Environ. Sci. Technol. **1998**, *32,* 2283−2287

Defluorination of Organofluorine Sulfur Compounds by *Pseudomonas* Sp. Strain D2

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Little is known of the potential for biodegradation of fluorinated sulfonates. To evaluate this potential, the following model compounds were selected: difluoromethane sulfonate (DFMS), trifluoromethane sulfonate (TFMS), 2,2,2-trifluoroethane sulfonate (TES), perfluorooctane sulfonate (PFOS), and 1H,1H,2H,2H-perfluorooctane sulfonate (H-PFOS). A laboratory isolate designated *Pseudomonas* sp. strain D2 completely defluorinated DFMS under aerobic, sulfur-limiting conditions in a defined mineral medium. Strain D2 utilized DFMS as the sole source of sulfur, but not as a source of carbon or energy. DFMS utilization was inhibited by other forms of sulfur, and noncompetitive inhibition kinetics were observed, with K_i values of 3−4 μM for sulfate, sulfite, methane sulfonate, and cystine. Strain D2 was subsequently used to evaluate degradation of other fluorinated sulfonates. Growth and defluorination were only observed for those compounds containing hydrogen (TES and H-PFOS). TFMS and PFOS were not degraded. TES was completely defluorinated, and H-PFOS was partially defluorinated. No volatile transformation products were detected for TES or DFMS, but six volatile products were detected for H-PFOS. All of the volatile products contained oxygen and fluorine, but not sulfur. This is the first report of defluorination of fluorinated sulfonates, a linkage between sulfur assimilation and defluorination, and generation of volatile fluorinated biotransformation products.

Introduction

The commercial use of organofluorine compounds has dramatically increased over the past few years. These compounds are used as propellants, surfactants, agrochemicals, adhesives, refrigerants, fire retardants, and medicines (*1*). One category with particularly useful properties is the fluorinated sulfonates. Perfluorinated sulfonates are used as industrial surfactants and as catalysts in synthetic chemistry. Perfluorooctane sulfonate (PFOS; $C_8F_{17}SO_3^-$) has excellent chemical and thermal stability and is important commercially as a surfactant and as a precursor of other fluorinated surfactants and pesticides (*2*). PFOS inhibits gap junction intercellular communication (GJIC) in rat liver epithelial cells cultured in vitro (*3*), and it is an uncoupler of phosphorylation in rat liver mitochondria (*4*). Shorter chained perfluorinated compounds, such as trifluoromethane sulfonate (TFMS; $CF_3SO_3^-$), are used as oligomerization or polymerization catalyst. TFMS is one of the strongest organic acids known, has great thermal stability, does not release fluoride in the presence of strong nucleophiles, and resists both oxidation and reduction (*5*). TFMS exhibits both chronic and acute toxicity, but is not known to be carcinogenic (*6*). Difluoromethane sulfonate (DFMS; $CHF_2SO_3^-$), 1H,1H,2H,-2H-perfluorooctane sulfonate (H-PFOS; $C_6F_{13}C_2H_4SO_3^-$), and 2,2,2-trifluoroethane sulfonate (TES; $CF_3CH_2SO_3^-$) are partially fluorinated analogues of the perfluorinated sulfonates. The toxicological properties of these compounds are not known. Because of the apparent stability of fluorinated organics, their bioactivity, and their potential for accumulation in the environment, it is important to understand their environmental fate and the mechanisms by which they might be degraded. We evaluated the biodegradability of TFMS, TES, PFOS, and H-PFOS using a *Pseudomonas* isolate that completely defluorinated DFMS under aerobic, sulfur-limiting conditions. Sulfur inhibition studies were used to determine whether transformation of DFMS is linked to sulfur metabolism.

Materials and Methods

Media and Chemicals. *Pseudomonas* sp. strain D2 was routinely cultivated in a defined mineral medium supplemented with an organofluorine sulfonate as the source of sulfur. The mineral medium contained (in grams per liter) glucose, 2.0; K_2HPO_4, 3.5; KH_2PO_4, 2.0; NH_4Cl, 1.0; $MgCl_2 \cdot 6H_2O$, 0.5; 22 g/L stock of $CaCl_2 \cdot 2H_2O$, 1.0 mL/L; and trace elements stock I and II, 1.0 mL/L. Trace elements stock I contained (in grams per liter) $FeCl_3$, 1.36; $CoCl_2 \cdot 6H_2O$, 0.2; $MnCl_2 \cdot 4H_2O$, 0.122; $ZnCl_2$, 0.07; $Na_2MoO_4 \cdot 2H_2O$, 0.036; $NiCl_2 \cdot 6H_2O$, 0.12; $B_3(OH)_3$, 0.062; and $CuCl_2 \cdot 2H_2O$, 0.017. Concentrated HCl was added at 2.5 mL/L to the trace elements solution I. Trace elements solution II contained (in grams per liter) $Na_2SeO_3 \cdot 5H_2O$, 0.006; $NaWO_4 \cdot 2H_2O$, 0.033; and $Na_2MoO_4 \cdot 2H_2O$, 0.024. Final pH of the defined mineral medium was 6.9−7.0.

The sodium salt of DFMS (CHF_2SO_3Na, 99% purity) was provided by 3M Company (St Paul, MN). The potassium salt of PFOS ($C_8F_{17}SO_3K$, >98% purity) and the acid form of H-PFOS ($C_6F_{13}C_2H_4SO_3H$, >90% purity) were obtained from ICN Pharmaceuticals Inc. (Costa Mesa, CA). 2,2,2-Trifluoroethane sulfonyl chloride (99.9% purity) was obtained from Sigma Chemical (St. Louis, MO). Trifluoroethane sulfonic acid ($CF_3CH_2SO_3H$) was generated hydrolytically, by autoclaving a sealed bottle containing 2,2,2-trifluoroethane sulfonyl chloride dissolved in water. Stoichiometric release of chloride was observed by ion chromatography, with recovery of 99% of the expected chloride. Purity of DFMS, TES, and H-PFOSA was confirmed by HPLC-electrospray ionization/MS. A 10 μL sample of each fluorinated organic was injected into an HPLC equipped with a Fluofix 120N column (NEOS Company LTD, Japan) and operated with an acetonitrile-water (1:1) mobile phase at an flow rate of 500 μL/min at 467 psi. Detection of mass fragments was accomplished on a Fisons VG Platform II mass spectrometer. For DFMS, a single peak with a major ion fragment of 131 amu was detected at 3.84 min; for TES, a single peak with

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S0013-936X(98)00012-1 CCC: $15.00
Published on Web 06/20/1998

a major ion fragment of 163 amu was detected at 9.27 min; for H-PFOS, a single peak was detected with a major ion fragment of 427 amu.

Medium XS contained the following (in grams per liter): $NaHCO_3$, 3.36; $K_2HPO_4 \cdot 3H_2O$, 0.35; $MgCl_2 \cdot 6H_2O$, 0.2; glucose, 0.5; sucrose, 0.5; lactose, 0.5; ribose, 0.5; xylose, 0.5; L-alanine, 0.021; L-arginine hydrochloride, 0.021; L-asparagine, 0.021; L-aspartic acid, 0.021; L-glutamic acid, 0.021; L-glutamine, 0.021; glycine, 0.021; L-histidine hydrochloride, 0.021; *trans*-4-hydroxy-L-proline, 0.021; L-isoleucine, 0.021; L-leucine, 0.021; L-lysine hydrochloride, 0.021; L-phenylalanine, 0.021; L-proline, 0.021; L-serine, 0.021; L-threonine, 0.021; L-tryptophan, 0.021; L-tyrosine, 0.021; L-valine, 0.021; folate, 0.00021; pyridoxine, 0.0021; nicotinate, 0.021; riboflavin, 0.0018; pantothenate, 0.0053; *p*-aminobenzoate, 0.00025; and cobalamin, 0.00025. The above chemicals were obtained from Sigma Chemical.

Isolation and Identification of Strain D2. Strain D2 was isolated from an enrichment that fortuitously contaminated a laboratory stock solution of medium XS containing 19.5 μM DFMS as the sole source of sulfur. The strain was isolated by streaking on nutrient agar plates (Difco, Detroit, MI). It was subsequently characterized as a Gram-negative rod that was motile, catalase positive, and oxidase positive. Optimal growth was observed at 30 °C. Fatty acid profiles were performed by Microbial ID, Inc. (MIDI), Newark, DE. This analysis gave a similarity index of 0.788 for *Pseudomonas chloroaphis* and a similarity index of 0.692 for *Pseudomonas fluorescens*. Biolog, Inc. (Hayward, CA), identified strain D2 as *P. fluorescens*.

Growth of *Pseudomonas* sp. Strain D2. Strain D2 was routinely maintained on nutrient agar plates. Single colonies were inoculated with a loop into 5 mL of nutrient broth (Difco, Detroit, MI) and incubated at 30 °C for 24 h. Nutrient broth culture was used as a 1% (by volume) inoculum for cultures in defined mineral media. Cells were grown aerobically at 30 °C and were shaken on a rotary shaker at 160 rpm. Growth controls consisted of inoculated medium without fluorinated sulfonate. Abiotic controls consisted of uninoculated medium with fluorinated sulfonate. Experiments with TES and H-PFOS were also performed as closed incubations, using 5 mL liquid samples in 60 mL glass vials sealed with Teflon-lined rubber septa.

To evaluate growth of strain D2 under denitrifying conditions, growth medium containing glucose or acetate as the source of carbon was amended with 10 mM sodium nitrate, degassed with a 98% N_2 and 2% H_2 gas mixture, and capped with Teflon-faced butyl rubber septa. Culture manipulations were performed in a Coy anaerobic glovebox (Coy Laboratories, Ann Arbor, MI).

Fluoride and DFMS Analysis. DFMS was separated from other anions in the growth medium using a Dionex ion chromatography model 2000i/sp fitted with an Dionex IonPac AS4A ion-exchange column and a Dionex IonPac AG4A guard column. DFMS detection was achieved using an anion micromembrane suppressor with a Dionex Conductivity Detector-II (CDM). The eluant was a carbonate buffer (1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$) at a flow rate of 2 mL/min. Quantification was performed by comparison to external standards prepared in mineral medium. The method detection limit was 0.65 μM DFMS. Fluoride was assayed by ion selective electrode (Orion 96-09 BN), as per the manufacturer's instructions.

Atomic Emission Analysis of Volatile Transformation Products. Headspace containing volatile transformation products of H-PFOS was analyzed by inserting a solid-phase microextraction (SPME) fiber assembly with a 100 μm poly(dimethylsiloxane) coating (Supelco Inc., Bellefonte, PA) through a Teflon-lined septum into the headspace of a sealed sample, equilibrating the fiber assembly with the headspace for 30 min, withdrawing the assembly and inserting it into the injection port of a Hewlett-Packard 5890 series II GC with a Hewlett-Packard 5921A atomic emission detector (AED). The AED was used to determine elemental composition of volatile transformation products. Operating conditions for the GC were as follows: flow of 30 cm/s linear velocity, initial temperature of 40 °C for 4 min followed by a 10 °C/min ramp to 200 °C, injector temperature of 250 °C, and transfer line temperature of 225 °C. The emission wavelengths and plasma gases used for the various elements were as follows: sulfur (181 nm, oxygen and hydrogen); carbon (496 nm, oxygen); hydrogen (486 nm, oxygen); fluorine (690 nm, hydrogen); and oxygen (777 nm, hydrogen with auxiliary gas of 10% methane/90% nitrogen).

Crude Cell Extracts. One liter cultures of strain D2 were grown aerobically from a 1% inoculum for approximately 36 h. Strain D2 was screened for defluorination activity by measuring transformation rates prior to preparation of cell extracts. Actively transforming cultures were transferred to 250 mL centrifuge tubes and centrifuged at 12100*g* for 15 min at 4 °C. Cells were washed twice and resuspended in 50 mL of buffered growth medium without DFMS. Thirty milliliters of this concentrated cell suspension was sonicated on ice for 10 min. The sonicate was centrifuged at 12100*g* for 1 h at 4 °C and divided into a supernatant and cell pellet. Whole cells, whole sonicate, supernatant, and cell pellet (resuspended in 30 mL medium) were used to assay DFMS transformation. DFMS was added to these samples at 32 and 20 μM. β-NADH was added to a subset of samples.

In addition to the sonicated cell extracts, cell extracts were also prepared by French press. Cells grown and harvested as described above were resuspended in 5 mL of 20 mM Tris-buffer (pH 7.0) with 1 mM EDTA, placed on ice, and supplemented with 1 μL/mL of leupeptin solution and 5 μL/mL aprotinin (protease inhibitors). The cells were then passed three times through a chilled French pressure cell at 1000−1200 psi. The cell extract was diluted to 20 mL. Three reaction mixtures were used to evaluate cell-free activity: growth medium amended with glucose and 1 mM DFMS; a solution containing 50 μM ascorbic acid, 50 μM $FeCl_2$, and 10 mM imidazole buffer (pH 6.75) amended with 1 mM DFMS; and 20 mM Tris-buffer (pH 7.0) amended with 1 mM DFMS. Three different cofactors, ATP, β-NADH, and α-ketoglutarate, were also evaluated. A portion of the cell extract was centrifuged at 27200*g* for 20 min, and 0.5 mL of the supernatant was added to 1.5 mL of reaction mixture with one of the three cofactors. Controls consisted of samples with no cofactor, samples with no cell extract, and cell extract that was not centrifuged. The 2 mL samples were placed into 12 mL screw-capped tubes and shaken for 20 min to 1 h at 21 °C.

Growth Conditions and Preparation of Cells for Inhibition Experiments. Cells were grown to the stationary phase in defined mineral medium containing DFMS as the sole source of sulfur. Cells were harvested by centrifugation (15 min at 12100*g* in a Beckman SS-34 rotor at 4 °C), washed in defined mineral medium without sulfur, and resuspended to one-tenth the original culture volume (1.0−1.5 μg protein/mL). One milliliter of the resulting 10× concentrated cell suspension was added to 4 mL of medium plus DFMS and an additional sulfur source (sulfate, sulfite, methane sulfonate, methionine, and cystine). Specific substrate utilization curves were determined by varying the concentration of inhibitory sulfur source (2−200 μM) and the concentration of DFMS (1.3−65 μM). Fluoride was measured over a 20 min time period, and total cell protein was determined by the modified Lowry method, with bovine serum albumin as the standard (*7*).

Modeling of Kinetic Parameters. Uptake of DFMS was modeled using saturation kinetics (eq 1):

TABLE 1. Defluorination of DFMS and Growth of *Pseudomonas* sp. Strain D2 under Different Test Conditions

cells tested	carbon source	sulfur source	electron acceptor	nitrogen source	growth	defluorination
growth from small inoculum	glucose	none	oxygen	ammonia	−	−
	glucose	DFMS	oxygen	ammonia	+	+
	acetate	DFMS	oxygen	ammonia	+	+
	glucose	sulfate	nitrate	ammonia	+	not evaluated (no DFMS)
	glucose	DFMS	nitrate	ammonia	−	−
	DFMS	DFMS	oxygen	ammonia	−	−
	DFMS	sulfate	oxygen	ammonia	−	−
washed cell suspensions grown with DFMS as the sulfur source	glucose	DFMS	oxygen	ammonia	not evaluated	+
	glucose	DFMS	oxygen	none	not evaluated	−
	none	DFMS	oxygen	ammonia	not evaluated	−

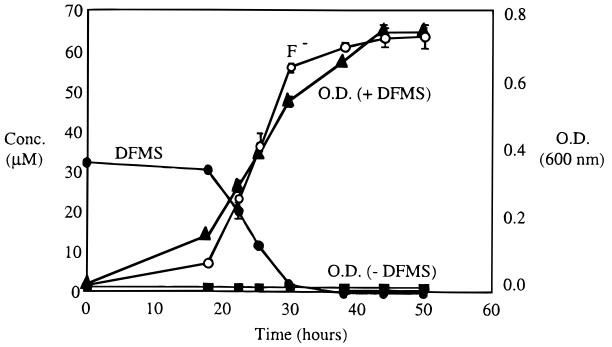

FIGURE 1. Defluorination of DFMS by *Pseudomonas* sp. strain D2. Error bars represent the standard deviation of triplicate samples.

$$q = (q_{max}S)/(K_s + S) \quad (1)$$

where q is the observed specific substrate utilization rate (μmoles of DFMS per milligram of protein per hour), q_{max} is the maximum specific rate of substrate utilization (μmoles of DFMS per milligram of protein per hour), S is concentration of DFMS (micromolar), and K_s is the half-saturation coefficient for DFMS (micromolar). The coefficients q_{max} and K_s were estimated by fitting initial specific rates of DFMS degradation for different DFMS concentrations to eq 1 using the nonlinear curve fitting routine of Systat 5.2.1.

Inhibition of DFMS transformation by other sulfur sources was quantified using conventional models for competitive inhibition (eq 2) and noncompetitive inhibition (eq 3) (*8*):

$$q = (q_{max}S)/(K_s(1 + I/K_i) + S) \quad (2)$$

$$q = (q_{max}S)/[(I/K_i + 1)(K_s + S)] \quad (3)$$

where I is the concentration of inhibitory sulfur source (micromolar), and K_i is the inhibition coefficient for the added source of sulfur (micromolar). Values for K_i were estimated by nonlinear curve fitting using values of q_{max} and K_s determined in the absence of inhibitory sulfur sources with initial specific rates of DFMS degradation for different initial concentrations of added sulfur. Inhibition of DFMS uptake was modeled using data for all samples except those for the highest levels of added inhibitory sulfur (100 and 200 μM), which proved to be completely inhibitory to uptake of DFMS.

Results

Biotransformation of Difluoromethane Sulfonate. Strain D2 was grown with DFMS as the sole source of sulfur. DFMS was defluorinated, with stoichiometric release of fluoride (2 mol of fluoride/mol of DFMS) and corrresponding growth of strain D2, as measured by change in optical density (Figure 1). No inorganic sulfur (sulfate or sulfite) was detected in the medium during growth. A yield of 25 ± 1 mg of protein/μmol sulfur as DFMS was obtained. No change in fluoride levels was observed in abiotic controls. In additional studies (Table 1), no growth was observed in (1) inoculated controls without DFMS, (2) controls in which DFMS was the sole source of carbon and sulfur, and (3) inoculated samples supplied with glucose and DFMS but with nitrate replacing oxygen as the electron acceptor. Growth under denitrifying conditions was only observed when sulfate was provided as the source of sulfur.

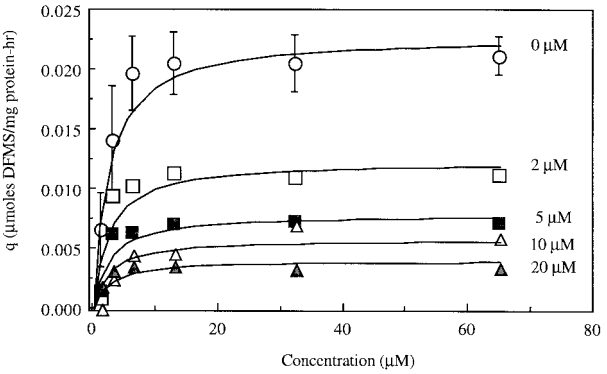

FIGURE 2. Noncompetitive inhibition of DFMS transformation by sulfate. Error bars represent the standard deviation of triplicate samples. Model fit parameters (eq 3): $q_{max} = 0.021 \pm 0.012$ μmol/mg h, $K_s = 3.14 \pm 0.28$ μM, $K_i = 3.24 \pm 0.92$ μM.

Noncompetitive Inhibition of Transformation by Other Sources of Sulfur. When sulfate, sulfite, methane sulfonate, methionine, and cystine were added to DFMS incubations, DFMS transformation was inhibited. The competitive inhibition model (eq 2) did not fit the data, but the noncompetitive inhibition model (eq 3) gave an excellent fit, with an average correlation coefficient of 0.98 ± 0.013. Figure 2 illustrates the fit for sulfate, where $K_i = 3.24 \pm 0.92$ μM, $K_s = 3.14 \pm 0.28$ μM, and $q_{max} = 0.021 \pm 0.001$ μmol of DFMS/mg of protein h. Values of the inhibition coefficient K_i were 3.59 ± 0.20 μM for sulfite, 2.87 ± 0.56 μM for methane sulfonate, and 3.64 ± 0.61 μM for cystine.

Biotransformation of Other Fluorinated Sulfonates. Strain D2 utilized DFMS, TES, and H-PFOS as sole sources of sulfur, but did not utilize TFMS or PFOS. Fluoride measurements indicated only partial defluorination of TES and H-PFOS. An alternative explanation for limited defluorination of TES and H-PFOS would be toxicity. However, at increasing concentrations of added sulfonate, toxicity should cause reduced fluoride release per mole of sulfonate added, and this was not observed. When increasing initial concentrations of TES (27.4−109.6 μM) were provided as the source of sulfur, 1.04 ± 0.14 mol of fluoride was released per mole of TES added (average for all concentrations), and this ratio was consistent for increasing concentrations of added

TES. For H-PFOS incubations that were closed to the atmosphere, 1.42 ± 0.04 mol of fluoride was released per mole of H-PFOS added, and this ratio was consistent for increasing concentrations of added H-PFOS. For incubations open to the atmosphere, only 0.90 ± 0.03 mol of fluoride was released per mole of H-PFOS added. The different stoichiometry for open and closed experiments was observed repeatedly (five times for each condition), indicating a real difference in the extent of defluorination under these conditions. Transformation of both TES and H-PFOS correlated with increased growth, indicating sulfur assimilation. The yield was 30 ± 2 mg of protein/μmol of sulfur as TES and 45 ± 2 mg of protein/μmol of sulfur as H-PFOS. For both H-PFOS and TES, growth continued for a brief period after fluoride concentrations had stabilized at their maximum level. No growth was observed in inoculated controls that did not contain TES or H-PFOS. No change in fluoride concentration was observed in abiotic controls.

Six volatile fluorinated products of H-PFOS transformation were detected by GC/AED. All of the volatile transformation products of H-PFOS contained carbon, oxygen, hydrogen, and fluorine. None contained sulfur. No volatile transformation products were detected for TES. This and the fluoride data suggest that TES transformation yields a nonvolatile product with two fluorine substituents.

Experiments with Crude Cell Extracts. Crude cell extract experiments investigated the hypothesis that an oxygenase played a role in the observed transformations, as proposed by others for other similar transformations (*9*−*12*). The possibility of an energy requirement was evaluated using ATP as a cofactor. Finally, α-ketoglutarate was used to determine if a dioxygenase similar to that encoded by the tfdA gene of *Alcaligenes eutrophus* was present (*13*). No defluorination was observed, even with added cofactors. Perhaps additional cofactors are required or critical membrane or cellular components were destroyed during cell lysis. Sulfur release from lysed cells may also have contributed to the negative results obtained.

Discussion

Several researchers have demonstrated that aliphatic sulfonates can serve as the sole sources of sulfur for growth (*11*, *14*−*17*), but we know of no previous reports indicating that fluorinated sulfonates can serve in that role. Fluorinated organics are generally perceived as refractory, with potential for accumulation (*1*). Therefore, it is important to understand the details of how these compounds are transformed and metabolized. This report establishes that certain hydrogen-substituted fluorinated sulfonates are at least partially degraded under aerobic conditions and that these transformations are growth-associated. On the basis of differences in observed yield (protein produced per mole of sulfur provided), most efficient growth was obtained on H-PFOS, next most efficient on TES, and least efficient on DFMS.

To further investigate the relationship between defluorination and sulfur scavenging, sulfur inhibition studies were performed with DFMS. Inhibition of DFMS utilization was observed when any of a chemically diverse set of sulfur sources were added to the growth medium. The kinetic data failed to fit a competitive inhibition kinetic model, but gave an excellent fit to a noncompetitive kinetic model. In the classical "textbook" description of the mechanism for noncompetitive inhibition, a single enzyme binds its target substrate at the active site and an inhibitor binds at some other site, leading to formation of enzyme−inhibitor complexes and enzyme−inhibitor−substrate complexes. It seems unlikely that different forms of sulfur would bind to a DFMS-degrading enzyme to the same extent at nearly the same concentration. On the other hand, the similarity of K_i coefficients (~3 μM) for different sulfur sources does suggest a commonality in the mechanism of inhibition. It seems possible that a noncompetitive pattern of inhibition in whole-cell experiments would result if intracellular sulfur levels regulate sulfur uptake. Our results are similar to those of Uria-Nickelsen et al. (*17*). These researchers found that sulfonate utilization was constitutive and that sulfate-S was used in preference to sulfonate-S when both were present. In a subsequent report (*18*), they proposed that sulfonate utilization may share some intermediates (e.g., sulfite) and regulatory features (repression by cysteine) of the assimilatory sulfate reductive pathway, but they found no evidence that the sulfonates they examined (cysteine, taurine, cysteate, and isethionate) exerted any regulatory effects on sulfate utilization. They concluded that as yet unrecognized regulatory mechanisms, such as specific transport mechanisms for sulfonates, must play an important role in sulfonate metabolism.

In our experiments, glucose was typically used as the source of carbon and energy, but its use was not essential for DFMS transformation; acetate also supported transformation and growth (Table 1). When DFMS was supplied alone or in combination with other sources of sulfur, there was no growth or transformation of DFMS, indicating that DFMS did not serve as a sole carbon and energy source. This is also similar to the findings of Uria-Nickelsen et al. (*17*) who reported that short-chain aliphatic sulfonates were not used as both carbon sources, even though the carbon compounds expected to result from oxidative cleavage of the C−S bond were known growth substrates. Also of interest were experiments performed with washed cells grown to stationary phase with DFMS as the source of sulfur (Table 1). When these cells were resuspended in medium containing glucose and ammonia, rapid DFMS transformation ensued. However, when these cells were resuspended in medium lacking either glucose or ammonium, DFMS was not transformed. These results suggest that something derived from the metabolism of glucose or acetate, such as carbon, energy, or electrons, is required for DFMS transformation. The results also suggest a role for ammonium at the reaction level.

Most researchers investigating sulfonate degradation have focused on aerobic conditions (*9*−*12*, *14*−*18*). However, Chien et al. (*19*) demonstrated that a fermenting *Clostridium* isolate was able to grow with taurine and isothionate as sulfur source, and that a fermenting *Klebsiella* isolate could grow with cysteate as the sulfur source. Although strain D2 grew under denitrifying conditions with sulfate as the sulfur source, it did not grow with DFMS as the sole source of sulfur. It is possible that molecular oxygen plays a role in growth upon DFMS. Kelly et al. (*10*) demonstrated that a methylotrophic bacterium degrades methanesulfonic acid with sulfite and formaldehyde as products. A NADH-dependent monooxygenase was identified as the responsible agent (*9*). A requirement for electrons as NADH might explain the glucose requirement in our studies. However, crude cell extract experiments with NADH failed to detect any defluorination activity so this hypothesis could not be verified.

Drawing on the above observations and assuming a common mechanism of transformation for fluorinated sulfonates, a general model capable of explaining our data might include (1) uptake of fluorinated sulfonates; (2) attack at the α-hydrogen or at the carbon−sulfur bond itself with cleavage of the carbon−sulfur bond and desulfonation; (3) generation of oxygenated products, including unstable intermediates, such as fluorinated aldehydes; (4) fluoro-elimination, hydrolysis, and rearrangement of unstable intermediates; and (5) assimilation of sulfur by existing

pathways for sulfonate assimilation. Evidence supporting such a mechanism includes noncompetitive inhibition of DFMS transformation by different sulfur sources, atomic emission data indicating the presence of oxygen and absence of sulfur in volatile fluorinated products from H-PFOS, increased fluoride release for closed incubations of H-PFOS versus open incubations (suggesting further defluorination of volatile intermediates in closed systems), failure to detect inorganic sulfur in the growth medium indicating sulfur uptake, and the observation that cells grown on TES and H-PFOS continued to grow after fluoride release had stopped.

Only those fluorinated compounds with one or more hydrogen atoms at the α-carbon were transformed. TFMS and PFOS did not degrade. It appears likely that sulfonates that are fully fluorinated will be persistent. Recalcitrance of such compounds can be attributed to the rigidity conferred by fluorine substituents (*20*) and to the absence of structures that are susceptible to electrophilic or nucleophilic attack. When hydrogen is present at the α-carbon, a site for attack is provided, and the carbon−sulfur bond becomes more accessible.

This study demonstrates that hydrogen-substituted fluorinated sulfonates are susceptible to biodegradation and defluorination and that they can support growth under sulfur-limiting and aerobic conditions. The sulfur inhibition studies establish a link between defluorination and sulfur metabolism. The results also suggest that nonvolatile fluorinated compounds can be converted into volatile fluorinated compounds. Until the identity of these volatile compounds is determined, it is unclear what effect, if any, they might have on the environment. Further insight into the mechanism of degradation of fluorinated sulfonates will require understanding of the specific nature of the enzyme(s) and cofactor(s) involved in the transformation. Such insight may facilitate design of fluorinated surfactants that are more readily degraded to harmless end products.

Acknowledgments

Financial support for this work was provided by the 3M Company and by the NSF Center for Microbial Ecology under Grant DEB-91Z0006. We thank James D. Johnson, Gregory N. Maisel, James B. Drake, and James K. Lundberg of 3M Environmental Technology and Safety Services Laboratories for their assistance.

Literature Cited

(1) Key, B. D.; Howell, R. D.; Criddle, C. S. *Environ. Sci. Technol.* **1997**, *31*, 2445−2454.
(2) Abe, T.; Nagase, S. In *Preparation, properties, and industrial applications of organofluorine compounds*; Banks, R. E., Ed.; John Wiley & Sons: New York, 1982; pp 19−44.
(3) Upham, B. L. Michigan State University, E. Lansing, MI, personal communication, 1996.
(4) Gadelhak, G. G. Ph.D. Dissertation, Michigan State University, 1992.
(5) Stang, P. J.; White, M. R. *Aldrichim. Acta* **1983**, *16*, 15−22.
(6) Material Data Safety Sheets for trofluoromethane sulfonic acid (CAS number 1493-13-6), Occupational Health Services, Inc., created April 17, 1991, revised June 30, 1994.
(7) Markwell, M. A.; Hass, S. M.; Tolbert, N. E.; Bieber, L. L. *Methods Enzymol.* **1981**, *72*, 296−301.
(8) Han, K.; Levenspiel, O. *Biotechnol. Bioeng.* **1987**, *32*, 430−437.
(9) Higgins, T. P.; Davey, M.; Trickett, J.; Kelly, D. P.; Murrell, J. C. *Microbiology* **1996**, *142*, 251−260.
(10) Kelly, D. P.; Baker, S. C.; Trickett, J.; Davey, M.; Murrell, J. C. *Microbiology* **1994**, *140*, 1419−1426.
(11) Thysee, G. J. E.; Wanders, T. H. *J. Microbiol.* **1974**, *40*, 25−37.
(12) Junker, F.; Leisinger, T. *Microbiol.* **1994**, *140*, 1419−1426.
(13) Fukumori, F.; Hausinger, R. *J. Biol. Chem.* **1993**, *268*, 24311−24317.
(14) Laue, H.; Field, J. A.; Cook, A. M. *Environ. Sci. Technol.* **1996**, *30*, 1129−1132.
(15) Seitz, A. P.; Leadbetter, E. R.; Godchaux, W., III. *Arch. Microbiol.* **1993**. *159*, 440−444.
(16) Thompson, A. S.; Owens, N. J. P.; Murrell, J. C. *Appl. Environ. Microbiol.* **1995**, *61*, 2388−2393.
(17) Uria-Nickelsen, M. R.; Leadbetter, E. R.; Godchaux, W., III. *J. Gen. Microbiol.* **1993**, *139*, 203−208.
(18) Uria-Nickelsen, M. R.; Leadbetter, E. R.; Godchaux, W., III. *Arch. Microbiol.* **1994**, *161*, 434−438.
(19) Chien, C. C.; Leadbetter, E. R.; Godchaux, W., III. *FEMS Microbiol. Lett.* **1995**, *129*, 189−194.
(20) Guo, W.; Brown, T. A.; Fung, B. M. *J. Phys. Chem.* **1991**, *95* (5), 1829−1836.

Received for review January 8, 1998. Revised manuscript received April 27, 1998. Accepted May 12, 1998.

ES9800129