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# Degradation of Hydrohalocarbons and Brominated Compounds by Methane- and Propane-Oxidizing Bacteria

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Concern over the use of chemicals that potentially contribute to ozone depletion and the greenhouse effect has led to a ban on chlorofluorocarbons (CFCs) and potential limitations on the use of methyl bromide and other brominated fumigants. The objective of this work was to test naturally occurring aerobic microorganisms for the ability to degrade brominated fumigants and the hydrohalocarbons that have been developed to replace CFCs. The bacterial strains used in this study degraded a significant percentage of the hydrohalocarbons and brominated fumigants tested, which were present at high substrate levels. Degradation was often rapid and complete. Ion chromatography results indicated the stoichiometric release of halogens for several of the compounds tested. Many of the compounds were readily biodegradable at high concentrations and were metabolized by all of the strains tested, while others were more recalcitrant. The presence of these microorganisms in the environment may represent a natural sink for some of these compounds; however, the high substrate and bacterial concentrations used in this study were chosen for bioreactor development. Information about the biodegradability of these compounds may make it easier to choose the most ecologically safe CFC replacements and fumigants.

## Introduction

The hydrohalocarbons, developed to replace the ozone-depleting chlorofluorocarbons (CFCs), are not environmentally benign. The ozone-depletion potential (ODP) of the hydrochlorofluorocarbons (HCFCs), while about one-tenth that of the CFCs (*1*), is still a significant source of concern. The Clean Air Act Amendments of 1990 mandate that the use of HCFCs be discontinued by the year 2015 and that methyl bromide use be phased out by the year 2001 in the United States. Under the United Nations Montreal Protocol on Substances that Deplete the Ozone Layer, production of HCFCs in developed countries ceased in 1996. The protocol also imposes continual reductions in the use of HCFCs, leading to a complete ban on use by the year 2030 (*2*), although the European Union (EU) would like to see this landmark moved up to 2015 (*3*). The European Commission has proposed even stricter deadlines for the reduction and eventual ban on ozone-depleting substances, which would prohibit the use of HCFCs in new air conditioning and refrigeration units by 2001, in solvents by 2003, and in insulating foams by 2004 (*4*). The hydrofluorocarbons (HFCs), which are also being investigated as possible CFC replacements, have an ODP of zero (*5*). Nonetheless, the HFCs are greenhouse gases with global warming potential (GWP), and these and any ozone-depleting compounds are being regulated as to their use and emissions as well. The Kyoto Protocol to the United Nations Framework Convention on Climate Change calls for the "implementation and/or further elaboration of measures to limit and/or reduce emissions of greenhouse gases not controlled by the Montreal Protocol", including the HFCs (*6*). Systems to control the amount of these chemicals released into the atmosphere may help to extend the useful lifetime of these important compounds.

There is no known aerobic terrestrial, aquatic, or atmospheric sink for CFCs other than solar UV decomposition; in fact, most of the total amount released since the 1930s is still present in the atmosphere (*7*). Although two of the CFCs, CFC-11 and CFC-12, have both been found to be biodegradable anaerobically, the conditions under which degradation takes place are very limited and therefore may not represent a natural sink (*8, 9*). Questions regarding the photoreactivity of the hydrohalocarbons in the lower atmosphere and the resulting products of these reactions have resulted in studies that have sought to identify natural sinks for these potentially harmful products (*10*). Trifluoroacetate (TFA) is a common product of hydroxyl radical attack of HCFCs and HFCs. Concerns over the accumulation and potential toxicity of TFA due to deposition on the earth's surface have decreased as bacteria in both aerobic and anaerobic environments have been found to degrade this compound (*11*). If the hydrohalocarbons can be destroyed before they are released to the troposphere, potential products would be of little concern.

The European Union protocol includes phasing out methyl bromide use in Europe by 2001 instead of the year 2005 as was originally stipulated in the Montreal Protocol (*4*). Methyl bromide and other brominated fumigants are used worldwide to control agricultural pests such as weeds, arthropods, nematodes, fungi, and bacteria, making it integral to the cultivation of numerous agricultural products such as strawberries and tomatoes. Discontinuing methyl bromide use without any comparable replacement will not only affect the quality and availability of certain agricultural products but will also be detrimental to the economies of the nations and individuals who rely on the growth and sale of these products as sources of income. Recently, there has been concern over the potential for stratospheric ozone depletion resulting from the emissions of methyl bromide. It has been estimated that as much as 50% of organic bromide that reaches the stratosphere may be attributable to methyl bromide (*12*). Several factors are important for estimating the importance of anthropogenic methyl bromide in the depletion of stratospheric ozone. These include the amount that reaches the atmosphere, the atmospheric lifetime, and the efficiency with which bromine destroys ozone. Natural sinks for methyl bromide include dry deposition on soils, removal at ocean surfaces, and biological destruction (*13*). The fact that a majority of the anthropogenic methyl bromide is applied to soils by direct injection suggests that soil adsorption and biological destruction by soil microbes may be of primary importance in minimizing atmospheric releases.

In previous work, an aerobic methanotrophic bacterium, *Methylosinus trichosporium* OB3b, was shown to degrade three out of five HCFCs and one out of three HFCs tested.

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Complete destruction of these compounds was verified using ion chromatography to measure the amount of halogen ions released during catalysis (*14*). In the work described here, the ability of three bacterial strains and one mixed culture to degrade a suite of halogenated compounds was examined to determine the potential for developing bioreactors designed to treat air emissions of these compounds. For one of the strains tested, the efficiency of degradation following growth on different inducing substrates was examined, which will also be useful in designing the most effective and cost-efficient type of catalyst-based bioreactor system.

## Materials and Methods

**Bacterial Strains.** *Methylosinus trichosporium* OB3b was selected for this study due to its production of soluble methane monooxygenase (sMMO). This enzyme has previously been shown to degrade chlorinated compounds such as 1,1-dichloroethane, 1,2-dichloroethane (*15*), dichlorofluoromethane (HCFC 21, 14), and chloroform (*16*). The methanotrophs ENV2040 and ENV2041 were selected presupposing that their methane-oxidizing enzyme would have a similarly broad substrate range and therefore degrade the HCFCs, HFCs, and brominated compounds. ENV2040 is a mixed methanotrophic culture with two members enriched from agricultural soil. Strain ENV2041, an unidentified methanotroph, was also obtained from local agricultural soil. *Mycobacterium vaccae* JOB5, which produces propane monooxygenase (PMO), has been shown to catabolize the chlorinated groundwater pollutant 1,2-dichloroethylene (*17, 18*) as well as 1,1-dichloroethylene and vinyl chloride (*17*).

**Media and Growth Conditions.** *M. trichosporium* OB3b, ENV2040, and ENV2041 were grown in 2-L shake flasks containing 1 L of OB3b medium (*19*). The flasks were plugged with Teflon-coated rubber stoppers and sealed with Parafilm. A sterile 16-gauge needle was passed through the stopper, which was then attached to a sterile 0.22-$\mu$m syringe filter (Nalge Nunc International Corp., Rochester, NY) and a three-way valve. A total of 180 mL of methane gas was added to the cultures daily through the filter after first removing the existing headspace using a vacuum pump. The remaining headspace was filled with filtered room air. *M. vaccae* JOB5 was grown on ATCC medium 990 (*20*) for starter cultures and was then subcultured in BSM (*21*) supplemented with propane in the headspace as described above. All cultures were incubated on a rotary shaker at 200 rpm at 30 °C.

**Chemicals.** HCFC 22, HCFC 123, HCFC 131, HCFC 141, HCFC 141b, HCFC 142b, HFC 134, HFC 134a, and HFC 143 were obtained from PCR, Inc. (Gainesville, FL). Trichloroethylene (TCE), methyl bromide, and HCFC 21 were purchased from Aldrich (Milwaukee, WI); 1,2,-dibromoethylene was purchased from Lancaster Synthesis (Wyndham, NH); and HCFC 124 and HFC 143a were obtained from Dupont (Wilmington, DE). The full chemical names of these compounds are listed in Table 1. 2-Propanol was purchased from Mallinckrodt (Paris, KY), and acetone was obtained from Fisher Scientific (Fair Lawn, NJ).

**Bottle Assays.** Degradation experiments were performed in 50-mL serum vials with Teflon-lined crimp-sealed tops. Cells were suspended in 20 mL of 25 mM 3-(*N*-morpholino)-propanesulfonic acid (MOPS, Sigma Chemical Co., St. Louis, MO), pH 7.4, containing 20 mM formate. Negative controls consisted of MOPS buffer plus formate, the test compound without cells, and heat-killed cells in MOPS to monitor abiotic losses. To test for the presence and proper activity and function of the appropriate enzyme, subsamples of cells used in the bottle assay were incubated with trichloroethylene (TCE), a substance known to be degraded by both sMMO (*22, 15*) and PMO (*17*). Only those cultures that were shown to degrade TCE were used to assess HCFC and methyl bromide degradation (data not shown). Target compounds were added by injection through the septa, and the vials were shaken at 150 rpm at 22−25 °C for a minimum of 15 min prior to sampling. Bottle assays were used to test the degradation of the target compounds at concentrations ranging from 2 to 200 $\mu$M and concentrations of the biocatalyst ranging from $1 \times 10^8$ to $1 \times 10^9$ cells/mL.

TABLE 1. Chemical Names of the Compounds Tested and Gas Chromatograph Conditions for Analysis[a]

| abbreviation | chemical name | column temp (°C) | retention time (min) |
|---|---|---|---|
| | **Hydrochlorofluorocarbons** | | |
| HCFC 21 | dichlorofluoromethane | 150 | 1.5 |
| HCFC 22 | chlorodifluoromethane | | 2.0 |
| HCFC 123 | 2,2-dichloro-1,1,1-trifluoroethane | 35 | 2.1 |
| HCFC 124 | 2-chloro-1,1,1,2-tetrafluoroethane | 35 | 1.3 |
| HCFC 131 | 1,1,2-trichloro-2-fluoroethane | 150 | 2.3 |
| HCFC 141 | 1,2-dichlorofluoroethane | 150 | 2.8 |
| HCFC 141b | 1,2-dichloro-1-fluoroethane | 150 | 1.5 |
| HCFC 142b | 1-chloro-1,1-difluoroethane | 150 | 1.5 |
| | **Hydrofluorocarbons** | | |
| HFC 23 | trifluoroethane | | 2.0 |
| HFC 125 | pentafluoroethane | | 2.0 |
| HFC 134 | 1,1,2,2-tetrafluoroethane | | 2.4 |
| HFC 134a | 1,1,1,2-tetrafluoroethane | | 2.3 |
| HFC 143 | 1,1,2-trifluoroethane | | 1.9 |
| HFC 143a | 1,1,1-trifluoroethane | | 2.0 |
| | **Other Chlorinated Compounds** | | |
| cDCE | *cis*−1,2-dichloroethylene | 150 | 1.8 |
| tDCE | *trans*−1,2-dichloroethylene | 150 | 1.9 |
| TCE | trichloroethylene | 150 | 2.0 |
| | **Brominated Compounds** | | |
| MeBr | methyl bromide | 150 | 1.5 |
| DBE | 1,2-dibromoethylene | 150 | 2.3 |

[a] All methods had an injector temperature of 180 °C and a detector temperature of 300 °C.

**Gas Chromatographic Analysis.** Gas chromatography (GC) was performed using direct on-column injections of headspace. The concentrations of the target compounds were measured using an electron-capture detector (ECD) or a flame ionization detector (FID) in a Varian model 3400 GC (Varian Instrument Division, Walnut Creek, CA) equipped with a Supelco VOCOL fused silica capillary column (30 m, 0.53 mm i.d., 3.0 $\mu$m film thickness). Optimal GC conditions were determined for each individual compound prior to analysis (Table 1). Concentrations of the target compounds were calibrated by injecting standards and constructing a standard curve.

**Ion Chromatographic Analyses.** Ion chromatography and the release of chloride, fluoride, and bromide ions were used to indicate complete mineralization. Serum vial experiments were set up as described above and incubated with the target compound until there was no detectable target compound in the headspace of the vials. For these experiments, cells were initially grown in standard medium and then washed and suspended in halogen-free medium. After degradation was complete, cells were removed from the reaction mix by centrifugation, and the supernatant fractions were filtered through a 0.22-$\mu$m filter to ensure complete removal of the cells and cellular material prior to injection. Dehalogenation was measured by evaluating the stoichiometry of chloride, fluoride, and bromide release with an ion chromatography system developed for inorganic separation. Anion analysis was performed using a Dionex Ion Chromatography series 4500i (Dionex Corp., Marlton, NJ). An IonPac AS9 ion exchange column provided the separation followed by a Dionex conductivity-II (CDM) detector. A conductivity detector coupled with this separation system is sensitive to concentrations of these ions as low as 10 ppb. Duplicate

TABLE 2. Results of Screening a Number of Bacterial Strains and Halogenated Substrates for Degradation[a]

| substrate | strain | | | |
|---|---|---|---|---|
| | *M. trichosporium* | *M. vaccae* JOB5 | ENV2040 | ENV2041 |
| HCFC21 | + | + | + | + |
| HCFC22 | | − | | |
| HCFC123 | − | ± | ± | + |
| HCFC124 | − | − | − | − |
| HCFC131 | + | + | ± | ± |
| HCFC141 | + | ± | ± | − |
| HCFC141B | + | + | − | − |
| HCFC142B | − | + | − | − |
| HFC23 | | − | | |
| HFC125 | | − | | |
| HFC134 | − | | | |
| HFC134A | − | − | | − |
| HFC143 | + | ± | | + |
| HFC143A | | − | | |
| MeBr | + | + | + | + |
| DBE | + | ± | ± | + |

[a] Complete degradation (+), incomplete degradation (±), and no degradation (−) are indicated for 24-h bottle assays. Blanks indicate no analysis done.

standards were run at the beginning and at the end of each sample batch.

**Inducing Substrate Assays.** The effectiveness of propane, 2-propanol, and acetone as inducing substrates for degradative activity by *M. vaccae* JOB5 was tested in bottle assays. The bottle assays used were performed in essentially the same manner as the bottle assays described above, except that they were performed using 5 mL of culture in BSM, grown on either propane, 2-propanol, or acetone, in 20-mL serum vials. Control cultures were killed using 600 $\mu$g/mL $HgCl_2$.

## Results

**Bottle Assays and Gas Chromatographic Analysis.** Several bacterial isolates were tested for the ability to degrade hydrohalocarbons and methyl bromide (Table 2). Four of the seven HCFCs, one of three HFCs, and both brominated compounds decreased in concentration when incubated with *M. trichosporium* OB3b. Complete loss by 72 h was observed for all of the compounds tested with this strain. When incubated with ENV2040, four of the seven HCFCs tested and both brominated compounds were either partially or completely transformed. ENV2041 incubations showed either complete or incomplete loss of substrate with three of the HCFCs tested and complete loss of one of the two HFCs tested. Six of the eight HCFCs, one of the five HFCs tested, and both brominated compounds decreased in concentration when incubated with *M. vaccae* JOB5. Transformation rates for the substrate/strain combinations in which complete loss of compound was observed are shown in Table 3. The rates varied widely, both by bacterial strain and by compound, with the brominated compounds exhibiting the highest rates of transformation.

Figure 1 shows the results of incubating HCFC 141 with *M. trichosporium* OB3b. After 24 h, only 37% of the compound remains. The increase in the substrate concentration in the control is most likely due to an increase in ambient temperature during the course of the assay. Figure 2 shows similar results obtained with ENV2041 and HCFC 21; within 24 h only 20% of the HCFC 21 remains. In both of these studies, complete loss was observed within 72 h, and there was no significant loss of the compounds in either of the no-cell controls.

Figures 3 and 4 are two examples of the results of incubating various concentrations of the target substrates with different concentrations of bacterial cells. The results appear to follow first-order kinetics, i.e., when the cell density was held constant and the substrate concentration was varied, increases in substrate concentration increased the rate of the reaction. For example, in the presence of *M. vaccae*, a starting concentration of 2.2 $\mu$mol of MeBr was transformed at a rate of 0.2 $\mu$mol/h in the first 10 h. However, a starting

TABLE 3. Degradation Rates in a Standard Bottle Assay[a]

| bacterial strain | compound | initial [substrate] ($\mu$mol/bottle) | degradation rate ($\mu$mol/h) |
|---|---|---|---|
| *M. trichosporium* OB3b | HCFC 21 | 2.2 | 0.2 |
| *M. trichosporium* OB3b | HCFC141 | 197 | 15.7 |
| *M. trichosporium* OB3b | MeBr | 45 | 10[b] |
| *M. trichosporium* OB3b | DBE | 6 | >3[b] |
| *M. vaccae* JOB5 | HCFC21 | 2.2 | 0.1 |
| *M. vaccae* JOB5 | HCFC141b | 2.2 | 0.1 |
| *M. vaccae* JOB5 | MeBr | 22.5 | 4.1 |
| ENV2041 | HCFC21 | 2.2 | 0.3 |
| ENV2041 | HCFC123 | 5.4 | 0.4 |

[a] Cell density ~$10^9$/mL, 24 °C, 150 rpm shaker for a total of 24 h. [b] All of the substrate degraded by the first time point.

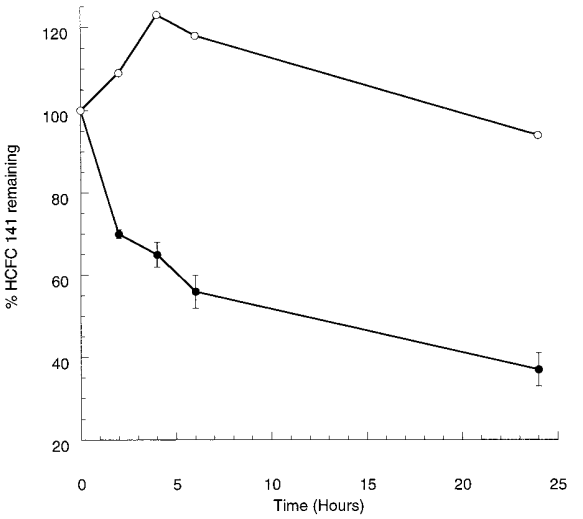


**FIGURE 1. Degradation of HCFC 141 by *M. trichosporium* OB3b. Solid circle, HCFC 141; open circle, HCFC 141 no-cell controls. Values are the average of duplicate assays; range is indicated with error bars.**

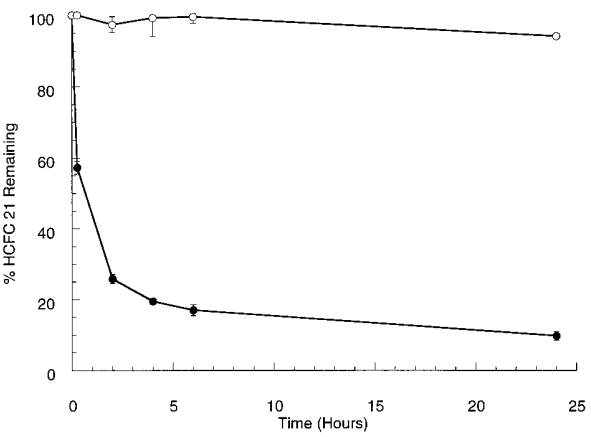


**FIGURE 2. Degradation of HCFC 21 by ENV2041. Solid circle, HCFC 21; open circle, HCFC 21 no-cell controls. Values are the average of duplicate assays; range is indicated with error bars.**

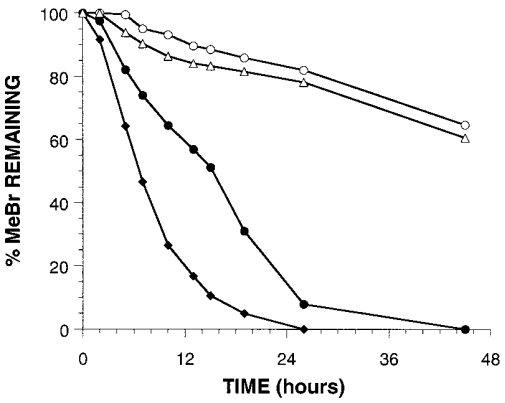


**FIGURE 3. Effect of substrate concentration on degradation of methyl bromide by *M. vaccae* JOB5 at $10^8$ cells/mL. Open circle, 106.0 $\mu$g/mL; solid circle, 21.2 $\mu$g/mL; solid diamond, 4.2 $\mu$g/mL; open triangle, killed control.**

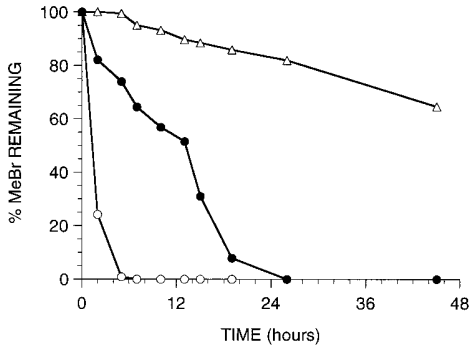


**FIGURE 4. Effect of cell concentration on degradation rate of methyl bromide (21.2 ug/mL) by *M. vaccae* JOB5. Open circle, $10^9$ cells/mL; solid circle, $10^8$ cells/mL; open triangle, killed control.**

amount of 0.4 $\mu$mol, five times lower, is transformed at a lower rate of 0.04 $\mu$mol/h (Figure 3). Similarly for HCFC141b, the rate at 2.2 $\mu$mol is 0.1 $\mu$mol/h, and the rate for 0.4 $\mu$mol is 0.04 $\mu$mol/h. For methyl bromide, concentrations of $10^9$ cells/mL transformed this compound so rapidly (within 30 min) that a rate was not calculated. The cell concentration was lowered to $10^8$ cells/mL in order to obtain a more accurate transformation rate. Rates shown in Figure 3 at a starting concentration of 4.5 $\mu$mol were 0.16 $\mu$mol/h, while for 0.9 $\mu$mol the rate was 0.06 $\mu$mol/h. Figures 3 and 4 show results obtained with M. vaccae JOB5, but they are representative of the results obtained with all of the bacterial strains tested (data not shown). This effect of substrate concentration on rate was measured for all of the substrate/strain concentrations tested, up to a critical concentration of substrate above which there was no observed degradation. This effect can be seen in Figure 3 where the highest concentration used (106 $\mu$g/mL) was not or was minimally degraded and essentially followed the same pattern as the killed control. A similar effect of biocatalyst concentration on rate is observed (Figure 4). Increasing the biocatalyst concentration while holding the substrate concentration constant increases the rate of the reaction. There was relatively little loss over time in controls containing either heat-killed cells or controls without added cells (Figures 1−4).

Ion Chromatographic Analysis. Some of the bacterial strain/substrate combinations that had complete loss of single or multiple peaks as determined by gas chromatography, were further analyzed by ion chromatography. There was a stoichiometric release of halogens for the compounds tested. Table 4 shows the expected values for the halogens and actual values measured in triplicate assays.

Inducing Substrate Assays. Results of the assays in which propane, 2-propanol and acetone were tested as possible inducing substrates for M. vaccae JOB5 are presented in Table 5. Growth of M. vaccae JOB5 was obtained on all three substrates. Three hydrohalocarbons were tested: methyl bromide, HCFC 21, and HFC 143. The order of inducing substrate effectiveness was as follows: propane > 2-propanol > acetone. Acetone was not shown to be an effective inducing substrate. Regardless of whether the inducer was propane or 2-propanol, the order of hydrohalocarbon degradation rate for this strain was MeBr > HCFC 21 > HFC 143. Enzyme induction was not checked separately since it was inferred from the work of Perry (23, 24). The same concentration of biomass was used for each experiment, but there was variability, presumably in the growth state of the cultures. Although grown by the same protocol each time, the observed variability required several experiments with each combination to confirm the results.

## Discussion

The aerobic microorganisms used in this study are common inhabitants of soil and aquatic systems and produce oxygenase enzymes known to attack compounds with molecular structures similar to the hydrohalocarbons and methyl bromide. Transformation of the hydrohalocarbons and brominated compounds was measured in 31 of the 54 substrate and bacterial strain combinations tested. Of the 29 HCFC and bacteria combinations tested, 17 resulted in complete or partial transformation, while 3 of the 10 HFCs tested were transformed. Although the HFCs are considered more environmentally benign than the HCFCs because of their lack of chlorine, which gives them an ODP of zero, their effect on global warming may be more important if they are more recalcitrant to biological degradation. Differences in substrate transformation may be attributable to the specific halogen substituents. Chang and Criddle (25) note that the rates of transformation for all of the fluorinated compounds tested were slower than rates of transformation for TCE. Of the halogens, the direct substitution of a C−Cl bond is the fastest (26). Incomplete transformation of these compounds may be attributable to the buildup of toxic byproducts that inhibit the activity of the organisms or simply a much slower rate of transformation. The results of killed and no-cell controls indicate that the transformation activity was biological rather than abiotic in nature.

For four of the compounds tested for which complete transformation was indicated by GC results, complete dehalogenation was indicated by ion chromatography results. For most compounds, the halogen values measured by ion chromatography were within a few percentage points of 100% recovery if all of the halogens were removed. Complete dehalogenation of these compounds would eliminate the possibility of a number of halogenated analogues of methanol, formaldehyde, and formate that are potential oxidation intermediates (27). In laboratory studies, trifluoroacetate (TFA), a fluorinated analogue of acetate and a product of hydroxyl attack of HCFCs and HFCs, has been shown to be incorporated into microbes and aquatic organisms, presumably through its transformation into biomolecules such as lipids and proteins (28). Although the biological effects of this type of incorporation are unclear, they are not a concern if TFA is not produced as a degradation byproduct. Jordan and Frank (29) have also found evidence that the TFA in the environment is derived from sources other than breakdown of HFCs or HCFCs. The most likely products of reactions that result in complete dehalogenation are environmentally benign with most of the carbon occurring as carbon dioxide, although the fate of the carbon was not determined in this study. Further carbon fate and ion chromatography analyses

TABLE 4. Results of Ion Chromatography for Four of the Compound/Strain Combinations That Had Complete Degradation of the Substrate As Indicated by GC Analysis[a]

| | substrate and expected values | | | |
|---|---|---|---|---|
| strain | HCFC21<br>2.2 $\mu$mol of fluoride<br>4.4 $\mu$mol of chloride | MeBr<br>4.5 $\mu$mol of bromide | DBE<br>11 $\mu$mol of bromide | HCFC141B<br>2.2 $\mu$mol of fluoride<br>4.4 $\mu$mol of chloride |
| *M. trichosporium* OB3b | Fl 2.4 ± 0.04<br>Cl 4.6 ± 0.3 | 4.5 ± 0.2 | 9.2 ± 1.3 | |
| ENV2041 | Fl 2.1 ± 0.1<br>Cl 4.2 ± 0.6 | 5.3 ± 0.5 | 14.1 ± 1.6 | |
| ENV2040 | Fl 1.6<br>Cl 3.9 | 4.4 ± 0.7 | | |
| *M. vaccae* JOB5 | Fl 2.0 ± 0.3<br>Cl 4.3 ± 2.1 | 3.0 | | Fl 2.3 ± 0.2<br>Cl 4.1 ± 2.3 |

[a] The expected values for complete dehalogenation are listed below each substrate. The values obtained for each halogen are listed next to each bacterial strain.

TABLE 5. Evaluation of Inducing Substrates Using *M. vaccae* JOB5[a]

| | propane | | | acetone | | | 2-propanol | | |
|---|---|---|---|---|---|---|---|---|---|
| | expt | relative degradation[b] | rate | expt | relative degradation[b] | rate | expt | relative degradation[b] | rate |
| MeBr | 1 | +++ | 1.34 | 1 | − | 0 | 1 | +++ | 1.34 |
| | 2 | ++++ | 12.50 | 2 | − | 0 | 2 | ++++ | 9.00 |
| HFC 143 | 1 | ++ | 0.40 | 1 | − | 0 | 1 | − | 0.00 |
| | 2 | ++ | 0.62 | 2 | − | 0 | 2 | ++ | 0.32 |
| | 3 | ++ | 0.25 | 3 | − | 0 | 3 | + | 0.16 |
| HCFC 21 | 1 | ++ | 0.76 | 1 | − | 0 | 1 | ++ | 0.40 |
| | 2 | +++ | 1.88 | 2 | − | 0 | 2 | ++ | 0.25 |
| | 3 | +++ | 1.40 | 3 | − | 0 | 3 | + | 0.16 |
| HCFC 21/HFC 143 | 1 | +++/++[c] | 1.44/0.84 | 1 | −/− | 0/0 | 1 | ++/++[c] | 0.32/0.40 |
| | 2 | ++/++[c] | 0.40/0.34 | 2 | −/− | 0/0 | 2 | ++/++[c] | 0.54/0.20 |
| | 3 | ++++/++[c] | 4.00/0.30 | | | | 3 | ++/++[c] | 0.20/0.06 |
| HFC 143 /MeBr | 1 | +/++++[c] | 0.08/2.14 | | | | | | |
| | 2 | −/++++[c] | 0.00/3.00 | | | | | | |

[a] Growth was obtained on all three inducing substrates, and the same amount of biomass was used for each experiment. The large variation between experiments was attributed to differences in the growth state of the culture at the time of the experiment, since it was difficult to ensure that shake flask cultures were grown up exactly the same way each time, thus each experiment was repeated 2−3 times. Values are the average of two replicates. Controls consisting of killed cells showed no degradation (data not shown). [b] −, no degradation observed. +, degradation rate < 0.2 $\mu$g/h. ++, degradation rate = 0.2−1 $\mu$g/h. +++, degradation rate = 1−2 $\mu$g/h. ++++, degradation rate > 2 $\mu$g/h. [c] Values to the left are for the first compound listed/values to the right are for the second compound listed.

should be performed on the other HCFCs that were degraded if they are to be considered for use in reactor-based systems.

In previously published studies, Oremland et al. (30) and Chang and Criddle (25) studied the degradation of HCFC 21 and HCFC 123. Oremland et al. (30) suggest in their studies with aerobic soils and sediments that HCFC 21 is more widely biodegradable than HCFC 123, and the results presented here with isolated strains agree with those findings. All four strains studied transformed HCFC 21, but only one fully and two incompletely transformed HCFC 123. Chang and Criddle (25) report the biotransformation of HCFC 123 by a methanotrophic mixed culture. Our methanotrophic mixed culture, ENV2040, did not transform HCFC 123, but the unidentified methanotroph, ENV2041 and M. trichosporium OB3b, both did. Chang and Criddle (25) also report biotransformation of HCFC 142b and HFC 134a by methanotrophs, two compounds that were not transformed by any of the strains that we tested. The differences in substrate degradation for M. trichosporium OB3b, ENV2040, and ENV2041 indicated that the enzymes responsible for degradation of these compounds and therefore the substrate specificities were different in all three strains.

M. vaccae JOB5 grows on numerous alkanes, ranging from C1 to C22 compounds (31). This strain has also been found to co-metabolically degrade a number of groundwater pollutants, including chlorinated compounds such as chlorobenzene (18). Poelarends et al. (32) have found a different strain of Mycobacterium, GP1, that is capable of utilizing 1,2-dibromoethane as a sole source of carbon and energy. However, to our knowledge, this is the first report of the degradation of HCFCs and HFCs by propane-oxidizing bacteria. M. vaccae JOB5 transformed both of the brominated compounds tested as well as 7 of 13 of the HCFCs and HFCs tested. In their studies with sandy soil, Toccalino et al. (33) estimate the number of propane-degrading bacteria to be less than 10 CFU/g of dry soil, but state that this number increases to 680 propane-degrading organisms/g if the soil is amended with propane. More research needs to be done to determine the prevalence of M. vaccae JOB5 and other propane-oxidizing bacterial strains in order to determine the extent of natural remediation that can be attributed to these strains globally.

Matheson et al. (34), in their work with Methylococcus capsulatus (Bath), found that this strain degraded HCFC 21 and HCFC 22 at low concentrations. At concentrations of 10 $\mu$M HCFC 21, however, they observed effective inhibition of sMMO (34). In our studies with M. vaccae JOB5, the same phenomenon was observed with inhibition occurring at 11 $\mu$M HCFC 21. Inhibition of degradative activity was also observed when M. trichosporium OB3b was incubated with concentrations of HCFC 21 five times higher than those used in our standard bottle assays (2.2 versus 11 $\mu$M; data not shown). Inhibition of degradation at relatively low concentrations is another factor that should be considered and

studied in further detail before choosing a particular CFC replacement.

The highest rates of degradation in this study were measured for the brominated compounds, methyl bromide and 1,2-dibromoethylene. These rates, coupled with the fact that all of the bacterial strains tested could degrade these compounds, indicate that soil microorganisms may represent a significant natural sink for these compounds. A large number of the uses for these brominated compounds involve application to soils. It is diffusion and volatilization of the brominated fumigants during and soon after their application to the soil that has resulted in the large amounts of bromine escaping into the atmosphere (35−37). The supplemental use of polyethylene films has been shown by Gan et al. (35) to significantly reduce the speed of volatilization and diffusion of methyl bromide from soils, thereby increasing the time in which the methyl bromide remains in the soil and is available to the bacteria for degradation. The amount of volatilization and diffusion that occurs differs for different soil types. The addition of the relatively inexpensive fertilizer ammonium thiosulfate (ATS; 36) has also been shown to significantly reduce the volatile loss of methyl bromide from soil and therefore have a positive effect on methyl bromide degradation in ATS-amended soil. Although atmospheric concentrations resulting from field application would not be as high as the concentrations used in this study, the application of brominated fumigants to fields combined with tarping may reach concentrations high enough to relate to the results presented here.

All of the bacterial cultures used in this study transformed a significant percentage of the hydrohalocarbons and brominated compounds tested. The presence of these microorganisms in the environment may represent a natural sink for some of these compounds. This study expanded on earlier work (14) including two additional bacterial isolates, a mixed culture, and a broader range of HCFCs and HFCs. Studies with pure or defined bacterial cultures with high concentrations of both the biocatalyst and the substrate can be used to design biological treatment systems to degrade these compounds in emissions from production and recycling facilities.

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