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Rates of Microbial Metabolism in Deep Coastal Plain Aquifers

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Rates of microbial metabolism in deep anaerobic aquifers of the Atlantic coastal plain of South Carolina were investigated by both microbiological and geochemical techniques. Rates of [2-^{14}C]acetate and [U-^{14}C]glucose oxidation as well as geochemical evidence indicated that metabolic rates were faster in the sandy sediments composing the aquifers than in the clayey sediments of the confining layers. In the sandy aquifer sediments, estimates of the rates of CO_2 production (millimoles of CO_2 per liter per year) based on the oxidation of [2-^{14}C] acetate were 9.4×10^{-3} to 2.4×10^{-1} for the Black Creek aquifer, 1.1×10^{-2} for the Middendorf aquifer, and $< 7 \times 10^{-5}$ for the Cape Fear aquifer. These estimates were at least 2 orders of magnitude lower than previously published estimates that were based on the accumulation of CO_2 in laboratory incubations of similar deep subsurface sediments. In contrast, geochemical modeling of groundwater chemistry changes along aquifer flowpaths gave rate estimates that ranged from 10^{-4} to 10^{-6} mmol of CO_2 per liter per year. The age of these sediments (ca. 80 million years) and their organic carbon content suggest that average rates of CO_2 production could have been no more than 10^{-4} mmol per liter per year. Thus, laboratory incubations may greatly overestimate the in situ rates of microbial metabolism in deep subsurface environments. This has important implications for the use of laboratory incubations in attempts to estimate biorestoration capacities of deep aquifers. The rate estimates from geochemical modeling indicate that deep aquifers are among the most oligotrophic aquatic environments in which there is ongoing microbial metabolism.

It has long been suspected that microbially catalyzed reactions could have a significant impact on the geochemistry of deep aquifer systems (3, 15), and a wide diversity of microorganisms have now been recovered from deep subsurface sediments (4, 5, 7, 8, 13, 37; D. R. Lovley, F. H. Chapelle, and E. J. P. Phillips, Geology, in press). Some of these microorganisms may be metabolically active in situ as evidenced by the observation that microorganisms produced carbon dioxide or methane from the metabolism of either natural sediment organic matter (7, 19, 29) or low concentrations of radiolabeled substrates (5, 16, 31) in laboratory incubations of relatively undisturbed deep subsurface sediments or sediment slurries. However, the in situ rates of microbial metabolism in deep subsurface environments have not been investigated in detail.

The microbiology of deep aquifers of the Atlantic coastal plain has been under intense study in recent years (4, 7, 12, 13, 16, 19, 23, 28, 29, 31, 37). Most of these studies (4, 12, 13, 16, 19, 28, 31, 35, 37) have focused on the recharge areas of these aquifers. This zone is hydrologically atypical. In these recharge areas, the rates of groundwater flow are high (up to 20 m/year), and the groundwater is relatively young and aerobic (35). However, most of the areal extent of these aquifers is downgradient from the recharge area, where the groundwater is anaerobic (22; F. Chapelle, unpublished data) and the groundwater flow rates are slow (ca. 1 m/year). Furthermore, in the recharge areas, the aquifers are most susceptible to transport of allochthonous microbial populations from soils or stream sediments. Therefore, microbial activity in the downgradient, anaerobic zones is more representative of the overall microbial metabolism in the aquifers.

The purpose of the study reported here was to estimate the in situ rate of microbially catalyzed carbon dioxide production in deep anaerobic aquifers of the Atlantic coastal plain. Carbon dioxide production is of interest, in part, because it provides an estimate of overall organic matter metabolism in the aquifers. Furthermore, it has a significant impact on the water quality and secondary porosity of this hydrologic system because carbon dioxide generated by microbial metabolism drives carbonate and silicate mineral dissolution (7, 8, 27). Our results indicated that although there is a significant potential for microbial carbon dioxide production in deep anaerobic subsurface sediments, the in situ rates of organic carbon oxidation are as slow or slower than those previously reported for such oligotrophic environments as deep ocean waters and sediments. The results also suggest that laboratory studies are not reliable indicators of the potential for in situ biodegradation in the deep subsurface since laboratory incubations of deep subsurface sediments may greatly overestimate in situ rates of microbial metabolism.

MATERIALS AND METHODS

Sediment sampling. The core holes were located near Myrtle Beach and Florence, S.C. (Fig. 1). Sediments from the Black Creek, Middendorf, and Cape Fear aquifers were sampled at each site (Fig. 2). Groundwater in these aquifers is anaerobic as determined by undetectable dissolved oxygen at both sites as well as the accumulation of dissolved Fe(II) in the aquifers at the Florence site (22; F. Chapelle, unpublished data). Fe(III) reduction is considered to be the predominant terminal electron-accepting process in the aquifers at the Florence site, and sulfate reduction, fed by a sulfate flux from the confining beds (Fig. 2), is considered to be the predominant terminal electron-accepting process in the aquifers at the Myrtle Beach site (F. H. Chapelle and D. R. Lovley, unpublished data).

Cores were obtained with a Christensen rotary-type core barrel equipped with an inner barrel for separating circulating drilling fluid from the fresh core. The core diameter was

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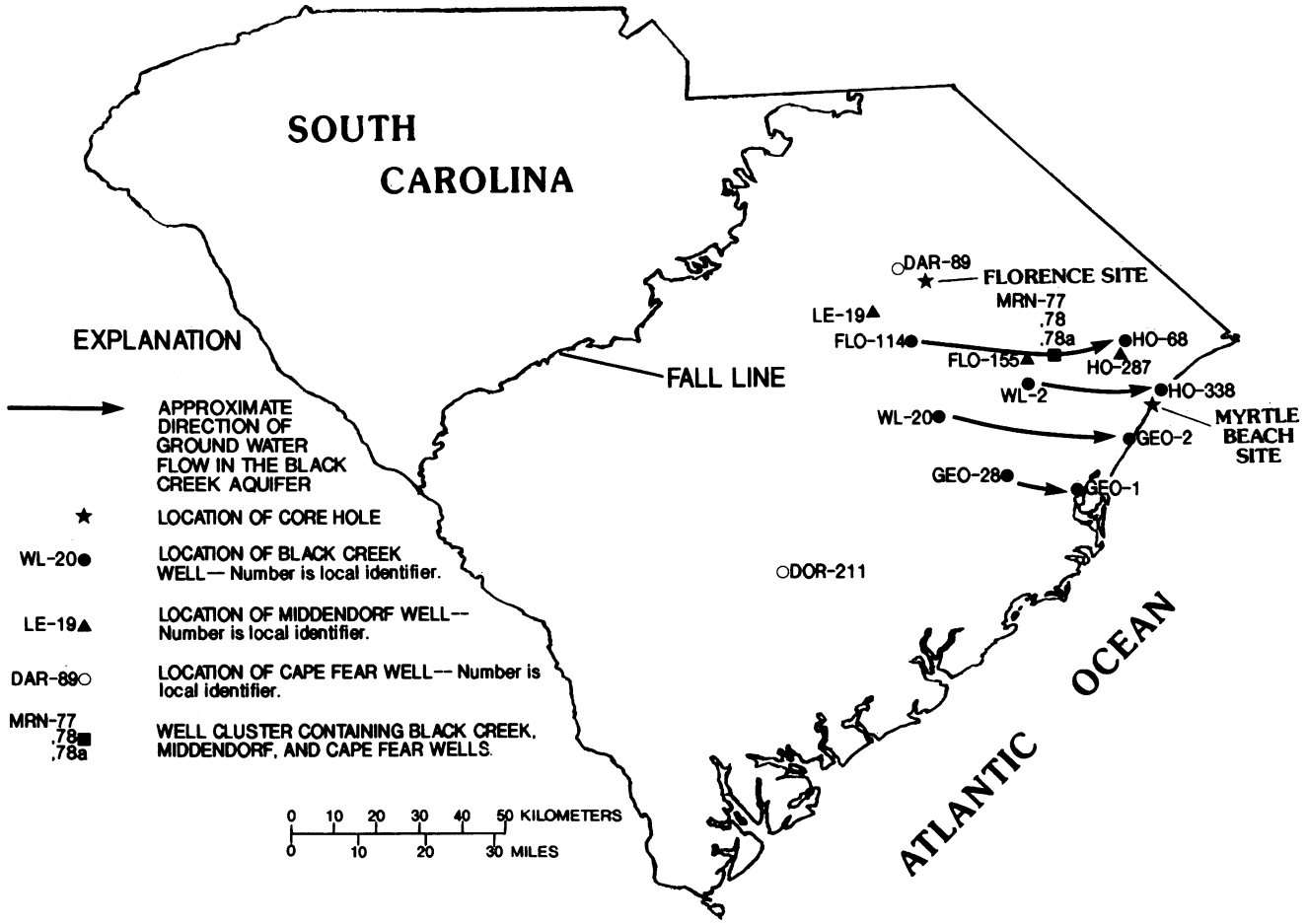

FIG. 1. Locations of study area, core holes, and wells.

either 8.2 cm (Myrtle Beach) or 5.0 cm (Florence). The coring apparatus was configured so that the shoe of the inner barrel protruded as much as 1 cm out of the core barrel. This design increased recovery of coarse-grained sands and gravels and reduced the possibility of core contamination by restricting penetration of drilling fluid into the sediments. At the Myrtle Beach site, barium concentrations, which are less than 10 μg/liter in the native groundwater (22) and ca. 10 mg/liter in the drilling fluid, were measured as a check for potential drilling fluid contamination. Cores were broken in half, and 1-ml subsamples of sediment were taken at 0.5-cm intervals in a traverse from the outside to the center of the core. Each 1-ml subsample was diluted 1:10 with deionized water, and barium was analyzed by direct current plasma atomic emission spectroscopy (detection limit, 2 μg/liter). For the Florence core hole, a tracer of carboxylated fluorescent microspheres (1-μm diameter; Polysciences Inc.) was added to the drilling fluid to provide a final concentration of 10^5 spheres per ml. Core subsamples were taken along a traverse, diluted as described above, and filtered onto a black filter (0.2-μm pore diameter; Nuclepore Corp., Pleasanton, Calif.). One-tenth of the total filter surface was scanned by epifluorescence microscopy (×1,200) for the presence of microspheres (detection limit, 10 spheres per ml). Except where specifically noted, only sediments in which the screening procedures demonstrated that the drilling fluid had penetrated less than 0.5 cm into the cores were used for rate measurements.

Radiotracer studies. Acetate oxidation was chosen as an estimate of CO_2 production in these sediments because with sulfate reduction or Fe(III) reduction as the terminal electron-accepting process, ca. 50% of the carbon flow proceeds through acetate (24, 26). Glucose oxidation to CO_2 was measured as an independent qualitative estimate of the relative metabolic potential in the sediments. Rates of [U-^{14}C]glucose metabolism to CO_2 cannot be readily translated into rates of glucose uptake because of the complicated kinetics of [U-^{14}C]glucose metabolism in anaerobic sediments (21).

Core samples to be used for radiotracer experiments were returned to the laboratory within 8 h of collection. A length of ca. 5 cm was cut from one end for analysis of drilling fluid penetration as described above. The remaining length of core was placed in an N_2-filled glovebag and broken in half. The radial center portion of the core was then subsampled with sterilized instruments. About 10 ml of sediment was placed into sterile 20-ml serum vials that had been previously flushed with N_2. The vials were sealed with thick black butyl stoppers. Abiological controls were generated by autoclaving the sediments (120°C, 1 h).

The sediments were injected with 100 μl of an anaerobic solution of [2-^{14}C]acetate (53 mCi/mmol) or [U-^{14}C]glucose (280 mCi/mmol) to provide 0.5 μCi per bottle. The bottles of sandy sediments were placed on a vortex mixer for ca. 15 s to mix the label solution into the sediments. For the highly cohesive clayey sediments, bottles were not shaken and the

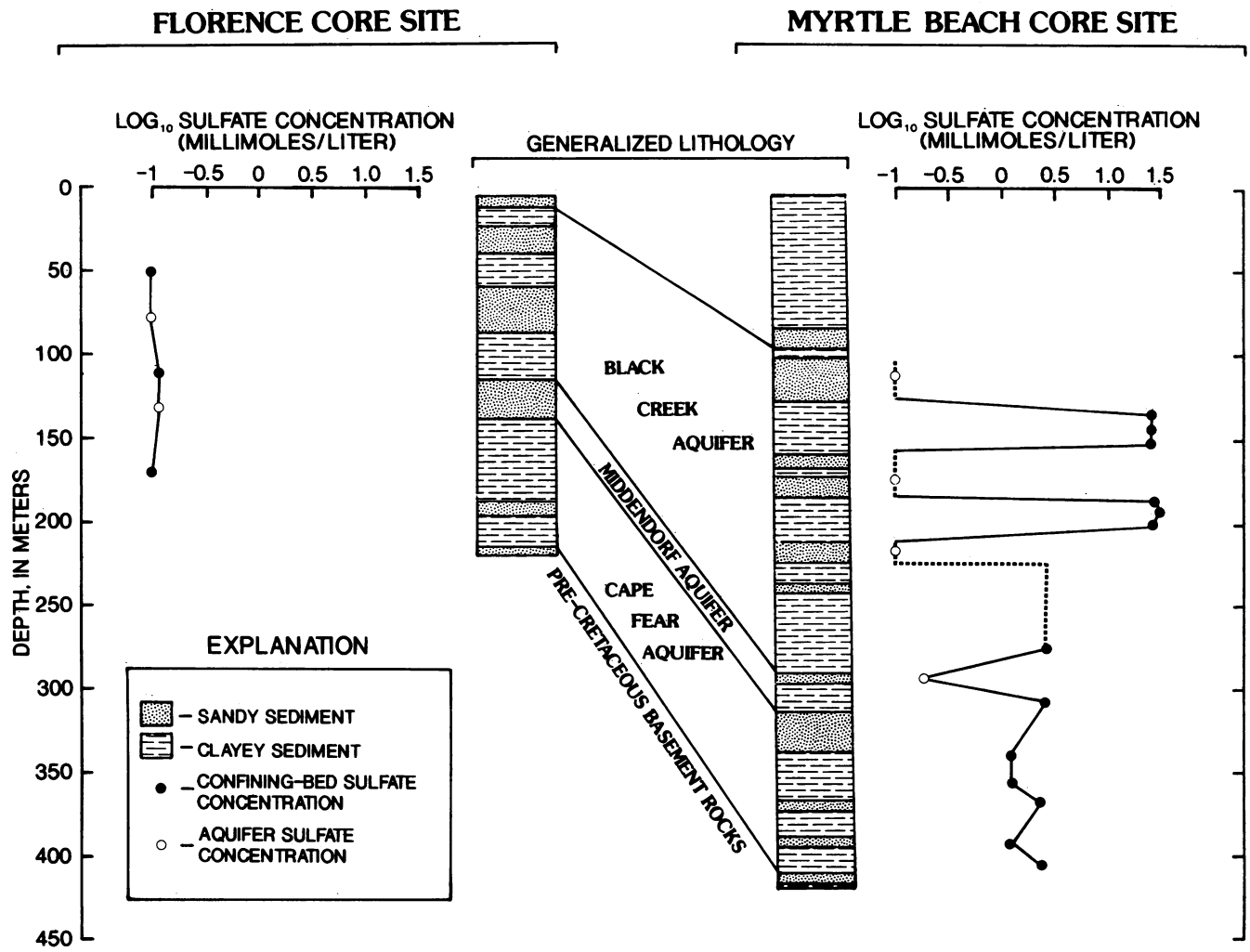

FIG. 2. Geohydrologic section showing the generalized lithology of the core holes and sulfate concentrations in the aquifers and confining beds. Zero designates the land surface.

label solution was allowed to absorb into the sediments. The sediments were incubated at the in situ temperature of 20°C in the dark. At each time point, duplicate vials were acidified with 2 ml of 5 N H_2SO_4. CO_2 was flushed from the bottles with a stream of N_2 and trapped in NaOH, and the $^{14}CO_2$ was quantified by liquid scintillation counting (26). The first-order turnover rate constant (k) was calculated as $k = f/t$, where f was the fraction of added label evolved as $^{14}CO_2$ over time (t) during the initial time points when $^{14}CO_2$ production was linear. The detection limit of this procedure was about 0.5% of total added label. For the maximum incubation time of 77 days, the lowest observable k was therefore 0.025/year. No methane production was expected in these sediments. However, the possibility of $^{14}CH_4$ production was examined as previously described (26) at a time point at which there was significant $^{14}CO_2$ production. $^{14}CH_4$ was not detected.

In the absence of methanogenesis, anaerobic oxidation of acetate yields 2 mol of carbon dioxide. Therefore, the rate of carbon dioxide production from acetate oxidation in the sediments (v_{CO_2}) was calculated as:

$$v_{CO_2} = k \times [\text{acetate}] \times 2 \quad (1)$$

where [acetate] is the molar concentration of acetate in the groundwater of the appropriate aquifer.

Water chemistry. All major-ion water chemistry data used for geochemical modeling in this study were collected by using standard water quality techniques (38, 42) and are available from the WATSTORE data base of the U.S. Geological Survey. Water samples for acetate analysis were taken from production wells tapping the appropriate aquifers. KOH (5 N) was added to each sample to raise the pH over 10. The samples were freeze-dried and reconstituted with 30% phosphoric acid. Organic acids were extracted from the acidic solution with diethyl ether and analyzed by capillary gas chromatography with a flame ionization detector (10). Blanks run with the reagents had acetate concentrations of less than 0.1 μM.

Anions in the pore water of the confining bed clays were measured by a method modified from that of Hendry et al. (17). Approximately 5 g of sediment was slurried with 10 ml of deionized water and centrifuged for 15 min, the supernatant was filtered through a 0.2-μm-pore-size filter, and anions were quantified by ion chromatography. Measurements of sediment moisture content were used to back-calculate

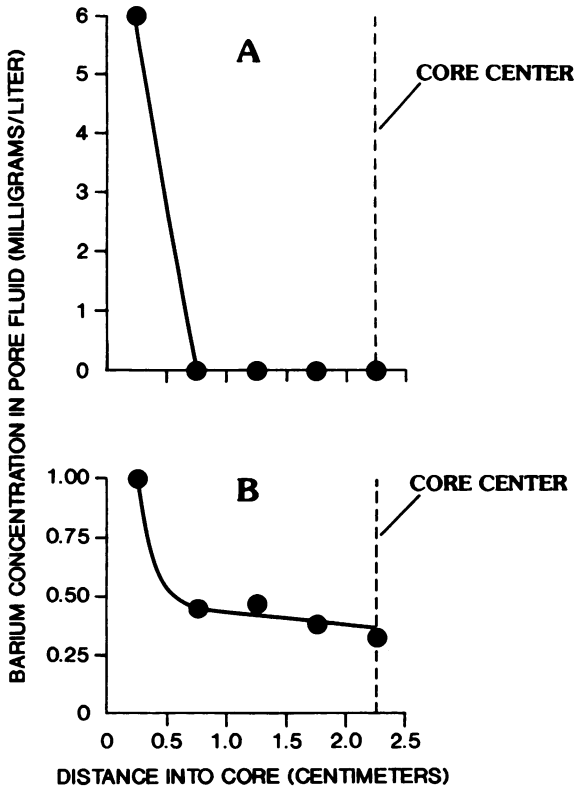

FIG. 3. Barium concentrations (A) in a typical Myrtle Beach sediment core (388-m depth) that was not contaminated with drilling fluids and (B) in a sediment core (295-m depth) that was contaminated with drilling fluids.

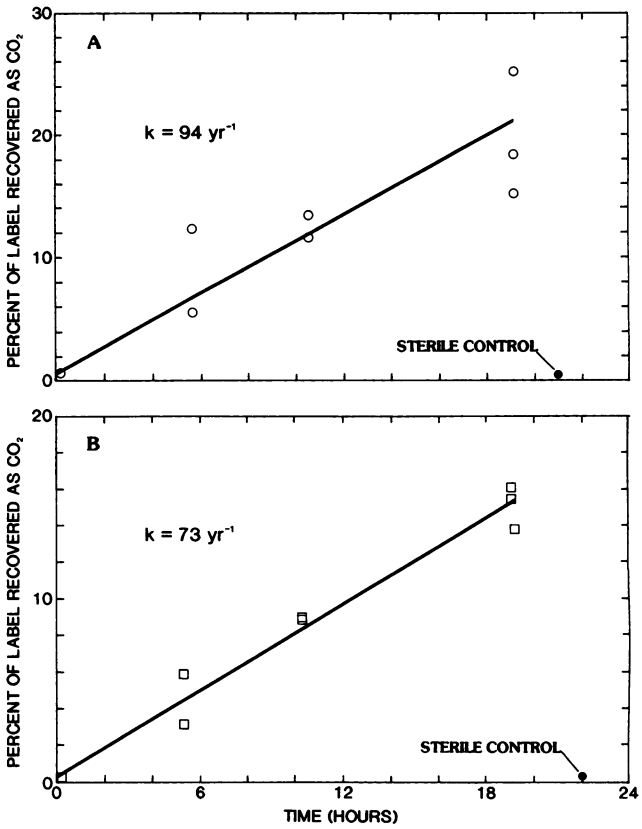

FIG. 4. Production of $^{14}CO_2$ from [2-^{14}C]acetate (A) and [U-^{14}C]glucose (B) in sandy sediments (123-m depth) of the Black Creek aquifer from the Myrtle Beach site. Each symbol represents the results from one sediment incubation that was acidified and flushed for $^{14}CO_2$ determination at the time designated. The line shown is the best-fit line as determined by a least-squares linear regression.

anion concentrations in the pore water. This method assumes that leachable sulfate-bearing minerals such as gypsum are absent from the sediments. X-ray analysis and petrographic microscopy of sediments from these horizons have shown no evidence of sulfate-bearing minerals (33).

Sediment properties. Total porosity, effective porosity, and the size distribution of pore throats of sediment samples were determined by K & A Laboratories (Tulsa, Okla.). Total porosity was measured by helium saturation. The pore throat radius distribution and interconnected pore space were measured by mercury injection. Effective porosity was calculated by taking the product of total porosity and interconnected pore space. To determine sediment organic matter, the sediments were first treated with 0.5 N HCl to remove carbonates and then were analyzed for total carbon with a CHN analyzer.

RESULTS AND DISCUSSION

Potential for contamination of cores with drilling fluids. Inappropriate sampling techniques can readily contaminate subsurface sediments with drilling fluids, and thus it is essential to ensure that sediment samples have not been contaminated before performing microbial analyses. All the sediment cores were intact with no visually apparent drilling fluid contamination. Most of the core samples were free of drilling fluid contamination as evidenced by a lack of fluorescent microspheres (data not shown) or detectable barium (Fig. 3A) more than 0.5 cm into the core. However, the sediments from the Middendorf aquifer at the Myrtle Beach site were contaminated with barium to the center of the core (Fig. 3B). Preliminary studies (F. Chapelle, unpublished observations) have suggested that penetration of drilling fluid into subsurface sediments is affected by such factors as drillstem pressure, coring rate, and downhole fluid pressure. These parameters must be constantly varied during drilling operations. Thus, the potential for contamination varies during coring operations. The results presented here emphasize that visual examination of cores is not an adequate check for potential drilling fluid contamination, and thus it is necessary to screen all sediment materials with sensitive drilling fluid tracers.

Rate estimates from laboratory incubations. Typical initial linear rates of $^{14}CO_2$ evolution that were used to estimate first-order turnover constants are shown in Fig. 4. Although all the sediments examined had detectable linear rates of $^{14}CO_2$ production from [U-^{14}C]glucose, production of $^{14}CO_2$ from [2-^{14}C]acetate was only detectable within 77 days of incubation in some sediments (Table 1). In all cases, the oxidation of acetate and glucose to CO_2 could be attributed to biological activity as there was no production of $^{14}CO_2$ when the microorganisms were killed with heat before the injection of the radiolabeled substrates (Fig. 4; data not shown).

At both core hole sites, the highest rates of $^{14}CO_2$ production from [2-^{14}C]acetate or [U-^{14}C]glucose were observed in sandy aquifer sediments (Table 1). Turnover rates were highest in the Black Creek aquifer and lowest in the Cape

TABLE 1. Acetate and glucose turnover and estimated rates of acetate oxidation to carbon dioxide

Site	Depth (m)	Hydrologic unit	Sediment texture	Turnover rate constant (yr^{-1})		Acetate concn in groundwater (μM)	Estimated CO_2 production from acetate turnover[a] (mmol $liter^{-1}$ yr^{-1})	Sediment organic carbon (%)
				Acetate	Glucose			
Florence	38	Black Creek (aquifer)	Sandy	118	174	1.0 ± 0.25	2.4×10^{-1}	0.46
Florence	92	Black Creek (confining bed)	Clayey	<0.025	NA[b]	NA	NA	1.70
Florence	113	Middendorf (aquifer)	Sandy	6.8	NA	0.8 ± 0.07	1.1×10^{-2}	0.040
Florence	141	Cape Fear (aquifer)	Sandy	<0.025	NA	0.5 ± 0.03	$<2.5 \times 10^{-5}$	0.025
Myrtle Beach	123	Black Creek (aquifer)	Sandy	94	73	1.8 ± 0.9	3.4×10^{-1}	NA
Myrtle Beach	165	Black Creek (aquifer)	Sandy	2.6	74	1.8 ± 0.9	9.4×10^{-3}	0.33
Myrtle Beach	240	Black Creek (confining bed)	Clayey	<0.025	0.05	NA	NA	NA
Myrtle Beach	295	Middendorf[c] (aquifer)	Sandy	<0.025	15	NA	NA	0.06
Myrtle Beach	305	Middendorf (confining bed)	Clayey	<0.025	7.7	NA	NA	0.09
Myrtle Beach	388	Cape Fear (aquifer)	Sandy	<0.025	0.2	1.4 ± 0.8	$<7 \times 10^{-5}$	0.21

[a] Calculated from equation 1 as described in Materials and Methods.
[b] NA, Not analyzed.
[c] Sample contaminated with drilling fluid.

Fear aquifer. Within the Black Creek aquifer at Myrtle Beach, acetate turnover rates varied an order of magnitude at two different depths, indicating that there could be considerable heterogeneity in metabolic rates within an aquifer. There was no clear relationship between sediment organic matter concentrations and the turnover rates of the acetate and glucose pools (Table 1). The sediments from the Middendorf aquifer at Myrtle Beach had low rates of acetate and glucose turnover despite being contaminated with drilling fluids (Table 1).

The differences in the rates of [U-^{14}C]acetate turnover in the sandy aquifer sediments appeared to be due to differences in the rates of acetate metabolism since the aquifers contained comparable acetate concentrations (Table 1). Acetate concentrations in the Fe(III)-reducing sediments of the Florence aquifers appeared to be slightly lower than those in the sulfate-reducing sediments of the Myrtle Beach aquifers, although the differences were not statistically significant. Slightly lower acetate concentrations would be expected in the Fe(III)-reducing sediments since Fe(III)-reducing bacteria can maintain lower steady-state concentrations of acetate than sulfate reducers (25).

Slow metabolic activity in clayey confining beds. All the clayey sediments of the confining beds had low rates of $^{14}CO_2$ production from [2-^{14}C]acetate or [U-^{14}C]glucose relative to those observed in the active sandy sediments from the Black Creek and Middendorf aquifers (Table 1). In recent studies on aerobic portions of some of the same aquifers as those studied here, it was commonly found that microbial numbers and activity were lower in the clayey layers than in the sandy sediments (4, 13, 16, 19, 31, 37). It was speculated that this might be attributed to lower pH (37), hydraulic conductivity (13), or water potential (13) in the clayey sediments.

Characterization of the clayey and sandy sediments from the Florence site (Fig. 5) suggests an additional mechanism for diminished microbial activity in the clayey sediments. More than 80% of the pore throats in the clayey sediments had diameters of 0.05 μm or less, whereas ca. 50% of the pore throats in the sandy sediments had diameters greater than 5 μm (Fig. 5). Furthermore, in the clayey sediments, only about 20% of the total porosity was interconnected, so that the effective porosity was about 7.8%. In contrast, the sandy sediments had an effective porosity of ca. 23%. Thus, even though the clayey sediments actually have a slightly greater porosity (43%) than the sandy sediments (32%), the small pore throats and lack of interconnection in the clayey sediments must greatly restrict microbial and nutrient mobility relative to the sandy aquifer sediments.

Rate estimates from geochemical modeling. Concentrations of dissolved inorganic carbon increased along the flowpaths of each aquifer (Table 2). This was associated with a corresponding molar increase in dissolved sodium and little change in dissolved calcium and magnesium. The progression of a calcium-magnesium water to a sodium bicarbonate water is typically observed in coastal plain aquifers (2) and reflects the reaction of microbially produced carbon dioxide with carbonate shell material:

$$CO_2 + Ca_xMg_{1-x}CO_3 + H_2O \rightarrow Ca_x + Mg_{1-x}^{2+} + 2HCO_3^- \quad (2)$$

as well as cation exchange of Ca^{2+} and Mg^{2+} for Na^+:

$$Ca^{2+} + Na_2(clay) \rightarrow Ca(clay) + 2Na^+ \quad (3)$$

$$Mg^{2+} + Na_2(clay) \rightarrow Mg(clay) + 2Na^+ \quad (4)$$

The observed downgradient increase in chloride concentrations results from mixing of fresh groundwater with seawater that has been incompletely flushed from marine sediments (39).

Equations describing these geochemical changes can be formulated (32). For example, the change in dissolved inorganic carbon along each flowpath segment (ΔM_C) is equal to the sum of carbon dioxide produced from organic matter oxidation (m_{CO_2}) and the carbonate shell material (m_{sm}) dissolved:

$$\Delta M_C = m_{CO_2} + m_{sm} \quad (5)$$

In these sediments, carbonate shell material contains about 98% calcite ($CaCO_3$) and 2% magnesite ($MgCO_3$) (P. McMahon and F. Chapelle, submitted for publication). Thus, the change in dissolved calcium along each flowpath segment (ΔM_{Ca}) is equal to $0.98m_{sm}$ minus the amount of calcium that is removed from solution by sorption onto clays ($m_{Ca(clay)}$):

$$\Delta M_{Ca} = 0.98m_{sm} - m_{Ca(clay)} \quad (6)$$

Similarly, the change in dissolved magnesium (ΔM_{Mg}) is given by:

$$\Delta M_{Mg} = 0.02m_{sm} - m_{Mg(clay)} \quad (7)$$

The change in sodium (ΔM_{Na}) along each flowpath segment is given by the sum of sodium coming from incompletely flushed seawater (m_{sw}) and sodium added to the solution from calcium-sodium ($2m_{Ca(clay)}$) and magnesium-sodium ($2m_{Mg(clay)}$) exchange:

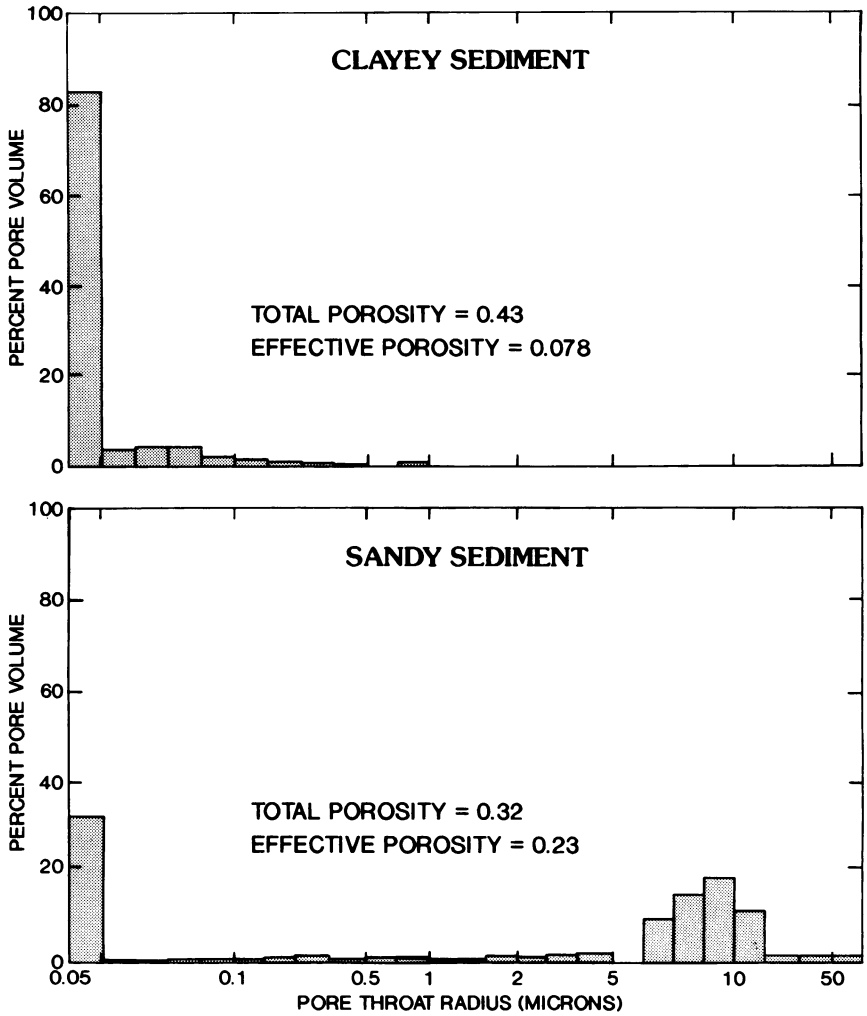

FIG. 5. Comparison of porosity and pore throat radius of clayey confining bed sediments (60-m depth) and sandy aquifer sediments (141-m depth) from the Florence site.

$$\Delta M_{Na} = m_{sw} + 2m_{Ca(clay)} + 2m_{Mg(clay)} \quad (8)$$

The change in chloride concentration (ΔM_{Cl}) indicates the amount of seawater mixing with fresh groundwater:

$$\Delta M_{Cl} = m_{sw} \quad (9)$$

As is typically done for geochemical studies on groundwater, these five mass transfer equations (equations 5 to 8) were solved with the program BALANCE (30) to determine the mass of carbon dioxide produced by organic matter oxidation (m_{CO_2}) between two points along groundwater flowpaths of the three aquifers under study. In this specific study, m_{CO_2} could also have been calculated directly from the equation:

$$m_{CO_2} = \Delta M_C - \Delta M_{Ca} - \Delta M_{Mg} - (\Delta M_{Na} - \Delta M_{Cl})/2 \quad (10)$$

However, the use of BALANCE was preferable as this program represents a general approach that may be applied to the geochemical modeling of a wide variety of groundwaters. Furthermore, BALANCE performs important checks on the model equations such as making sure that all the proposed independent parameters are not inadvertently dependent and ensuring that there are appropriate mineral phases specified for all the dissolved constituents.

The approximate velocity (R) of groundwater flow in these aquifers may be estimated from Darcy's equation: $R = K(dh/dL)/P$, where K is the hydraulic conductivity, dh/dL is the hydraulic gradient, and P is the aquifer porosity. The parameters necessary to calculate groundwater flow rates for these aquifers are available from a regional digital simulation of this aquifer system (Table 3). From the values for the mass of carbon dioxide produced from organic matter oxidation (m_{CO_2}), rates of groundwater flow (R), and the length of each flowpath segment (L), rates of CO_2 production from organic matter oxidation ($OMCO_2$) are given by: $OMCO_2 = (m_{CO_2})(R)/L$. With this method, the estimated rates of CO_2 production from organic matter oxidation in the aquifer sediments were 10^{-4} to 10^{-6} mmol of CO_2 per liter per year (Table 3).

Comparison of rate estimates. In those instances in which the production of $^{14}CO_2$ was sufficiently rapid to permit an estimate of the rate of [2-^{14}C]acetate turnover, the estimated rates of CO_2 production were 2 to 4 orders of magnitude faster than rates estimated by geochemical modeling. Not factored into this difference is the fact that only ca. one-half the CO_2 production in these sediments probably results from acetate oxidation. In a parallel study, rates of CO_2 produc-

TABLE 2. Water chemistry data for Black Creek, Middendorf, and Cape Fear aquifers

Well	pH	mmol/liter						
		Dissolved inorganic carbon	Cl	SO_4^{2-}	SiO_2	Ca^{2+}	Mg^{2+}	Na^+
Black Creek aquifer								
GEO-28	8.7	5.9	0.37	0.05	0.20	0.07	0.029	7.0
GEO-1	8.3	7.6	1.3	0.03	0.20	0.05	0.037	9.6
WL-20	8.7	2.7	0.13	0.06	0.40	0.03	0.008	3.0
GEO-2	8.7	10.2	1.5	0.20	0.20	0.11	0.067	13.0
WL-2	8.6	6.5	0.87	0.24	0.23	0.21	0.15	7.0
HO-338	8.0	10.9	6.2	0.03	0.25	0.075	0.104	16.0
FLO-114	6.9	1.13	0.037	0.09	0.58	0.23	0.11	0.15
MRN-77	8.7	5.2	0.15	0.06	0.23	0.035	0.01	5.2
HO-68	8.2	9.4	1.7	0.02	0.21	0.088	0.12	10.4
Middendorf aquifer								
LE-19	6.7	0.52	0.056	0.80	0.23	0.14	0.045	0.074
FLO-155	8.3	5.0	0.85	0.17	0.50	0.010	0.053	6.1
MRN-78	8.1	6.3	1.6	0.14	0.40	0.04	0.015	7.8
HO-287	8.6	7.6	2.5	0.12	0.21	0.04	0.022	10.4
Cape Fear aquifer								
DAR-89	7.2	1.4	1.0	0.21	0.25	0.2	0.17	1.7
MRN-78a	7.6	14.1	11.3	1.3	0.57	0.3	0.144	25.2
DOR-211	8.5	7.1	3.9	1.0	0.30	0.058	0.017	13.9
Core hole	8.1	14.0	33.4	0.63	0.28	0.28	0.23	56.5

tion were estimated by incubating slurries of sediments from the Myrtle Beach site and measuring the accumulation of dissolved inorganic carbon over time (P. McMahon and P. M. Williams, Ground Water, in press). The rate estimates from those incubations were 3 to 6 orders of magnitude greater than the rate estimates from [2-^{14}C]acetate turnover. Similarly high rates of CO_2 production were measured in laboratory incubations of other deep subsurface sediments from the Atlantic coastal plain (7, 29). These high rates were observed whether the sediments were slurried (29; McMahon and Williams, in press) or not (7).

Given the wide discrepancy between the various rate estimates, which estimates most closely correspond to in situ rates? Particulate organic matter is considered to be the primary source of organic matter for microbial metabolism in these sediments. This is because there is no net decrease in dissolved organic carbon along the flowpaths studied (22). Furthermore, even if all the dissolved organic carbon in the groundwater was metabolized, the concentration of dissolved organic carbon (ca. 0.1 mM organic carbon) (22) is too low to account for the accumulation of dissolved inorganic carbon (0.75 to 4.8 mM inorganic carbon) that was

TABLE 3. CO_2 production rates from geochemical modeling

Flowpath segment	M_{CO_2} (mmol/liter)	Flowpath distance (m)	Hydraulic conductivity[a] (m/yr)	Hydraulic gradient[a] (10^4)	Porosity[a]	Flow rate (m/yr)	CO_2 production rate (mmol/liter per yr)
GEO-28 to GEO-1 (Black Creek aquifer)	0.77	3.2×10^4	2.7×10^3	2.4	0.25	2.6	6.3×10^{-5}
WL-20 to GEO-2 (Black Creek aquifer)	2.9	7.7×10^4	2.7×10^3	1.9	0.25	2.1	7.9×10^{-5}
WL-2 to HO-338 (Black Creek aquifer)	2.2	5.6×10^4	2.7×10^3	1.6	0.25	1.7	6.7×10^{-5}
MRN-77 to HO-68 (Black Creek aquifer)	2.1	3.2×10^4	2.7×10^3	1.4	0.25	1.5	9.8×10^{-5}
FLO-114 to MRN-77 (Black Creek aquifer)	1.9	6.0×10^4	2.7×10^3	2.5	0.25	2.7	8.6×10^{-5}
LE-19 to FLO-155 (Middendorf aquifer)	1.9	6.4×10^4	1.1×10^3	2.4	0.25	1.0	3.0×10^{-5}
FLO-155 to MRN-78 (Middendorf aquifer)	0.75	1.3×10^4	1.1×10^3	1.1	0.25	0.5	2.9×10^{-5}
MRN-78 to HO-287 (Middendorf aquifer)	0.48	2.9×10^4	1.1×10^3	1.0	0.25	0.4	6.6×10^{-6}
DOR-89 to MRN-78a (Cape Fear aquifer)	4.8	7.2×10^4	5.0×10^2	2.1	0.25	0.4	3.0×10^{-5}
DOR-211 to core hole (Cape Fear aquifer)	3.5	1.6×10^5	5.0×10^2	2.0	0.25	0.4	8.6×10^{-6}

[a] Values from reference 1.

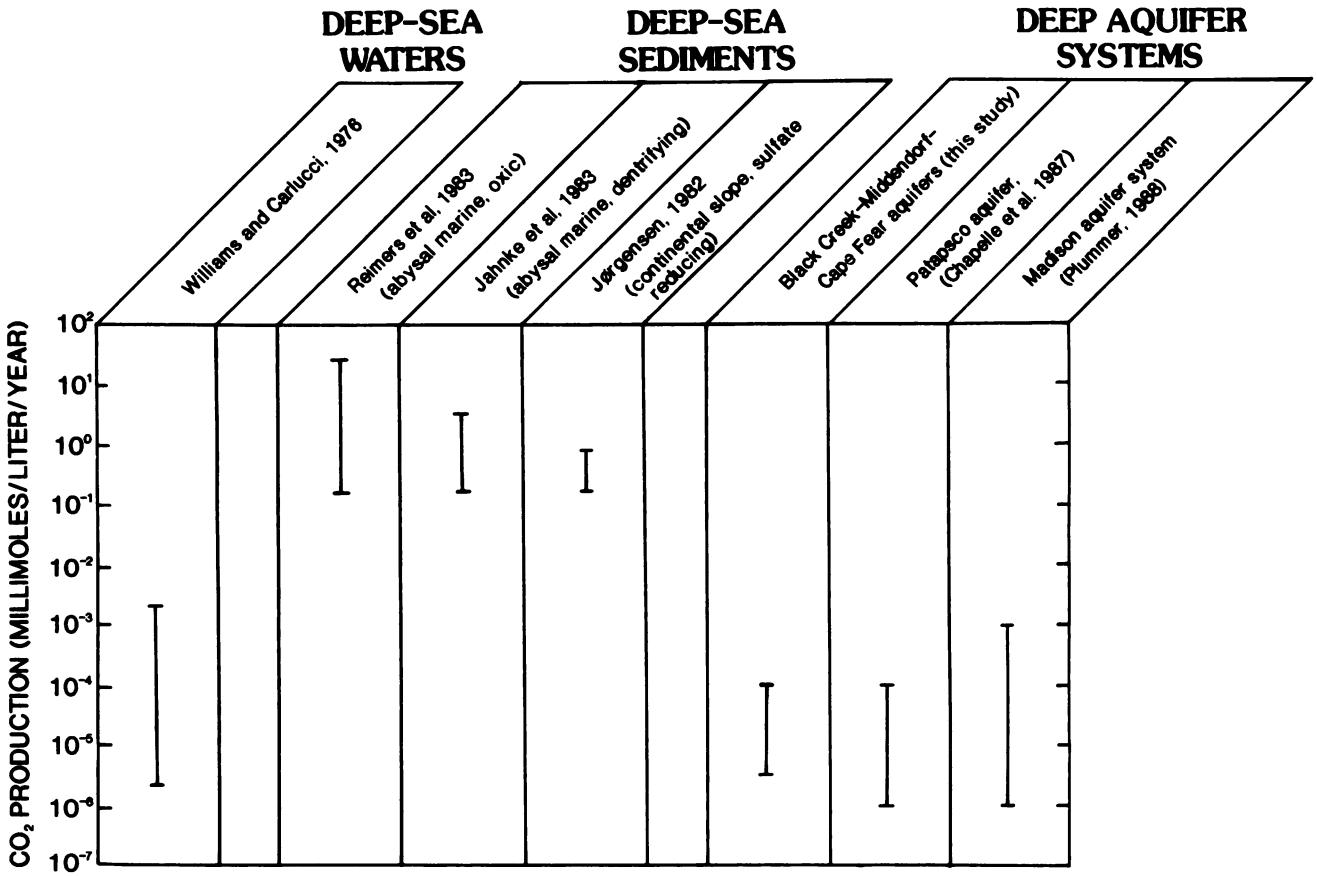

FIG. 6. Comparison of carbon dioxide production rates from deep seawaters (41), deep sea sediments (18, 20, 34), and deep aquifer systems (8, L. N. Plummer, EOS **69**:1182, 1988; this study). Estimates for sulfate-reducing deep sea sediments were calculated from sulfate reduction rates (20) and the assumption that 2 mol of carbon dioxide are produced per mol of sulfate reduced.

observed along the flowpath segments. The sediments that compose this hydrologic system were deposited between 70 and 80 million years ago (14), and they still contain metabolizable organic material (Table 1). It can be calculated that even if the sediments contained as much as 5% organic carbon at the time of deposition, an average carbon dioxide production rate as low as 2×10^{-4} mmol/liter per year would consume the organic material within 70 million years. Since the rate of organic matter oxidation is expected to slow over time as more-labile constituents are metabolized (6), it seems likely that the rates of carbon dioxide production in the aquifers can be no more than ca. 10^{-4} mmol/liter per year. All the rate estimates from geochemical modeling are lower than this estimated upper limit.

For horizons where $^{14}CO_2$ production from [2-^{14}C]acetate was not detectable with extended incubation, the maximum estimated limit for carbon dioxide production was also ca. 10^{-4} mmol/liter per year or less. However, for horizons where $^{14}CO_2$ production could be quantified, the rate estimates were significantly greater than 10^{-4} mmol/liter per year and must be overestimates. For example, at the Florence site, if the estimated rates of acetate oxidation to carbon dioxide approximated the in situ rates of organic matter oxidation in the Black Creek and Middendorf aquifers, all the organic material that is now present would be completely oxidized within 1,000 (Middendorf) or 5,000 (Black Creek) years. If the estimates from measuring carbon dioxide accumulation over time in laboratory incubations (McMahon and Williams, in press) were correct, all the organic carbon in the sediments would be consumed in a matter of years. These considerations indicate that the geochemical modeling technique provided the more consistently reliable rate estimates.

The reasons for the apparent overestimation of in situ rates in some instances with [2-^{14}C]acetate and the consistent gross overestimates that are obtained by measuring CO_2 accumulation over time in laboratory investigations were not investigated. Although the addition of the [2-^{14}C]acetate may have increased the acetate pool size ca. twofold, such an increase could not account for the extent of the apparent CO_2 production rate overestimates. It has been previously observed that measurements of [^{14}C]acetate turnover may overestimate the rate of organic material decomposition in sediments (9, 36) and that laboratory incubations of sediment often overestimate in situ metabolic rates (11). The high rates of metabolism in the laboratory incubations cannot be attributed to contamination of the sediments with drilling fluids because in the one case in which the sediments were contaminated, the rate of [2-^{14}C]acetate turnover was undetectable (Table 1). In a study of microbial activity in anaerobic deep sea sediments, it was considered that the rates of microbial metabolism that were measured in laboratory incubations were probably higher than the in situ metabolic rates and that this might be due, in part, to slower rates of microbial metabolism at the higher pressures the microorganisms must tolerate in situ (40). However, pressure effects

seem unlikely to explain the high rates of [2-^{14}C]acetate oxidation that were measured in the Black Creek and Middendorf aquifer sediments. These sediments were collected from depths no greater than 165 m, and thus, the in situ pressure is estimated to be no more than ca. 17 atm (1,721.9 kPa) greater than at land surface.

The finding that the [2-^{14}C]acetate technique greatly overestimated the probable in situ rates of CO_2 production at some sites but not at others and the potential for considerable heterogeneity with depth even within the same aquifer at one site suggest that it would be difficult to develop correction factors that could be used to convert metabolic rates in laboratory incubations to in situ rates.

Comparison of deep subsurface metabolic rates with other environments. The results of geochemical modeling for other deep aquifers (8; L. N. Plummer, EOS **69**:1182, 1988) yield rates comparable to those of the aquifers studied here (Fig. 6). The rates of metabolism in these sediments are extremely slow compared with most other sedimentary environments. Even sediments of the deep ocean have CO_2 production rates orders of magnitude higher than those in the terrestrial deep subsurface (Fig. 6). The metabolic rates in deep aquifers are comparable to the lowest rate estimates for highly oligotrophic deep ocean waters (Fig. 6). It is interesting that measurements of the metabolism of [^{14}C]organics in deep ocean waters also give estimates of CO_2 production that are greater than those obtained through geochemical modeling (41). Even when the rate estimates from [2-^{14}C]acetate turnover are considered, it is apparent that deep aquifer systems are among the most oligotrophic aquatic environments yet described that still contain biological activity.

In summary, geochemical modeling as well as considerations of sediment age and organic content indicate that rates of organic carbon metabolism in deep subsurface aquifers are extremely slow and are on the order of 10^{-4} to 10^{-6} mmol of C per liter per year. Furthermore, the available evidence suggests that there are even lower metabolic rates in the clayey sediments of the confining beds. Despite these slow rates of organic matter decomposition, the sediments remain metabolically active. The microorganisms in these aquifer systems could be remnants from the microbial populations that were present at the time of deposition (70 to 80 million years ago) or may be transported in with the groundwater, which has been underground for 10,000 to 50,000 years. In either case, these results suggest that the subsurface microbial populations can remain metabolically active for long periods while metabolizing organic matter at very slow rates.

In some sediments, estimates of the rates of CO_2 production with radiotracer techniques appeared to significantly overestimate in situ rates. In all the aquifers that were studied, measurements of CO_2 accumulation in bottle incubations greatly overestimated the rates of in situ metabolism. A major reason for investigating microbial metabolism in the deep subsurface is to estimate the potential for biorestoration of deep aquifers. The results reported here demonstrate that studies on the metabolic potential of microorganisms in deep aquifer systems must use extreme caution in extrapolating from laboratory-derived metabolic rates to in situ rates.

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