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Biodegradation of [^{14}C]Phenol in Secondary Sewage and Landfill Leachate Measured by Double-Vial Radiorespirometry

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Double-vial radiorespirometry was used to estimate the biodegradation rates of ^{14}C-labeled phenol in a landfill leachate and a secondary treated domestic wastewater. Rates were found to be comparable for each material at each of the three concentrations tested. Sewage microorganisms immediately began biodegrading the [^{14}C]phenol; landfill leachate microorganisms required a lag period before maximum biodegradation of the [^{14}C]phenol. The apparent rate of [^{14}C]phenol biodegradation was 2.4 times faster in the sewage than in the landfill leachate. Double-vial radiorespirometry was shown to be an effective method for screening biodegradation rates in aquifers.

Recent investigations have found substantial numbers of microorganisms in shallow water-table aquifers (9). Since these microorganisms may biodegrade specific organic compounds, their importance must be considered together with physical and chemical processes when one is predicting the transport and fate of synthetic organic chemicals which might be found as contaminants in ground water.

Environmental problems may result from the slow degradation or complete recalcitrance of pollutants to microbial breakdown. Therefore, testing for the biodegradability of organic chemicals in ground water is an important component of evaluating their transport and fate for risk assessment. However, many of the more common tests used to assess rates of biodegradation involve chemical concentrations far in excess of those likely to be found in ground water and do not utilize indigenous organisms under in situ conditions. Insufficient data exist to allow for extrapolation from the high levels (milligrams per liter) commonly used in laboratory studies to the far lower levels (micrograms per liter) characteristic of polluted ground water (7).

Radiometric methods are among the most sensitive of analytical procedures and have been used to measure the biodegradation of organic pollutants in environmental samples (3, 4, 7, 8). The application of these methods can be accomplished by supplying ^{14}C-labeled substrates, followed by collecting and measuring the radioactivity of the evolved gases or the compounds remaining in the solution.

A two-compartment scintillation vial method developed by Buddemeyer (1) has been used successfully to determine the biodegradation rates of ^{14}C-labeled substrates contained in composting cow manure, forest soil, and arctic lake sediment microbiota (5). Modifications to the method designed to simplify sample preparation and improve counting efficiency have been suggested (2).

The purpose of this study was to use the method of double-vial radiorespirometry to estimate the biodegradation of [^{14}C]phenol by microorganisms indigenous to a secondary treated domestic wastewater and a landfill leachate. The success of the technique indicates its applicability for examining the biodegradation potential of synthetic organic pollutants by subsurface microorganisms.

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MATERIALS AND METHODS

Radiorespirometer. The double-vial respirometer consisted of a 1-dram (3.7-ml) vial (outer diameter, 1.5 cm; height, 4.5 cm; Kimble Div., Owens-Illinois, Inc., Toledo, Ohio) which was cemented inside a standard (20-ml) borosilicate glass scintillation vial (Kimble). The two-compartment vials were sterilized by autoclaving at 121°C for 15 min. The outer vial was lined on the inside with a strip of Whatman no. 1 filter paper (3 by 8 cm) which had been soaked in the scintillation mixture. The filter paper strip within the radiorespirometer was dried under a steam of nitrogen. The radiorespirometer vial was then tightly capped and stored in the dark.

Scintillation mixture. The scintillation mixture was prepared by combining 10 g of 2,5-diphenyloxazole, 0.125 g of 1,4-bis-(5-phenyloxazolyl)benzene (Packard Instrument Co., Downers Grove, Ill.), and 25 ml of methanolic NaOH in 100 ml of PCS (Amersham Corp., Arlington Heights, Ill.). Methanolic NaOH was prepared by adding 8 g of reagent grade NaOH to 100 ml of methanol (Fisher Scientific Co., Pittsburgh, Pa.).

Efficiency determination. Efficiency was determined by adding 0.0038 μCi (0.0308 μg) of [^{14}C]phenol (Pathfinder Laboratories, St. Louis, Mo.) directly to the filter paper impregnated with scintillation fluor. The vials were capped and placed into a scintillation counter at ambient temperature (Beckman model LS 300, C-14 Isoset). The counting efficiency was 43.3 ± 2.2%, compared with a previously reported value of 43.7 ± 2.7% (5).

Background counts from prepared vials without [^{14}C]phenol were relatively low (32 to 46 cpm). Control experiments containing unlabeled phenol (Chem Services, Inc., West Chester, Pa.) also had low counts (51.7 to 85.0 cpm), indicating that the controls had no significant metabolic luminescence and any activity seen in the experiments was produced by ^{14}C-labeled degradation products of [^{14}C]phenol.

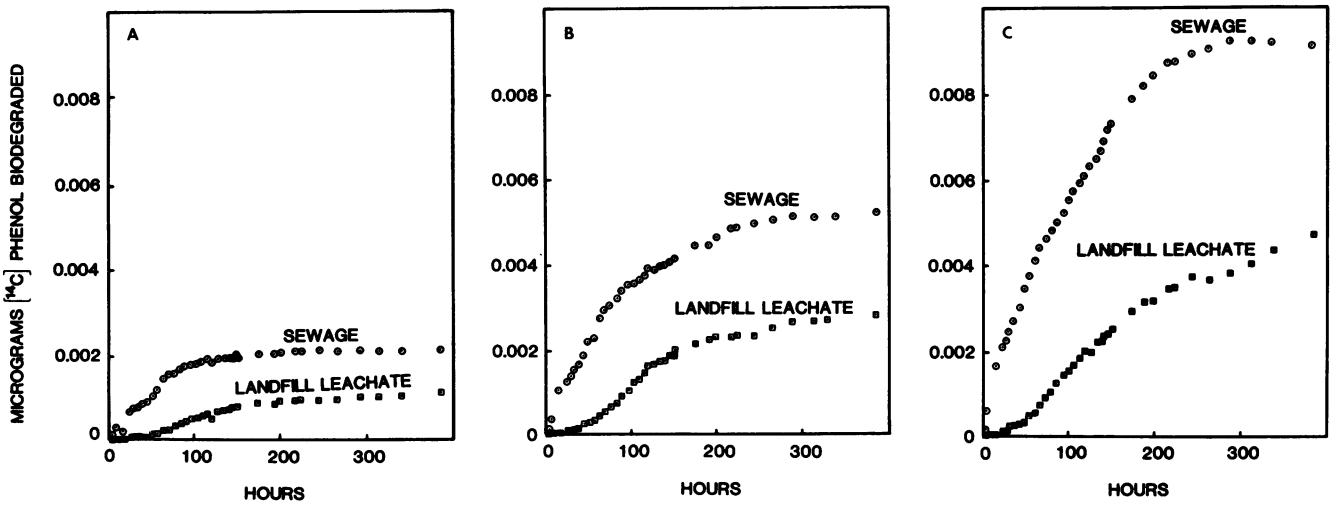

FIG. 1. [^{14}C]phenol biodegradation products released from solutions containing secondary sewage or landfill leachate after the addition of (A) 0.0308 μg, (B) 0.0616 μg, and (C) 0.0924 μg of [^{14}C]phenol.

Environmental samples. A domestic wastewater sample from the final clarifier of a waste treatment plant (Norman, Okla.) was collected into a sterilized 500-ml glass jar. Subsurface material contaminated by leachate was collected from a site located 3 m downgradient from an active landfill (Norman, Okla.) documented to contain organic pollutants (6). The water table was encountered at 1 m below the surface by excavating a 3-m^2 area. A sterilized core boring device (2.5 cm diameter by 30 cm long) was then used to collect the sample at a depth of 10 cm below the water table. The sample was placed into a sterilized 500-ml glass jar and refrigerated (4°C) until needed.

Biodegradation experiments. Two milliliters of subsurface suspension or secondary sewage was pipetted under sterile conditions into the inner vial of the radiorespirometer for each experiment. An aqueous solution of [^{14}C]phenol was added to provide 0.0038 μCi (0.0308 μg), 0.0076 μCi (0.0616 μg), or 0.0114 μCi (0.0924 μg) in the samples for a concentration of 15.4, 30.8, or 46.2 μg/liter, respectively. Abiotic controls for each experiment were prepared with a sterilized sample. Duplicate vials were prepared for all samples and controls. No additional substrates or nutrients were added to the samples because the objective of the study was the detection of potential biodegradation under actual environmental conditions. The experiments were performed under aerobic conditions; however, anaerobic conditions could be maintained, when necessary, by preparing the vials in a glove box.

The vials were counted at various intervals for 624 h. Initial intervals were short (3 to 6 h) but lengthened (>24 h) when count rates began to stabilize.

Results of the abiotic controls were subtracted from the treatments to correct for counts which may have resulted from processes other than biodegradation such as the volatilization of labeled phenol. The resulting data are a measure of [^{14}C]phenol biodegradation products released from solution and are reported as micrograms of [^{14}C]phenol biodegraded.

RESULTS

The results (Fig. 1) are expressed as micrograms of [^{14}C]phenol biodegraded over a period of 400 h for each of the three starting concentrations. For all three cases, the sewage curve showed immediate biodegradation of [^{14}C]phenol. Biodegradation proceeded linearly and then approached a maximum as the reaction neared completion. The absence of an initial lag period may have been caused by the presence of organisms already acclimated to phenol, organisms acclimated to similar compounds and thus able to accept phenol, or organisms able to become acclimated to phenol very quickly. The variety and large number of organisms present in secondary treated sewage make it an excellent positive control in aerobic biodegradation studies.

The landfill leachate curves consist of a lag period followed by a linear portion and finally a leveling off. This may reflect an insufficient number or initial absence of acclimated microorganisms capable of immediately biodegrading phenol, or a diffusion-limited process within the sample. For all three concentrations tested, linear biodegradation began at ca. 50 h. The lower degree of degradation in the landfill leachate curves indicates a nutrient limitation in the sample, suggesting the possibility of using this assay for determining optimum nutrient levels for in situ underground biological treatment of polluted ground water.

The average maximum rate of [^{14}C]phenol biodegradation was determined from the maximum slope of each curve, and normalized per microgram of [^{14}C]phenol initially added. The average normalized rates were 6.4×10^{-14} h^{-1} for sewage and 2.7×10^{-14} h^{-1} for landfill leachate. The normalized rates for each of the three starting concentrations are nearly identical within each type sample (Table 1), indicating that similar processes were occurring without limiting factors such as toxic or insufficient levels of phenol being present within the range tested.

TABLE 1. Maximum rates of [^{14}C]phenol biodegradation in sewage and landfill leachate

[^{14}C]phenol added (μg)	Biodegradation rate (h^{-1})[a]	
	Sewage	Leachate
0.0308	6.5×10^{-4}	2.7×10^{-4}
0.0616	6.3×10^{-4}	2.8×10^{-4}
0.0924	6.5×10^{-4}	2.6×10^{-4}

[a] Normalized per microgram of [^{14}C]phenol initially added.

DISCUSSION

Our investigation indicates that phenol is biodegraded by organisms indigenous to both sewage and landfill leachate. For each material, biodegradation rates are comparable within the range of concentrations tested. [^{14}C]phenol biodegrades at a faster rate in sewage than in landfill leachate for the samples tested.

Double-vial radiorespirometry is shown to be an effective screening method for estimating the biodegradation of trace levels of organic pollutants in subsurface environments. The procedure may, therefore, be useful for determining whether the subsurface beneath a proposed disposal site has a potential for biodegrading organic pollutants, or whether a presently active or abandoned site is providing biodegradation of pollutants of concern. By the performance of tests with various nutrients, optimum conditions for in situ underground biological treatment may be established because of an ability to monitor reaction rates in a continuous and non-destructive manner.

Further studies are being performed with other organic pollutants and subsurface materials. Sites previously exposed to pollutants are being selected together with pristine areas for measurement of the ability of indigenous microorganisms to cope with introduced organic pollutants.

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LITERATURE CITED

1. **Buddemeyer, E. U.** 1974. Liquid scintillation vial for cumulative and continuous radiometric measurement of in vitro metabolism. Appl. Microbiol. **28**:177–180.
2. **Coveney, M. F., and R. G. Wetzel.** 1984. Improved double-vial radiorespirometric technique for mineralization of ^{14}C-labeled substrates. Appl. Environ. Microbiol. **47**:1154–1157.
3. **Fina, L. R., and A. M. Fiskin.** 1980. The anaerobic decomposition of benzoic acid during methane fermentation. II. Fate of carbons one and seven. Arch. Biochem. Biophys. **91**:163–165.
4. **King, G. M., and W. J. Wiebe.** 1980. Tracer analysis of methanogenesis in salt marsh soils. Appl. Environ. Microbiol. **39**:877–881.
5. **McKinley, V. L., T. W. Federle, and J. R. Vestal.** 1983. Improvements in and environmental applications of double-vial radiorespirometry for the study of microbial mineralization. Appl. Environ. Microbiol. **45**:255–259.
6. **Robertson, J. M., C. R. Toussaint, and M. R. Jorque.** 1974. Organic compounds entering ground water from a landfill. U.S. Environmental Protection Agency Report EPA-660/2-74-077.
7. **Rubin, H. E., R. V. Subba-Rao, and M. Alexander.** 1982. Rates of mineralization of trace concentrations of aromatic compounds in lake water and sewage samples. Appl. Environ. Microbiol. **43**:1133–1138.
8. **Subba-Rao, R. V., H. E. Rubin, and M. Alexander.** 1982. Kinetics and extent of mineralization of organic chemicals at trace levels in freshwater and sewage. Appl. Environ. Microbiol. **43**:1139–1150.
9. **Wilson, J. T., J. F. McNabb, D. L. Balkwill, and W. C. Ghiorse.** 1983. Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer. Ground Water **21**:134–142.