (LSS) using a Packard 1900TR (Meriden, CT) liquid scintillation spectrometer. Total radioactivity in the headspace was determined by LSS on a duplicate 0.5 mL headspace sample, and the fractions of labeled and unlabeled methane were calculated.

Determination of Volatile ^{14}C-Dimethenamid and Metabolites. Dimethenamid volatilization and estimation of the radioactivity associated with volatile ^{14}C-metabolites were assessed using polyurethane foam plugs (PUFs) with the method of Mervosh et al. (*24*). To obtain these samples, the headspace of each microcosm was displaced with N_2 gas into a 20 mL syringe that contained a PUF plug. The PUF was transferred to a scintillation vial for LSS.

Processing Microcosm Slurries. *Redox Potential*. Upon opening each microcosm, the contents were quickly transferred to a 50 mL Oak Ridge centrifuge tube (polyallomer), and the Eh was immediately measured with a polished combination platinum AgCl/Ag redox electrode (Orion model 9678BN) connected to a portable Orion pH/ISE meter (model 290A). The effect of transferring the slurries to centrifuge tubes on Eh measurement was determined by comparing triplicate Eh in situ measurements with triplicate Eh measurements after transfer. The results were -1 ± 3 and -3 ± 6 mV, respectively; thus, the effect of the transfer on the anaerobic integrity of the system was negligible.

Determination of ^{14}C-Distribution. The solution in each CO_2 collection vial was quantitatively transferred to a scintillation vial, and $^{14}CO_2$ was quantified via LSS. Aqueous, extractable (sorbed), and unextractable (bound) ^{14}C were also quantified with LSS after sample processing as follows. After redox measurement, the solid and liquid phases of the soil slurry were separated by centrifugation (15 min, 4000*g*). Aqueous samples were removed and filtered (PVDF, 0.2 μm), a portion was stored at 4 °C for TLC, and 1.5 mL portions were frozen in microcentrifuge tubes at −10 °C for eventual $(NO_3^- + NO_2^-)$−N, aqueous Fe^{2+}, and SO_4^{2-} analyses. Aqueous aliquots for liquid scintillation counting were prepared in duplicate. One was treated with saturated $BaCl_2$ to precipitate CO_2/HCO_3^- and was centrifuged (4 min, 12 000*g*), and the supernatant was drawn off for LSS. The non-$BaCl_2$-treated sample represented the total aqueous radiocarbon (^{14}C-dimethenamid + ^{14}C-metabolites + $H^{14}CO_3^-$). The difference in ^{14}C between the $BaCl_2$-treated and the untreated samples was reported as $H^{14}CO_3^-$ (or aqueous $^{14}CO_2$).

Soil was extracted with ethyl acetate/acetone for 48 h with horizontal shaking. After the mixture was centrifuged (15 min, 4000*g*), a 15 mL portion of the ethyl acetate was transferred to a glass vial. An aliquot was removed for LSS (to quantify extractable ^{14}C), and the remainder was evaporated to dryness and redissolved in 2 mL of ethyl acetate/5% acetone for TLC. Sorbed ^{14}C was calculated as extractable minus solution ^{14}C still remaining in the soil following removal of most of the aqueous fraction. After the soil was air-dried and pulverized (with mortar and pestle), bound (unextractable) ^{14}C-residues were quantified by combustion (Harvey Biological Oxidizer OX500, R. J. Harvey Instruments, Hillsdale, NJ) and LSS of collected $^{14}CO_2$.

TLC Analysis of Parent and Metabolites. The ratio of ^{14}C-dimethenamid to ^{14}C-metabolites was quantified in aqueous and organic extracts using TLC. Aqueous samples were first extracted 1:6 (v/v) with acetone-fortified ethyl acetate (5%, v/v), and the organic portion was concentrated by evaporating to dryness and dissolving in 100 μL of ethyl acetate for TLC. A mobile phase composition of ethyl acetate:toluene:formic acid (concentrated):H_2O of 87:3:5:5 (Sandoz Agro., personal communication) was employed with Adsorbosil Plus 1 P TLC plates (Alltech Associates, Inc., Deerfield, IL). The plates were developed with ascending chromatography, and ^{14}C detection was conducted with an Ambis 4000 Radioanalytic Imaging Detector and Ambis version 4.31 software (Scanalytics, Inc., Billerica, MA).

HPLC Analysis of Parent and Metabolites. Soil extract samples containing [^{14}C]dimethenamid and [^{14}C]metabolites were analyzed using HPLC equipped with a Hewlett-Packard 1050 series autosampler instrument, using both ^{14}C (Packard Radiomatic Flo-one/beta detector) and UV (254 nm) detectors. Several HPLC conditions were used to separate metabolites dimethenamid oxalamide and dimethenamid sulfonate. A mobile phase with composition 55:45 acetonitrile:water (v/v) was continuously sparged with He and was delivered at 1.0 mL min^{-1} to a C_{18} RP Econosil column (5 μm, 250 mm × 4.6 mm; Alltech Associates, Deerfield, IL). For each treatment, triplicate soil extracts from day 142 of the incubation were combined and evaporated and then resuspended in 500 μL of methanol for HPLC analysis. [^{14}C]-dimethenamid, dimethenamid oxalamide, and dimethenamid sulfonate standards were dissolved in methanol and stored at 4 °C. Samples of 100 μL were injected into the instrument.

pH Measurement. Colorphast Indicator Strips (pH ranges of 7.5−14 ± 0.5, 4.0−7.0 ± 0.3, and 6.5−10.0 ± 0.3, EM Science, Gibbstown, NJ) were used to determine the pH of the supernatant soil solution after the transfer to the microcentrifuge tubes. The accuracy of the indicator strips was confirmed by comparing pH values with those obtained with a pH electrode.

Determination of Terminal Electron-Accepting Processes. Methanogenesis was determined as described under the Analysis of Methane section above. Denitrification, Fe^{3+} reduction, and SO_4^{2-} reduction were monitored by measuring the appropriate chemical species in aqueous samples using the following assays. Assays were conducted in microtiter plates, and spectrophotometric measurements of microtiter plates were obtained with a CERES model UV900HDi plate reader (Bio-Tek Instruments, Winooski, VT). Denitrification was evaluated by measuring (nitrate + nitrite)−nitrogen with the modified indophenol blue method of ref *25*.

Aqueous Fe^{2+} formation (an indicator of Fe^{3+} reduction) was analyzed with a microtiter plate modification of Stookey (*26*): a 250 μL aliquot of an aqueous sample was pipetted into a 96 well plate, and 5 μL of the ferrozine reagent (Sigma Chemical Co., St. Louis, MO) was added. The plate was mixed for 1 min on a vortex mixer fitted with a microtiter plate adapter, 5 μL of ammonium acetate buffer was added, and the plate was mixed and allowed to develop for 10 min. The absorbance was measured at 590 nm. Further evidence of sulfate reduction was qualitatively assessed by the appearance of FeS_2 precipitation (black deposits in the microcosms) as well as H_2S evolution (characteristic odor upon opening microcosms). High levels of sulfide cause a positive interference in the ferrozine method of Fe(II) quantification (*27*). In the present study, aqueous sulfide was analyzed in microcosms after 32 and 142 days with the methylene blue method described by Lindsay and Baedecker (*28*). The sulfide concentrations were below the detection limit (<31.2 μM) and thus would not be expected to influence quantification of Fe(II) with the ferrozine method.

Sulfate was determined photometrically with a microscale adaption of the barium precipitation procedure adapted by Cypionka and Pfennig (*29*) from Tabatabai (*30*). In the microscale analysis of sulfate, 125 μL of sample was added to a 96 well microtiter plate (flat bottom, polystyrene, Corning, NY). One hundred microliters of citric acid−glycerol solution (5%, w/v, citric acid·H_2O in 60%, v/v, glycerol) was added to each sample well, and the plate was mixed on a vortex shaker with an attached plate adapter for 1 min. After the solution was mixed, 25 μL of barium reagent (1%, w/v, $BaCl_2 \cdot 2H_2O$ in 10% w/v, citric acid) was added to each sample well, and the plate was shaken again (1 min); after 30 min, the plate was mixed again, and turbidity was measured at 430 nm. The potential for sulfide oxidation during sulfate quantification was considered since the assay is exposed to air. To test this, sulfide was added to standards to final concentrations of 0.0025 and 0.000 25%, and there was no difference in sulfate concentrations at the lower concentration. For this reason, the reoxidation of sulfide under assay conditions was concluded to be inconsequential.

Data Treatment. The statistical program SAS/STAT for Windows (*31*) was used to calculate means and standard error ($n = 3$), and the percent of total radiocarbon applied that was recovered as mineralized, sorbed (parent and metabolites), aqueous (parent and metabolites), and bound residue ^{14}C at each sample date. Linear regression was applied

Table 1. Eh and pH throughout the 142 Days of Incubation[a]

	biometer Eh and pH							
	glucose-pretreated		unamended		$NO_3^- + SO_4^{2-}$		autoclaved, $NO_3^- + SO_4^{2-}$	
time (DAT)	Eh	pH	Eh	pH	Eh	pH	Eh	pH
0.25	80 ± 1	7.2 ± 0.1	66 ± 11	7.5 ± 0	164 ± 3	7.5 ± 0	−4 ± 47	6.5 ± 0
1	40 ± 12	7.5 ± 0	81 ± 23	7.5 ± 0	183 ± 13	7.5 ± 0	−176 ± 58	7.7 ± 0.2
2	8 ± 10	7.5 ± 0	62 ± 0	7.5 ± 0	138 ± 7	7.5 ± 0	98 ± 0	7.5 ± 0.3
4	−37 ± 0	7.5 ± 0	76 ± 34	7.3 ± 0.2	155 ± 2	7.5 ± 0	−84 ± 0	7.3 ± 0.2
8	−50 ± 3	7.8 ± 0.2	7 ± 19	7.2 ± 0.3	39 ± 21	7.5 ± 0	62 ± 29	7.0 ± 0
16	−78 ± 13	7.7 ± 0.2	−68 ± 10	7.8 ± 0.6	−68 ± 3	7.7 ± 0.2	1 ± 21	7.2 ± 0.2
32	−74 ± 6	7.0 ± 0	−67 ± 3	7.5 ± 0	−78 ± 1	7.7 ± 0.2	−22 ± 13	6.5 ± 0
142	−283 ± 9	7.5 ± 0	−299 ± 4	7.5 ± 0.1	−316 ± 18	8.0 ± 0.1	−323 ± 22	7.7 ± 0.2

[a] Mean of triplicate samples ± standard error.

to the natural log of the percent of total recovered dimethenamid to estimate first-order kinetics.

RESULTS AND DISCUSSION

Biometer Redox Conditions and Microbiological Characteristics. Decreasing Eh values demonstrated that the saturated soil in the microcosms became progressively more reduced during the incubation (**Table 1**). Conditions were favorable for the anaerobic microbial processes of denitrification, Fe^{2+} formation, and sulfate reduction (**Figure 2a−d**) as well as methanogenesis, which are all expected in anaerobic soil and water (*5*, *32*−*34*). Thus, the presence of dimethenamid was not inhibitory to anaerobic microbial populations. Nitrate imparts higher Eh values to aqueous systems, and this was observed in the present study. Following (nitrate + nitrite)−N removal (**Figure 2c**), Eh values continued a decreasing trend similar to that observed in glucose-pretreated and unamended microcosms (**Table 1**).

Glucose-Pretreated. Aqueous Fe^{2+} concentrations peaked on days 2 and 32 (**Figure 2a**). The glucose-pretreated microcosms had initial sulfate concentrations of 2.5 mM as a result of the soluble salts of metals and minerals in the basal salts medium. Sulfate depletion occurred through day 16 (**Figure 2a**). Methane accumulated to 5.8% of headspace gases by 142 days, indicating that redox conditions and substrate availability were appropriate for methanogenic populations. Less than 0.001% of the methane contained the radiolabel; therefore, substrates for methanogenesis were derived from other than [^{14}C]dimethenamid and the $H^{14}CO_3$ impurity in the herbicide preparation. Substrates for methanogenesis exist in soil as a result of soil organic matter turnover. In the present study, CO_2 and acetate resulting from glucose consumption prior to herbicide treatment may have also been used in methane formation, although some escape of headspace gases would have occurred upon opening biometers to add the herbicide.

Unamended. As in the glucose-pretreated microcosms, unamended microcosms had initial sulfate concentrations of 2.5 mM, and Fe^{2+} accumulation was followed by sulfate and Fe^{2+} depletion, after which Fe^{2+} accumulated (**Figure 2b**). Methane was not detected in headspace samples of these microcosms.

$NO_3^- + SO_4^{2-}$-Treated. Although periods of nitrate removal and Fe^{2+} were evident, sulfate depletion was the most prevalent anaerobic process during the study (**Figure 2c**). Sulfate reduction was accompanied by FeS precipitation (evident as black deposits) and H_2S evolution. Methane accumulated to 3.4% in the headspace of these microcosms, of which less than 0.001% was $^{14}CH_4$.

Autoclaved Treatment. Elevated concentrations of Fe^{2+} and sulfate (**Figure 4d**) as compared with other treatments suggest

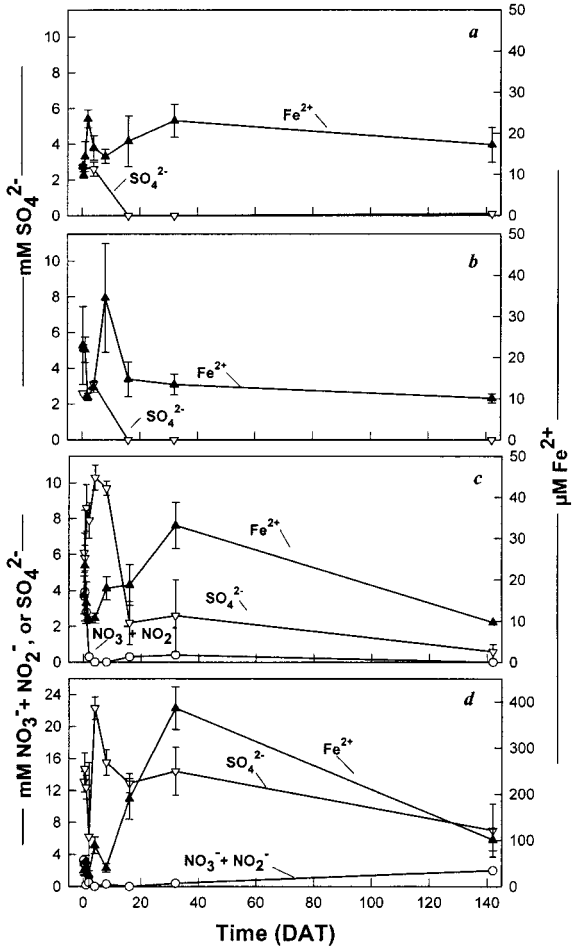

Figure 2. Time course microbial terminal electron-accepting processes in (**a**) glucose-pretreated microcosms, (**b**) unamended microcosms, (**c**) nitrate + sulfate-amended microcosms, and (**d**) autoclaved microcosms. Fe^{2+}, ▲; SO_4^{2-}, ▽; and NO_3^-, ○. Each data point represents the mean of triplicate samples ± standard error.

that these species were liberated into the soil solution during autoclaving, which is consistent with previous reports (*35*, *36*). Sulfate concentrations fluctuated but were mostly unchanged throughout the incubation. However, a net depletion of (nitrate + nitrite)−N and accumulation of methane (to 13.5% by day 142) indicated incomplete sterilization and partial regrowth of the microbial population in the microcosms. However, the continued presence of $NO_3^- + NO_2^-$ and SO_4^{2-} at 142 days suggests that populations were at least diminished. For this reason, the autoclaved control still provides a control in that it

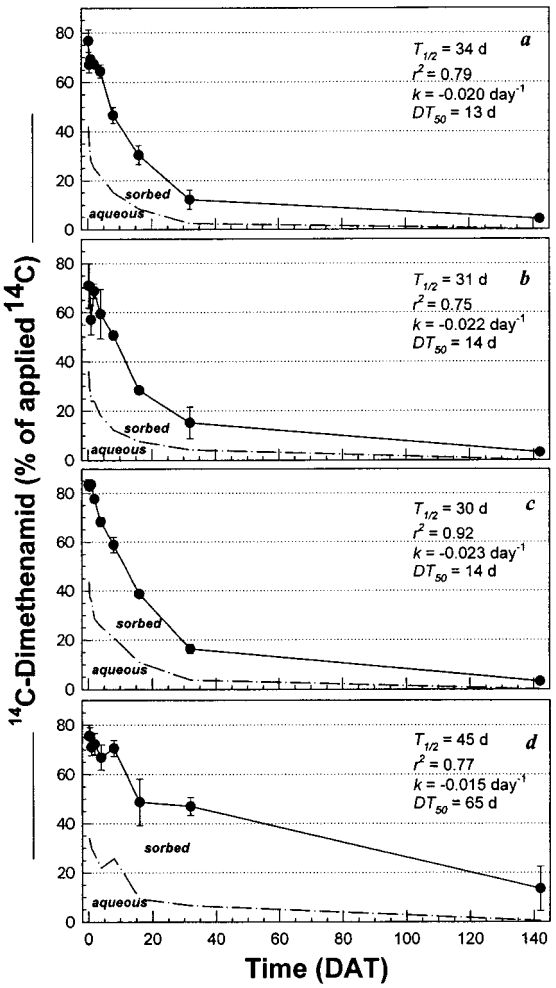

Figure 3. Average distribution of [^{14}C]dimethenamid, first-order $T_{1/2}$ and r^2, and observed half-lives (DT_{50}) in (**a**) glucose-pretreated microcosms, (**b**) unamended microcosms, (**c**) nitrate + sulfate-amended microcosms, and (**d**) autoclaved microcosms. – • – shows the distribution of [^{14}C]dimethenamid in the aqueous (below the line) and sorbed (above the line) fractions. Each data point represents the mean of triplicate samples ± standard error.

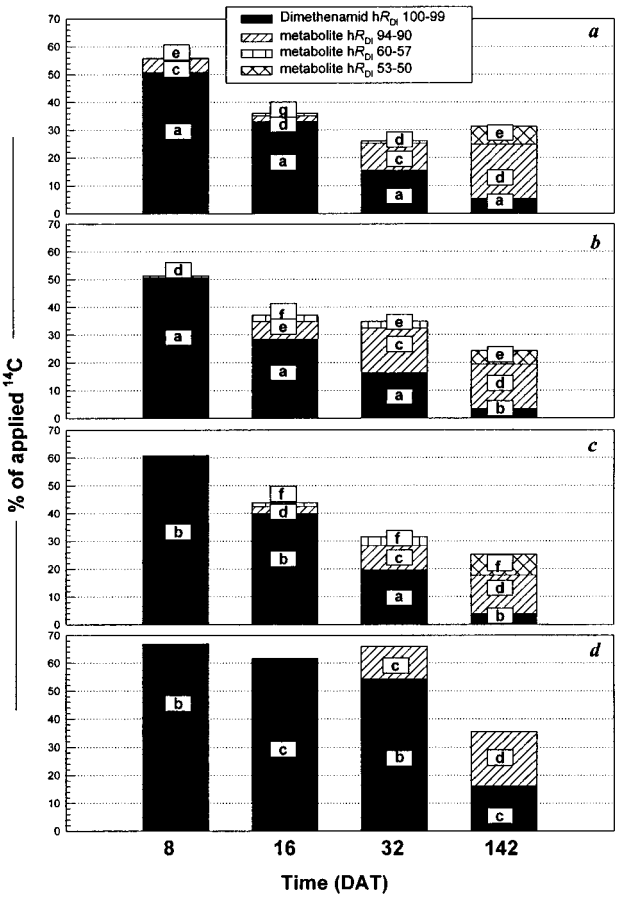

Figure 4. Distribution of [^{14}C]dimethenamid, nonpolar, and polar ^{14}C-metabolites in the glucose-amended, unamended, NO_3^- + SO_4^{2-}-amended, and autoclaved treatments at 8, 16, 32, and 142 DAT. The boxed letters are for comparing treatments on the same day and denote differences at the 5% probability level (n = 3). (**a**) Glucose-pretreated microcosms, (**b**) unamended microcosms, (**c**) nitrate + sulfate-amended microcosms, and (**d**) autoclaved microcosms.

harbors less biological activity, particularly less sulfidogenesis, than the nonautoclaved microcosms.

Discussion of Microbial Data. The higher levels of methane in the glucose-pretreated as compared with the nitrate + sulfate-amended biometers were expected since acetate, a product of glucose fermentation, is a substrate for methanogenesis. Methane was not detected in the unamended biometers, which were otherwise the same as the nitrate + sulfate-amended systems. The processes of denitrification and sulfate reduction may have provided CO_2 or other C1 substrates for methanogenesis. The lack of $^{14}CH_4$ in the headspace implies that substrates for methanogenesis did not include the impurity of the radiolabel or the single ^{14}C of dimethenamid (**Figure 1**). The main effect of autoclaving appeared to be to inhibit sulfidogenesis. Methane production was greatest in the autoclaved biometers and may reflect regrowth in the presence of elevated substrate levels of CO_2 or other C1 compounds due to their release during autoclaving.

The decrease in aqueous Fe^{2+} concentrations after initial increases may indicate the formation of insoluble reduced iron precipitates such as FeS, which would be favored under the reduced conditions of the microcosms (*32*). Other studies have noted reoxidation of Fe(II) during transformations of nitro-substituted aromatics (*13*, *37*). In some biometers, a reddish-brown precipitate was observed on the aqueous surface−headspace interface after 142 days and was presumed to be oxidized iron species. A decrease in the Fe^{2+} concentration relative to the previous sample time always corresponded to a pH increase to 7.7 or above (**Table 1**).

[^{14}C]Dimethenamid Recovery and Transformations. *Mass Balance*. Recovery of ^{14}C in all treatments ranged from 89 to 108%, which is typical for soil biometer studies (*13*, *24*, *38*−*41*). The average recovered radiocarbon (n = 27, expressed as % of applied) over the 142 day incubation for each treatment was 95 ± 2% in unamended, 96 ± 1% in the glucose-pretreated microcosms, 100 ± 1% in the nitrate + sulfate-amended, and 94 ± 1% in the autoclaved microcosms (**Tables 2**−**5**). Elevated levels of $H^{14}CO_3$ (7−9%) after only 6 h of incubation were mostly ascribed to the 6% radiolabel impurity.

Radiocarbon Distribution. The radiocarbon distribution over time in the mineralized, aqueous, sorbed, and bound fractions of the soil−water slurries was similar in each treatment (**Tables 2**−**5**). In general, radiocarbon was depleted from the aqueous phase before losses from the sorbed phase were evident. In the nonautoclaved treatments, ^{14}C bound residue represented over 50% of applied radiocarbon at the end of the incubation, and in the autoclaved treatment, 45% of applied radiocarbon was associated with this fraction. Neither radiolabeled parent nor metabolites were trapped as volatiles during the study. Only a

Table 2. Product Distribution and Material Balance for Microcosms Pretreated with Glucose for 8 days Prior to [^{14}C]dimethenamid Amendment

	% of applied radiocarbon						
time (DAT)	aqueous[a]	sorbed[a]	CO_2	HCO_3^{-}[b]	bound	total[c]	total recovered
0.25	46 ± 7[c]	42 ± 5	0 ± 0	7 ± 1	6 ± 0	94 ± 4	96 ± 1
0.5	39 ± 5	54 ± 5	0 ± 0	10 ± 1	3 ± 1	93 ± 9	(*n* = 27)
1	32 ± 2	49 ± 2	0 ± 0	8 ± 1	11 ± 4	91 ± 5	
2	30 ± 1	51 ± 1	0 ± 0	4 ± 0	19 ± 2	100 ± 1	
4	27 ± 1	51 ± 2	0 ± 0	5 ± 0	18 ± 2	96 ± 4	
8	20 ± 1	44 ± 3	0 ± 0	2 ± 0	35 ± 2	100 ± 6	
16	14 ± 2	36 ± 4	0 ± 0	3 ± 0	49 ± 8	95 ± 3	
32	9 ± 2	20 ± 3	0 ± 0	1 ± 0	61 ± 5	90 ± 9	
142	12 ± 0	30 ± 2	1 ± 0	1 ± 0	63 ± 3	106 ± 1	

[a] Includes ^{14}C-parent + ^{14}C-metabolites. [b] $H^{14}CO_3^{-}$ present at 0.25 DAT reflects a 6% radiolabel impurity. [c] Total = aqueous + sorbed + CO_2 + bound, since $H^{14}CO_3^{-}$ is included in the aqueous sample. Each value represents the mean of triplicate samples ± standard error.

Table 3. Product Distribution and Material Balance for Anaerobic Unamended Microcosms Containing [^{14}C]dimethenamid

	% of applied radiocarbon						
time (DAT)	aqueous[a]	sorbed[a]	CO_2	HCO_3^{-}[b]	bound	total[c]	total recovered
0.25	44 ± 5[c]	42 ± 6	0 ± 0	9 ± 1	8 ± 1	94 ± 8	95 ± 2
0.5	36 ± 6	47 ± 4	0 ± 0	7 ± 1	11 ± 1	95 ± 11	(*n* = 27)
1	33 ± 2	47 ± 0	0 ± 0	6 ± 1	14 ± 1	94 ± 3	
2	32 ± 1	53 ± 2	0 ± 0	5 ± 1	6 ± 3	91 ± 1	
4	29 ± 0	50 ± 8	0 ± 0	5 ± 0	20 ± 3	100 ± 11	
8	19 ± 3	49 ± 3	0 ± 0	3 ± 1	21 ± 4	89 ± 4	
16	17 ± 1	36 ± 2	0 ± 0	2 ± 0	36 ± 8	89 ± 8	
32	14 ± 0	31 ± 1	0 ± 0	2 ± 1	61 ± 0	106 ± 2	
142	11 ± 2	27 ± 7	1 ± 0	1 ± 0	57 ± 3	96 ± 12	

[a] Includes ^{14}C-parent + ^{14}C-metabolites. [b] $H^{14}CO_3^{-}$ present at 0.25 DAT reflects a 6% radiolabel impurity. [c] Total = aqueous + sorbed + CO_2 + bound, since $H^{14}CO_3^{-}$ is included in the aqueous sample. Each value represents the mean of triplicate samples ± standard error.

Table 4. Product Distribution and Material Balance for [^{14}C]dimethenamid-, NO_3^{-}-, and SO_4^{2-}-Amended Anaerobic Microcosms

	% of applied radiocarbon						
time (DAT)	aqueous[a]	sorbed[a]	CO_2	HCO_3^{-}[b]	bound	total[c]	total recovered
0.25	47 ± 2[c]	47 ± 4	0 ± 0	9 ± 1	6 ± 2	101 ± 2	100 ± 1
0.5	41 ± 1	53 ± 2	0 ± 0	8 ± 1	7 ± 4	101 ± 4	(*n* = 27)
1	40 ± 0	57 ± 1	0 ± 0	6 ± 1	4 ± 4	101 ± 4	
2	33 ± 0	57 ± 3	0 ± 0	5 ± 0	8 ± 2	98 ± 5	
4	29 ± 0	50 ± 2	0 ± 0	6 ± 1	19 ± 6	98 ± 8	
8	24 ± 2	45 ± 2	0 ± 0	2 ± 1	39 ± 17	108 ± 19	
16	17 ± 1	35 ± 1	0 ± 0	3 ± 0	46 ± 5	99 ± 5	
32	11 ± 0	26 ± 1	0 ± 0	3 ± 0	55 ± 7	92 ± 6	
142	12 ± 1	26 ± 4	1 ± 0	1 ± 0	63 ± 6	101 ± 3	

[a] Includes ^{14}C-parent + ^{14}C-metabolites. [b] $H^{14}CO_3^{-}$ present at 0.25 DAT reflects a 6% radiolabel impurity. [c] Total = aqueous + sorbed + CO_2 + bound, since $H^{14}CO_3^{-}$ is included in the aqueous sample. Each value represents the mean of triplicate samples ± standard error.

trivial amount of $^{14}CO_2$ was trapped in KOH (**Tables 2**−**5**). Dimethenamid was labeled at the 3-thienyl carbon; thus, mineralization of this particular carbon was inconsequential in anaerobic dissipation. The *Herbicide Handbook* (*1*) states that oxalamide formation is the main result of soil metabolism with no distinction of aerobic or anaerobic conditions, and there is no mention of mineralization in this or the only other report of dimethenamid degradation (*2*). In general, degradation of chloroacetamide and chloroacetanilide herbicides results in the formation of metabolites rather than mineralization (*14*, *15*); thus, metabolite formation is a primary concern (*4*, *17*, *42*, *43*).

Table 5. Product Distribution and Material Balance for Autoclaved [^{14}C]dimethenamid-Amended Anaerobic Microcosms

	% of applied radiocarbon						
time (DAT)	aqueous[a]	sorbed[a]	CO_2	HCO_3^{-}[b]	bound	total[c]	total recovered
0.25	37 ± 1[c]	49 ± 4	0 ± 0	9 ± 1	5 ± 2	92 ± 2	94 ± 1
0.5	36 ± 1	51 ± 1	0 ± 0	10 ± 1	9 ± 1	96 ± 1	(*n* = 27)
1	33 ± 0	50 ± 3	0 ± 0	11 ± 0	11 ± 4	94 ± 4	
2	30 ± 1	55 ± 4	0 ± 0	8 ± 1	11 ± 6	96 ± 4	
4	27 ± 1	53 ± 3	0 ± 0	7 ± 1	11 ± 2	91 ± 4	
8	29 ± 1	53 ± 3	0 ± 0	4 ± 1	9 ± 2	90 ± 2	
16	29 ± 1	46 ± 3	0 ± 0	4 ± 1	18 ± 0	93 ± 4	
32	21 ± 0	47 ± 2	0 ± 0	6 ± 1	25 ± 5	94 ± 3	
142	19 ± 2	34 ± 3	0 ± 0	2 ± 1	45 ± 3	99 ± 1	

[a] Includes ^{14}C-parent + ^{14}C-metabolites. [b] $H^{14}CO_3^{-}$ present at 0.25 DAT reflects a 6% radiolabel impurity. [c] Total = aqueous + sorbed + CO_2 + bound, since $H^{14}CO_3^{-}$ is included in the aqueous sample. Each value represents the mean of triplicate samples ± standard error.

^{14}C-Dimethenamid. Initially, dimethenamid was distributed almost equally between aqueous and sorbed fractions (**Figure 3a**−**d**). Dimethenamid was depleted to less than 20% of applied within 32 days. Degradation of dimethenamid occurred under all anaerobic conditions (**Figure 3a**−**d**), with similar rates in nonautoclaved treatments, although the types and duration of anaerobic microbial processes varied (**Tables 2**−**5**). The degradation of dimethenamid to <5% of applied by day 142 occurred in all treatments except the autoclaved. Aqueous phase herbicide was depleted to <5% with metabolite formation before a loss of herbicide from the sorbed fraction was observed (**Figures 3** and **4**). ^{14}C-metabolites together with bound residue represented the majority of applied radiocarbon after 32 days in the nonautoclaved treatments; however, over 50% of applied dimethenamid remained in the autoclaved treatment at this time (**Tables 2**−**5** and **Figures 3** and **4**). Biological activity enhanced bound residue formation. The nitrate data demonstrated that the sterility of the autoclaved microcosms had been compromised by day 142; however, on the basis of the herbicide data, it was concluded that substantial microbial regrowth did not occur until after day 32. Thus, the autoclaved treatment provides a reasonable comparison of biological vs abiotic degradation through day 32.

Depletion of dimethenamid from the sorbed (extractable) phase was slower than its depletion from the aqueous phase (**Figure 3a**−**d**) and probably reflects a bioavailability limitation. The deviation of predicted first-order half-lives from the length of time observed for 50% parent depletion (DT_{50}) is probably due to this biphasic decay (*41*, *44*). The apparent Kd (% sorbed dimethenamid/%aqueous dimethenamid) further illustrates the nonequilibrium between sorbed and solution phase dimethenamid (**Figure 5a**−**d**).

^{14}C-Metabolite Formation. As dimethenamid was depleted, metabolites accumulated (**Figure 4a**−**d**), representing over 20% of applied ^{14}C at study termination. A common metabolite was recovered in aqueous and organic extracts, between days 8 and 142 under all treatment conditions (**Figure 4a**−**d**). This metabolite was designated by its TLC mobility as hR_{DI} 94−90 (hR_{DI} = hR_f of metabolite × 100/hR_f of dimethenamid). The accumulation of a second metabolite with TLC characteristics of hR_{DI} 60−57 was observed in all nonautoclaved treatments through day 32. By 142 days, the second metabolite was not detected and a third metabolite, hR_{DI} 53−50, was evident. Two minor metabolites (<1% of applied), hR_{DI} 86 and hR_{DI} 75, were also detected in some of the nonautoclaved treatments. Me-

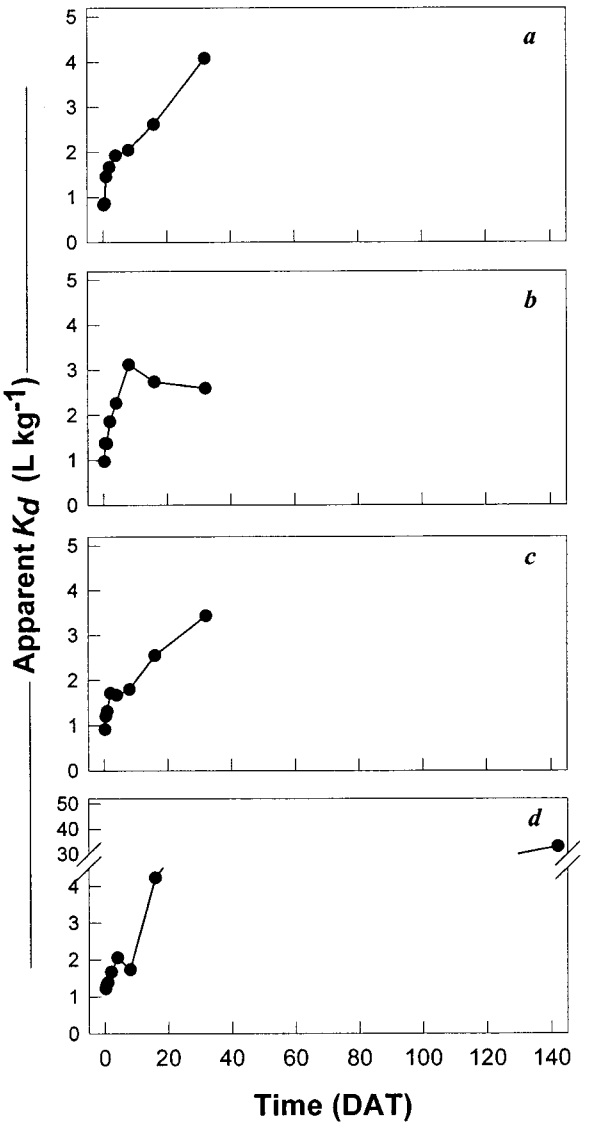

Figure 5. Apparent Kd (sorbed dimethenamid/solution dimethenamid) over time. Data are plotted only for days in which solution phase dimethenamid was still detected.

tabolite hR_{DI} 94−90 was the only metabolite detected in the autoclaved treatment, and it did not appear until day 32. In the autoclaved microcosms, degradation of dimethenamid and metabolite formation were delayed until day 32, which further suggests that microbial regrowth had not occurred at this time. Slower degradation of dimethenamid and the lack of metabolite formation prior to regrowth of the system indicate that abiotic processes were secondary to biologically mediated processes of dimethenamid transformation.

The HPLC retention times of the two major metabolites at study termination, hR_{DI} 94−90 and hR_{DI} 53−50, were 7.7 and 4.8 min, respectively. Neither metabolite coeluted with metabolite standards dimethenamid oxalamide or dimethenamid sulfonate. These results are in agreement with Novak et al. (*15*), who found that the anaerobic soil metabolism of alachlor under denitrifying, sulfate-reducing, and methanogenic conditions resulted in partial breakdown products other than the OXA and sulfonic acid metabolites. The oxanilic and sulfonic acid metabolites of chloroacetamide herbicides are of particular concern because their concentrations in groundwaters, surface waters, and soils are usually much greater than those of the parent herbicides (*4*, *42*, *43*). They are readily formed as a result of glutathione conjugation, which is a common mechanism of detoxification in microorganisms, plants, and animals. As yet, formation of these metabolites has been observed under aerobic aquatic conditions (*42*, *43*) but not under anaerobic conditions.

Bound Residue Formation. Bound (unextractable) residues accounted for the majority of applied ^{14}C at the end of the study (**Tables 2−5**). The nonautoclaved microcosms demonstrated similar trends in bound residue formation, whereas formation in the autoclaved microcosms was substantially slower (**Tables 2−5**). As with dimethenamid depletion and metabolite formation, substantial bound residue formation did not occur in these microcosms until after day 32. The higher percentage of bound residue formation in the nonautoclaved microcosms as compared with the autoclaved microcosms suggests that anaerobic microbial activities contributed to the formation of bound residues. Although mechanisms of anaerobic bound residue formation have not been described, it is likely that anaerobic reductive processes contribute to the integration of herbicide byproducts into soil organic matter. Herbicides and their transformation products resemble reactants in soil organic matter formation, and biological activities may catalyze this process (*45*, *46*). The lack of available technology for the determination of chemical species retained on soil has hindered the characterization of soil bound herbicide residues (*47*, *48*).

The preparation of the microcosms (aerobic with glucose pretreatment, anaerobic, and anaerobic with nitrate or sulfate amendment) influenced the type of microbial metabolism but not the net loss of dimethenamid. In particular, elevated levels of methane observed in the glucose-pretreated as compared with the unamended and nitrate + sulfate-amended suggest that the anaerobic aquatic metabolism protocol selects for methanogens. Sulfate amendment resulted in a prolonged period of sulfidogenesis and formation of a metabolite that was not observed in other treatments.

Glucose pretreatment is expected to result in the degradation of glucose with organic acid formation and CO_2/HCO_3^-, all of which may be substrates for methanogenesis once the microcosms are anaerobic. It is important to determine herbicide persistence under methanogenic conditions; however, using a methanogenic environment exclusively to represent all anaerobic conditions neglects other important redox processes, such as iron reduction, that occur prior to methanogenesis following soil flooding. In some cases, as with the dinitroaniline herbicide trifluralin, iron-reducing conditions are sufficient to invoke dramatic changes in herbicide fate (*13*). Most land in crop production undergoes temporarily flooded conditions and therefore may not become methanogenic prior to drying, whereas iron reduction would begin almost immediately upon flooding. With this in mind, the methanogenic environment created by the EPA protocol does not allow the natural succession of redox processes beginning from more oxidized to more reduced to occur; rather, it targets degradation that occurs only under the most reducing condition. The EPA protocol is intended to be a conservative estimate of degradation in natural environments; thus, it makes sense to include microcosm conditions in which terminal electron acceptors are limiting and glucose consumption byproducts are not available. Of the treatments described here, the unamended protocol would serve this purpose. Describing the nature of the anaerobic environment achieved by the microcosms provides a context in which dissipation is observed. Knowing the conditions under which degradation and metabolite formation occur is necessary since many anaerobic processes are possible and have the potential to result in different herbicide transformations. The

current EPA protocol does not include analysis of anaerobic microbial processes; however, understanding herbicide behavior and metabolite formation under various microbial redox processes should make results obtained more descriptive and meaningful by placing them in an environmental context, thereby improving predictions of herbicide fate.

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