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# *Pseudomonas* sp. Strain 273 Degrades Fluorinated Alkanes

Yongchao Xie, Gao Chen, Amanda L. May, Jun Yan, Lindsay P. Brown, Joshua B. Powers, Shawn R. Campagna, and Frank E. Löffler*

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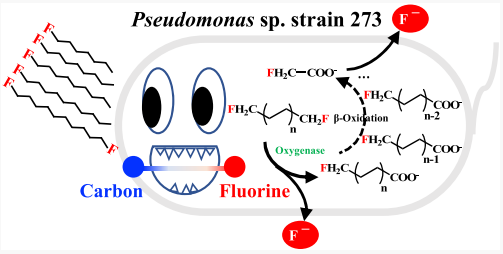
**ABSTRACT:** Fluorinated organic compounds have emerged as environmental constituents of concern. We demonstrate that the alkane degrader *Pseudomonas* sp. strain 273 utilizes terminally monofluorinated $C_7$−$C_{10}$ alkanes and 1,10-difluorodecane (DFD) as the sole carbon and energy sources in the presence of oxygen. Strain 273 degraded 1-fluorodecane (FD) (5.97 ± 0.22 mM, nominal) and DFD (5.62 ± 0.13 mM, nominal) within 7 days of incubation, and 92.7 ± 3.8 and 90.1 ± 1.9% of the theoretical maximum amounts of fluorine were recovered as inorganic fluoride, respectively. With *n*-decane, strain 273 attained (3.24 ± 0.14) × $10^7$ cells per μmol of carbon consumed, while lower biomass yields of (2.48 ± 0.15) × $10^7$ and (1.62 ± 0.23) × $10^7$ cells were measured with FD or DFD as electron donors, respectively. The organism coupled decanol and decanoate oxidation to denitrification, but the utilization of (fluoro)alkanes was strictly oxygen-dependent, presumably because the initial attack on the terminal carbon requires oxygen. Fluorohexanoate was detected as an intermediate in cultures grown with FD or DFD, suggesting that the initial attack on the fluoroalkanes can occur on the terminal methyl or fluoromethyl groups. The findings indicate that specialized bacteria such as *Pseudomonas* sp. strain 273 can break carbon−fluorine bonds most likely with oxygenolytic enzyme systems and that terminally monofluorinated alkanes are susceptible to microbial degradation. The findings have implications for the fate of components associated with aqueous film-forming foam (AFFF) mixtures.




## INTRODUCTION

The widespread use of fluorinated organic chemicals as refrigerants, surfactants, lubricants, pharmaceuticals, pesticides, aqueous film-forming foam (AFFF) mixtures, etc. has resulted in the release of these compounds into the environment.[1−4] Many fluorinated organic chemicals are recalcitrant, and recent studies demonstrated that a specific group of fluorinated organics, the so-called per- and polyfluoroalkyl substances (PFAS), is globally distributed and may impact environmental and human health.[5−7] Recalcitrance of fluorinated compounds has been explained by the lack of naturally occurring fluoroorganics and the strength of carbon−fluorine (C−F) bonds.[8] Fluorinated organics have been considered xenobiotics; however, there is clear evidence that geologic[9−11] and biologic systems[12−15] generate C−F bonds. For example, certain plants and some members of the Gram-positive *Actinomycetales* order synthesize monofluoroacetate and ω-fluorinated fatty acids.[16] A fluorinase that incorporates fluoride into organic molecules has been identified and characterized in *Streptomyces cattleya*.[13] The observation that the C−F bond is not solely anthropogenic implies that fluorinated organics have long been part of the biosphere, and microorganisms have had ample time to evolve strategies for breaking C−F bonds.

The strength of the C−F bond has served as an argument against direct enzymatic attack and microbial degradation of fluorinated compounds;[17,18] however, a number of studies have demonstrated that naturally occurring microorganisms possess enzyme systems that break C−F bonds at neutral pH and at room temperature.[19−21] Bacteria can catalyze C−F bond cleavage via oxygenolytic,[21] hydrolytic,[22] reductive,[23−25] and hydration[26] mechanisms. In one instance, evidence for organohalide respiration with monofluoroacetate was obtained,[27] suggesting that microbes can conserve energy from breaking C−F bonds via reductive defluorination.[28] Under oxic conditions, some bacteria and fungi with specific hydrolytic haloacid dehalogenases degrade monofluoroacetate.[29,30] *Pseudomonas* sp. strain D2 was reported to perform defluorination reactions to access the sulfur in certain fluorinated aliphatic sulfonates under oxic, sulfur-limiting growth conditions.[31] Reductive defluorination of certain PFAS has been demonstrated *in vitro* using reduced corrinoids, which are biomolecules.[32−36] Further, cometabolic defluorination linked to aerobic methanotrophy has been reported.[37] *Aureobacterium* sp. strain RHO25 possesses an enzyme system that removes the fluorine substituent from 4-fluorobenzoate to form 4-hydroxybenzoate.[38] This organism was unable to grow with 4-chlorobenzoate, 4-bromobenzoate, and 4-iodobenzoate, sug-

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gesting that the initial enzyme system releasing the para fluorine substituent specifically evolved to catalyze a defluorination reaction. These observations demonstrate that microbes can catalyze C−F bond cleavage, thereby refuting the perception that bond strength prevents biological systems from breaking these bonds.

Terminally and partially fluorinated alkanes have industrial and medical applications.[4,39] Certain plant species, in particular members of the family Dichapetalaceae, synthesize monofluorinated fatty acids, and 1-fluorodecane (FD) has been detected in the abdominal glands of African weaver ants.[40,41] A potentially significant source of partially fluorinated alkanes stems from the application of AFFF and the transformation of PFAS mediated by aggressive physical−chemical treatments[42−46] or biological defluorination.[47,48] To date, the environmental fate of alkanes with a low degree of fluorination is unclear.

The soil isolate *Pseudomonas* sp. strain 273 has been shown to grow with $C_5$ to $C_{12}$ $\alpha,\omega$-dichloroalkanes under oxic conditions.[49,50] During growth, stoichiometric amounts of inorganic chloride were released, and preliminary data implicated the involvement of a monooxygenase in dechlorination.[49] Dechlorinating enzyme systems often also cleave C−Br and C−I bonds but not C−F bonds.[51] Unexpectedly, we observed growth of strain 273 with $C_7$ to $C_{10}$ fluorinated alkanes as the sole source of carbon and energy under oxic conditions with concomitant fluoride release, indicating that this organism has the ability to break C−F bonds. Experiments with *Pseudomonas* sp. strain 273 demonstrated that fluorinated alkanes are not inert and susceptible to defluorination and degradation under oxic conditions.

## MATERIALS AND METHODS

**Chemicals.** 1-Fluorodecane (FD) (purity, >97%), 1-fluorononane (>98%), 1-fluorooctane (>99%), 1-fluoroheptane (>99%), and 1-fluorohexane (>99%) were obtained from SynQuest Labs, Inc., Alachua, FL, USA. 1,10-Difluorodecane (DFD) (>97%) was custom-synthesized by Carbosynth, Newbury, UK. Isocetane (>98%), sodium decanoate, disodium sebacate, sodium fluoride, sodium acetate, sodium trifluoroacetate (TFA), boron trifluoride, nitrous oxide ($N_2O$, >99%), and fatty acid methyl ester (FAME) standards were obtained from Sigma-Aldrich, St. Louis, MO, USA. *n*-Decane (decane, >99%) and 1,10-dichlorodecane (DCD) (>98%) were obtained from Acros Organics, Fair Lawn, NJ, USA. *n*-Hexane (hexane, >99.8%), methanol (>99.9%), and sodium nitrite were obtained from Fisher Chemical, Pittsburgh, PA, USA. Sodium monofluoroacetate (MFA) was obtained from MP Biomedicals, Solon, OH, USA. Dinitrogen, helium (both ultra-high purity), and acetylene (>99.99%) were obtained from Airgas, Radnor Township, PA, USA. A Bradford 1x dye reagent was obtained from Bio-Rad Laboratories, Hercules, CA, USA. Bovine serum albumin was obtained from Fisher Bioreagents, Pittsburgh, PA, USA. All other chemicals used were of analytical reagent grade or higher.

**Cultivation of *Pseudomonas* sp. Strain 273.** Strain 273 was isolated from garden soil and taxonomically characterized as a pseudomonad.[49] Strain 273 was grown in a completely synthetic, defined mineral salt medium that comprised (per liter) 6.34 g of $Na_2HPO_4$, 1.33 g of $KH_2PO_4$, 1.0 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4{\cdot}7H_2O$, the Wolin vitamin mix,[52] and a trace metal solution.[49] To avoid precipitate formation, a 10-fold concentrated phosphate buffer stock was autoclaved separately and added to the medium after the solutions had cooled to room temperature. The final medium pH was 7.3. The vitamins were added from a filter-sterilized (0.2 $\mu$m polyethersulfone membrane filter, Thermo Scientific, Waltham, MA, USA) stock solution. The culture was routinely grown in 160 mL serum bottles containing 50 mL of the medium and 110 mL of air headspace. The bottles were closed with black butyl rubber stoppers held in place with aluminum crimps (Bellco Glass, Inc., Vineland, NJ, USA). Neat hydrocarbons (e.g., decane) were added to the autoclaved medium with 5 $\mu$L glass microsyringes (Hamilton, Reno, NV, USA) to reach initial (nominal) substrate concentrations ranging from 0.5 to 5 mM, which is higher than the maximum aqueous phase solubility of the hydrocarbons tested (Table S1). The headspace of each 160 mL culture vessel was flushed daily with air to ensure oxygen was not limiting hydrocarbon oxidation. Due to immiscibility of the medium-chain hydrocarbons with water, the quantification of $C_{10}$ hydrocarbons required sacrificial sampling and hexane extraction of the entire incubation vessel. For time-series quantification, triplicate culture vessels (i.e., 20 mL glass vials containing 2 mL of the medium) were prepared for each anticipated sampling time point. The vials were closed with Teflon-lined butyl rubber septa held in place with aluminum crimps. To ensure that oxygen availability was not limiting alkane degradation (Table S2), the vials were pressurized by injecting 18 mL of air (~2 atm). Independent experiments with larger incubation vessels and a decreased liquid-to-headspace volume ratio (i.e., 160 mL serum bottles with 10 mL of the medium) verified the results obtained with the 20 mL glass vials. All vessels were shaken at 120 rpm in an upright position and incubated at 30 °C.

**Substrate Range.** The maximum aqueous phase solubility of the fluorohydrocarbons differed according to chain length ($\mu$M values for 25 °C in parentheses): FD (15.7), 1-fluorononane (51.9), 1-fluorooctane (172.5), 1-fluoroheptane (573.6), and 1-fluorohexane (1910.4) (Table S1). To test the range of fluorinated alkanes that strain 273 could utilize as growth substrates, cultures received fluoroalkanes dissolved in a water immiscible carrier phase (i.e., isocetane). This procedure avoided potential toxicity of shorter fluoroalkanes due to their higher aqueous phase solubility. Stock solutions of fluoroalkanes (14.48−15.97 mg) in isocetane (2 mL) were prepared gravimetrically, and fluoroalkane stock concentrations ranged from 49.3 to 69.5 mM (Table S1). Stock solutions of fluoroalkanes dissolved in isocetane (0.5 mL) were transferred to 20 mL glass vials containing 10 mL of the sterile medium and inoculated (1% vol/vol). The aqueous phase concentrations of fluoroalkanes (0.32−40.45 $\mu$M) at equilibrium were calculated based on published *n*-octanol/water partition coefficient ($K_{OW}$) values (Table S1). Triplicate cultures were established including control cultures that received only isocetane. The vials were covered with aluminum foil and shaken at 120 rpm at 30 °C. Growth occurred in positive control cultures that received FD dissolved in the carrier phase, indicating that 0.5 mL of isocetane did not prevent growth with FD. No growth was observed in cultures that received only isocetane.

To investigate the range of fatty acids that strain 273 could utilize as carbon sources, stock solutions (prepared in Milli-Q water) of sodium decanoate, disodium sebacate, MFA, or TFA were autoclaved and added to the medium with sterile 1 mL plastic syringes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The utilization of MFA (1 and 2 mM) and TFA (2 mM) was also tested with decane (5 mM, nominal) supplied as a primary substrate.



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To determine if strain 273 is a complete denitrifier, the organism was grown in an anoxic medium (50 mL) with nitrate (2 mM, 100 μmol), nitrite (2 mM, 100 μmol), or $N_2O$ (0.42 mM, 100 μmol) as an electron acceptor in 160 mL glass serum bottles. Acetate (10 mM, 500 μmol) was provided as an electron donor. Control cultures received acetylene (11 mL, 10% of headspace) to inhibit $N_2O$ reduction.[53]

To test if oxygen was required for fluoroalkane degradation, strain 273 was grown under anoxic conditions with 2 mM nitrite as an electron acceptor and decanoate, decane, or FD (1 mM, nominal) provided as electron donors (four replicate cultures each). To remove oxygen, the headspace of the 160 mL serum bottles was evacuated (−10 psig for 30 s) and purged with oxygen-free nitrogen (three cycles). Four replicate cultures of strain 273 with FD and an air headspace served as positive controls. Four additional control cultures were maintained under anoxic conditions with 2 mM nitrite and FD for 7 days before the dinitrogen headspace was replaced with air. All cultures were inoculated (1% vol/vol) from a strain 273 culture grown with decanoate and nitrite under anoxic conditions.

**Resting Cell Assays.** Strain 273 was grown with decane (5 mM, nominal) under oxic conditions in two 2 L glass bottles each containing 1.5 L of the medium. The bottles were covered with aluminum foil to allow gas exchange and shaken at 120 rpm at 30 °C. The cells were harvested after 6 days ($OD_{600nm}$ readings of about 0.1) by centrifugation at 11,325 × *g* at 20 °C for 20 min. The cell pellets were suspended in 200 mL of the sterile mineral salt medium, and 2 mL of the cell suspension (0.7 mg protein $mL^{-1}$, Figure S1) was transferred to three sets of 20 mL glass vials, each set receiving 2 μL of decane (5.1 mM), FD (4.9 mM), or DFD (4.7 mM, all nominal). The vials were sealed with Teflon-lined butyl rubber septa held in place with aluminum crimps and pressurized (∼2 atm) with air. At each sampling event, triplicate vials from each set were sacrificed for liquid extraction and quantification of hydrocarbon remaining.

**Growth Yields.** To compare growth yields with different (halo)carbon substrates, strain 273 cultures received decane (15.5 ± 0.7 μmol, 1.55 ± 0.07 mM), FD (14.6 ± 0.4 μmol, 1.46 ± 0.04 mM), DFD (15.3 ± 0.6 μmol, 1.53 ± 0.06 mM), DCD (14.4 ± 0.7 μmol, 1.44 ± 0.07 mM), acetate (69.6 ± 1.8 μmol, 6.96 ± 0.18 mM), sodium decanoate (12.6 ± 0.2 μmol, 1.26 ± 0.02 mM), or disodium sebacate (12.3 ± 0.4 μmol, 1.23 ± 0.04 mM) in 160 mL serum bottles containing 10 mL of the medium and closed with black butyl rubber stoppers. Dedicated 5 μL glass microsyringes were used to add 3 μL of the different hydrocarbons, and gravimetric measurements determined the exact amounts of hydrocarbons added. The amount of oxygen in the headspace allowed complete oxidation of the carbon substrates (i.e., electron acceptor present in at least 5-fold excess of the theoretical demand) (Table S2). After a 7 day incubation period, 2 mL of culture suspension samples was collected for liquid extraction to quantify hydrocarbons remaining in the medium. Cell yields were determined with qPCR and the Bradford assay (see the Supporting Information for details). For qPCR, the forward primer (5′-3′) F-GCTAGTCTAACCTTCGGGGG ($T_m$ = 59 °C) and reverse primer R-TCCCCTACGGCTACCTTGTT ($T_m$ = 60 °C) were designed using Primer-BLAST NCBI to amplify a consensus region of the strain 273 five 16S rRNA genes.[54]

**Identification of $C_{10}$ Hydrocarbon Metabolites.** Strain 273 cultures were grown with decane, FD, or DFD as the sole carbon source (7 mM, nominal) in closed 160 mL serum bottles containing 50 mL of the medium and 110 mL of air headspace, which was replaced daily. Following a 4 day incubation period ($OD_{600nm}$ > 0.08), 10 mL cell suspension samples were centrifuged at 8,000 × *g* for 15 min at room temperature and washed once with the sterile medium. To identify potential metabolites generated from $C_{10}$ hydrocarbons, fatty acids were extracted from cell pellets and derivatized for gas chromatography−high-resolution mass spectrometry (GC−HRMS) analysis (see the Supporting Information for details).

**Analytical Procedures.** Aliphatic hydrocarbons were extracted from the culture medium with hexane and quantified with an Agilent 7890A GC−MS system equipped with an autosampler, a DB-624 column, and a mass-selective detector (Agilent, Santa Clara, CA, USA) (see the Supporting Information for details). Fluoride was quantified with an ICS-2100 (Dionex, Sunnyvale, CA, US) ion chromatography system equipped with a 4 mm × 250 mm IonPac AS18 hydroxide-selective anion-exchange column and a conductivity detector. MFA, TFA, and acetate were measured with an Agilent 1200 series HPLC system (Agilent, Santa Clara, CA, USA) equipped with an Aminex HPX-87H ion exclusion column (Bio-Rad Lab., Hercules, CA, USA) and a G1365D multiwavelength detector (Agilent, Santa Clara, CA, USA). Decanoate and sebacate were quantified as methyl esters, and FAME was analyzed by GC−HRMS following an established method (see the Supporting Information for details).[55] Oxygen and $N_2O$ in culture headspace samples were quantified with an Agilent 7890A GC system equipped with an HP-PLOT/Q column (30 m length, 0.32 mm inner diameter, and 20.0 μm film thickness) and a microelectron capture detector. Optical density ($OD_{600nm}$) readings were obtained after gentle vortexing 1 mL of the culture suspension using a 20D+ spectrophotometer (Thermo Scientific, Waltham, MA, USA).

**Statistical Analysis.** Significant differences between growth yields with different hydrocarbons were examined by Duncan's multiple range test using SPSS Statistics (Version 26, SPSS, Inc., Chicago, IL). The calculation of the Pearson correlation coefficient between the cell number and protein concentration measurements and the linear regression of $OD_{600nm}$ and protein concentration measurements were both performed using SPSS Statistics (Version 26).

## RESULTS

**Growth of *Pseudomonas* sp. Strain 273 with Fatty Acids, Alkanes, and Fluoroalkanes.** *Pseudomonas* sp. strain 273 grew with 1-fluorodecane (FD) as the sole source of carbon and energy with concomitant release of inorganic fluoride in the presence of oxygen (Figure 1). Cultures completely consumed 5.97 ± 0.22 mM (11.94 ± 0.44 μmol) FD within 1 week and produced 5.53 ± 0.23 mM (11.06 ± 0.46 μmol) fluoride, which accounted for 92.7 ± 3.8% of the total fluorine contained in the substrate (Figure 1A). Growth and defluorination were also observed in cultures amended with 1-fluorononane, 1-fluorooctane, and 1-fluoroheptane (Table 1). No growth occurred in cultures that received 0.5 mM 1-fluorohexane or 1-fluorohexane dissolved in an inert isocetane carrier phase that lowered the aqueous phase concentration of 1-fluorohexane to ∼40 μM. Strain 273 grew with the α,ω-fluorinated alkane DFD, and cultures completely consumed 5.62 ± 0.13 mM (11.24 ± 0.26 μmol) DFD within 1 week with concomitant release of 10.14 ± 0.22 mM (20.28 ± 0.44 μmol) fluoride, which accounted for 90.1 ± 1.9% of the total fluorine contained in the initial amount of DFD (Figure 1B). Neither defluorination nor


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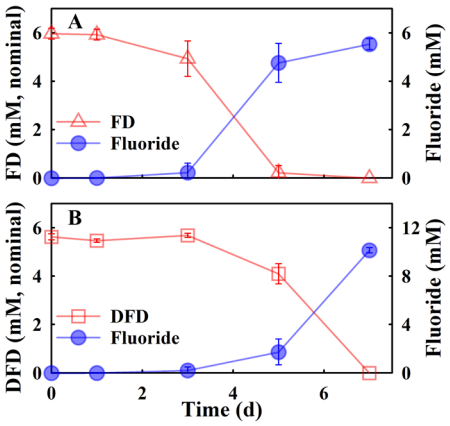


**Figure 1.** Degradation of $C_{10}$ fluorodecanes by *Pseudomonas* sp. strain 273 and the concomitant release of inorganic fluoride. Degradation of FD (panel A) and of DFD (panel B). The data represent the averages of triplicate cultures, and the error bars represent the standard deviations.

growth occurred in autoclaved control cultures or in vessels without inoculum.

Growth also occurred with 1,10-dichlorodecane (DCD), decane, decanol, decanoate, sebacate, and acetate as the sole carbon and energy sources in the presence of oxygen (Table 1). No growth was observed in cultures amended with monofluoroacetate (MFA) or trifluoroacetate (TFA) as the sole carbon source; however, partial MFA degradation occurred in cultures that received decane as the primary substrate in the presence of oxygen. Over the course of a 17 day incubation period, 0.15 ± 0.03 mM MFA (7.35 ± 1.30 μmol or 12.4 ± 2.7% of the initial amount) was consumed with the concomitant release of 0.15 ± 0.03 mM (7.26 ± 1.59 μmol) fluoride (Figure S2). Defluorination of TFA was not observed.

Strain 273 cultures that received decanoate, sebacate, or acetate (i.e., fatty acids) as a carbon substrate produced (10.55 ± 0.82) × $10^7$, (9.71 ± 1.15) × $10^7$, and (5.22 ± 1.34) × $10^7$ cells per μmol of carbon consumed, respectively (Table 2). Among all the alkane substrates, the highest growth yield of (3.24 ± 0.14) × $10^7$ cells per μmol of carbon consumed was observed in cultures grown with decane. A slightly lower growth yield of (2.48 ± 0.15) × $10^7$ cells per μmol of carbon consumed was observed with FD. Cultures grown with DFD or DCD reached significantly lower cell densities, and (1.62 ± 0.23) × $10^7$ and (1.61 ± 0.61) × $10^7$ cells per μmol of carbon consumed were determined, respectively. Protein quantification was used as an independent measure of growth and corroborated the growth yields determined with qPCR (Table 2). Strain 273 produced 5.35 ± 0.32, 5.03 ± 0.21, and 2.53 ± 0.14 μg of protein per μmol of carbon consumed in cultures that were fed with decanoate, sebacate, or acetate, respectively. With decane, FD, DCD, or DFD as a carbon substrate, 2.04 ± 0.31, 1.89 ± 0.43, 1.51 ± 0.75, and 1.24 ± 0.58 μg of protein per μmol of carbon consumed were measured, respectively (Table 2). Cell and protein yield measurements showed a significant correlation (Pearson correlation coefficient, $r$ = 0.95, $p$ < 0.01) (Figure S3), supporting the observation of lower growth yields with halogenated alkanes.

**Resting Cell Experiments.** Dense cell suspensions of strain 273 obtained with decane as a carbon source consumed decane, FD, and DFD without an apparent lag phase (Figure 2). DFD was consumed at the highest rate of 0.89 ± 0.09 μmol $h^{-1}$ $mg^{-1}$ protein, and 10.30 ± 0.14 μmol of DFD was completely consumed over a 12 h incubation period (Figure 2). FD and decane were consumed at lower rates of 0.55 ± 0.08 and 0.63 ± 0.05 μmol $h^{-1}$ $mg^{-1}$ protein, respectively.

**Table 1. Growth and Defluorination Observed in Cultures of *Pseudomonas* sp. Strain 273 with Different Electron Donor/Electron Acceptor Combinations[g]**

| electron donor (mM) | electron acceptor (mM) | growth[a] | defluorination[b] |
|---|---|---|---|
| decane (1[c], 5[c]) | oxygen | + | NA |
| decane (1[c]) | nitrite (2) | – | NA |
| 1-fluorodecane (0.5[c], 1[c], 5[c], carrier[d]) | oxygen | + | + |
| 1-fluorodecane (0.5[c], 1[c]) | nitrite (2) | – | – |
| 1-fluorodecane (0.5[c]) | nitrous oxide (0.42) | – | – |
| 1,10-difluorodecane (1[c], 5[c]) | oxygen | + | + |
| 1-fluorononane (0.5[c], carrier[d]) | oxygen | + | + |
| 1-fluorooctane (0.5[c], carrier[d]) | oxygen | + | + |
| 1-fluoroheptane (0.5, 1[c])[e] | oxygen | + | + |
| 1-fluorohexane (0.5, carrier[d]) | oxygen | – | –[f] |
| 1,10-dichlorodecane (1[c]) | oxygen | + | NA |
| acetate (2, 5, 10) | oxygen | + | NA |
| acetate (5, 10) | nitrite (1, 2) | + | NA |
| acetate (5, 10) | nitrate (2) | + | NA |
| acetate (5, 10) | nitrous oxide (0.42) | + | NA |
| monofluoroacetate (1, 2) | oxygen | – | – |
| trifluoroacetate (2) | oxygen | – | – |
| monofluoroacetate (1) + decane (5[c]) | oxygen | + | + |
| trifluoroacetate (2) + decane (5[c]) | oxygen | + | – |
| decanol (1[c]) | oxygen | + | NA |
| decanol (1[c]) | nitrite (2) | + | NA |
| decanoate (1) | oxygen | + | NA |
| decanoate (1) | nitrite (2) | + | NA |
| decanoate (1) | nitrate (2) | + | NA |
| sebacate (1) | oxygen | + | NA |

[a]Growth was determined by the increase in culture turbidity and the degradation of substrates. [b]Defluorination was determined by the detection of released fluoride in a medium. [c]Nominal concentration. [d]An isocetane carrier phase was used to sustain a low concentration of electron donors in the aqueous phase. [e]Independent experiments demonstrated growth with 0.5 and 1 mM 1-fluoroheptane but not in systems with an isocetane carrier phase. [f]Some fluoride release from 1-fluorohexane was observed in cultures that received decane as a primary substrate. [g]NA: not applicable.

**Oxygen Requirement for (Halo)Alkane Degradation.** Growth without oxygen was tested in the anoxic medium with nitrate, nitrite, or $N_2O$ as electron acceptors and excess acetate as electron donor (Table S3). Complete consumption of 104.2 ± 4.5 μmol (2.08 ± 0.09 mM) of nitrite was coupled with the oxidation of 42.7 ± 1.1 μmol (0.85 ± 0.02 mM) of acetate. No $N_2O$ was detected in the cultures at the end of the 7 day incubation period. In cultures that received 100.0 ± 11.6 μmol (0.42 ± 0.05 mM) of $N_2O$ as an electron acceptor, $N_2O$ was completely reduced, and 27.8 ± 4.4 μmol (0.56 ± 0.09 mM) of acetate was consumed over a 7 day incubation period, indicating that strain 273 possesses a functional *nos* operon responsible for the reduction of $N_2O$ to $N_2$. In cultures that received 112.0 ± 9.2 μmol (2.24 ± 0.18 mM) of nitrite as an electron acceptor and


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Table 2. *Pseudomonas* sp. Strain 273 Growth Yields with Different Carbon Substrates[c]

| carbon substrate (electron donor)[a] | carbon substrate consumed (mM) | carbon consumed ($\mu$mol) | growth yield[b] | |
|---|---|---|---|---|
| | | | cell numbers ($\times 10^7$/$\mu$mol carbon) | protein ($\mu$g/$\mu$mol carbon) |
| acetate | 6.96 ± 0.18 | 139.18 ± 3.54 | 5.22 ± 1.34$^{B}$ | 2.53 ± 0.14$^{B}$ |
| decanoate | 1.26 ± 0.02 | 125.86 ± 1.64 | 10.55 ± 0.82$^{A}$ | 5.35 ± 0.32$^{A}$ |
| sebacate | 1.23 ± 0.04 | 123.37 ± 4.49 | 9.71 ± 1.15$^{A}$ | 5.03 ± 0.21$^{A}$ |
| decane | 1.55 ± 0.07 | 154.60 ± 7.03 | 3.24 ± 0.14$^{C}$ | 2.04 ± 0.31$^{B,C}$ |
| FD | 1.46 ± 0.04 | 145.56 ± 3.60 | 2.48 ± 0.15$^{C,D}$ | 1.89 ± 0.43$^{B,C,D}$ |
| DFD | 1.53 ± 0.06 | 153.30 ± 6.48 | 1.62 ± 0.23$^{D}$ | 1.24 ± 0.58$^{D}$ |
| DCD | 1.44 ± 0.07 | 143.62 ± 7.23 | 1.61 ± 0.61$^{D}$ | 1.51 ± 0.75$^{C,D}$ |

[a]The carbon substrates were completely consumed after a 1-week incubation period. [b]Cell numbers were determined with qPCR and protein amounts were estimated after alkaline cell lysis using the Bradford assay. The yield data represent the net increase of cells and protein per $\mu$mol of carbon consumed. For both the qPCR and the protein datasets, the Duncan test ($\alpha = 0.05$) observed significant differences between A, B, C, and D. [c]The data represent the averages and the standard deviations of triplicate cultures.

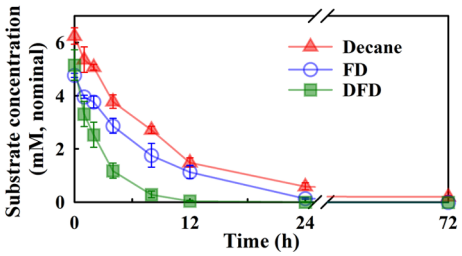


**Figure 2.** Consumption of decane, FD, and DFD in resting cell suspension assays using *Pseudomonas* sp. strain 273 cells grown with decane. The data represent the averages of triplicate incubations, and the error bars represent the standard deviations.

the $N_2O$ reductase inhibitor acetylene, 60.0 ± 6.2 $\mu$mol (0.25 ± 0.03 mM) of $N_2O$ was measured after a 7 day incubation period indicating stoichiometric conversion of nitrite to $N_2O$. Electron recoveries calculated for cultures that received nitrite or $N_2O$ as electron acceptors corroborated the involvement of the canonical denitrification pathway (Table S3). In cultures amended with nitrite, nitrite plus acetylene, or $N_2O$, 91.5 ± 4.0, 84.8 ± 7.0, and 89.9 ± 10.4% of the electrons released from acetate oxidation were accounted for in the amounts of electron acceptors consumed, respectively. No electron recoveries were calculated for nitrate-amended cultures because phosphate in the medium interfered with nitrate quantification.

In addition to acetate, strain 273 utilized decanol and decanoate as electron donors for nitrate and nitrite reduction (Table 1). With decanoate (1 mM, 50 $\mu$mol) as an electron donor, 1.79 ± 0.04 mM nitrite (89.5 ± 2.0 $\mu$mol) was consumed over a 7 day incubation period (Figure 3A). In anoxic cultures amended with nitrite as an electron acceptor and decane as an electron donor, neither growth, nitrite consumption, nor decane degradation occurred (Figure 3B). Similar observations were made with FD as an electron donor, and no inorganic fluoride was released in anoxic incubations with nitrite as an electron acceptor (Figure 3C). Conversely, both growth and fluoride release occurred in positive control vessels that received FD with oxygen as an electron acceptor (Figure 3D). In anoxic cultures amended with nitrite, the consumption of FD and the release of inorganic fluoride emerged after the nitrogen headspace had been replaced with air (Figure 3E).

**Detection of (Halo)Alkane Fatty Acid Metabolites.** Microbes metabolize alkanes via $\beta$-oxidation to shorter chain fatty acids.[56] GC−HRMS was used to profile the free fatty acid content of strain 273 grown with decane, FD, or DFD at one time point during active growth (Table S4). Nonfluorinated $C_5$ to $C_{10}$ saturated fatty acids were detected in decane-grown cells. Even-chain fluorinated fatty acids, including fluorodecanoate, fluorooctanoate, and fluorohexanoate, were detected in cells grown with DFD, and fluorohexanoate was measured in cells grown with FD (Figure 4).

## DISCUSSION

Only a few organisms have been described that degrade halogenated alkanes, and the substrate range for most of the characterized halogenated alkane degraders has been restricted to chlorinated, brominated, and iodinated alkanes.[57−59] In contrast, *Pseudomonas* sp. strain 273, the focus of the current study, utilizes $C_7$ to $C_{10}$ monofluoroalkanes and DFD as growth substrates with concomitant release of inorganic fluoride. Apparently, this organism possesses one or more enzyme systems that lead to C−F bond breakage in alkanes with fluorine substitution in one or both terminal methyl groups.

**Oxidation of the Terminal Carbon and the Degradation Pathway of (Halo)Alkanes in Strain 273.** The growth studies demonstrated that alkane degradation in strain 273 only occurred in the presence of oxygen. Presumably, oxygen is a required cosubstrate for the initial attack of the terminal carbon atom and conversion to the respective alcohols via a monooxygenase-catalyzed reaction.[60] Prior work demonstrated that strain 273 metabolizes decane and DCD, presumably using the same monooxygenase enzyme system for the initial attack.[49] The lack of an apparent lag phase when dense cell suspensions of decane-grown cells were challenged with FD or DFD (Figure 2) suggested that a *de novo* enzyme biosynthesis was not required to initiate FD and DFD degradation. Therefore, it is likely that the same monooxygenase enzyme system is responsible for the initial attack on decane, FD, and DFD. An alternate explanation would be the constitutive expression of a monooxygenase that causes C−F bond cleavage, and detailed expression studies under various growth conditions would be needed to reveal if a single or multiple monooxygenase enzyme systems are involved in (halo)alkane degradation.

In light of the experimental observations, a single pathway is likely utilized for the degradation of (halo)alkanes in strain 273 (Figure 5). The detection of fluorinated fatty acids (e.g., fluorohexanoate) in cells grown with FD and DFD suggests that the initial oxidation can occur at either the terminal methyl ($-CH_3$) or fluoromethyl ($-CH_2F$) carbon. With decane as a growth substrate, the initial oxidation product would be the corresponding alcohol (i.e., decanol), which would then be


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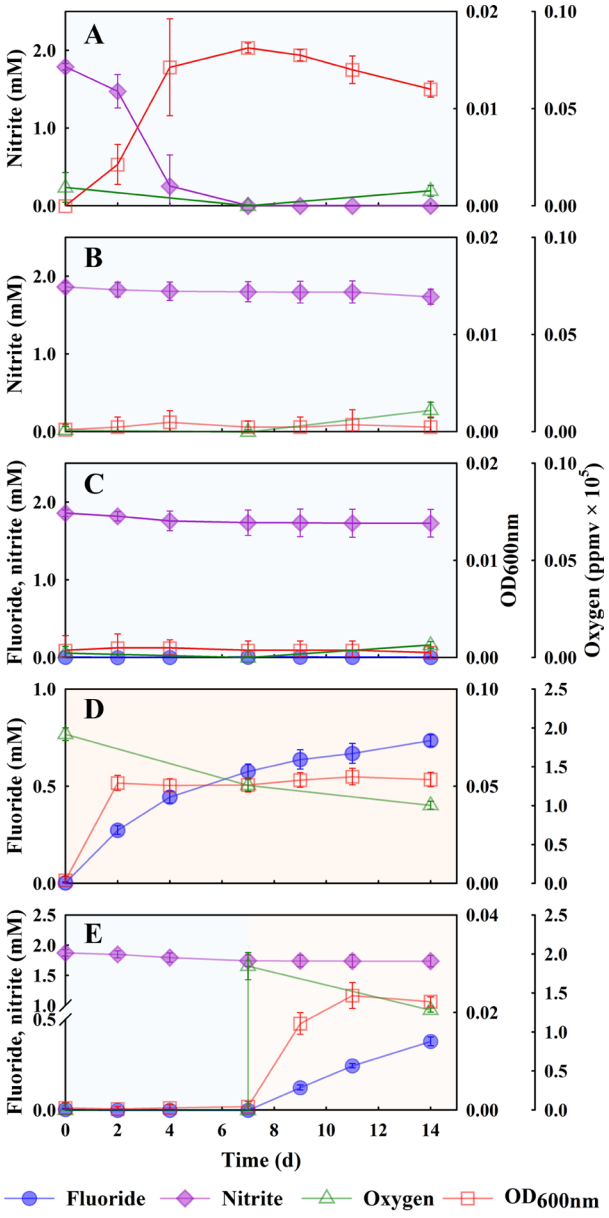


**Figure 3.** Utilization of $C_{10}$ (fluoro)alkanes and decanoate by strain 273 under oxic (light red background) and anoxic (light gray background) conditions. Cultures were supplied with different electron donor/acceptor combinations including nitrite (2 mM), decanoate (1 mM), and nitrogen headspace (panel A); nitrite (2 mM), decane (1 mM), and nitrogen headspace (panel B); nitrite (2 mM), FD (1 mM), and nitrogen headspace (panel C); FD (1 mM) and air headspace (panel D); and FD (1 mM), nitrite (2 mM), and nitrogen headspace, which was replaced with air after 7 days (panel E). The data represent the averages of four replicate cultures, and the error bars represent the standard deviations.

oxidized to a carboxylic acid to undergo $\beta$-oxidation or channeled into anabolic pathways. Analogously, the initial attack of a monooxygenase on the terminal fluoromethyl group carbon would generate an unstable geminal fluorohydrin that would spontaneously eliminate fluoride to form the corresponding aldehyde, which would be further oxidized to the corresponding carboxylic acid.[19,61] No monooxygenase enzyme system of strain 273 has been characterized, and it is unknown whether the enzyme(s) has (have) a preference for the terminal methyl group carbon or the fluoromethyl group carbon. For both FD and DFD, $\beta$-oxidation could lead to the formation of fluoroacetyl-CoA, which is potentially toxic (Figure 5). Utilization of fluoroacetyl-CoA by the tricarboxylic acid (TCA) cycle would lead to the formation of fluorocitrate, a known inhibitor of aconitase, a key TCA cycle enzyme.[62−64] Although MFA did not support growth of strain 273, some fluoride release was observed with decane serving as the primary substrate, suggesting that strain 273 has inherent mechanisms to defluorinate MFA and potentially other $\omega$-fluorofatty acids (or the respective CoA thioesters).

Theoretically, both terminal carbon atoms of the alkane could be attacked by the monooxygenase,[19,65] which would transform FD and DFD to the corresponding dicarboxylates. Such a mechanism would avoid the formation of fluoroacetyl-CoA. A purified monooxygenase from the alkane degrader *Pseudomonas oleovorans* could not only hydroxylate *n*-alkanes to the corresponding primary alcohols but also convert fatty acids to their $\omega$-hydroxy derivatives,[66,67] suggesting that attacks on both ends of the alkane molecule by the same monooxygenase enzyme system are feasible. However, the GC−HRMS analysis provided no evidence for the formation of $C_4$ to $C_{10}$ dicarboxylic acids, suggesting that an attack on both ends of the molecule did not occur (Figure 5).

**Metabolic Burden of (Fluoro)Alkane Degradation.** Strain 273 attained approximately 3-fold more biomass per $\mu$mol of carbon utilized with decanoate or sebacate compared to decane as a carbon substrate (Table 2). Even lower growth yields were measured in cultures grown with halogenated alkanes, which declined about 50% with DFD and DCD compared to the yield with decane (Table 2). The standard Gibbs free energy changes associated with complete oxidation of decane, halogenated $C_{10}$ alkanes, decanoate, and sebacate are in a similar range (Table S5); thus, thermodynamics alone cannot explain the measured growth yield differences. Possible explanations for the lower growth yields with decane and (halo)alkanes compared to the corresponding fatty acids may include the required synthesis of the monooxygenase enzyme system(s) and/or the production of a biosurfactant(s) needed for emulsifying the sparingly soluble (halo)alkanes. Strain 273 efficiently solubilizes water-insoluble alkanes with a yet uncharacterized biosurfactant(s).[49] The production of biosurfactants by prolific oil hydrocarbon-degrading bacteria is a well-studied phenomenon, and several *Pseudomonas* spp. were shown to produce rhamnolipids to emulsify hydrocarbons with a low solubility in water.[68−70] Conversely, growth with water-soluble decanoate and sebacate does not burden the cells with the synthesis of the monooxygenase enzyme system and biosurfactant(s). This reasoning cannot explain the lower growth yields with FD and DFD compared to cultures that were grown with decane. The GC−HRMS analysis detected $\omega$-fluorofatty acids as intermediates during growth with both FD and DFD. $\omega$-Fluorofatty acids are precursors of potentially inhibitory fluoroacetyl-CoA. For FD, preferential initial oxidation of the fluoromethyl carbon would avoid the formation of $\omega$-fluorinated intermediates and fluoroacetyl-CoA. In this case, cultures fed with FD or decane should exhibit similar growth yields; however, the experimental data determined about 23.4 ± 4.7% lower growth yields with FD. This observation suggests that the monooxygenase may not discriminate between the $\alpha$ and the $\omega$ positions of FD and oxidizes both the methyl and the fluoromethyl group carbon atoms. In the case of DFD, the initial attack must occur on one of the two fluoromethyl groups, and the formation of fluorinated metabolites (e.g., $\omega$-


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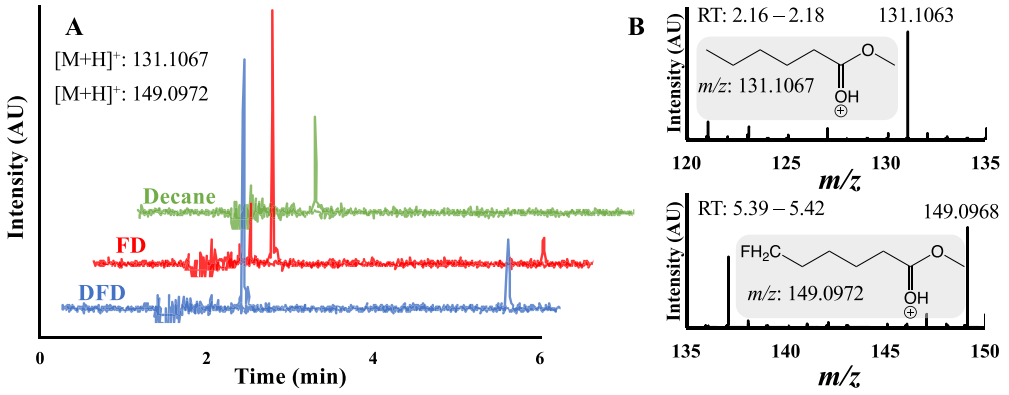


**Figure 4.** Detection of fluorohexanoate as an intermediate of FD and DFD metabolism in strain 273. (A) Merged extracted ion chromatograms of methyl hexanoate ($[M + H]^+ = 131.1067\ m/z$) and methyl fluorohexanoate ($[M + H]^+ = 149.0972\ m/z$) in cultures of strain 273 grown with decane, FD, or DFD. (B) GC−HRMS identification of methyl hexanoate and methyl fluorohexanoate with a mass accuracy tolerance of 3 ppm to the monoisotopic mass of the putative structure illustrated.

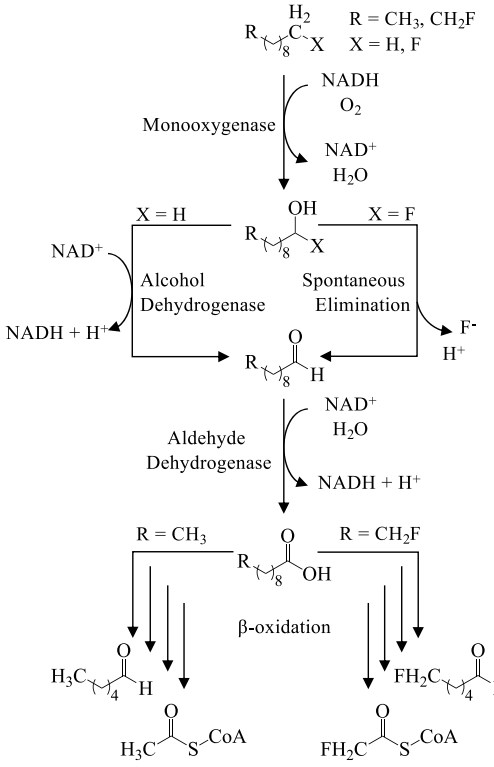


**Figure 5.** Proposed pathways for decane, FD, and DFD catabolism in *Pseudomonas* sp. strain 273. Fluorohexanoate and hexanoate were identified by GC−HRMS analysis and are shown as intermediates.

fluorofatty acids and fluoroacetyl-CoA) could explain the observed reduction in growth yields with DFD (Table 2). Fluoride toxicity offers another potential explanation for the lower growth yields with FD and DFD, which would only occur during growth with fluorinated electron donors.[71]

**Implications for the Environmental Fate of Fluorinated Compounds.** Knowledge about the presence and the distribution of organofluorines in environmental systems is rapidly evolving, and an improved understanding of the processes that affect the fate and longevity of fluorinated compounds is needed. Physical,[45] chemical,[43] and biological[72,73] approaches, as well as combined treatments,[74] have been shown to transform PFAS and generate partially defluorinated alkyl products. The data herein demonstrate that $\alpha,\omega$-fluorinated alkanes are susceptible to microbial degradation, and organisms with defluorinating oxygenase enzyme systems may contribute to the degradation of AFFF components and partially defluorinated PFAS transformation products (i.e., compounds with nonperfluorinated carbon atoms) generated during aggressive remedial treatment. Therefore, future efforts should explore whether organisms like *Pseudomonas* sp. strain 273 have value in sequential treatment (i.e., combined remedy) approaches and achieve more complete fluoride release.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c04029.

Detailed experimental procedures including protein measurements, cell enumeration with qPCR, derivatization of decanoate, sebacate, and FAME analysis, and supplemental analytical procedures; (Table S3) electron recovery in strain 273 cultures grown with nitrite or nitrous oxide as electron acceptors; (Table S4) FAME metabolites detected in cultures of strain 273 grown with decane, FD, or DFD; (Figure S1) linear regression of $OD_{600nm}$ readings versus protein concentration measurements; (Figure S2) cometabolism of MFA with decane as the primary substrate by strain 273; (Figure S3) Pearson correlation between cells produced and protein yields per $\mu$mol of carbon consumed during growth of *Pseudomonas* sp. strain 273 with different carbon substrates (PDF)
(Table S1) Solubility and *n*-octanol/water partition coefficient data for the carbon substrates tested, (Table S2) oxygen demand for complete oxidation of different carbon substrates, and (Table S5) Gibbs free energy changes associated with the complete oxidation of different carbon substrates (XLSX)

## ■ AUTHOR INFORMATION

### Corresponding Author

**Frank E. Löffler** − *Department of Civil and Environmental Engineering, Center for Environmental Biotechnology, Department of Microbiology, and Department of Biosystems Engineering and Soil Science, University of Tennessee, Knoxville, Tennessee 37996, United States; Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States;* orcid.org/0000-0002-



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9797-4279; Phone: (865) 974-4933; Email: frank.loeffler@utk.edu

## Authors

**Yongchao Xie** − *Department of Civil and Environmental Engineering and Center for Environmental Biotechnology, University of Tennessee, Knoxville, Tennessee 37996, United States; Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, United States*

**Gao Chen** − *Department of Civil and Environmental Engineering and Center for Environmental Biotechnology, University of Tennessee, Knoxville, Tennessee 37996, United States*

**Amanda L. May** − *Center for Environmental Biotechnology, University of Tennessee, Knoxville, Tennessee 37996, United States*

**Jun Yan** − *Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, Liaoning 110016, China*

**Lindsay P. Brown** − *Department of Chemistry, University of Tennessee, Knoxville, Tennessee 37996, United States*

**Joshua B. Powers** − *Department of Chemistry, University of Tennessee, Knoxville, Tennessee 37996, United States*

**Shawn R. Campagna** − *Department of Chemistry and Biological and Small Molecule Mass Spectrometry Core, University of Tennessee, Knoxville, Tennessee 37996, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.0c04029

## Notes

The authors declare no competing financial interest.

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