

## Biodegradation of Dimethylsilanediol in Soils

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**The biodegradation potential of [<sup>14</sup>C]dimethylsilanediol, the monomer unit of polydimethylsiloxane, in soils was investigated. Dimethylsilanediol was found to be biodegraded in all of the tested soils, as monitored by the production of <sup>14</sup>CO<sub>2</sub>. When 2-propanol was added to the soil as a carbon source in addition to [<sup>14</sup>C]dimethylsilanediol, the production of <sup>14</sup>CO<sub>2</sub> increased. A method for the selection of primary substrates that support cometabolic degradation of a target compound was developed. By this method, the activity observed in the soils was successfully transferred to liquid culture. A fungus, *Fusarium oxysporum* Schlechtendahl, and a bacterium, an *Arthrobacter* species, were isolated from two different soils, and both microorganisms were able to cometabolize [<sup>14</sup>C]dimethylsilanediol to <sup>14</sup>CO<sub>2</sub> in liquid culture. In addition, the *Arthrobacter* sp. that was isolated grew on dimethylsulfone, and we believe that this is the first reported instance of a microorganism using dimethylsulfone as its primary carbon source. Previous evidence has shown that polydimethylsiloxane is hydrolyzed in soil to the monomer, dimethylsilanediol. Now, biodegradation of dimethylsilanediol in soil has been demonstrated.**

Domestic consumption of silicone fluids in 1991 was estimated at  $155 \times 10^6$  to  $160 \times 10^6$  lb (ca.  $70.3$  to  $72.6 \times 10^6$  kg) (26). The bulk of this material is polydimethylsiloxane (PDMS), illustrated in Fig. 1 (II to IV). Linear polymers and cyclics are used in many consumer products and therefore are released into the environment through municipal wastewater treatment facilities and direct evaporation into the air. PDMS has very low water solubility, decreasing with increasing molecular weight. For example, the solubility for the end-capped dimer (Fig. 1, II;  $n = 0$ ) is 930 ppb, and that for the linear end-capped pentamer (Fig. 1, II;  $n = 3$ ) is 0.070 ppb (29). Since most of the siloxane material that leaves the wastewater treatment plant has a high polymer content, it is found in the sludge. Most of the sludge is land-filled or incinerated, and the remainder is applied to soils as fertilizer.

There are very few published reports that claim biological transformation of methyl-silicon compounds. Rats that were fed phenyltrimethylsilane reportedly excreted hydroxyphenyl and hydroxymethyl derivatives. Although the Si-phenyl bond was prone to cleavage, yielding hexamethyldisiloxane, the Si-methyl bond remained intact (11). In 1978, the catalysis of hydrolysis of ethoxysilanes by soil bacteria and their growth on the resulting ethanol were reported by Heinen (13). It was reported that in cells that had been grown on tetraethoxysilane (TES) and fed dimethyldiethoxysilane as the carbon source, small amounts of inorganic silicate were detected. In addition, the level of oxygen consumption was higher than when an equimolar concentration of ethanol was used as the carbon source. However, Heinen also reported that TES-grown cells washed free of TES continued to produce silicate. One possible explanation for this is that partially hydrolyzed TES resided in the cells and may have produced the silicate, which led to increased oxygen consumption when the cells were fed dimethyldiethoxysilane. A follow-up paper addressing these issues has never appeared in the literature.

The growth of a pure culture of *Pseudomonas putida* on silicone oils as the carbon source was recently reported (30). However, the paper did not identify products from the silicones and did not report evidence for the purity of the silicones. In addition, the paper claimed that growth was best on high-molecular-weight silicones. The increased biodegradability of synthetic polymers with increasing molecular weight is unprecedented in the literature and is difficult to understand.

Previous work with PDMS indicated that these polymers are hydrolyzed and rearranged in dry soils to cyclics and linear siloxanediols (4). More-recent work has shown that PDMS is hydrolyzed quickly (within days) in soil containing 2 to 3% moisture to low-molecular-weight oligodimethylsiloxane- $\alpha,\omega$ -diols (5, 18). Lehmann et al. (18) report that these soil moisture levels occurred several times in the first 2.5 cm of soil, even during a wet summer season in Bay County, Mich. At lower depths (2.5 to 10 cm) the soil moisture remained above 9%, so degradation of the PDMS occurred much more slowly. Another study indicated that these oligomer diols hydrolyze to the monomer diol, dimethylsilanediol (Fig. 1, I) and that the equilibrium in water lies far toward the monomer diol at the low concentrations that are observed in the environment. In addition, dimethylsilanediol was identified in an environmental sample, compost from a municipal wastewater treatment plant (25).

Since PDMS hydrolyzes to dimethylsilanediol in soils under environmentally attainable conditions and the water-soluble dimethylsilanediol is likely to be more biodegradable than its parent polymer compounds, studies were initiated to study the fate of [<sup>14</sup>C]dimethylsilanediol added directly to soils. The hypothesis that dimethylsilanediol is biodegradable is consistent with earlier research that indicated that [<sup>14</sup>C]PDMS in soils resulted in the production of <sup>14</sup>CO<sub>2</sub> under conditions that led to the hydrolysis of PDMS to water-soluble silanols (19). The production of <sup>14</sup>CO<sub>2</sub> followed a period of soil drying and rewetting, in which the drying led to the hydrolysis of the PDMS to dimethylsilanediol (identified by gas chromatography-mass spectrometry) and the rewetting led to biological activity. The study that is the subject of this paper was initiated to confirm that dimethylsilanediol is biodegradable in soil and

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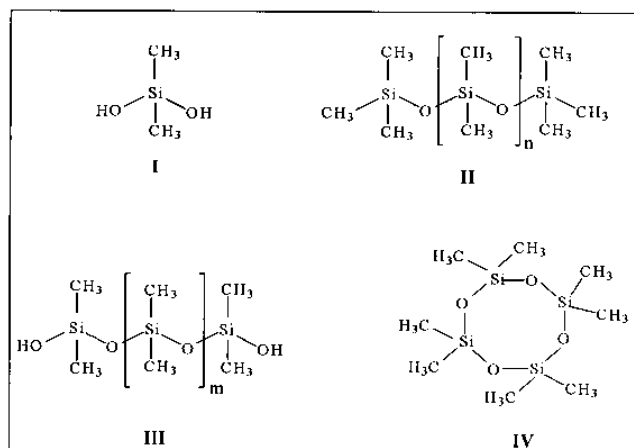


FIG. 1. Dimethylsilanediol (I) and examples of PDMS (II, linear end-capped PDMS; III, oligodimethylsiloxane- $\alpha,\omega$ -diol; and IV, cyclic PDMS).

to isolate and identify soil microorganisms capable of biodegrading dimethylsilanediol.

#### MATERIALS AND METHODS

**Materials.** [ $^{14}\text{C}$ ]dimethylsilanediol (specific activity of 275  $\mu\text{Ci/g}$ ) was prepared as described previously (6). Therefore, 0.04% of all dimethylsilanediol molecules were labeled with a  $^{14}\text{C}$  atom, and on a labeled molecule only one of the two methyl groups carries the  $^{14}\text{C}$  label. Nonlabeled dimethylsilanediol was also prepared as described previously (7). Opti Fluor and Hionic Fluor were obtained from Packard Instruments (Downer's Grove, Ill.). Modified Gledhill flasks (Fig. 2) were obtained from ACE Glass (Vineland, N.J.). Chemicals used as carbon sources or for preparation of medium were all reagent grade or better. The antibiotics vancomycin and nisin and the fungicide hygromycin B were obtained from Sigma (St. Louis, Mo.), and the fungicide carbendazim was obtained from E. I. DuPont de Nemours and Co. (Wilmington, Del.). BBL R2A medium was purchased from Becton Dickinson (Cockeysville, Md.) and was prepared as described on the package. Minimal medium was prepared by combining all solutions and compounds in Table 1, except the trace metal solution, in a flask with a stir bar. The flask and its contents were autoclaved for 20 min. After the flask was cooled to 60°C, with stirring, the trace metal solution, which had been

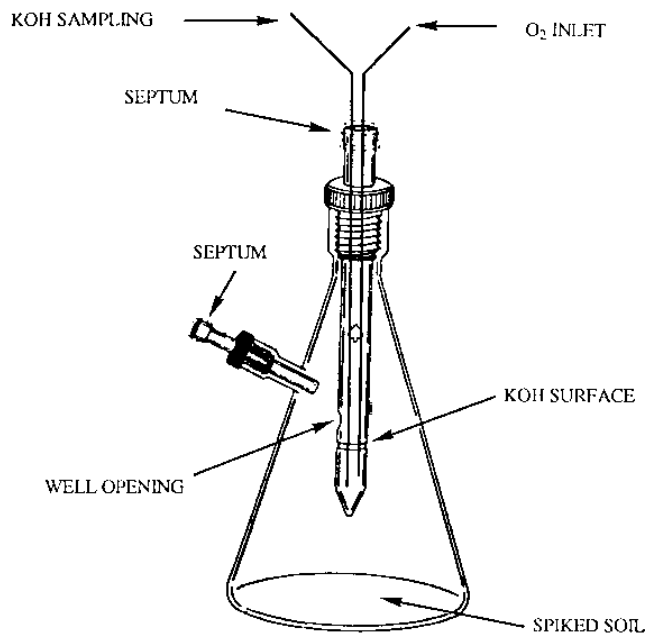


FIG. 2. Schematic drawing of a Gledhill flask.

TABLE 1. Preparation of minimal media used for liquid culture experiments and for plating of isolated organisms

Component	Amt <sup>a</sup>
<b>Minimal medium</b>	
Metal stock.....	4 ml
NH <sub>4</sub> Cl (50 g/liter).....	10 ml
Yeast extract.....	50 mg
MgSO <sub>4</sub> · 7H <sub>2</sub> O.....	200 mg
CaCl <sub>2</sub> .....	11 mg
K <sub>2</sub> HPO <sub>4</sub> .....	5.65 g
NaH <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O.....	2.70 g
DI water <sup>b</sup> .....	Amt to bring to 1 liter
Noble agar <sup>c</sup> .....	15 g
<b>Trace metal stock</b>	
FeSO <sub>4</sub> · 7H <sub>2</sub> O.....	1 g
ZnCl <sub>2</sub> .....	189 mg
MnCl <sub>2</sub> · 4H <sub>2</sub> O.....	18 mg
H <sub>3</sub> BO <sub>3</sub> .....	15 mg
NiCl <sub>2</sub> · 6H <sub>2</sub> O.....	10 mg
EDTA.....	1.04 g
CoCl <sub>2</sub> · 6H <sub>2</sub> O.....	50 mg
CuCl <sub>2</sub> · 2H <sub>2</sub> O.....	5 mg
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> · 4H <sub>2</sub> O.....	193 mg

<sup>a</sup> For minimal media, amount used for 1 liter; for trace metal stock, amount used for 4 liters.

<sup>b</sup> DI, deionized.

<sup>c</sup> Added when agar plates were made.

filter sterilized (0.2- $\mu\text{m}$  pore size) was added. If dimethylsulfone, dimethylsilanediol, antibiotics, or fungicides were to be included, they were also added at this point. For liquid medium, the solution was transferred to a sterile plastic flask and stored at 4°C. For agar plates, 20-ml aliquots of minimal medium were transferred to petri dishes by sterile technique. After the agar had solidified, the agar plates were stored at 4°C. When possible, plastic labware was used to store solutions and to grow biological cultures. This was done to prevent the introduction of inorganic silicate into our liquid cultures so that we could analyze for silicate production, as evidence for mineralization, at a later date.

**Analytical instruments.** Gas chromatography analysis for water-soluble carbon sources was done on a Hewlett-Packard (San Fernando, Calif.) 5890 or a Varian (Sugar Land, Tex.) 6000 gas chromatograph equipped with a flame ionization detector with an autoinjector, using a Tenax column (1/8 in. by 6 ft. [ca. 0.3 by 200 cm]; 60/80 mesh) from Supelco (Bellefonte, Pa.). High-performance liquid chromatography-inductively coupled argon plasma emission spectrometry (HPLC-ICP) analysis was done with either a Varian 5000 or a Waters (Milford, Mass.) 6000 liquid chromatography pump and a Jobin-Yvon Instruments S.A. (Longjumeau, France) 38 Plus inductively coupled plasma sequential optical emission spectrometer, monitoring the 251.611-nm emission line of silicon. Liquid scintillation measurements were done on an LKB-Wallac (Turku, Finland) model 1214 RackBeta liquid scintillation counter. Combustion analysis of soil for residual  $^{14}\text{C}$  counts was done with a Packard (Meriden, Conn.) model 307 sample oxidizer.

**Measuring of KOH traps.** The entire contents or an aliquot of the KOH trap were withdrawn periodically with a sterile needle and transferred to a screw-cap vial. Fresh KOH was added to the traps immediately. One-milliliter aliquots of the KOH trap were measured into each of two scintillation vials. Scintillation cocktail (10 ml) was immediately added to one vial, and 0.5 ml of 6 N HCl, to release CO<sub>2</sub>, was added to the other vial. After CO<sub>2</sub> release was allowed for at least 2 h, scintillation fluid (10 ml) was added to the acidic vial. Separate control experiments have shown that [ $^{14}\text{C}$ ]dimethylsilanediol is not evaporated by this technique.  $^{14}\text{C}$  in all samples was measured for 5 min. The counts attributable to  $^{14}\text{CO}_2$  were determined by subtracting the residual counts in the acidic vial from the total  $^{14}\text{C}$  counts in the original vial. If only an aliquot of the trap had been withdrawn, the  $^{14}\text{CO}_2$  accumulated in the trap and the carryover between time points was calculated. Initially, 0.2 M KOH was used in the CO<sub>2</sub> traps, and for these analyses Opti Fluor scintillation cocktail was used. When the KOH concentration was increased to 2 M KOH to increase CO<sub>2</sub> trapping capacity, Hionic Fluor scintillation cocktail was used.

To test the effectiveness of removing CO<sub>2</sub> by acidification, this method was occasionally compared with the BaCl<sub>2</sub> method (18). Instead of acid, a 20% solution of BaCl<sub>2</sub> was added to the 0.2 M KOH sample. The resulting BaCO<sub>3</sub> precipitate was removed by centrifugation, and the  $^{14}\text{C}$  in 1 ml of supernatant was counted with 10 ml of Opti Fluor. There was no difference in the two

TABLE 2. Analysis of [<sup>14</sup>C]dimethylsilanediol for radiochemical purity

Peak	Fraction	Retention time (min)	% of total counts	% of counts in peak	ICP standard retention time (min)
1	Total peak	4.62–7.59	97.9	76.2	5.23 (for monomer diol)
	Maximum	4.95–5.28			
2	Total peak	13.53–14.85	1.9	81.2	14.07 (for dimer diol)
	Maximum	13.86–14.19			
3	Total peak	15.18–15.84	0.15	90.7	15.72 (for trimer diol)
	Maximum	15.50–15.84			
4	Total peak	16.17–16.83	0.03	78.2	
	Maximum	16.50–16.83			

methods (BaCl<sub>2</sub> versus acidification) used to calculate the amount of <sup>14</sup>CO<sub>2</sub> produced.

**Determination of radiochemical purity of [<sup>14</sup>C]dimethylsilanediol.** A 25- $\mu$ l sample of a 2,000-ppm solution of [<sup>14</sup>C]dimethylsilanediol was chromatographed on a YMC-Pack (YMC, Wilmington, N.C.) polymer C<sub>18</sub> column (4.6 by 250 mm) by HPLC-ICP, as described previously (9). The column was held at 50°C, and the mobile phase was a gradient of water to acetonitrile.

The eluant from the column was collected in 66- $\mu$ l fractions in polypropylene tubes with a fraction collector. Each fraction was transferred to a glass scintillation vial by rinsing the polypropylene tubes three times with 5 ml of Opti Fluor scintillation cocktail. The completeness of transfer was tested with known amounts of [<sup>14</sup>C]dimethylsilanediol and was found to be 98%. Blanks were also prepared; they consisted of 15 ml of Opti Fluor scintillation fluid with either 66  $\mu$ l of water or 66  $\mu$ l of acetonitrile. <sup>14</sup>C in samples and blanks was measured for 15 min.

Pure standards, dimethylsilanediol (monomer diol), 1,1,3,3-tetramethyltrisiloxane-1,3-diol (dimer diol), and 1,1,3,3,5,5-hexamethyltrisiloxane-1,5-diol (trimer diol), were prepared as described previously (7) and chromatographed in the manner described above. However, instead of the eluant being collected in a fraction collector, it was passed through an ICP detector to determine the retention times of these authentic standards.

**Soils.** Four soils were taken from either a residential garden or a sludge disposal area and tested for their ability to biodegrade [<sup>14</sup>C]dimethylsilanediol. Two soils, a sandy soil from Santa Barbara, Calif., and a mixture of forest and residential garden soils and composted cow manure from Cobleskill, N.Y., were not believed to have been previously exposed to PDMS. The mixture that composed the Cobleskill soil was prepared to maximize biological diversity and activity by providing a rich environment, and it was not meant to represent a particular soil. The remaining two soils were taken from a residential garden (Guilderland, N.Y.) and a sludge disposal area (Glendale, Ohio). The Guilderland soil had been amended with compost (presumably containing PDMS) from a municipal wastewater treatment plant 3 years earlier. The Glendale soil was taken from an area that was used to store sludge from a municipal wastewater treatment plant prior to its removal. It had been exposed to PDMS-containing municipal sludge within the last year. Because of its use in another experiment, the Cobleskill soil was stored at 20% moisture for 3 months at 30°C prior to use. The remaining three soils were taken from the field and stored at room temperature over water, ensuring that the soils were never allowed to dry out. The soils were used within a few weeks. Each soil was sieved prior to the start of the experiment to remove any large particles or debris in the soil.

**Setup of Gledhill flasks.** A subsample of each soil (50 g, moist), 2.5 ml of a 2,000-ppm solution of [<sup>14</sup>C]dimethylsilanediol, and 100  $\mu$ l of ammonium chloride (50 g/liter) were mixed together in a 500-ml modified Gledhill flask (Fig. 2). Ammonium chloride was added to eliminate the possibility of nitrogen limitation in the soils. Prior to weighing, the moisture content of the soil was measured and then adjusted so that the moisture contents were 14% in the Santa Barbara soil, 20% in the Cobleskill mixture, and 40% in the Glendale soil. The moisture level was not measured in the Guilderland soil, but the soil was very moist when collected. So that the soil would be at the same moisture level for the experiment that it had been in the field, an appropriate amount of water was removed from the soil by blowing air over the sample prior to the addition of dimethylsilanediol and ammonium chloride. Sterilized controls of both the Cobleskill and the Santa Barbara soils were prepared by autoclaving the soils for 1 h on each of three successive days, adding the soil to the Gledhill flasks, and the next day autoclaving the soil-containing flask for an additional hour. In addition, sodium azide (2 ml of a 2.5% aqueous solution) was also added to the autoclaved controls.

The Gledhill CO<sub>2</sub> traps were filled with 10 ml of 0.2 M KOH, although in some cases this was increased to 2 M KOH. Oxygen was supplied by diffusion. A 12-in. (ca. 30-cm) 20-gauge needle was inserted through the septum at the top of the

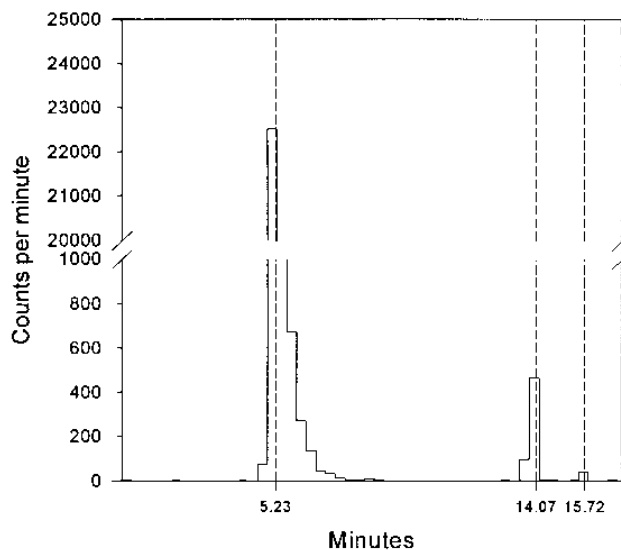


FIG. 3. Radiochemical purity of [<sup>14</sup>C]dimethylsilanediol as determined by HPLC analysis. Counts per minute in collected fractions (—) and retention times of authentic samples by HPLC-ICP (---) of dimethylsilanediol (5.23 min), dimer diol (14.07 min), and trimer diol (15.72 min) are shown.

trap, and the needle was pushed through until it rested on the bottom of the trap. The needle was connected to an oxygen manifold so that the flask could draw in oxygen as required to make up for CO<sub>2</sub> trapped. The flasks were kept in the dark by being covered with foil.

For each of the four soils, duplicate Gledhill flasks were prepared and the production of <sup>14</sup>CO<sub>2</sub> was monitored. Additional flasks (two each) were also prepared for the Guilderland soil, to which 2-propanol was added as a carbon source, and for the Santa Barbara soil, to which cow manure was added. Propanol was introduced to the soils in the vapor phase by adding 50 mg of 2-propanol to a cotton plug positioned in the sidearm of the Gledhill flask. Additional 2-propanol was added at each sampling point. After either 72 (Glendale soil) or 107 (Cobleskill soil and Santa Barbara soil with cow manure) days, 2-propanol was added to one of the duplicate flasks. Although it is possible that some propanol could have diffused out of the Gledhill flask, much of it must have remained to have produced the positive response observed with its addition. Dimethylsilanediol, however, could not have diffused out of the flask. Since dimethylsilanediol is acidic and not very volatile, any that evaporated from the soil would have been trapped in the KOH trap.

**Determination of PDMS concentrations in soil samples.** To determine the amount of PDMS in each soil sample, a subsample of each soil was extracted with tetrahydrofuran (THF) to remove PDMS as described previously (17). Each extracted sample was then analyzed in duplicate by HPLC-ICP, using size exclusion chromatography, for quantification of PDMS (9).

**Determination of radioactivity balance in soil samples.** At the end of the experiment, approximately 3 g of soil (each sample was done in duplicate) was added to a 25-ml polypropylene centrifuge tube and extracted by being shaken with 5 to 10 ml of THF for 5 min. The sample was then centrifuged at 3,000 rpm (ca. 2,000  $\times$  g) for 5 min, and the supernatant was decanted into a 25-ml volumetric flask. The THF extraction was repeated an additional three times. The THF extracts were combined, and the volume was brought to 25 ml with THF. Each soil sample was then extracted overnight with 10 ml of 0.1 M HCl. The sample was centrifuged as described above, and the HCl was decanted into a 10-ml volumetric flask and diluted to the mark, if necessary, with water. Each soil sample was then extracted overnight with 10 ml of 0.5 M KOH. The sample was centrifuged, decanted into a 10-ml volumetric flask, and, if necessary, brought to the mark with water. The THF, HCl, and KOH extractions were analyzed by liquid scintillation counting for <sup>14</sup>C counts. Multiple fractions (100 to 200 mg each) of each extracted soil sample were combusted to determine the amount of <sup>14</sup>C remaining in the soil.

**Soil characterization.** Soil textural classification (percent sand, silt, and clay) was done by A and L Great Lakes Laboratories (Fort Wayne, Ind.). Soil analyses (pH and organic carbon) were done by Cornell Nutrient Analysis Laboratory (Ithaca, N.Y.).

**Screen for primary carbon sources.** A 22-ml vial containing 5 g of either Guilderland or Santa Barbara soil and a second vial with 0.2 M KOH were placed into a 500-ml centrifuge bottle. The soil contained 100 ppm of [<sup>14</sup>C]dimethylsilanediol and 100 ppm of NH<sub>4</sub>Cl. Simple carbon sources were added at a concentration of approximately 1,000 ppm. Acids and bases were neutralized with NaOH or HCl prior to their addition to the soil. Complex carbon sources were

TABLE 3. Properties of soil samples used to study the biodegradability of [ $^{14}\text{C}$ ]dimethylsilanediol

Soil	% Sand	% Silt	% Clay	Textural classification	pH	Organic carbon (%)	PDMS (ppm)
Glendale	54	32	14	Sandy loam	6.4	12.1	361
Guilderland	76	14	10	Sandy loam	6.4	18.6	53
Santa Barbara	68	20	12	Sandy loam	6.3	3.1	0.8
Cobleskill Garden	48	18	34	Sandy clay Loam	6.9	4.7	0.4 <sup>a</sup>
Forest Control	32	42	26	Loam	5.8	3.7	0.5

<sup>a</sup> The mixed Cobleskill soil was analyzed for PDMS.

finely ground or chopped and added at a much higher level. Alfalfa, locally grown in upstate New York, was added at ~24,000 ppm, and leaf litter from the floor of a local deciduous forest floor was added at ~20,000 ppm. Cellulose (cotton) was added at ~40,000 ppm. The centrifuge bottles were capped (the caps contained an O-ring for a leakproof seal) and placed in a darkened area. After 14 days, the KOH traps from both sets of samples were analyzed for production of  $^{14}\text{CO}_2$ .

**Transfer of activity to liquid culture.** The contents of the vials from the most active samples from the carbon source screening (Guilderland soil, acetone and 2-propanol; Santa Barbara soil, 2,3-butanediol, dimethylsulfone, and 2-propanol) were taken and shaken with glass beads and a 3 $\times$  volume of minimal medium. After brief settling, the cloudy supernatant (5 ml) was used to inoculate 20 ml of minimal medium containing 500 or 1,000 ppm of the corresponding carbon source and 100 ppm of [ $^{14}\text{C}$ ]dimethylsilanediol. Cultures were transferred every few days (10% inoculum) to remove any remaining soil or soil contaminants.

For liquid cultures, 250-ml Erlenmeyer presterilized plastic flasks with screw caps were used. Air was supplied to the cultures when the flasks were opened to remove traps, sample the culture, or add a carbon source. A plastic, presterilized test tube standing upright inside the flask and containing 3 ml of 2 M KOH was used as the  $\text{CO}_2$  trap. Since the steady-state vapor concentration of  $^{14}\text{CO}_2$  or dimethylsilanediol over a KOH trap is low, there would be little loss of either when the flask was opened. To the extent that there were such losses, we would have underestimated the rate of dimethylsilanediol biodegradation. All liquid culture flasks were incubated at room temperature and were continuously shaken (except during sampling) on a bench-top laboratory shaker.

**Passaging of active liquid cultures.** In order to ensure continued activity, cultures were passaged as necessary. A new flask was set up with liquid minimal medium (25 ml) and [ $^{14}\text{C}$ ]dimethylsilanediol (100 ppm). From this flask, 2.5 ml of medium was withdrawn with a sterile needle and syringe, and the syringe was set aside. From the active, live culture, 2.5 ml of the culture suspension was withdrawn and added to the new flask. The liquid that had been set aside from the new flask was then added to the old culture. This procedure allowed the start of a new liquid culture and the continuation of the old culture without a change in volume or total  $^{14}\text{C}$  counts. Live cultures were maintained by adding additional carbon source as it was consumed, as determined by gas chromatography.

**Isolation of active microorganisms.** The most active liquid cultures were spread on agar plates containing the same minimal medium used in liquid cultures and nonlabeled dimethylsilanediol (100 ppm). For cultures receiving dimethylsulfone, the agar was made with 500 ppm of dimethylsulfone. For culture plates receiving propanol, 2-propanol was added in the vapor phase by placing a small vial of 2-propanol (20 mg per plate) in a closed container with the plates. In addition, biocides (1,000 ppm) were sometimes added to the agar medium of the plates for the Guilderland microorganisms in order to separate fungus and bacteria in a mixed culture.

A few days after inoculation, the plates were examined for growth. Single colonies were picked and replated. Picking and replating were continued until the colonies on the plates were homogeneous. Single colonies from homogeneous plates were picked from the minimal medium plates and started in sterile plastic tubes containing 1 ml of minimal medium, nonlabeled dimethylsilanediol (100 ppm), and an additional carbon source (1,000 ppm of 2-propanol or 500 ppm of dimethylsulfone). In some cases, for the Guilderland microorganisms, vancomycin was added to fungal cultures and carbendazim was added to bacterial cultures. After 1 week, the tubes were checked for obvious growth or consumption of the carbon source. Those tubes with growth or consumption of the carbon source were used to inoculate 10 ml of liquid medium, containing 100 or 1,000 ppm of [ $^{14}\text{C}$ ]dimethylsilanediol. After 5 to 10 days, the KOH traps were monitored for the production of  $^{14}\text{CO}_2$ , and cultures that had activity were increased to 25 ml. Cultures were plated, picked, and restarted in liquid culture to ensure purity and to ensure that activity was not lost during the purification. This cycle was repeated three times before the microorganisms were considered isolated, pure, and active.

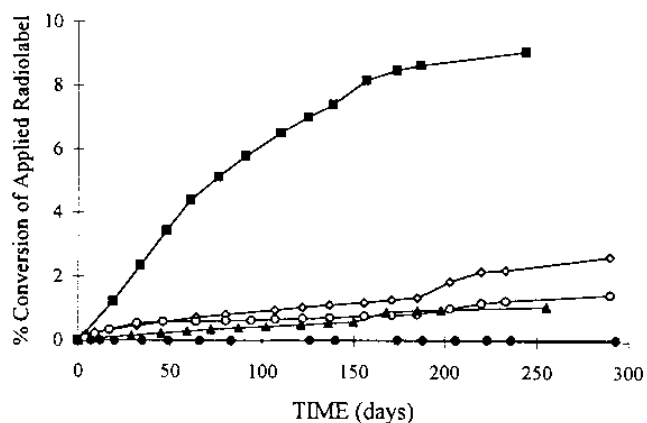


FIG. 4. Biodegradation of [ $^{14}\text{C}$ ]dimethylsilanediol in unamended soil samples. ■, Guilderland soil; ◇, Santa Barbara soil; ○, Cobleskill soil; ▲, Glendale soil; ●, Cobleskill sterile control.

**Identification and characterization of isolated organisms.** Once the isolated microorganisms were pure, they were plated on BBL R2A medium slants (without biocides) and allowed to grow at room temperature. After adequate growth had occurred, the slants were sent to American Type Culture Collection (ATCC) (Rockville, Md.) for identification and characterization.

## RESULTS

**Purity of [ $^{14}\text{C}$ ]dimethylsilanediol.** The fractions that were collected from the HPLC contained  $^{14}\text{C}$  counts that corresponded to the monomer, dimer, trimer, and possibly tetramer diols. The results of the analysis are shown in Table 2 and Fig. 3. Most of the radioactivity for each peak was eluted in one fraction, as indicated in Table 2. The retention start and end times for the total peak, as well as for the fraction which contained the majority of the counts in the peak, are listed in Table 2. The elutions of the  $^{14}\text{C}$  counts are an excellent match to the retention times of the standards run under the same HPLC conditions but with ICP detection. These data indicate that the [ $^{14}\text{C}$ ]dimethylsilanediol is at least 99.9% pure dimethylsilanediol and oligomers. An injection that bypassed the column and was collected in a single tube was also made. The number of counts in that injection matched the total counts in all of the fractions within 2% (which is the reproducibility of the injector).

**Soil characterization and PDMS analysis.** Each of the four soils was analyzed for textural content, pH, and organic carbon, and the results are shown in Table 3. Forest and residential garden soils, which were mixed to provide the Cobleskill soil, were analyzed separately. Each of the soils was also analyzed for its PDMS concentration.

**Unamended-soil experiments.** The production of  $^{14}\text{CO}_2$  from [ $^{14}\text{C}$ ]dimethylsilanediol in the unamended soils is shown in Fig. 4. Each of the four soils tested was able to convert [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$ . Although the soil samples were done in duplicate, only one curve is shown for each. The Santa Barbara soil for which results are shown was amended with cow manure, but the rate of  $^{14}\text{CO}_2$  production was not different from that of Santa Barbara soil not amended with cow manure (data not shown). The results for the Cobleskill sterile control are also shown in Fig. 4; the other sterile control (Santa Barbara soil) had a similar curve (data not shown).

The Guilderland soil was able to convert [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  much faster than the other three samples, and 2.1% of the total  $^{14}\text{C}$  counts was converted to  $^{14}\text{CO}_2$  in the first month. This rate slowed after about 4 months, so that after

TABLE 4. Effect on the conversion of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  with the addition of a primary carbon source to the soil<sup>a</sup>

Carbon source	% Deviation from the mean conversion <sup>b</sup>	
	Guilderland soil	Santa Barbara soil
None	-15	
Acetate	23	-57
Acetone	<b>91</b>	-46
Alfalfa	29	23
2,3-Butanediol	-32	<b>113</b>
<i>tert</i> -Butanol	-36	19
<i>t</i> -Butyl methyl ether	-1	
Cellulose	2	-68
Diglyme	-67	
Dimethylamine	-23	-78
Dimethyldimethoxysilane	-6	
Dimethylhydroxylamine	-53	
Dimethylphosphinic acid	-3	
Dimethylsulfone	4	<b>322</b>
Glucose	-2	-24
Leaf litter	8	
Methanol	16	-49
Methyl sulfoxide	-45	-78
Polypropylene glycol	-40	
Pristane	-28	-68
1,2-Propanediol	-4	19
2-Propanol	<b>185</b>	<b>243</b>
Trimethylamine	0	-71

<sup>a</sup> Measured after 14 days. Significant results are indicated in boldface.

<sup>b</sup> The mean conversions of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  were 1.71% for Guilderland soil samples and 0.28% for Santa Barbara soil samples.

244 days, 9% of the total  $^{14}\text{C}$  counts added to the Guilderland flask had been converted to  $^{14}\text{CO}_2$ . The other three soils converted the total  $^{14}\text{C}$  counts to  $^{14}\text{CO}_2$  at a much lower rate during the first month (Santa Barbara, 0.42%; Cobleskill, 0.51%; and Glendale, 0.17%). The rate of conversion in all of the soils slowed over the course of the experiment so that the final conversions to  $^{14}\text{CO}_2$  were 2.64% (Santa Barbara, 290 days), 1.5% (Cobleskill, 290 days), and 1.1% (Glendale, 255 days). The slight increase in the rate of conversion for the Santa Barbara soil and Cobleskill soil at day 180 and for the Glendale soil at day 150 coincides with the addition of 100 ppm of sodium trimetaphosphate, which was added to ensure that the soils were not phosphate limited. Trimetaphosphate was used as the phosphate source since it does not bind to the soil and remains available for soil microorganisms (2).

Although the tenth-of-one-percent increases between time points shown in Fig. 4 are small, this activity is real, since it is above the control and  $^{14}\text{CO}_2$  was steadily produced over the course of the experiment. It is important to remember that we are actually measuring  $^{14}\text{C}$  counts produced as  $^{14}\text{CO}_2$ . The background measurement of our liquid scintillation counter is less than 60 disintegrations per minute (dpm). Typically, 1 ml of a KOH trap in an active flask would contain at least 10 times this amount of total  $^{14}\text{C}$  counts, and often even greater amounts, the majority of which were driven off as  $^{14}\text{CO}_2$  with the addition of acid. Therefore, in a 10-ml trap, there would be several thousand dpm, as  $^{14}\text{CO}_2$ , and although the percentage of the total  $^{14}\text{C}$  counts might be small, the measurements are valid and convincing. However, in a sterile control flask the total (entire KOH trap)  $^{14}\text{C}$  counts attributable to  $^{14}\text{CO}_2$  would range from 0 to 250 dpm. In addition, in some instances with the Gledhill flasks, only a portion of the trap was removed at each time point, allowing the  $^{14}\text{C}$  produced as  $^{14}\text{CO}_2$  to accumulate in the trap. After 83 days, the Santa Barbara soil

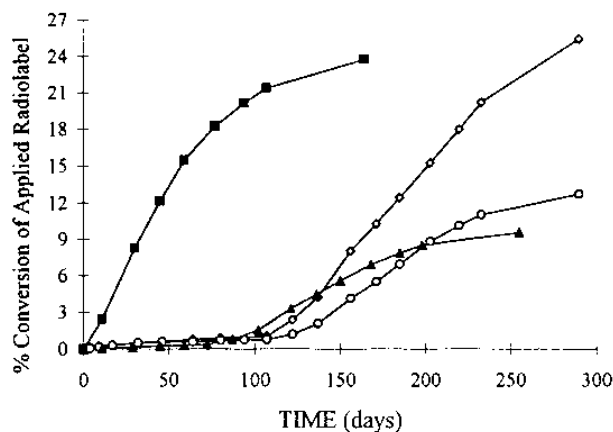


FIG. 5. Biodegradation of [ $^{14}\text{C}$ ]dimethylsilanediol in soil with carbon source amendment. 2-Propanol was added to the following soils on the indicated days: Guilderland, day 0 (■); Cobleskill, day 107 (○); Santa Barbara with manure, day 107 (◇); and Glendale, day 72 (▲).

had produced 65,000 dpm of accumulated  $^{14}\text{CO}_2$ , which is a conversion of 1.72%. However, a sterile control of the same soil produced only 925 dpm of accumulated  $^{14}\text{CO}_2$ , which is equivalent to a conversion of 0.02%. Thus, even though the percent conversion rates are not large, the  $^{14}\text{C}$  data supporting the rates are convincing.

**Screen for a primary carbon source.** In order to isolate soil microorganisms capable of biodegrading dimethylsilanediol, the activity observed in the soil samples needed to be transferred to liquid culture. However, before this could be done, a primary carbon source that could support the growth of the soil microorganisms in liquid culture and that allowed the continued conversion of dimethylsilanediol had to be identified. Numerous carbon sources were screened for their ability to enhance the biodegradation of [ $^{14}\text{C}$ ]dimethylsilanediol in soil. These carbon sources were added to separate samples of either Guilderland or Santa Barbara soil which were spiked with [ $^{14}\text{C}$ ]dimethylsilanediol, and the production of  $^{14}\text{CO}_2$  was monitored.

Carbon sources were chosen for several reasons: analogies in structure to dimethylsilanediol (e.g., dimethylsulfone, 2-propanol, 2,3-butanediol, and acetone), one-carbon units available as a carbon source (e.g., methanol), and prevalence in a soil environment (e.g., leaf litter and alfalfa). The results after 14 days are shown in Table 4. All results are expressed as percent deviation from the mean (the average percent conversion of total  $^{14}\text{C}$  to  $^{14}\text{CO}_2$  of all samples for that soil). The average conversions in the Guilderland and Santa Barbara soils were 1.7 and 0.28%, respectively. No entry indicates that the carbon source was not tested.

Many of the carbon sources did not enhance the rate of  $^{14}\text{CO}_2$  production. However, for each soil type, at least two carbon sources increased the production of  $^{14}\text{CO}_2$  greater than 90% above the mean. For the Guilderland soil, these carbon sources were acetone and 2-propanol, and for the Santa Barbara soil, the carbon sources that enhanced activity were 2,3-butanediol, 2-propanol, and dimethylsulfone. Putting the soil samples in a  $\text{N}_2$  atmosphere or adding  $\text{NH}_4\text{NO}_3$  (as a nitrogen source and as an anaerobic electron acceptor) in a  $\text{N}_2$  atmosphere resulted in no conversion of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$ , indicating that there was no activity under anaerobic conditions, and the observed activity in the Gledhill flasks was aerobic.

TABLE 5. Distribution of  $^{14}\text{C}$  counts in soil samples after extraction and total radioactivity balance

Soil	Description	% of theoretical $^{14}\text{C}$ count <sup>a</sup>			$^{14}\text{C}$ balance (%)
		THF	Acid	Base	
Guilderland	Unamended	79.6	2.4	2.1	2.3
	With 2-propanol	74.7	3.7	3.6	3.4
Santa Barbara	Unamended	81.6	6.8	1.6	1.3
	With 2-propanol	72.1	13.6	3.7	9.8
Glendale	Unamended	77.9	4.8	5.4	2.7
	With 2-propanol	63.5	6.2	8.4	4.1
Cobleskill	Unamended	87.2	6.8	1.9	0.8
	With 2-propanol	69.3	9.4	3.3	1.1

<sup>a</sup> Expected in the soil, after accounting for  $^{14}\text{C}$  counts found in the KOH trap.

**Soils with 2-propanol.** Since the addition of 2-propanol to the Guilderland soil in the Gledhill flask had increased the rate of conversion of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  compared with that of the soil without 2-propanol, and since the screening test had suggested that the Santa Barbara soil would also respond to the addition of 2-propanol, it was added to one of each of the duplicate flasks used in the experiment whose results are shown in Fig. 4. The results are shown in Fig. 5. The Guilderland soil which had been amended with 2-propanol starting at day 0 of the experiment was able to convert [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  much faster than when 2-propanol was not present. The rate of conversion of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  during the first month was 8.3%, and 23.7% of the total  $^{14}\text{C}$  was converted to  $^{14}\text{CO}_2$  after 164 days.

The remaining soils served as duplicates to the flasks monitored in the experiment whose results are shown Fig. 4, until 2-propanol was added on either day 72 or day 107. Monitoring of the duplicates indicated that the conversion rates before the addition of 2-propanol were nearly identical between replicates. When 2-propanol was added, either at day 72 or day 107, the production of  $^{14}\text{CO}_2$  increased significantly. The Santa Barbara soil, which had converted 1.0% of the total  $^{14}\text{C}$  to  $^{14}\text{CO}_2$  during the first 107 days, was able to convert an additional 24.4% of the total  $^{14}\text{C}$  to  $^{14}\text{CO}_2$  during the next 183 days with the addition of 2-propanol. The Cobleskill soil, which had converted 0.8% of the total  $^{14}\text{C}$  to  $^{14}\text{CO}_2$  after the first 107 days, converted an additional 11.9% of the total  $^{14}\text{C}$  to  $^{14}\text{CO}_2$  during the next 183 days with the addition of 2-propanol. The Glendale soil converted 0.4% of the total  $^{14}\text{C}$  to  $^{14}\text{CO}_2$  in the first 72 days but with the addition of 2-propanol converted an additional 9.2% of the total  $^{14}\text{C}$  to  $^{14}\text{CO}_2$  in the next 183 days.

**Radioactivity balance in Gledhill flasks.** Each soil sample was sequentially extracted with THF, acid, and then base, and each extraction was analyzed with scintillation counting. After extraction, the soil samples were combusted to determine the

TABLE 6. Results of plating the mixed Guilderland culture on agar plates with and without biocides

Biocide	Growth <sup>a</sup>	
	Bacteria	Fungus
None	+	+
Vancomycin	-	+
Nisin	+	+
Carbendazim	+	-
Hygromycin B	-	-

<sup>a</sup> -, no or little growth; +, growth.

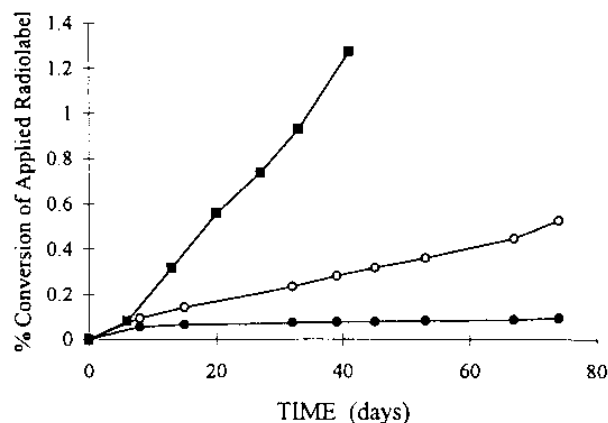


FIG. 6. Biodegradation of [ $^{14}\text{C}$ ]dimethylsilanediol (100 [■] or 1,000 [○] ppm) in liquid culture by *F. oxysporum* Schlecht. A control flask (●) containing liquid medium and 1,000 ppm of [ $^{14}\text{C}$ ]dimethylsilanediol but no fungus was also monitored for conversion to  $^{14}\text{CO}_2$ .

residual  $^{14}\text{C}$  counts in the soil. The percentages of counts in each extraction and the combusted soil, based on the theoretical counts remaining in the soil after  $^{14}\text{CO}_2$  release, are shown in Table 5. The final  $^{14}\text{C}$  balance for each soil sample is also shown in Table 5. All values are based on the average of two determinations, except for the Santa Barbara samples, which are based on one analysis. Radioactive mass balance was between 82 and 99% for all of the soil samples. Less than 100%  $^{14}\text{C}$  balance may be attributable to  $^{14}\text{CO}_2$  leaking from the septum of the Gledhill flasks or heterogeneous distribution in the soil.

#### Transfer of activity from Guilderland soil to liquid culture.

The Guilderland soil samples, with 2-propanol and acetone as the carbon sources, from the carbon screening test were used to inoculate liquid medium to enable isolation of a soil organism(s) capable of biodegrading [ $^{14}\text{C}$ ]dimethylsilanediol. Initially, after activity was transferred from the Guilderland soil, the cultures were maintained in minimal medium and received either acetone or 2-propanol as the primary carbon source. Since 2-propanol-containing cultures were slightly more active than cultures with acetone, only the 2-propanol cultures were continued. These cultures were transferred frequently in order to remove any remaining soil particles and contaminants.

Initially, mixed liquid cultures from the Guilderland soil converted [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  at a steady rate of about 1% per month, while metabolizing 2-propanol. Plating of the mixed cultures indicated that there were both bacteria and a fungus present in the liquid culture. Two antibiotics (vancomycin and nisin) and two fungicides (carbendazim and hygromycin B) were tested individually for their ability to inhibit growth of the microorganisms on agar plates. The results are shown in Table 6.

Mixed liquid cultures containing vancomycin produced  $^{14}\text{CO}_2$  at the same rate as the mixed cultures without an antibiotic present, but mixed cultures with carbendazim produced almost no  $^{14}\text{CO}_2$ . These results indicated that a fungus (susceptible to carbendazim) was likely responsible for the conversion of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  in these liquid cultures inoculated with Guilderland soil.

The fungal culture and two bacterial cultures were separated by plating, with the aid of either vancomycin or carbendazim, to confirm that a fungus was responsible for the observed activity in liquid culture. Once the fungus and bacteria were separated and transferred separately back to liquid culture,

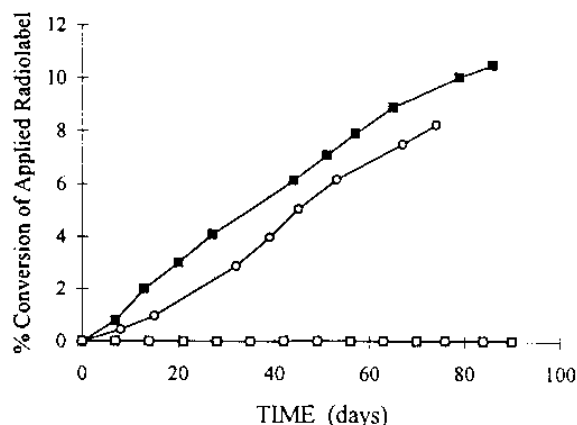


FIG. 7. Conversion of [ $^{14}\text{C}$ ]dimethylsilanediol (100 [■] or 1,000 [○] ppm) to  $^{14}\text{CO}_2$  in liquid culture by the Santa Barbara *Arthrobacter* bacterium. A control flask (□) containing liquid medium and 100 ppm of [ $^{14}\text{C}$ ]dimethylsilanediol but no bacterium was also monitored for conversion to  $^{14}\text{CO}_2$ .

only the fungal culture had the ability to convert [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$ . The two bacterial cultures had no activity above background. The pure fungal culture, while metabolizing 2-propanol, converted [ $^{14}\text{C}$ ]dimethylsilanediol (100 ppm) to  $^{14}\text{CO}_2$  at a rate of about 0.9% per month, about equivalent to that of the mixed liquid cultures. If the [ $^{14}\text{C}$ ]dimethylsilanediol concentration was increased to 1,000 ppm, the rate of conversion of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  was about 0.3% per month. The fungal culture was identified by ATCC as *Fusarium oxysporum* Schlechtendahl. A typical *F. oxysporum* Schlechtendahl curve of  $^{14}\text{CO}_2$  production from [ $^{14}\text{C}$ ]dimethylsilanediol (at 100 or 1,000 ppm) with 2-propanol as the primary carbon source is shown in Fig. 6. The curve for a sterile control flask which contained medium, [ $^{14}\text{C}$ ]dimethylsilanediol, and 2-propanol is also shown. An *F. oxysporum* Schlechtendahl culture that was allowed to continue for a longer period converted 19% of [ $^{14}\text{C}$ ]dimethylsilanediol (100 ppm) to  $^{14}\text{CO}_2$  in 240 days. Similar results were obtained by growing *F. oxysporum* Schlechtendahl on acetone. Growth on alfalfa did not result in [ $^{14}\text{C}$ ]dimethylsilanediol degradation activity; however, the addition of 2-propanol resulted in the production of  $^{14}\text{CO}_2$  from [ $^{14}\text{C}$ ]dimethylsilanediol.

**Transfer of activity from Santa Barbara soil to liquid culture.** Santa Barbara soil samples, with 2,3-butanediol, 2-propanol, or dimethylsulfone as the carbon source, from the carbon screening test were used to inoculate liquid medium to enable isolation of a soil organism(s) capable of biodegrading of [ $^{14}\text{C}$ ]dimethylsilanediol. Biodegradation of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  was achieved with mixed liquid cultures from the Santa Barbara soil in minimal medium, with either 2,3-butanediol, 2-propanol, or dimethylsulfone as the primary carbon source. Since cultures with dimethylsulfone produced  $^{14}\text{CO}_2$  5 to 10 times faster than those with the other carbon sources, only the cultures metabolizing dimethylsulfone were pursued.

Mixed liquid cultures started from the Santa Barbara soil produced  $^{14}\text{CO}_2$  from [ $^{14}\text{C}$ ]dimethylsilanediol much faster than those from the Guilderland soil. Conversion of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  proceeded at a rate of about 2.5% per month in mixed cultures containing 100 ppm of [ $^{14}\text{C}$ ]dimethylsilanediol. This was similar to the conversion rates observed for Santa Barbara soil in the Gledhill flasks and in the centrifuge bottles. Plating of the liquid cultures on agar plates containing dimethylsilanediol indicated that there were several

types of bacteria present. Purification (by repeated plating) and testing of these various bacteria led to the isolation of one that had the ability to convert [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  in liquid culture. This pure bacterial culture, while metabolizing dimethylsulfone, converted [ $^{14}\text{C}$ ]dimethylsilanediol (100 ppm) to  $^{14}\text{CO}_2$  at a rate of about 4% per month. This bacterium was identified by ATCC as an *Arthrobacter* sp., with the closest, but not an exact, match to *Arthrobacter ilicis*.

Experiments with the *Arthrobacter* bacterium and 1,000 ppm of [ $^{14}\text{C}$ ]dimethylsilanediol indicated that the conversion rate to  $^{14}\text{CO}_2$  was about 3.5% per month. A typical curve for the conversion of [ $^{14}\text{C}$ ]dimethylsilanediol (100 or 1,000 ppm) to  $^{14}\text{CO}_2$  by the *Arthrobacter* culture, while metabolizing dimethylsulfone, is shown in Fig. 7. The curve for a control flask without the bacterium but containing [ $^{14}\text{C}$ ]dimethylsilanediol, minimal medium, and dimethylsulfone is also shown. When trimethylamine was the primary carbon source, there was very little [ $^{14}\text{C}$ ]dimethylsilanediol degradation. However, the conversion of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  occurred with the addition of dimethylsulfone but not with the addition of 2-propanol.

## DISCUSSION

Dimethylsilanediol was found to be biodegraded in four different soil samples, confirming that it is biodegradable and suggesting that this activity is widespread. Two microorganisms capable of cometabolizing dimethylsilanediol in liquid culture were isolated and purified from two of the soil samples. The production of  $^{14}\text{CO}_2$  from [ $^{14}\text{C}$ ]dimethylsilanediol in soil and the isolation of organisms from the soil capable of biodegrading [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  in liquid culture provide the definitive evidence that dimethylsilanediol is biodegradable.

In Table 4, we report that the addition of acetone or 2-propanol to Guilderland soil and the addition of dimethylsulfone, 2,3-butanediol, or 2-propanol to Santa Barbara soil increased the rate of degradation of dimethylsilanediol compared with the average conversion for all of the carbon sources tested. All of these compounds are structural analogs of dimethylsilanediol. It is interesting that nonanalogous  $\text{C}_1$  compounds (e.g., methanol and trimethylamine), although excellent biological carbon sources, are not effective.

We believe that the enhanced rates of degradation of dimethylsilanediol in soil caused by the addition of the dimethylsilanediol analogs are a biological, and not a chemical, effect. The evidence for biological degradation is, first, that dimethylsilanediol is stable in aqueous solution in combination with either 2-propanol or dimethylsulfone but not in the presence of certain specific organisms isolated from the soil. Although absolute stability of dimethylsilanediol in soil is not demonstrable (100% recovery is not possible because of binding), we have demonstrated (by HPLC-ICP) the long-term stability of dimethylsilanediol in liquid culture controls. In a sterile control flask containing 125 ppm of [ $^{14}\text{C}$ ]dimethylsilanediol, (99  $\pm$  6)% of the dimethylsilanediol was recovered after 1 year. Since active cultures of *F. oxysporum* Schlecht. converted 19% of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  after 240 days and cultures of the *Arthrobacter* sp. converted more than 10% of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  after 90 days, there can be no doubt that the chemical degradation (if any) of dimethylsilanediol played no significant role in the liquid cultures. Second, even if one imagines a reaction between 2-propanol and dimethylsilanediol that produces a low equilibrium level of a  $^{14}\text{C}$ -compound that microorganisms can degrade, it would be very difficult to explain why neither methanol nor *tert*-butanol reacts in

the same manner with dimethylsilanediol. The dimethylsilanediol analogs, 2-propanol and dimethylsulfone, that are required for the biodegradation of dimethylsilanediol in liquid culture also enhance the rate of degradation in soil. Other carbon sources that are considered good for soil microorganism growth did not enhance the rate of dimethylsilanediol biodegradation. Third, dimethylsulfone was effective on Santa Barbara, but not on Guilderland, soil. Hence, Santa Barbara soil must contain a specific catalyst that brings about degradation of dimethylsilanediol in the presence of dimethylsulfone. Since we were able to isolate an active organism from Santa Barbara soil that can grow on dimethylsulfone as its sole carbon and energy source, this is readily explained biologically. As far as we know, this is the first reported instance of a microorganism using dimethylsulfone as its primary carbon source.

The simplest explanation for these observations is that the effective compounds, 2-propanol, dimethylsulfone, 2,3-butanediol, and acetone, acted as carbon and/or energy sources for organisms with the required activities and/or induced those activities. In the cases of both 2-propanol in the Guilderland soil and dimethylsulfone in the Santa Barbara soil, the primary carbon sources acted as both a growth substrate and an inducer. The case for enzyme induction is supported by the fact that in liquid culture the active fungus grew on alfalfa alone but was able to convert very little of the [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  (0.04% in 24 days). However, once 2-propanol was added to the culture, the conversion of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$  increased to 1% per month. Likewise, in liquid culture, the *Arthrobacter* sp. grew on trimethylamine, but there was very little conversion of [ $^{14}\text{C}$ ]dimethylsilanediol to  $^{14}\text{CO}_2$ . If dimethylsulfone was added, the rate of conversion increased, but if 2-propanol (which was able to induce the activity in the *Fusarium* cultures) was added to the culture, the rate of conversion did not increase.

*F. oxysporum* Schlecht., the microorganism isolated from the Guilderland soil, is the predominant fungus found on many food commodities and in soil in many food-producing areas (1, 31). It is a well-studied microorganism and has been found to transform or biodegrade many chemical compounds, particularly pesticides, such as atrazine (15) and monolinuron and solan (10). It is likely that some of these biotransformations are accomplished via hydroxylation. For example, *F. oxysporum* Schlecht. has been reported to hydroxylate monochloroaniline, a common pesticide residue, by *ortho* ring hydroxylation (12). This fungus has also been reported to convert cyclopropanecarboxylic acid to  $\gamma$ -hydroxybutyric acid through ring cleavage and hydroxylation (22, 23). However, there have been no previously published reports of *F. oxysporum* Schlecht. mediating a  $\text{CH}_3\text{-Si}$  bond cleavage.

We do not know the mechanism of the  $\text{CH}_3\text{-Si}$  bond cleavage exhibited by the fungus, but methyl hydroxylation followed by oxidation of the hydroxymethyl to a formyl or a carboxylic acid group would probably lead to a hydrolytic C-Si bond cleavage. Reviews in the literature indicate that acylsilanes are extremely sensitive to traces of aqueous base and are converted to the corresponding silanol and aldehyde (8, 20). The proposed mechanism involves addition of hydroxide to the carbonyl group followed by a 1,2-migration of the Si from carbon to oxygen to give a silyl-hemiacetal which spontaneously decomposes to give the silanol and aldehyde. Formyl silanes are particularly unstable (21). Formyltrimethylsilane has been successfully isolated only very recently (28), even though attempts at synthesis have been reported as far back as 1954 (24). The successful synthesis gave an isolated yield of 12% after reactions run at  $-78$  and  $0^\circ\text{C}$ . Previous attempts showed that formyltrimethylsilane is highly unstable under a variety of re-

action conditions and decomposes above  $25^\circ\text{C}$  (21). Therefore, if dimethylsilanediol were enzymatically hydroxylated and further oxidized to formyltrimethylsilanediol, we would expect a ready transformation to methylsilanetriol and formaldehyde.

The bacterium isolated from the Santa Barbara soil was identified by ATCC as belonging to the genus *Arthrobacter*, on the basis of its rod-coccus growth cycle, lack of diamino pimelic acid in the cell wall, and the absence of acid production from glucose and other carbohydrates. Species identification is difficult, since the *Arthrobacter* genus is not well defined (14). All of the characterization tests of the Santa Barbara bacterium matched those of *A. ilicis*, with the exception of citrate utilization and starch hydrolysis, for which the Santa Barbara bacterium tested positive and *A. ilicis* tested negative.

*Arthrobacter* bacteria are widely distributed in soil and are known to degrade or transform several chemical compounds. Many of these degradative pathways contain hydroxylation steps. For example, *A. ilicis* transforms longifolene to sativic acid via a hydroxylation step (3). Other *Arthrobacter* species have been reported to degrade 3-aminophenol (16) and picolinic acid (27). Both of these metabolic pathways are known to involve one or more hydroxylation steps. There have been no previously published reports of an *Arthrobacter* bacterium mediating a  $\text{CH}_3\text{-Si}$  bond cleavage. As with the fungus, methyl hydroxylation by the *Arthrobacter* bacterium and subsequent oxidation to a carbonyl function would probably lead to C-Si bond cleavage.

Previous evidence has shown that PDMS is hydrolyzed in soil to the monomer dimethylsilanediol. Now, biodegradation of dimethylsilanediol in soil has been demonstrated. Current work in our laboratory is focused on identifying the Si-containing products of dimethylsilanediol biodegradation in order to determine if dimethylsilanediol is completely mineralized to inorganic silicate.

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#### REFERENCES

1. Abbas, H. K., C. J. Mirocha, and W. T. Shier. 1984. Mycotoxins produced from fungi isolated from foodstuffs and soil: comparison of toxicity in fibroblasts and rat feeding tests. *Appl. Environ. Microbiol.* **48**:654-661.
2. Aggarwal, P. K., J. L. Means, and R. E. Hinchee. 1991. Formation of nutrient solutions for *in situ* bioremediation, p. 51-66. In R. E. Hinchee and R. F. Olfenbutte (ed.), *In situ* bioreclamation. Butterworth-Heinemann, Stoneham, Mass.
3. Bhosle, S., S. M. Kaliwal, S. K. Paknikar, and S. Mavinkurve. 1993. Molecular rearrangement of longifolene by *Arthrobacter ilicis* T<sub>2</sub>. *Appl. Environ. Microbiol.* **59**:1691-1693.
4. Buch, R. R., and D. N. Ingebrigstson. 1979. Rearrangement of poly(dimethylsiloxane) fluids on soil. *Environ. Sci. Technol.* **13**:676-679.
5. Carpenter, J. C., J. A. Cella, and S. B. Dorn. 1994. Study of the degradation of polydimethylsiloxanes on soil. *Environ. Sci. Technol.* **29**:864-868.
6. Cella, J. A., and J. C. Carpenter. 1994. Preparation of  $^{14}\text{C}$ -labeled dimethylsilane-1,1-diol. *J. Labelled Compound Radiopharm.* **34**:427-430.
7. Cella, J. A., and J. C. Carpenter. 1994. Procedures for the preparation of silanols. *J. Organomet. Chem.* **480**:23-26.
8. Cirillo, P. F., and J. S. Panek. 1992. Recent progress in the chemistry of acylsilanes. A review. *Org. Prep. Proc. Int.* **24**:553-582.
9. Dorn, S. B., and E. M. Skelly-Frame. 1994. Development of a high-performance liquid chromatographic-inductively coupled plasma method for speciation and quantification of silicones: from silanols to polysiloxanes. *Analyst* **119**:1687-1694.
10. Englehardt, G., P. R. Wallnöfer, and W. Ziegler. 1979. Degradation of the phenylamide herbicides monolinuron and solan by *Fusarium oxysporum* Schlecht. *Chemosphere* **10**:725-730.
11. Fessenden, R. J., and R. A. Hartman. 1970. Metabolic fate of phenyltrimethylsilane and phenyldimethylsilane. *J. Med. Chem.* **13**:52-54.
12. Fletcher, C. L., and D. D. Kaufman. 1979. Hydroxylation of monochloroaniline pesticide residues by *Fusarium oxysporum* Schlecht. *J. Agric. Food Chem.* **27**:1127-1129.



13. **Heinen, W.** 1977. Biodegradation of silicon-oxygen-carbon- and silicon-carbon bonds by bacteria—a reflection on the basic mechanisms for the biointegration of silicon, p. 129–146. In G. Bendz and I. Lindqvist (ed.), *Biochemistry of silicon and related problems*.
14. **Holt, J. G., et al.** 1994. *Bergey's manual of determinative bacteriology*, 9th ed. The Williams and Wilkins Co., Baltimore.
15. **Kaufman, D. D., and J. Blake.** 1970. Degradation of atrazine by soil fungi. *Soil Biol. Biochem.* **52**:73–80.
16. **Lechner, U., and G. Straube.** 1988. Degradation of 3-aminophenol by *Arthrobacter* spec. mA3. *J. Basic Microbiol.* **28**:629–637.
17. **Lehmann, R. G.** 1993. Extraction of silicones from soil. *Environ. Tox. Chem.* **12**:1851–1853.
18. **Lehmann, R. G., S. Varaprath, and C. L. Frye.** 1994. Degradation of silicone polymers in soil. *Environ. Tox. Chem.* **13**:1061–1064.
19. **Lehmann, R. G., S. Varaprath, and C. L. Frye.** 1994. Fate of silicone degradation products (silanols) in soil. *Environ. Tox. Chem.* **13**:1753–1759.
20. **Page, P. C. B., S. S. Klair, and S. Rosenthal.** 1990. Synthesis and chemistry of acylsilanes. *Chem. Soc. Rev.* **19**:147–195.
21. **Ricci, A., and A. Degl'Innocenti.** 1989. Synthesis and synthetic potential of acylsilanes. *Synthesis* **9**:647–660.
22. **Schiller, J., and A. E. Chung.** 1970. The metabolism of cyclopropanecarboxylic acid. *J. Biol. Chem.* **245**:5857–5864.
23. **Schiller, J. G., and A. E. Chung.** 1970. Mechanism of cyclopropane ring cleavage in cyclopropane carboxylic acid. *J. Biol. Chem.* **245**:6553–6557.
24. **Sommer, L. H., D. L. Bailey, G. M. Goldberg, C. E. Buck, T. S. Bye, F. J. Evans, and F. C. Whitmore.** 1954. Vinylsilanes, chlorovinylsilanes and  $\beta$ -styryltrimethylsilane. Further studies on the  $\alpha$ -silicon effect and  $\beta$ -eliminations involving silicon. *J. Am. Chem. Soc.* **76**:1613–1618.
25. **Spivack, J., and S. B. Dorn.** 1994. Hydrolysis of oligodimethylsiloxane- $\alpha,\omega$ -diols and the position of hydrolytic equilibrium. *Environ. Sci. Technol.* **28**:2345–2352.
26. **SRI International.** 1994. *Chemical economics handbook*. SRI International, Menlo Park, Calif.
27. **Tate, R. L., and J. C. Ensign.** 1974. A new species of *Arthrobacter* which degrades picolinic acid. *Can. J. Microbiol.* **20**:691–694.
28. **Trommer, M., and W. Sander.** 1996. Photochemistry and photooxidation of acylsilanes. *Organometallics* **15**:189–93.
29. **Varaprath, S., C. L. Frye, and J. L. Hamelink.** 1996. Aqueous solubility of permethylsiloxanes (silicones). *Environ. Tox. Chem.* **15**:1263–1265.
30. **Wasserbauer, R., and Z. Zadak.** 1990. Growth of *Pseudomonas putida* and *P. fluorescens* on silicone oils. *Folia Microbiol.* **35**:384–393.
31. **Windels, C. E., and T. Kommedahl.** 1974. Population differences in indigenous *Fusarium* species by corn culture of prairie soil. *Am. J. Bot.* **61**:141–145.