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Biodegradation and Biotransformation of Dicamba under Different Reducing Conditions

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We examined the anaerobic biodegradability and transformation of dicamba under denitrifying, iron Fe(III) reducing, sulfate reducing, and methanogenic conditions. Anaerobic microcosms were established with dicamba treated agricultural soil and golf course drainage stream sediments, which were each spiked with dicamba as the sole carbon source. Transformation of dicamba to 3,6-dichlorosalicylate via demethylation of the methoxy substituent (O-demethylation) occurred in cultures under methanogenic conditions in cultures from both sites. In methanogenic enrichments from golf course sediments, the O-demethylated product (3,6-dichlorosalicylate) was reductively dechlorinated to 6-chlorosalicylate and subsequently to salicylate, which was ultimately mineralized to CH_4 and CO_2. Sulfate reducing cultures from golf course drainage stream sediments O-demethylated dicamba and partially dechlorinated 3,6-dichlorosalicylate to 6-chlorosalicylate (<10%). Neither denitrifying nor iron reducing conditions promoted the biotransformation of dicamba. This study shows the effect of different electron acceptors on the transformation of dicamba in anaerobic environments and demonstrates the degradation pathway under methanogenic conditions. The work suggests that the electron acceptors present will influence in situ degradation of herbicides in anoxic soils and sediments.

Introduction

Dicamba is primarily used as a post-emergence broadleaf herbicide, which interferes with normal plant auxin function, subsequently causing uncontrolled growth and the inhibition of the phototropic and geotropic function. Cumulative response results in plant death. The success of auxinic analogues such as dicamba and 2,4-dichlorophenoxyacetic acid in weed control has led to widespread manufacturing and use. Estimated U.S. production for dicamba was 11 million lbs in 1990 (*1*).

The possibility for transport of dicamba in soils, resulting in subsequent groundwater pollution, is potentially high. Both dicamba and its initial transformation product 3,6-dichlorosalicylate have pK_a values of 1.95 (*2, 3*). The high solubility of these weak acids at neutral to high pH makes them feasibly mobile in lime treated or neutral pH soils. In the field, dicamba has been found to leach to a depth of 1 m over a 2-month period following application in a Missouri clay pan soil (*4*). In addition, dicamba was discovered in approximately 2% of pesticide monitoring wells tested in Iowa (*5*) and was one of six pesticides found in the shallow aquifers on the Delmarva Peninsula in Maryland (*6*). Furthermore, dicamba was detected in 21% of groundwater samples taken during a field study on pesticide leaching from historically sprayed agricultural plots (*7*). Dicamba was also found in over 4% of 45 wells tested in 1992 by the U.S. Geological Survey (*8*).

The occurrence of dicamba in groundwater at sites of herbicide application and drainage is an impetus for studying the fate of this herbicide in anoxic environments. Anaerobic microbial respiration in aquatic sediments and aquifers can take place via a variety electron acceptors (*9*−*12*), and microbial degradation of herbicides and substituted aromatic compounds (herbicide metabolites) can be influenced by the type of electron acceptors present (*13*−*17*). In general, the electron acceptors are utilized in order of their relative energy potential following the sequence O_2, NO_3^-, Mn(IV), Fe(III), SO_4^-, HCO_3^-, and the redox zones in anoxic aquifers and sediments can become stratified (*18*).

Biodegradation of dicamba in the presence of oxygen, through the O-demethylated product 3,6-dichlorosalicylate, is well documented (*19*−*23*). The metabolite 2,5-dihydroxy-3,6-dichlorosalicylate has been reported as an intermediate after O-demethylation of dicamba (*24*), but the pathway for degradation of 3,6-dichlorosalicylate has not been investigated in detail. Under anaerobic, methanogenic conditions, transformation of dicamba through O-demethylation and subsequent reductive dehalogenation of 3,6-dichlorosalicylate to 6-dichlorosalicylate has been observed (*25*). The metabolite 6-chlorosalicylate was resistant to further degradation and anaerobic mineralization has not been demonstrated. Furthermore, the biodegradability of dicamba under different anaerobic conditions has not been investigated. This study examines the effect of different electron acceptors on the anaerobic transformation and degradation of dicamba and demonstrates the mineralization of dicamba to CO_2 and CH_4.

Materials and Methods

Microcosm Culture Experiments. Denitrifying, iron reducing, sulfate reducing, and methanogenic microcosms were established under strictly anaerobic conditions (*17*), using as inoculum sediment from the Rutgers golf course drainage stream (characterized as a sandy loam with an organic content of 6.65%) in Piscataway, NJ and a dicamba treated agricultural soil (sandy loam with an organic content of 1.15%) from Wyoming (kindly provided by Dr. P. Colberg). In addition, methanogenic and denitrifying cultures were set up using inoculum from golf course sediments, tee cores, and a cornfield soil from the Rutgers University campus. Sediment slurries (between 10% and 20% vol/vol) were prepared in minimal salts medium consisting of KCl (1.3 g/L), KH_2PO_4 (0.2 g/L), NaCl (1.17 g/L), NH_4Cl (0.5 g/L), $CaCL_2$·2H2O (0.10 g/L), $MnCl_2$·6H2O, and $NaHCO_3$ (2.8 g/L) (*26*) and amended with trace salts and vitamins (*27*). The medium was deoxygenated by boiling for 15 min and cooled under argon or N_2:CO_2 (70:30). The medium was then either left methanogenic ($NaHCO_3$ 30 mM) or amended with either KNO_3 (20 mM), amorphous ferric oxyhydroxide (Fe(III) 200 mM), or Na_2SO_4 (20 mM), as an electron acceptor and the

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sediment inoculum added. Relatively high concentrations of the electron acceptors were used to ensure the development of methanogenic, denitrifying, Fe(III)-reducing or sulfidogenic conditions, respectively. Fifty milliliter aliquots were divided under argon (denitrifying) or N_2:CO_2 (70:30; methanogenic, sulfidogenic, iron-reducing) into 60 mL bottles and sealed with thick rubber stoppers and aluminum crimps. Triplicate cultures were then spiked with dicamba (100 μM) to study the anaerobic biotransformation and biodegradation of this herbicide under the four reducing conditions. Sterile controls were established by autoclaving cultures for 1 h on three consecutive days before spiking with substrate. Samples were taken for chemical analysis following strict anaerobic techniques. At each sampling point the cultures were rigorously shaken and sampled with sterile syringes flushed with either argon or N_2:CO_2 (70:30). Cultures were incubated in the dark at 20 °C.

^{14}C Mineralization Studies. Methanogenic microcosms were established using 50% (vol/vol) Rutgers golf course sediment as described previously. A 25 mL slurry was incubated at 30 °C in a 37.5-mL sealed bottle with a 12.5 mL headspace of N_2:CO_2 (70:30). Triplicate cultures were each fed 0.348 μCi of uniformly ^{14}C ring-labeled dicamba, and the headspace was monitored over the 175-day experimental period for ^{14}C in either the CO_2 or CH_4 fraction by gas chromatography and radioactive gas analysis. After $^{14}CO_2$ and $^{14}CH_4$ generation dissipated, cultures were acidified with 0.5 mL 12 N HCl to drive off $H^{14}CO_3$ remaining in solution, which was then trapped as $^{14}CO_2$ in Oxisol scintillation cocktail for analysis on a liquid scintillation counter. Residual organic ^{14}C in the sediment was analyzed using a 0.5 mL sample of the slurry in 10 mL of Redisafe scintillation cocktail. Sediment quench was determined by standard addition of 40 000 dpm ^{14}C labeled toluene. $^{14}CO_2$ and $^{14}CH_4$ were quantified using $^{14}CO_2$ external standards.

Chemicals. All aromatic compounds were > 98% pure. Analytical grade dicamba and 3,6-dichlorosalicylate were a gift from Sandoz Agro. Inc. (Des Plaines, IL). ^{14}C-ring-labeled dicamba was a gift from Dr. David Purdy courtesy of Sandoz Agro Inc. Salicylate and 3-, 4-, and 5-chlorosalicylate were obtained from Aldrich Chemical Co. (Milwaukee, WI). 6-Chlorosalicylate was synthesized by Berry & Associates, Inc. (Ann Arbor, MI). Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was from Sigma (St. Louis, MO). Radioactive standards ($^{14}C\ HCO_3^-$ and ^{14}C toluene) were a gift from Dr. Lily Young, Rutgers University (New Brunswick, NJ).

Analysis. Liquid samples for chemical analysis were filtered through 0.45 μm pore size filters (Millipore, Bedford, MA). Aromatic compounds were analyzed by HPLC using a Shimadzu LC-10AS HPLC, SCL-10A System Controller, SPD-10A UV−vis Detector, and SIL-10A Autoinjector (Shimadzu Scientific Instruments, Columbia, MD), equipped with a Spherisorb ODS 2 column (250 by 4.6 mm, particle size 5 μm, Phenomonex, Torrance, CA). UV detection was at 280 nm. Chromatograms were recorded with a Spectra-Physics Chrome Jet integrator (Spectra-Physics, San Jose, CA) and quantified using external standards. The mobile phase for the separation of dicamba, 3,6-dichlorosalicylate, and salicylate consisted of 75% methanol, 23% distilled water, and 2% acetic acid at a flow rate of 1 mL/min. Separation of dicamba, all of the chlorosalicylate isomers, and salicylate required a solvent gradient consisting of 10% methanol and 2% acetic acid held constant over a 24 min gradient of distilled water (decreasing from 64.8 to 51.8%) and acetonitrile (increasing from 23.2 to 36.2%) at a flow rate of 1 mL/min. Retention times (minutes) were as follows: dicamba (18.3), 3,6-dichlorosalicylate (19.4), 3-chlorosalicylate (20.5), 4-chlorosalicylate (26.9), 5-chlorosalicylate (23.5), 6-chlorosalicylate (9.5), and salicylate (12.2).

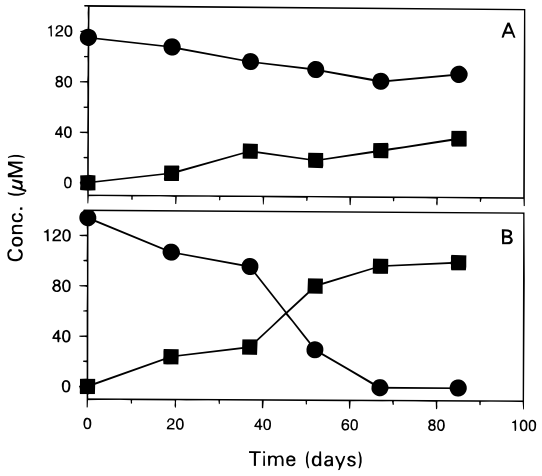

FIGURE 1. O-Demethylation of dicamba (●) to 3,6-dichlorosalicylate (■) in anaerobic microcosms of cornfield (10% inoculum) soil in the presence (A) or absence (B) of nitrate. Values represent the means of triplicate cultures (standard deviation ± 10 μM).

Nitrate and sulfate were measured using a DX-100 ion chromatograph (Dionex, Sunnyvale, CA.), equipped with a IonPac Asg-Sc (Dionex) column using a carbonate buffer (1 mM Na_2CO_3, 0.75 mM $NaHCO_3$) mobile phase and a sulfuric acid (25 mM) column regenerant. All compound concentrations were quantified with external standards using a Spectra-Physics Chrome Jet integrator.

Gas chromatography−mass spectrometry was done on a Hewlett-Packard 5890 GC equipped with a DB-5MS capillary column (J&W Scientific, Folsom, CA) and a HP 5971 Mass Selective Detector. Helium was used as the carrier gas at a column head pressure of 50 kPa. Injector temperature was 280 °C. Column temperature was held constant at 60 °C for 1 min and then increased at a rate of 14.67 °C/min to 280 °C. Samples (0.5 mL) were salted and acidified (pH 2) with saturated NaCl solution and 1 N HCl. Samples were then extracted into pentane (0.5 mL) twice, and the combined extract evaporated under N_2. Dried samples were derivatized using 100 μL of bis(trimethylsilyl)trifluoroacetamide (BSTFA), (Sigma-Aldrich, St. Louis, MO), then analyzed by GC-mass spectrometry, and compared to silylated standards.

Methane and carbon dioxide gas chromatography as well as ^{14}C gas analysis was conducted on a Varian 3300 GC, equipped with a Porapak-Q 100/120 column (Supelco, Supelco Park, Bellefonte, PA) and a TCD detector (Varian Instruments, Sugar Land Texas) in series with a Raytest RAGA (radioactive gas analyzer) (Raytest USA, Inc., New Castle, DE). Helium was used as carrier gas for the system and argon: methane (90:10) as detector gas for the RAGA (Matheson gas products, Bridgeport, NJ). Liquid ^{14}C analysis was done on a Beckman LS 5000TD liquid scintillation counter (Beckman Instruments, Nuclear Systems Operation, Fullerton, CA).

Results and Discussion

Effect of Nitrate on Anaerobic O-Demethylation of Dicamba. Anaerobic microcosms using inoculum (10%) from three different sites, a cornfield soil, a golf course soil, and a golf course drainage sediment, were established in the presence or absence of 30 mM nitrate. Figure 1 shows the effect of nitrate on O-demethylation of dicamba by anaerobic microcosms from the cornfield site. 3,6-Dichlorosalicylate was identified as the product by comparison of retention times in HPLC to external standards. Methanogenic cultures (in the absence of nitrate) completely O-demethylated dicamba to 3,6-dichlorosalicylate within 80 days, while cultures containing nitrate only transformed 20% of dicamba. O-Demethylation was inhibited in the presence of nitrate in

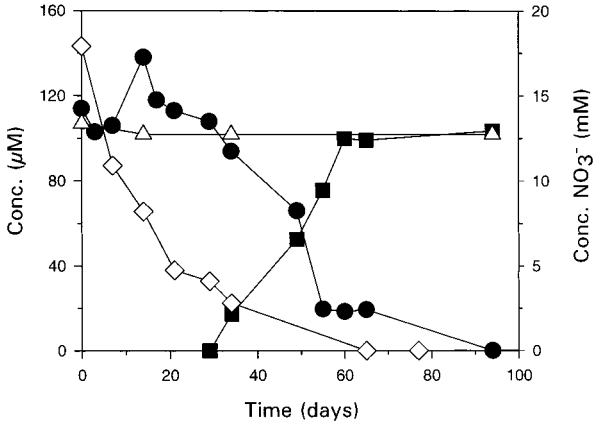

FIGURE 2. Anaerobic transformation of dicamba by drainage stream sediment cultures in the presence of 20 mM nitrate. Nitrate inhibited O-demethylation until reduced to a concentration below 4 mM. Symbols: (●) dicamba, (■) 3,6-dichlorosalicylate, (◇) nitrate, (△) dicamba sterile control. Values represent the means of triplicate cultures (standard deviation was equal to or less than ±15 μM for organics and ±3.3 mM for nitrate).

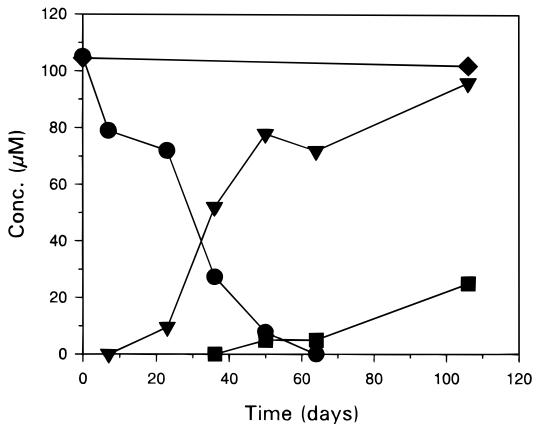

FIGURE 3. Anaerobic biotransformation of dicamba in the presence of 20 mM sulfate in golf course sediment microcosms. Symbols: (●) dicamba, (▼) 3,6-dichlorosalicylate, (■) 6-chlorosalicylate, (◆) dicamba sterile control. Values represent the means of triplicate cultures (standard deviation ±5 μM). The concentration of sulfate was 15.23 ± 1.15 mM on day 108.

enrichments from all three sites tested (data not shown), and O-demethylation occurred to a greater extent in enrichments without nitrate over the 80 day course of the experiment. No loss of dicamba occurred in sterile controls.

The transformation of dicamba under denitrifying conditions was examined more closely with microcosms from the golf course drainage stream. Figure 2 depicts the time course for dicamba transformation in the presence of 20 mM initial nitrate. O-Demethylation was inhibited in the denitrifying cultures until nitrate concentrations were reduced to below 4 mM. O-Demethylation of dicamba was first observed on day 34 (25 μM 3,6-dichlorosalicylate in two of three replicates, with corresponding nitrate levels of 0.0 and 1.8 mM). O-Demethylation had not occurred in the third replicate, which contained 7.2 mM nitrate. On day 60, no nitrate remained and dicamba had been completely O-demethylated to 3,6-dichlorosalicylate in all three cultures. O-Demethylation in enrichments without nitrate was stoichiometrically complete by day 14, indicating that nitrate inhibited the anaerobic O-demethylation of dicamba.

O-Demethylation of nonchlorinated methoxyaromatics has previously been demonstrated under denitrifying conditions (*28*), though nitrate in the medium increased initial lag times compared to methanogenic and sulfidogenic enrichments. In contrast, O-demethylation of vanillate and *m*-anisate was shown to be nitrate dependent in the facultative denitrifying bacterium, strain PN-1 (*29*). Other examples of nitrate reduction coupled to O-demethylation include the organism *Moorella thermoacetica* which uses nitrate as a preferred electron acceptor during growth on vanillate (*30*). Our experiments showed that the presence of 30 mM nitrate did not inhibit O-demethylation of vanillate by the acetogen *Eubacterium limosum* (unpublished results). In addition, the degradation of nonchlorinated *o*-anisate took place within 10 days in cultures containing 30 mM nitrate from the Rutgers golf course sediment cultures (data not shown). O-Demethylation of anisate to salicylate was observed, though end point nitrate concentrations were not measured. This suggests that nitrate mediated inhibition of O-demethylation may be specific to chlorinated anisic acids such as dicamba. Recently, Pacel et al. reported that the presence of nitrate (ranging from 0.47 to 2.3 mM) inhibited anaerobic transformation of dicamba in soil microcosms (*31*).

Anaerobic Transformation of Dicamba under Four Reducing Conditions. The preceding data suggested that the presence of alternative electron acceptors to carbonate may influence the anaerobic biodegradation of dicamba. Anaerobic enrichment cultures were established from a golf course drainage stream sediment and dicamba treated agricultural soil under four conditions to further evaluate the influence of different electron acceptors on the biotransformation and degradation of this herbicide. O-Demethylation of dicamba to 3,6-dichlorosalicylate occurred in cultures from both sites under methanogenic conditions (data not shown). In drainage stream sediment cultures, O-demethylation was complete within 40 days, while the methanogenic cultures from the agricultural soil O-demethylated approximately 40% of dicamba within 80 days. O-Demethylation also occurred in sulfate reducing cultures from the golf course sediment within 50 days (Figure 3). Denitrifying enrichments from both sites, maintained at 20 mM NO_3^-, did not transform dicamba over the 160 day incubation period. O-Demethylation occurred in iron reducing cultures from the golf course drainage stream, but methane was produced in the cultures indicating that iron reduction was no longer the primary microbial respiratory process. Sterile controls under each condition showed no substrate loss or transformation of dicamba over time.

After O-demethylation had occurred, the methanogenic golf course drainage stream cultures dehalogenated 3,6-dichlorosalicylate to 6-chlorosalicylate within 110 days. Under sulfate reducing conditions approximately 25% of 3,6-dichlorosalicylate formed from dicamba was dehalogenated to 6-chlorosalicylate by day 106 (Figure 3). The remaining sulfate concentration was 15 ± 1.5 mM on day 106. O-Demethylation of dicamba under sulfate reducing conditions has also been observed with the Wyoming agricultural soil (P. J. S. Colberg, personal communication). The reductive dehalogenation of substituted aromatic compounds has been shown to be thermodynamically feasible under both methanogenic and sulfidogenic conditions (*32, 33*). While inhibition of dehalogenation of monoaromatics in the presence of sulfate is well documented (*13, 14, 34*), dehalogenation in our cultures of 3,6-dichlorosalicylate to 6-chlorosalicylate is consistent with recent observations of chlorophenol dehalogenation and degradation in sulfidogenic systems (*35, 36*–*38*). No dehalogenation of 6-chlorosalicylate was observed under sulfate reducing conditions in our cultures.

O-Demethylation and Complete Dehalogenation of Dicamba under Methanogenic Conditions. The extent of dicamba O-demethylation and dehlaogenation was examined in more detail with methanogenic drainage stream sediment cultures. The time course for the biotransformation of

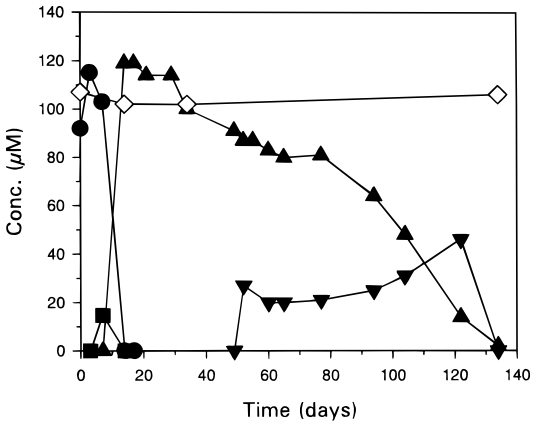

FIGURE 4. O-Demethylation and dechlorination of dicamba under methanogenic conditions. In methanogenic golf course sediment microcosms (20% inoculum), dicamba (●) was O-demethylated to 3,6-dichlorosalicylate (■), 6-chlorosalicylate (▲), and salicylate (▼), which was depleted by day 135. There was no loss in the dicamba sterile control (◇). Values represent the means of triplicate cultures (standard deviation ±10 μM).

TABLE 1. Methanogenic Mineralization of ^{14}C Ring Labeled Dicamba[a]

	% of recovered ^{14}C		
culture #	$^{14}CO_2$	$^{14}CH_4$	^{14}C in sediment
1	52	39	9.0
2	50	36	14
3	80	16	3.8
av	61 ± 17	30 ± 12	8.9 ± 5.1

[a] Activity recovered 100.4 ± 36.6% of 0.348 μCi added. Total recovery for each replicate was 0.412, 0.444, and 0.206 μCi. $^{14}CO_2$ and $^{14}CH_4$ are total amounts recovered over a 175 day period. Residual ^{14}C in sediment was measured on day 175 after CO_2 was purged from the slurry.

dicamba is shown in Figure 4. Dicamba was rapidly O-demethylated to 3,6-dichlorosalicylate, which was then reductively dechlorinated to 6-chlorosalicylate within 14 days, in stoichiometric concentrations to the dicamba spike. 6-Chlorosalicylate was subsequently dehalogenated over the next 120 days with transient accumulation of salicylate which was eventually depleted. No other metabolites were detected by HPLC. All metabolites were identified by comparison of their retention times in HPLC and GC and their mass spectra to authentic compounds. The mass spectra of silylated metabolites compared to silylated standards are shown in Figure 5, confirming O-demethylation of dicamba and reductive dehalogenation of 3,6-dichlorosalicylate and 6-chlorosalicylate, leading to salicylate.

The dehalogenation of 3,6-dichlorosalicylate and recalcitrance of 6-chlorosalicylate under methanogenic conditions is consistent with a previous report (*25*). The present study shows that 6-chlorosalicylate can be further dehalogenated under methanogenic conditions to salicylate. The second dehalogenation was clearly the rate limiting step and dependent on the inoculum and length of incubation. More extensive dehalogenation was generally observed with high inoculum density, possibly due to available electron donors (evidenced by elevated methanogenesis) provided by the increased organic carbon content.

The initial dehalogenation of dicamba at the meta position to the carboxy group and ortho to the hydroxy group of 3,6-dichlorosalicylate and the recalcitrance of 6-chlorosalicylate is similar to the observed specificity for dehalogenation of chlorobenzoates and chlorophenols under methanogenic conditions (for reviews, see refs *39*, and *40*). Initial dehalogenation ortho to the hydroxyl group of chlorophenols seems to be the dominant pathway under methanogenic conditions (*17*, *35*, *41*−*43*), although the microbial community clearly influences activity, and initial meta and para dechlorination has in some cases been reported (*43*−*45*). Conversely, dehalogenation of chlorobenzoates generally proceeds in the order meta > ortho > para (*17*, *46*−*48*). Although the sequence of dechlorination of 3,6-dichlorosalicylate is consistent with these observations, the enzymes for dehalogenation may be different for both compound classes and may also be affected by both a carboxyl and hydroxyl group on the aromatic ring.

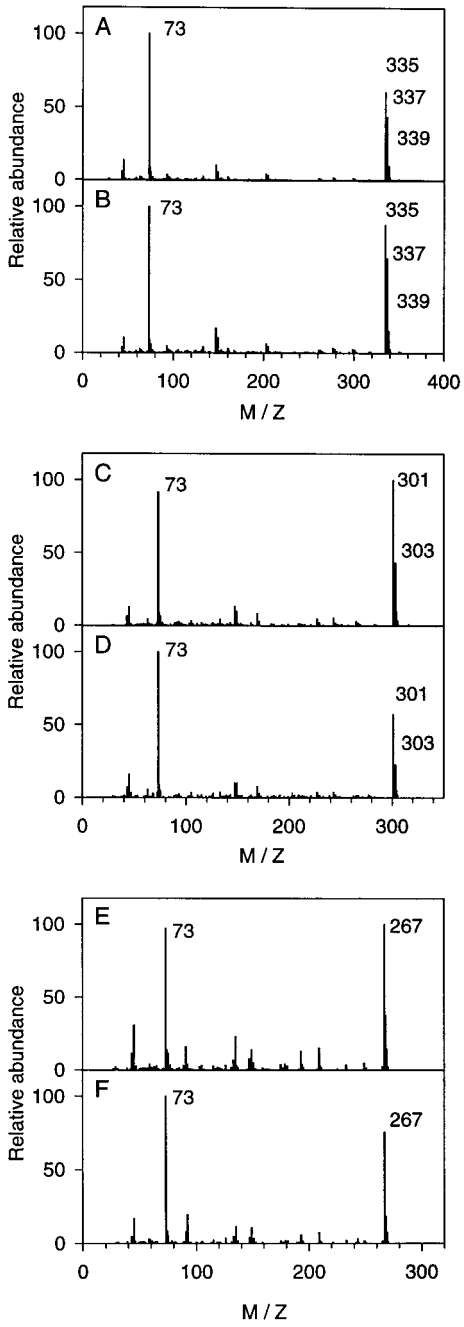

FIGURE 5. Mass spectra of silylated standards and corresponding metabolites produced from dicamba during methanogenic degradation: (A) 3,6-dichlorosalicylate standard, (B) first metabolite, (C) 6-chlorosalicylate standard, (D) second metabolite, (E) salicylate standard, and (F) third metabolite.

Methanogenic Mineralization of Dicamba. To determine whether dicamba was mineralized under methanogenic conditions, anaerobic enrichments were established with golf

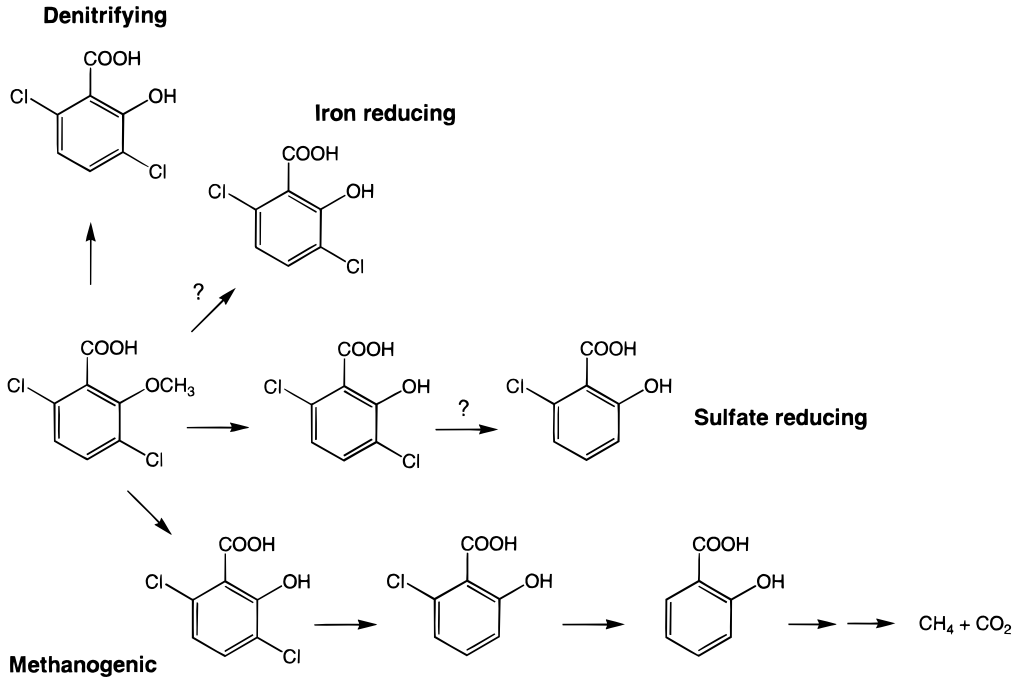

FIGURE 6. Transformation and degradation pathways of dicamba under different reducing conditions.

course sediment inoculum (50% vol/vol) in methanogenic medium. The recovery of ^{14}C, that was added as ring labeled dicamba, was 100 ± 36% of the total spiked (0.348 μCi). The variation in recovery was assumed to be the result of poor solubility of ^{14}C-dicamba prior to feeding which resulted in variation of the amount added to cultures. The proportion of recovery was 61% $^{14}CO_2$, 30% $^{14}CH_4$, and 9% of residual nonvolatile organics (Table 1). Recovery of ^{14}C labeled methane from the headspace of dicamba degrading cultures is evidence that methanogenesis is the terminal electron accepting process after ring-cleavage of dicamba. In two out of three replicates the proportion of $^{14}CH_4$ recovered was 36% and 39%, while $^{14}CO_2$ recovered was 50% and 52%, respectively. These CH_4 and CO_2 ratios are in accordance with the stoichiometric balanced equation for methanogenic mineralization of dicamba: $C_8H_6Cl_2 + 5.5H_2O \rightarrow 4.25CO_2 + 3.75CH_4 + 2HCl$. Recovery in the third replicate was 80% $^{14}CO_2$ and with only 16% $^{14}CH_4$. Total methane production was lower in the third replicate by 5% as well. These results demonstrate that the anaerobic O-demethylation and reductive dechlorination of dicamba ultimately results in mineralization to CH_4 and CO_2 under methanogenic conditions.

Conclusions. This study revealed that the predominant electron accepting process can effect the rate and extent of dicamba degradation in anaerobic environments. The degradation activity depended on the anaerobic condition and ranged between complete inhibition of biotransformation and mineralization of the herbicide. The degradation and transformation pathways that were observed under different reducing conditions are summarized in Figure 6. Mineralization of dicamba was demonstrated under methanogenic conditions and the degradation pathway elucidated. Methanogenic enrichments O-demethylated dicamba to 3,6-dichlorosalicylate that was reductively dechlorinated to 6-chlorosalicylate and to salicylate, which was further degraded to CH_4 and CO_2. Transformation of dicamba under sulfate reducing conditions does occur, but the extent of dehalogenation after O-demethylation remains unclear. Anaerobic O-demethylation was a prerequisite in all our cultures before dicamba could be degraded, and reductive dehalogenation of the dichloroanisic acid prior to O-demethylation was not observed.

The finding that nitrate can inhibit the anaerobic transformation of dicamba may have environmental implications, especially in agricultural areas where dicamba is used extensively and where nitrogen from nitrate often exceeds the EPA maximum contaminant level of 10 mg/L in the groundwater. This suggests that applications of dicamba where nitrate levels in groundwater are high may risk prolonging the anaerobic half-life of the herbicide in the aquifer. The observed accumulation of 6-chlorosalicylate may indicate that assessment of the toxicity and the recalcitrance of this chlorinated aromatic compound in anaerobic environments may be of more relevance than that of dicamba. These data provide clear evidence that anaerobic respiratory conditions must be taken into consideration when performing degradation feasibility studies and determining herbicide application practices in the future.

Acknowledgments

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