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# Biotransformation of HCFC-22, HCFC-142b, HCFC-123, and HFC-134a by methanotrophic mixed culture MM1

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## Abstract

This research investigated the potential for methanotrophic biotransformation of three HCFCs – chlorodifluoromethane (HCFC-22); 1-chloro-1,1-difluoroethane (HCFC-142b); and 1,1-dichloro-2,2,2-trifluoroethane (HCFC-123); and one HFC – 1,2,2,2-tetrafluoroethane (HFC-134a). All of these compounds were biotransformed to differing degrees by methanotrophic mixed culture MM1. Rates of transformation were obtained by monitoring disappearance of the target compounds from the headspace in batch experiments. Henry's constants were determined over a range of conditions to enable estimation of the intrinsic rates of transformation. Intrinsic rates of transformation were obtained by combining a second order rate expression with an expression describing loss of transformation activity due to either endogenous decay or product toxicity. For HCFC-123 and HFC-134a, the independently measured endogenous decay rate for mixed culture MM1 (0.594/day) was sufficient to account for the observed loss of transformation activity with time. However, the endogenous decay rate did not account for the loss of transformation activity for HCFC-22 and HCFC-142b. A model based on product toxicity provided a reasonable representation of the loss of transformation activity for these compounds. The order of reactivity was HCFC-22 > HCFC-142b > HFC-134a > HCFC-123, with second order rate coefficients of 0.014, 0.0096, 0.00091, and 0.00054 l/mg-day, respectively. Transformation capacities for HCFC-22 and HCFC-142b were 2.47 and 1.11 $\mu$g substrate/mg biomass, respectively.

## Introduction

Chlorofluorocarbons (CFCs) are widely used refrigerants and aerosols in industry and domestic life. Over the past decade, they have been implicated as agents of depletion of stratospheric ozone and as contributors to global warming (Molina & Roland 1974; Rowland & Molina 1975). As a result, worldwide production of CFCs will be banned under the terms of Montreal Protocol. Nevertheless, CFCs will continue to be released into the environment due to past production and continued use. In aerobic aquatic environments, CFCs are recalcitrant, but they are transformed anaerobically (Lesage et al. 1990; Denovan & Strand 1992; Lovley & Woodward 1992; Lesage et al. 1992; Semprini et al. 1992). The ban on CFCs has inspired a major research effort to assess two classes of CFC substitutes – the hydrochlorofluorocarbons (HCFCs) and the hydrofluorocarbons (HFCs). HCFCs and HFCs are one- and two-carbon aliphatics, similar in structure and physical properties to the CFCs, but containing one or more hydrogen atoms. The presence of hydrogen makes HCFCs and HFCs more susceptible to tropospheric oxidation than the CFCs, and thus less likely to migrate into the stratosphere. To date, there is relatively little information on the fate of HCFCs or HFCs in aquatic environments. Lesage et al. (1992) reported transformation of HCFC-123a to HCFC-133 and HCFC-133b under methanogenic conditions. DeFlaun et al. (1992) reported aerobic transformation of three HCFCs and one HFC by *Methylosinus trichosporium* OB3b.

In this article, we report on the methanotrophic biotransformation of three HCFCs (HCFC-22:

chlorodifluoromethane, HCFC-142b: 1-chloro-1,1-difluoroethane, and HCFC-123: 1,1-dichloro-2,2,2-trifluoroethane) and one HFC (HFC-134a: 1,2,2,2-tetrafluoroethane) in a defined methanotrophic mixed culture. The headspace method was used to monitor disappearance of target compounds. To obtain intrinsic kinetic data using this method, Henry's constants are needed. Because these constants were not available for the compounds studied, we first measured Henry's constants, then monitored disappearance of the target compounds in methanotrophic mixed cultures. Henry's constants were measured over a range of ionic strengths to enable use of these measurements in environments beyond those of the present study, such as seawater. Knowledge of methanotrophic transformations should assist in selecting environmentally acceptable HCFCs and HFCs, modeling environmental fate, developing treatment technologies for fugitive manufacturing emissions, and remediating future wastewater and groundwater contamination.

## Materials and methods

### *Chemicals*

HCFC-22, HCFC-142b and HFC-134a were obtained from Asahi Glass Co., Ltd. (Yokohama, Japan). HCFC-123 was obtained from Allied-Signal, Inc. (Morristown, NJ, USA). The properties and purity of these chemicals are summarized in Table 1. All chemicals used in media preparation were ACS grade, and all water used was 18 megaohm resistance or greater.

### *Analytical techniques*

The study compounds were analyzed by withdrawing 0.5 ml of headspace from the test bottles using a Precision gas-tight syringe and injecting the sample onto a Perkin Elmer 8500 Gas Chromatograph (GC) equipped with a squalene packed column and a flame ionization detector. The GC was operated isothermally at 90° C with helium as carrier. Concentrations were obtained from an external standard calibration curve bracketing the concentration range of interest.

### *Measurement of Henry's constants*

The modified EPICS procedure (Gossett 1987) was used to determine Henry's constants for each of the target compounds. Pure compounds were dissolved in methanol as stock solutions. To examine possible cosolvent interferences, five serum bottles with same amounts of HCFC-134 but with different methanol content (0 to 5%) were prepared. The result showed no significant cosolvent effect for methanol levels below 2%. Therefore, all subsequent measurements were conducted under this condition. For each compound, Henry's constant was measured in six 158.8 ml serum bottles: three containing 100 milliliters of distilled water, and three containing 25 milliliters. Both sets of bottles were sealed with Teflon/rubber septa and aluminum crimp caps. HCFCs/HFC solutions were injected into each bottle using a 0.5 ml gas-tight syringe. The bottles were then incubated at inverted position for 24 hrs at the desired temperature (6, 12, 22, 30 and 40° C, all $\pm$ 0.2° C) on a temperature-controlled shaker, and headspace samples were analyzed by gas chromatography. To assess the effects of ionic strength on the Henry's constant, six serum bottles were filled with 100 ml solution, each with different concentration of KCl (0, 0.2, 0.4, 0.6, 0.8, 1.0 M). These bottles were then analyzed by headspace gas chromatography.

### *Culture conditions*

Mixed culture MM1, a methanotrophic enrichment obtained from aquifer material at Moffett Field, California, was used for these experiments (Henry & Grbić-Galić 1991). This culture is a stable consortium consisting of one methanotroph and three or four heterotrophs containing predominantly Grain-negative pleomorphic coccobacilli and prosthecates as well as some Grain-negative bacilli and cocci. The methanotroph in the mixed culture expresses soluble MMO similar to that of *Methylosinus trichosporium* OB3b under similar growth conditions (Henry & Grbić-Galić 1991).

Mixed culture MM1 was grown in Whittenbury Mineral Medium containing (per liter of deionized water): 1.0 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of $KNO_3$, 200 mg of $CaCl_2 \cdot 2H_2O$, 3.8 mg of FeEDTA, 0.5 mg of $Na_2MoO_4 \cdot 2H_2O$, 0.5 mg of $FeSO_4 \cdot 7H_2O$, 0.4 mg of $ZnSO_4 \cdot 7H_2O$, 0.02 mg of $MnCl_2 \cdot 4H_2O$, 0.05 mg of $CoCl_2 \cdot 6H_2O$, 0.01 mg of $NiCl_2 \cdot 6H_2O$, 0.015 mg of $H_3BO_3$, 0.25 mg of EDTA, 260 mg of $KH_2PO_4$, and 330 mg of $Na_2HPO_4$. One liter of culture was grown at room temperature ($\sim$ 21° C) in a continuously stirred 4-liter bottle supplied 30% methane in air at 68 ml/min. Growth curves were monitored and as stationary phase approached, approximately 10 ml of culture was transferred to a 1 liter of fresh Whittenbury Medium. Cells

*Table 1.* Properties and purity of HCFCs and HFC evaluated in this work.

| Compound | Chemical name | Solubility in water, wt% @ 25° C | Boiling point, ° C, @ 760 mm Hg | Density g/cm$^3$ @ 25° C | Purity % |
|---|---|---|---|---|---|
| HCFC-22 | chlorodifluoromethane | 0.30 | - 40.8 | 1.194 | 99.9788 |
| HCFC-142b | 1-chloro-1,1-difluoroethane | 0.14 | - 9.2 | 1.108 | 99.9609 |
| HCFC-123 | 1,1-dichloro-2,2,2-trifluoroethane | 0.21* | 27.9 | 1.48* | ** |
| HFC-134a | 1,2,2,2-tetrafluoro-ethane | ** | - 26.2 | 1.206 | 99.8483 |

* @ 21.1° C.
* Data unavailable from manufacturers.

*Table 2.* Measured values of Henry's constant vs. temperature.

| Compound | Temperature ° C | Hc (-) | H m$^3$ · atm/mol | CV* % |
|---|---|---|---|---|
| HCFC-22 | 6 | 0.622 | 0.0142 | 8.00 |
| | 12 | 1.277 | 0.0298 | 5.36 |
| | 22 | 1.679 | 0.0406 | 5.57 |
| | 30 | 2.358 | 0.0586 | 3.87 |
| | 40 | 3.535 | 0.0907 | 1.99 |
| HCFC-142b | 6 | 1.390 | 0.0318 | 4.33 |
| | 12 | 1.749 | 0.0409 | 8.24 |
| | 22 | 2.432 | 0.0588 | 4.75 |
| | 30 | 3.213 | 0.0798 | 2.24 |
| | 40 | 3.926 | 0.101 | 2.83 |
| HCFC-123 | 6 | 0.571 | 0.0131 | 3.26 |
| | 12 | 0.825 | 0.0193 | 2.96 |
| | 22 | 1.057 | 0.0256 | 4.26 |
| | 30 | 1.463 | 0.0364 | 5.02 |
| | 40 | 1.979 | 0.0508 | 1.19 |
| HFC-134a | 6 | 1.190 | 0.0272 | 5.92 |
| | 12 | 1.528 | 0.0357 | 2.47 |
| | 22 | 2.067 | 0.0500 | 0.99 |
| | 30 | 2.199 | 0.0546 | 3.86 |

* Percent coefficient of variation = 100 (SD/mean). Triplicate measurements were performed for each compound and temperature.

were harvested in mid-log growth phase for biotransformation experiments.

*Batch biotransformation experiments*

HCFCs/HFC degradation studies were performed using 158.8 ml serum bottles sealed with Teflon/rubber septa and aluminum crimp caps. These bottles were incubated with 100 ml of Whittenbury Mineral Media plus culture. Appropriate amount (measured as dry weight) of mixed culture MM1 was added to each test bottle. Some bottles were autoclaved after cell addition (autoclaved cell controls) and others were filled with 100 ml pure water (water controls). HCFCs or HFC solutions (dissolved in water) were added to each bottle using Precision gas tight syringes, then vigorously shaken upside-down on a rotary shaker (250 rpm). Headspace samples were periodically analyzed by GC as described previously.

*Modeling transformation of HCFCs/HFCs*

To quantify the cometabolic transformation of HCFCs and the HFC studied, a second order rate expression was combined with an expression describing loss of activity due to endogenous decay (b) and product toxicity (q/T):

$$q = k' C_L \tag{1}$$

$$\mu = \frac{dX/dt}{X} = -b - \frac{q}{T} \tag{2}$$

where:

q = specific rate of transformation (mg substrate/mg cell-d)
$k'$ = second order rate coefficient (l/mg cell-d)
$C_L$ = liquid phase concentration of the substrate (mg/L)
$\mu$ = specific growth (or decay) rate ($d^{-1}$)
X = active organism concentration (mg/l)
b = endogenous decay coefficient ($d^{-1}$)

T = theoretical or true biomass transformation capacity (mg substrate/mg cell)

The endogenous decay term b includes loss of activity caused by cell death and by depletion of reducing power required for monooxygenase activity. A more extensive discussion of these processes and of equations 1 and 2 is provided by Criddle (1993). For batch transformation of a volatile cometabolic substrate, a mass balance at equilibrium gives:

$$-\frac{dM}{dt} = qXV_L \quad (3)$$

$$M = C_L(V_L + H_c V_G) \quad (4)$$

where:
M = mass of substrate (mg)
$H_c$ = Henry's constant (-)
$V_L$ = liquid volume (L)
$V_G$ = gas volume (L)

Batch cometabolic transformation can be described by equations 1–4. These equations can be solved simultaneously using a Runge-Kutta algorithm. Two simplifying cases should be noted. The first occurs when product toxicity is absent or insignificant, b>>q/T and $\mu$ = - b. For this case, equations 1–4 can be combined and integrated to give the mass of substrate M as a function of time:

$$M = M_o e[\frac{k'AX_o}{b}(e^{-bt} - 1)], \quad (5)$$

where:
$M_o$ = initial mass of substrate (mg)
$X_o$ = initial active biomass concentration (mg/l)
$A = \frac{V_L}{V_L + H_c V_G}$

The second simplifying case is also obtained when product toxicity is the dominant factor causing loss of transformation activity. For this case, q/T>>b, and $\mu$ = q/T, and equations 1–4 can be combined and integrated to give:

$$M = M_o \frac{F\exp(-k'AFt)}{X_o - \frac{C_{Lo}}{T}\exp(-k'AFt)} \quad (6)$$

where:
$C_{Lo}$ = initial concentration of substrate in the aqueous phase (mg/l)
$F = X_o - C_{Lo}/T$

*Table 3.* Temperature regression for Henry's constant.*

| Compound | H = exp (A-B/T) | | |
|---|---|---|---|
| | A | B | $r^2$ |
| HCFC-22 | 11.66 | 4387 | 0.956 |
| HCFC-142b | 7.363 | 3011 | 0.995 |
| HCFC-123 | 7.805 | 3373 | 0.990 |
| HFC-134a | 5.714 | 2588 | 0.979 |

* Computed using values given in Table 2. Units of Henry's constant are $m^3$-atm/mole; T is in degrees Kelvin.

*Table 4.* Salting-out coefficients (22° C).*

| Compound | log $\gamma$ = kI | |
|---|---|---|
| | k, L/mole | $r^2$ |
| HCFC-22 | 0.118 | 0.996 |
| HCFC-142b | 0.0838 | 0.960 |
| HCFC-123 | 0.0860 | 0.997 |
| HFC-134a | 0.0761 | 0.972 |

* Based upon measurements from 0 to 1.0 M KCl solution. Salting-out coefficients were determined by plotting $\log_{10}$ (activity coefficient) vs. ionic strength: $\log_{10}\gamma$ = kI where: $\gamma$ = activity coefficient (-), k = salting-out coefficient (L/mole), I = ionic strength (M).

Disappearance of the target compounds was modeled with both equations 5 and 6. Kinetic parameters were estimated by nonlinear regression using Systat 5.1 (Systat, Inc.). For all modeling with equation 5, an endogenous decay rate b of 0.594/day was assumed. This value was independently obtained by Clowater (1991) for loss of trichloroethylene (TCE) transformation activity in aerated batch cultures of mixed culture MM1. Cultures of MM1 were aerated in the absence of methane and periodically assayed to determine the TCE transformation rate. The endogenous decay coefficient b was then computed as the slope taken from a plot of the logarithm of specific TCE transformation rate vs. aeration time.

## Results

### *Henry's Law constants*

Measured Henry's Law constants are provided in Table 2, along with coefficients of variation. With two

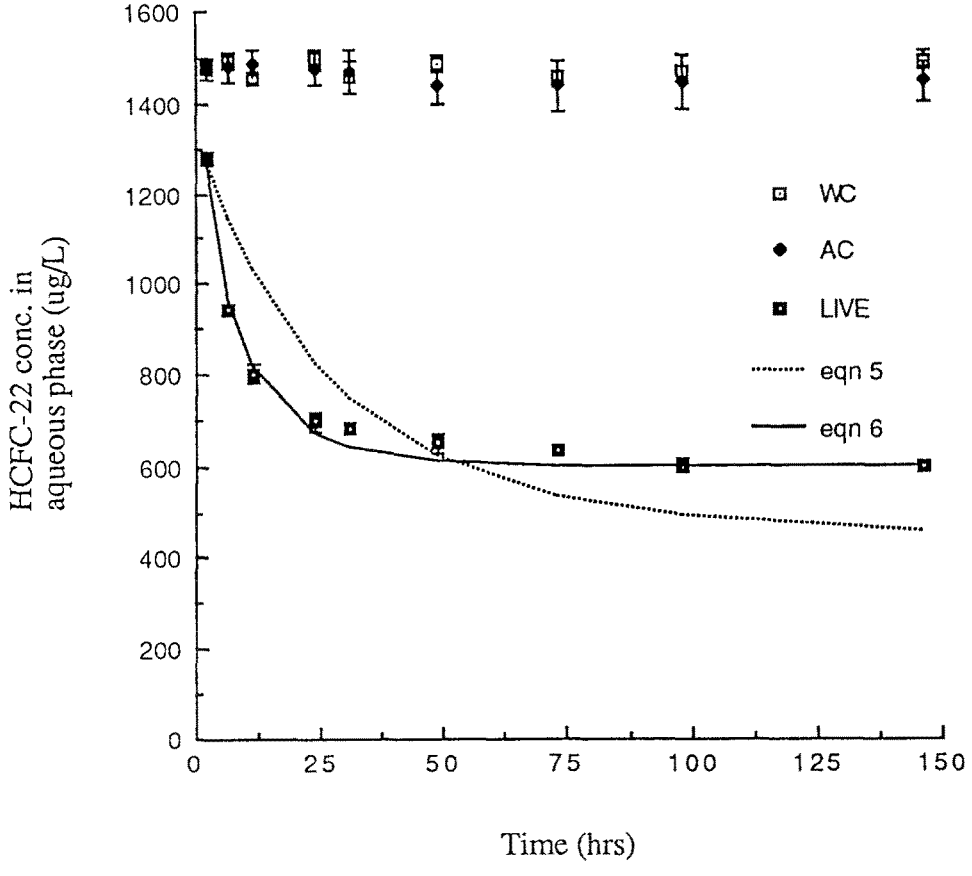


*Fig. 1.* Biotransformation of HCFC-22 by methanotrophic mixed culture MM1. Fitting parameters are summarized in Tables 5 and 6. Error bars give standard deviations for three samples. WC = water control (no cells), AC = autoclaved control, LIVE = 275 mg/l MM1 (dry weight).

*Table 5.* Kinetic coefficients for HCFC/HFC transformation by methanotrophic mixed culture MM1. Best fit for the parameters of equation 5: comparison with TCE.

| Compound | b ($day^{-1}$) | k′ (l/mg-day)[a] | Correlation coefficient $r^2$ |
|---|---|---|---|
| HCFC-123 | 0.594[b] | 0.00054 ± 0.00014 | 0.888 |
| HFC-134a | 0.594[b] | 0.00091 ± 0.00002 | 0.915 |
| HCFC-142b | 0.594[b] | 0.0030 ± 0.0002 | 0.580 |
| HCFC-22 | 0.594[b] | 0.0043 ± 0.0001 | 0.611 |
| TCE | 0.594[b] | 1.4 ±0.23[c] | 0.998[c] |

[a] Determined by triplicate samples at 95% confidence interval.
[b] Independently determined by Clowater (1992).
[c] Clowater (1992).

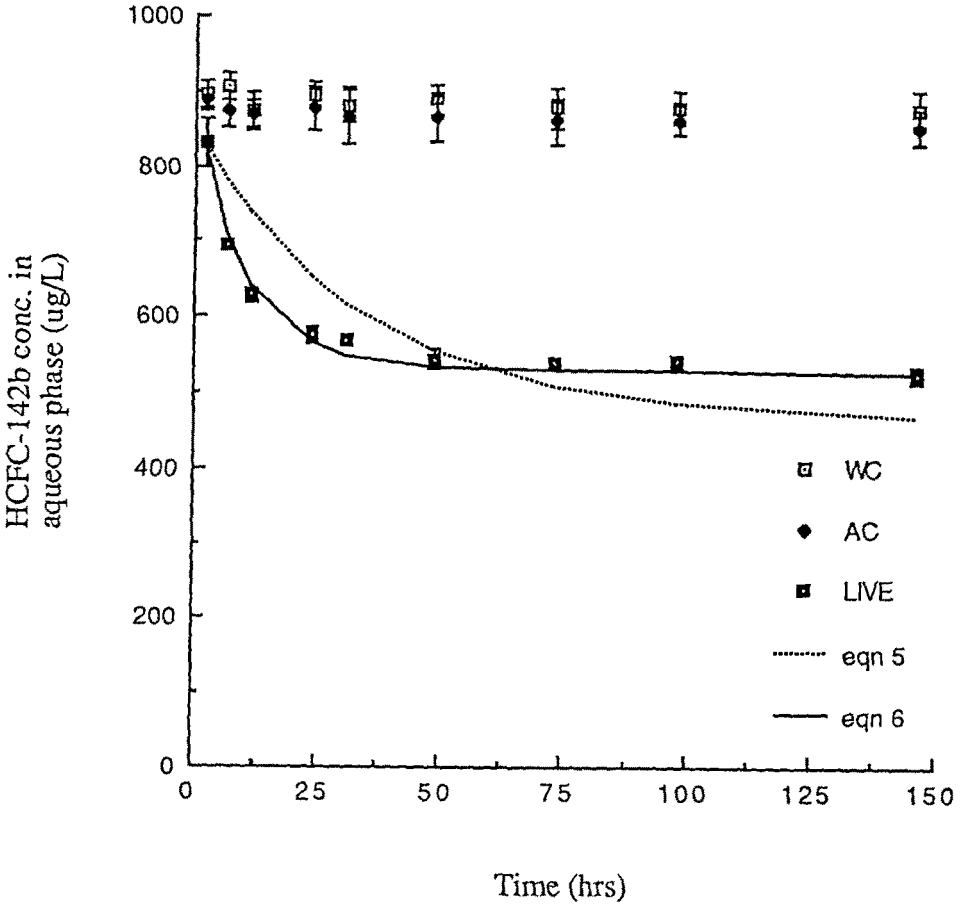


*Fig. 2.* Biotransformation of HCFC-142b by methanotrophic mixed culture MM1: model fit based on equation 5 and 6, respectively. Fitting parameters are summarized in Tables 5 and 6. Error bars give standard deviations for three samples. WC = water control (no cells), AC = autoclaved control, LIVE = 275 mg/l MM1.

*Table 6.* Kinetic coefficients for HCFC/HFC transformation by methanotrophic mixed culture MM1. Best fit for the parameters of equation 6: comparison with TCE.

| Compound | T ($\mu$g substrate/mg cell)[a] | k′ (l/mg-day)[b] | Correlation coefficient $r^2$ |
|---|---|---|---|
| HCFC-123 | 1.166 ± 0.264 | 0.00090 ± 0.00011 | 0.934 |
| HFC-134a | 1.636 ± 0.051 | 0.0011 ± 0.0001 | 0.937 |
| HCFC-142b | 1.113 ± 0.068 | 0.0096 ± 0.0016 | 0.981 |
| HCFC-22 | 2.468 ± 0.050 | 0.014 ± 0.002 | 0.984 |
| TCE | 47 ±0[c] | 1.33 ± 0.24[c] | 0.997[c] |

[a,b] Determined by triplicate samples at 95% confidence interval.
[c] Clowater (1992).

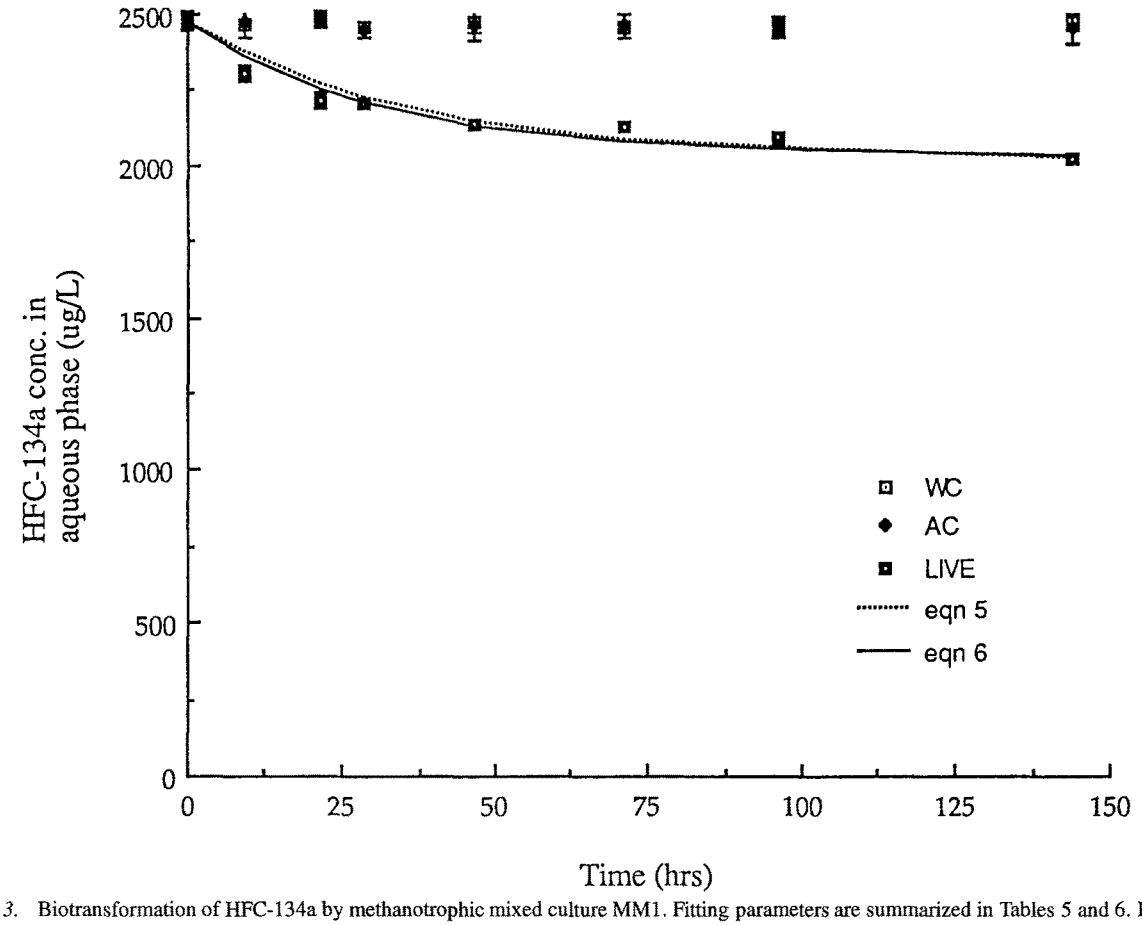


*Fig. 3.* Biotransformation of HFC-134a by methanotrophic mixed culture MM1. Fitting parameters are summarized in Tables 5 and 6. Error bars give standard deviations for three samples. WC = water control (no cells), AC = autoclaved control, LIVE = 275 mg/l MM1.

exceptions, all coefficients of variation were less than 6%. The effects of temperature on Henry's constant followed the van't Hoff relationship (Gossett 1987). Results from a linear regression of ln H vs. $T^{-1}$ (H in $m^3$-atm/mole; T in K) are provided in Table 3. Salting-out coefficients are listed in Table 4. Henry's constants were relatively insensitive to salinity. For the most sensitive compound studied (HCFC-22), the ionic strength must exceed 0.35 M to cause a greater than 10% increase in the apparent Henry's constant.

*HCFCs/HFC transformation rates*

Figures 1–4 illustrate the methanotrophic transformation of the target compounds. All four compounds were degraded to different degrees over the concentration range studied (900–3000 $\mu$g/l). Model fits obtained using equations 5 and 6 are also illustrated in Figs 1–4. Estimates for the kinetic parameters used to describe the transformation of each compound are summarized in Tables 5 and 6.

## Discussion

Different degrees of transformation were obtained for the compounds studied. DeFlaun et al. (1992) reported that HCFC-123, HCFC-142b, HCF-134a were not degraded by the pure culture *Methylosinus trichosporium* OB3b. In our study, HCFC-123 and HFC-134a degraded slowly, while HCFC-142b was transformed at a somewhat higher rate by mixed culture MM1. A possible explanation for the difference between this work and the DeFlaun study is that the MM1 methanotroph may possess enzymes with greater reactivity toward HCFCs and HFCs. It is also possible that the heterotrophs present in mixed culture MM1 facilitated transformation. Uchiyama (1992) found that TCE

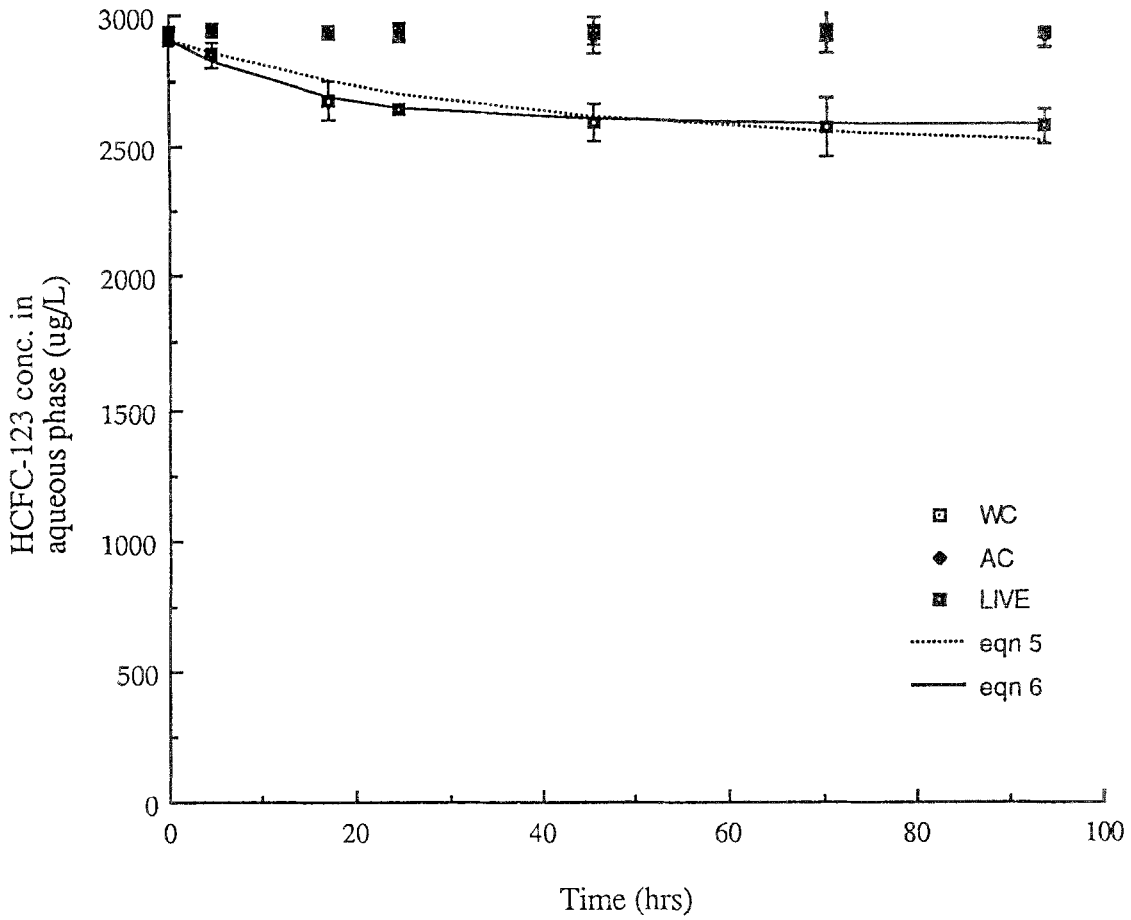


*Fig. 4.* Biotransformation of HCFC-123 by methanotrophic mixed culture MM1. Fitting parameters are summarized in Tables 5 and 6. Error bars give standard deviations for three samples. WC = water control (no cells), AC = autoclaved control, LIVE = 275 mg/l MM1.

was mineralized to a greater extent by a mixed culture containing heterotrophs.

Pathways of transformation for the compounds evaluated in this study are not known. Presumably, in each case, oxygen is inserted at the carbon-hydrogen bond yielding an alcohol intermediate. Halogenated alcohols undergo further hydrolysis and elimination in aqueous systems giving rise to a variety of products. For HFC-134a, one of the possible products is trifluoroacetic acid. Trifluoroacetic acid is thought to be stable in aqueous environments, and it is an expected oxidation product in the troposhpere. Analysis of the culture medium by ion chromatography after completion of HFC-134a transformation revealed a peak at a run time corresponding to that of trifluoroacetic acid. Additional analysis is needed to confirm this tentative identification.

For two of the target compounds – HCFC-123 and HFC-134a, use of equation 5 and the independently measured endogenous decay rate of 0.594 $day^{-1}$ provided a reasonable fit to the data. Thus, for these compounds, there is no reason to invoke another mechanism besides endogenous decay (such as product toxicity) to explain the loss of transformation activity with time. It should be noted, however, that the model based on product toxicity (equation 6) also fit the data well, indicating that conclusions about the mechanism for loss of transformation activity cannot be based on model fit alone.

As shown in Table 5, equation 5 provided a poor fit for HCFC-22 and HCFC-142b indicating that, for these compounds, another mechanism (besides endogenous decay) apparently contributes to the loss of transformation activity with time. As shown in Figs 1 and 2, equation 6 provided a good fit to these data. Thus, product toxicity may explain the loss of transformation activity for these compounds.

To assist in the interpretation of data, we compared the transformation of the targeted fluorocarbons with trichloroethylene (TCE). TCE is a useful bench mark for comparison because many researchers have evaluated methanotrophic transformation of TCE, and there is an extensive dataset on its transformation kinetics. As indicated by Table 6, rates of transformation for all of the fluorinated compounds studied were considerably slower than rates of transformation for TCE. Second order rate coefficients were 100 to 1000 times smaller for mixed culture MM1. Transformation capacities for HCFC-142b and HCFC-22 were ten to twenty times smaller than the values reported for TCE. The rapid loss of activity for HCFC-22 seems reasonable inasmuch as this compound is structurally similar to chloroform, a compound previously known to exhibit product toxicity in methanotrophic mixed cultures (Alvarez-Cohen & McCarty, 1991). For chloroform, the toxic byproduct is believed to be carbonyl chloride (phosgene). An analogous carbonyl may be formed from HCFC-22. Alvarez-Cohen and McCarty (1991) reported a chloroform transformation capacity of 6.5 $\mu$g/mg cell, a value somewhat higher than that observed for HCFC-22.

The results of this work suggest that methanotrophic transformation is not likely to be a significant sink for the removal of HFCs and HCFCs globally. As indicated in Table 5, the fastest second order rate observed in this study was 0.014 l/mg cell-day for HCFC-22. Typical microbial densities in the soil and marine environment are on the order of $10^6$–$10^7$ organisms per $cm^3$ of soil or water. Assuming that active methanotrophs constitute about 1% of these communities, first order rates of $10^{-10}$ to $10^{-9}$ $s^{-1}$ would be possible for HCFC-22. In order to have a significant affect (> 2–3%) on global lifetime estimates, first order rates exceeding $10^{-8}$ $s^{-1}$ are required (Rodriguez et al. 1991).

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