Comparison Between Geochemical and Biological Estimates of Subsurface Microbial Activities

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Abstract. Geochemical and biological estimates of in situ microbial activities were compared from the aerobic and microaerophilic sediments of the Atlantic Coastal Plain. Radioisotope time-course experiments suggested oxidation rates greater than millimolar quantities per year for acetate and glucose. Geochemical analyses assessing oxygen consumption, soluble organic carbon utilization, sulfate reduction, and carbon dioxide production suggested organic oxidation rates of nano- to micromolar quantities per year. Radiotracer time-course experiments appeared to overestimate rates of organic carbon oxidation, sulfate reduction, and biomass production by a factor of 10^3–10^6 greater than estimates calculated from groundwater analyses. Based on the geochemical evidence, in situ microbial metabolism was estimated to be in the nano- to micromolar range per year, and the average doubling time for the microbial community was estimated to be centuries.

Introduction

The presence, abundance, and activity of microorganisms residing within subsurface sediments have received increased interest in recent years [1, 5, 6, 11–14, 16–19, 21, 32, 36]. Diverse microbial communities have been detected in vadose zones [1, 9, 13, 32, 36], aerobic and anaerobic saturated sediments [6, 7, 12, 17], and sediment interbeds between basalts [9]. Subsurface microorganisms have also

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been associated with alterations of groundwater chemistry [21, 25, 26, 33]. In the early twentieth century, subsurface microorganisms were credited with methane accumulation in Illinois groundwater [4]. More recently, subsurface microbial communities have been associated with carbon dioxide production [6, 7, 25], denitrification [12], sulfate reduction [17, 18, 33], iron reduction [21, 25], and volatile fatty acid production [17, 24].

An association between subsurface microorganisms and changes in groundwater chemistry requires that the organisms be active. While geochemical data and models have been used to estimate in situ carbon dioxide production by subsurface microorganisms [6, 7, 25], direct measures of microbial activity have proved elusive. Thorn and Ventullo [37] calculated growth rates based on [^{3}H]thymidine incorporation into DNA, and Madsen reported mineralization rates for glucose [22] and contaminants [23]. Other investigators have reported microbial activities as percent of radioisotopes transformed or disintegrations per minute [16, 17, 28, 30], in part due to their inability to close mass balances. Nearly all investigators have observed rapid utilization of radiolabeled substrates, suggesting turnover times of days to weeks. However, the findings from radioisotope time-course experiments may not accurately reflect carbon and energy flow in subsurface environments and may be incompatible with geochemical data, nutrient resources, and oxygen availability.

Discrepancies between microbial activities estimated by geochemical modeling and radioisotope time-course experiments pose a problem to understanding nutrient flux through subsurface aquifers. Chapelle and Lovley [5] reported that estimates of microbial respiration rates based upon geochemical modeling more accurately reflected in situ microbial activities than did estimates based upon radiotracer mineralization experiments. This communication focuses on the aerobic to microaerophilic Eastern Coastal Plain sediments and uses estimates of microbial growth efficiencies and metabolism to substantiate and expand the findings of Chapelle and Lovley [5].

Materials and Methods

Field Studies

The study site was located on the Eastern Coastal Plain surrounding the Savannah River Site (SRS) in South Carolina (Fig. 1). The SRS is located approximately 32 km southeast of the fall line that separates the piedmont from the coastal plain [35]. Substantial information is available on the hydrology and geology beneath the SRS [2, 3, 25, 35]. Unconsolidated sediments extend to depths of 250–400 m and are underlain by crystalline metamorphic rock or consolidated mudstone. The Middendorf is the deepest Cretaceous aquifer in the region. The overlying Black Creek Formation forms a confining unit between the Middendorf and Pee Dee aquifers. The Congaree is an important aquifer in the overlying Tertiary sediments.

Groundwater samples were collected from the Middendorf aquifer at well locations on and surrounding the SRS. The major recharge to the Middendorf aquifer occurs in the outcrop area that extends from the fall line to near Aiken, SC (shaded area in Fig. 1). Near the SRS, flow in the Middendorf aquifer is to the southwest, toward the Savannah River (as approximated by the flow lines in Fig. 1). Deeper aquifers, where available, represented older groundwaters with less dissolved oxygen (DO). Groundwater in the Middendorf aquifer exhibits an upward hydraulic head >50 m, excluding oxygen contamination of the aquifer during groundwater sampling. Groundwaters were pumped through developed
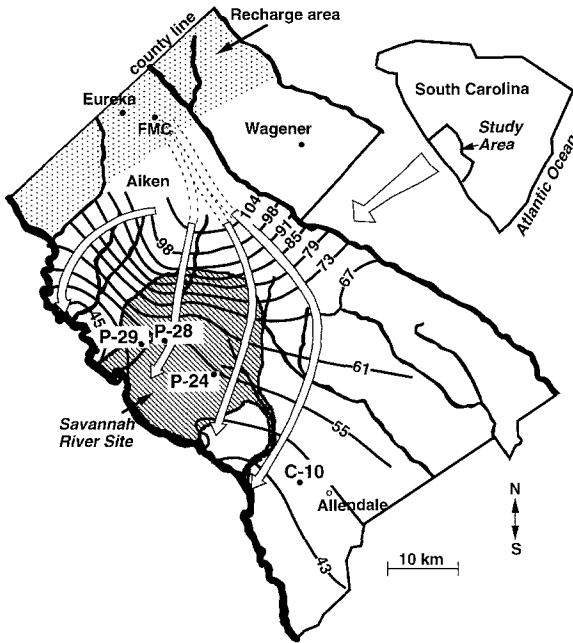
Fig. 1. Study area. Depicts sites sampled in the Atlantic Coastal Plain near Aiken and Allendale, S.C. Water level contours are in meters and approximate groundwater flow paths are shown by the *arrows.*

wells for multiple volumes until pH, temperature, conductivity, and DO concentrations reached steady-state, and values were recorded. Groundwater samples were filtered through a 0.2 μm polycarbonate filter installed in the pump line and analyzed for major inorganic ions, dissolved organic carbon (DOC), and carbon isotopes. Details of groundwater analyses and the geochemical modeling used to determine groundwater age from the ^{14}C activity of the dissolved inorganic carbon (DIC) have been reported by Murphy et al. [25].

In addition to the groundwater samples, sediment samples were obtained from the P-24, P-28, P-29, and C-10 sites (Fig. 1). Boreholes were drilled [31, 34] using a rotary bit (Graves Well Drilling, Inc., Jackson, S.C.) and were continuously flushed with recirculated sodium bentonite viscosifying drilling fluid (Quik-gel, NL Baroid/NL Industries, Houston, Texas), which contained ionic and fluorescent dye tracers for quality assurance tests [31, 34]. The concentration of fluorescent dye tracers in retrieved subsurface samples served as an on-site quality assurance measure. Only samples which exhibited more than 3 orders of magnitude reductions from drilling fluid tracer concentrations were examined. Retrieved sedimentary materials were removed from the sampling tools, the core liners carried into the mobile laboratory, and the sediments extracted. As materials exited the core liners, they automatically entered a N_2-flushed anaerobic glove bag which contained two pairs of sleeved gloves, an extruder port, and an air lock (Coy, Ann Arbor, MI). All manipulations were performed in the bag using aseptic procedures and sterile gloves [31, 34].

Gases, Chemicals and Isotopes

Nitrogen and $N_2:CO_2$ (90:10) were more than 99.9% pure. All chemicals were of reagent grade and were obtained from Mallinckrodt (Paris, Ky.) or Sigma (St. Louis, Mo.). Resi-analyzed, glass distilled

solvents and reagents were purchased from J. T. Baker Chemical Co. (Phillipsburg, N.J.). The following were purchased from New England Nuclear Corp. (Boston, Mass.): $1-^{14}C$-acetate (56 mCi mmol^{-1}), ^{3}H-acetate (3.3 Ci mmol^{-1}), and ^{35}S-sulfate (481 mCi mmol^{-1}). The following were obtained from Amersham Corp. (Arlington Heights, Ill.): $2-^{14}C$-acetate (56 mCi mmol^{-1}) and $U-^{14}C$-glucose (2.8 mCi mmol^{-1}).

Activity Experiments

Isotope solutions (1–50 μCi) were frozen prior to use, thawed, and transferred with gas-tight syringes (Hamilton Co., Reno, Nev.). Sediment aliquots were inoculated for aerobic and anaerobic activity experiments within 30 min of core extrusion. Time course experiments were performed in duplicate. Sterile polypropylene centrifuge tubes were used for aerobic isotope incorporation experiments. Anaerobic crimp-top tubes (Bellco Glass Co., Vineland, N.J.) were used for mineralization experiments or anerobic experiments. Incubations were at ambient conditions which were similar to the 21–27°C in situ temperatures.

Acetate incorporation experiments used 2.0 g sediment, 5.0 μCi $1-^{14}C$-acetate or ^{3}H-acetate, and 1.0 ml sterile distilled water. Final acetate concentrations in experimental tubes were 1.7 μg g^{-1} and 30 ng g^{-1}, respectively. At t_0 and appropriate time points, duplicate tubes were inhibited with 4.5 ml of a phosphate-buffered chloroform-methanol solution and frozen [32, 38]. Time-points of 0, 30 min, 2 h, 8 h, 1 day, and 4 days, generally provided linear rates within three or more time-points. Sulfate reduction experiments utilized 2.0 μCi of ^{35}S-SO$_4$ suspended in 1.0 ml sterile water and 2.0 g sediments. Reactions were inhibited with 0.5 ml of 2.0 M anaerobic sodium hydroxide. Mineralization experiments contained 2.0 g sediment, 1.0 ml sterile water, and 1.0 μCi of carrier-free $2-^{14}C$-acetate (0.4 μg g^{-1} experiment) or $U-^{14}C$-glucose (20 μg g^{-1} experiment) in 25-ml crimp-top tubes. Experiments were performed in duplicate with ten time points, ranging from t_0, 1 h, and up to 1 month. At selected times tubes were inhibited with 0.5 ml of 2.0 M sodium hydroxide.

Analytical Procedures

In the laboratory, acetate incorporation experiments were extracted with chloroform-methanol [38]. The radioactivity incorporated into microbial lipids was determined by scintillation counting [32]. Radioactive sulfide from sulfate-reducing experiments was recovered into acidified zinc acetate, and radioactivity was determined by scintillation counting [30]. The earliest time points yielding measurable results were used to calculate a linear rate that was extrapolated to disintegrations per minute per day. Radioactive $^{14}CO_2$ and $^{14}CH_4$ from mineralization experiments were examined by gas chromatography-gas proportional counting [30, 32]. A Shimadzu 8A gas chromatograph (GC) equipped with a thermal conductivity detector was connected to a Packard 894 gas proportional counter. One hour before analysis, tubes were acidified with 0.5 ml of 6 M hydrochloric acid. Transformation rate constants were estimated from product accumulation rates [28], rather than rates of substrate disappearance used for turnover rate constants. When assessing metabolic activities of microorganisms in low nutrient subsurface samples, the rates of product accumulation can be quantified more accurately and in less time than the rates of substrate disappearance. Furthermore, transformation rate constants have been shown to be similar to turnover rate constants [28].

Procedures Used for Estimating Biomass, Oxygen Requirements, Doubling Times and Carbon Dioxide Evolution

Microbial colony forming units (cfu) were enumerated using spread plate procedures and five-tube most-probable-number dilution series. The liquid medium contained 10 mg liter^{-1} of peptone, yeast extract, and glucose, plus trace minerals, vitamins, and a 2.0 mM carbonate-phosphate buffer [32].

Table 1. Microbial colony forming units and transformation times for acetate and glucose in subsurface water-producing formations[a]
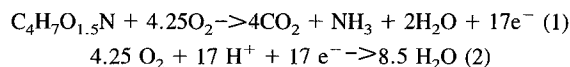
Subsurface formation (depth in m)	Biomass log cfu g^{-1}	Transformation time $(h)^b$			
		aerobic		anaerobic	
		glucose	acetate	glucose	acetate
Ellenton (200 m)	6.6	19 ± 7	30 ± 30	43 ± 24	110 ± 98
Pee Dee (290 m)	6.6	22 ± 8	27 ± 7	18 ± 1	97 ± 46
Middendorf (410 m)	5.6	35 ± 35	15 ± 5	125 ± 140	220 ± 140
Cape Fear (450 m)	4.6	22	177	25	180

[a] Values based on four or more samples, except the Cape Fear samples
[b] Transformation time estimates were determined from transformation rate constants based on product formation rates and the initial rate of ^{14}C-isotope utilization during time-course experiments

Spread plates utilized the same medium plus 20 g liter^{-1} Noble agar. Microbial enumeration experiments were incubated for 1 month at ambient temperature.

Aquifer sands less than 300 m in depth typically contained 10^6–10^7 cfu g^{-1} sediment [1, 13, 14, 32, 36] (Table 1). Unless stated otherwise in the text, calculations considered a case of 4×10^6 microorganisms g^{-1} of sandy sediments. Assuming 10^{13} cells gram^{-1} dry weight, the biomass was estimated to be 400 ng g^{-1}. Based on work of Harris and Adams [15], who estimated the carbon-bound electron composition of bacterial cultures by using dichromate oxidation techniques, calculations in the text assume a cellular formula of $C_4H_7O_{1.5}N$ with an approximate 104 g formula weight. Equation 1 illustrates the oxygen requirement for biomass oxidation [15] and Eq. 2 illustrates proton and electron balances:

$$C_4H_7O_{1.5}N + 4.25O_2 \rightarrow 4CO_2 + NH_3 + 2H_2O + 17e^- \quad (1)$$
$$4.25\ O_2 + 17\ H^+ + 17\ e^- \rightarrow 8.5\ H_2O \quad (2)$$

Calculations in the text will focus on Eq. 1. A microbial community of 4×10^6 microbes g^{-1}, with a biomass of 400 ng g^{-1}, could represent approximately 4 nmol of biomass.

Groundwaters from these study sites contained 0–5 mg liter^{-1} DO [25], corresponding to <1.3 µg or <40 nmol oxygen g^{-1} of sediment at 25% porosity (Fig. 2). These groundwaters contained <400 µg liter^{-1} DOC, and the DOC was dominated by hydrophobic neutral compounds [25, 26]. Glucose and acetate pool sizes were below the detectable limits of 100 µg liter^{-1}. Since acetate and glucose would have represented minor contributions to the <400 µg liter^{-1} DOC, estimated concentrations of 10 ng g^{-1} were used for calculations.

CO_2 accumulation models, using the geochemical code PHREEQE/CSOTOP [8, 27], were used to simulate inorganic mineral dissolution and evolution of CO_2 from organic carbon oxidation. The computer code PHREEQE is designed to model geochemical reactions along groundwater flow paths. Selected reactions are based on the mineralogy of the formation and groundwater chemistry. The accuracy of the selected reactions is determined by how well the modeled groundwater chemistry values match the measured values along the flow path. The version of PHREEQE used in this study included the CSOTOP subroutine which calculated the carbon isotope fractionation. The PHREEQE/CSOTOP model takes into account all non-decay sources and sinks of carbon that would alter the original ^{14}C activity along a designated groundwater flow path. Therefore, the remaining difference between the measured ^{14}C activity at a given well and the model-corrected initial ^{14}C activity in the recharge water is due to radioactive decay, and a groundwater age could be calculated. Groundwater ages, calculated from the ^{14}C-activity of the DIC, ranged from modern (Fig. 2) to approximately 11,500 years before present (yBP) [25]. The term "modern" meant that, within the errors of analyses
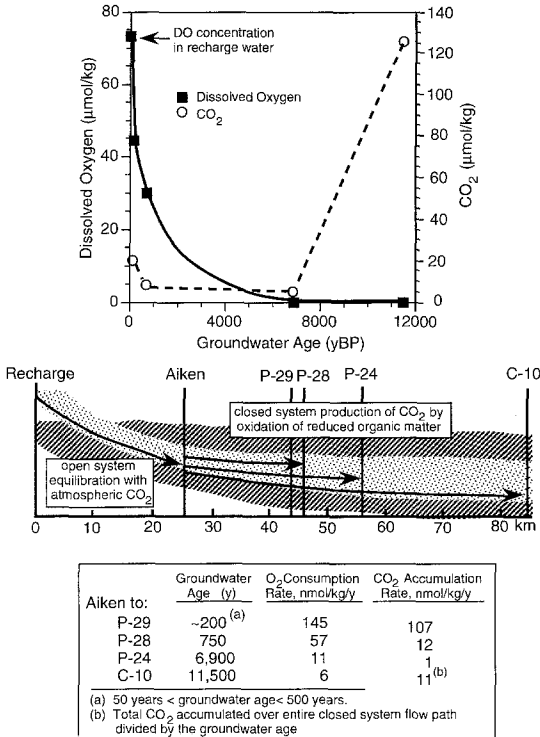
Fig. 2. Dissolved oxygen (■) and CO_2 (○) contents of well waters from the four boreholes. Analyses were obtained from developed wells at varying distances (km) from the recharge area. The groundwater flow paths were open to atmospheric CO_2 equilibration between recharge and Aiken. Downgradient of Aiken the flow paths are closed to atmospheric CO_2 equilibration. Consumption and accumulation rates were calculated by dividing the concentration by groundwater age.

and modeling, no decay of ^{14}C was detected (^{14}C half-life $=$ 5730 years). The absence of ^3H (half-life $=$ 12.3 years) suggested that these "modern" groundwaters were at least 50 yBP, but less than 500 yBP based on the ^{14}C activity and modeling. The oxygen consumption rate (nmol kg^{-1} y^{-1}) was calculated by dividing the DO concentration by the groundwater age and a porosity of 25%. Details of groundwater geochemical modeling used to determine groundwater age from the ^{14}C-activity of the DIC have been published [25].

Results

Utilization of Electron Donors and Acceptors

Microbial colony forming units (CFU) and transformation times for acetate and glucose which were estimated from ^{14}C-radioisotope time-course mineralization experiments are shown in Table 1. Aquifer sands less than 300 m in depth contained 10^6–10^7 cfu g^{-1} sediment. Experimental tubes contained 0.4 μg g^{-1} of carrier-free acetate or 20 μg g^{-1} of carrier-free glucose, which was greater than or equal to the readily usable short-chained organics present in the groundwater DOC. Even at

Table 2. Sulfate reducing activity observed from subsurface sediments[a]

Formation depth (m)	Activity (^{35}SH dpm $\times$ 10^3day^{-1})	Sulfate conc. (mg kg^{-1})	Estimated sulfate reduction rate (μmol kg^{-1} day^{-1})
Ellenton (213)	25[b]	26[c]	34[d]
Pee Dee (256)	7	8[d]	3[e]
Pee Dee (290)	<1	8[d]	<0.4[e]
Middendorf (406)	26[b]	11.5[d]	16[e]
Middendorf (416)	<0.1	11.5[d]	<0.1
Cape Fear (463)	9	6	3

[a] Procedure: one μCi ^{35}S-SO$_4$ plus 3 g of sediment were incubated in duplicate sets for 0.0 to 240 h. Sulfide was trapped, collected, and radioactivity determined by scintillation counting. Rates were determined by multiplying the rate constants by the pool size
[b] Results reflect stimulation of activities after long incubation times, 6-h values = 0
[c] Sulfate concentration was determined from pore waters expressed from cored sediments which were likely contaminated by sulfate present in the drilling fluids
[d] Sulfate concentrations of groundwaters sampled from recently developed wells
[e] Results are likely overestimates as a consequence of b or c above

these elevated concentrations, the transformation times for glucose and aerobic acetate utilization were 1–2 days (Table 1). Deeper aquifers (>350 m) contained fewer than 10^6 microorganisms g^{-1} sediment, which typically required longer times to transform acetate and glucose.

Anaerobic microbial activities in the microaerophilic subsurface sediments were also examined. Acetate and glucose were utilized more slowly under anaerobic conditions (Table 1), and sulfate reduction rates ranged from <0.1–34 nmol g^{-1} day^{-1} (Table 2). Many factors may have contributed to overestimates of the sulfate reduction rate. In the Ellenton and Middendorf sands the sulfate reducers were likely enriched after long incubation times, while Pee Dee and Middendorf sands may have contained elevated levels of sulfate from the drilling fluids [31].

Estimation of Microbial Generation Times Based on Radioisotope Experiments

It may be possible to estimate generation times for microorganisms residing within subsurface sediments with knowledge of substrate availability and assumptions concerning microbial growth efficiencies. Estimates of biomass generation times based on radioisotope time-course experiments are shown in Table 3. Values were calculated following the assumptions in the Methods section and assuming that the isotope solution provided the major carbon substrate. If 2% of the 0.4 μg g^{-1} acetate pool provided by the added ^{14}C-acetate entered biomass with a transformation time of 1 day, then 8 ng of biomass could have been produced daily per gram of sediment. Calculations based on the rapid transformation of ^{14}C-radioisotopes suggest that the 400 ng g^{-1} biomass could aerobically double in 50 days. At a 150 h substrate transformation time, 1 year could be required anaerobically (Tables 1 and 2). If 2% of the 20 μg g^{-1} ^{14}C-glucose pool was assimilated with a 1-day transformation time, then theoretically, 400 ng g^{-1} biomass could be produced each day. ^{3}H-Acetate experiments added 30 ng acetate g^{-1} and exhibited 3-day aerobic transformation times, enabling 0.2 ng of biomass production daily for an approxi-

Table 3. Approximate doubling times (days) for microorganisms in subsurface aquifers based on estimated transformation rate constants[a]

Based on	Aerobic		Anaerobic	
	glucose	acetate	glucose	acetate
[14]C-isotope experiments	1	50	2	320
[3]H-isotope experiments	nd[b]	2000	nd[b]	13000
Estimated natural pool sizes	>2000	6000	>2000	>10000
Estimated years of oxygen availability	<3	<7	NA[b]	NA[b]

[a] Substrate pool sizes in experimental tubes were 20 μg g^{-1} for ^{14}C-glucose and 400 ng g^{-1} and 30 ng g^{-1} for ^{14}C- and ^{3}H-acetate, respectively. Biomass with a formula of $C_4H_7O_{1.5}N$ at 104 g mole^{-1} and a cellular density of 10^6 g^{-1} (400 ng g^{-1}) was used to calculate growth rates assuming 2% efficiency of the substrates entering biomass. Natural pool sizes were estimated to be <10 ng g^{-1}. Oxygen was <5 mg liter^{-1} O_2 or <1.3 μg O_2 g^{-1} sediment at 25% porosity and slow velocity (<3 m year^{-1})
[b] NA = not applicable and nd = not determined

mate 2000-day doubling time. Under anaerobic conditions, biomass doubling would have been considerably slower. Using radioisotope transformation rate constants and estimated natural pool sizes of 10 ng g^{-1}, biomass generation times of 5–30 years could be estimated (Table 3).

One indication of error in the transformation rate and biomass doubling calculations was a faster transformation rate for ^{14}C-acetate than ^{3}H-acetate. Experiments utilizing ^{3}H-acetate increased the pool size 30 ng g^{-1} versus 400 ng g^{-1} for ^{14}C-acetate, a concentration equal to the groundwater DOC and which certainly violated steady-state assumptions commonly used in determining turnover rates. A large stimulation of microbial activity may have enabled the more rapid transformation rate observed with the larger ^{14}C-acetate substrate pool. These results suggested that alternative estimates of in situ metabolism should be sought.

Estimation of Microbial Generation Times Based on Oxygen Availability

If a 400 ng g^{-1} biomass was utilizing glucose for carbon and energy with a 2% efficiency of glucose incorporation into new cells, ~20,000 ng of glucose (110 nmol) would be utilized to produce 400 ng (2% of 20,000) of cells. An approximate reaction based on Eq. 1 could be: 110 nmol glucose + 660 nmol O_2 4 nmol $C_4H_7O_{1.5}N$ (400 ng) + 656 nmol (CO_2 + 658 nmol H_2O + 4.25 nmol e$^-$. Sediment contained <40 nmol O_2 g^{-1} (5 mg liter^{-1} DO at 25% porosity is <1.3 μg or <40 nmol oxygen g^{-1} of sediment), <10% of the O_2 required for biomass doubling. Glucose oxidation rates of 10 ng g^{-1} day^{-1} could eliminate oxygen availability in <3 years (Table 3). If one assumed 10% efficiency of glucose assimilation, a biomass doubling could require 4000 ng of glucose (22 nmol) and 130 nmol O_2 g^{-1} or more than 3 times the O_2 available per gram of sediment. Once the DO is utilized, it is not replenished, and all downgradient environments would be anero-

bic. Figure 2 reveals that oxygen is available for centuries in these subsurface sediments, suggesting that O_2 utilization and microbial growth rates in the subsurface are much slower or that alternative substrates are used.

An alternative substrate potentially available to microorganisms could be preexisting biomass. Even assuming a 25% efficiency of incorporating existing biomass into new cells: 4 nmol $C_4H_7O_{1.5}N$ + 13 nmol $O_2 \rightarrow$ 1 nmol $C_4H_7O_{1.5}N$ + 12 nmol CO_2 + 3 nmol NH_3 + 5.6 nmol e^-, 400 ng g^{-1} biomass could use 13 nmoles of oxygen to produce a new nanomole of cells. Consequently, a gram of soil containing 4 × 10^6 cells g^{-1}, with a generation time of 1000 days, could consume 13 pmol O_2 per day and exhaust the oxygen supply in less than 10 years.

Geologic Measurements

An alternative means of assessing microbial metabolic rates in subsurface sediments could be based on groundwater age and DO concentrations in a confined portion of the major water bearing unit, the Middendorf aquifer. The vertical hydraulic gradients were upward in this Cretaceous aquifer [2], so no significant diffusion of oxygen from the higher tertiary aquifers was expected. Figure 2 shows concentrations of DO as a function of groundwater age in the Middendorf aquifer. Groundwaters in the recharge zone were nearly saturated with oxygen. Concentrations of DO decreased along the flow path, approaching anaerobic conditions ~50 km downgradient from the recharge area (P-24, Fig. 2). Further downgradient the aquifers were anerobic [5, 25]. Groundwater ages, as calculated from the ^{14}C activity of the DIC, ranged from modern (P-29, Fig. 2) to approximately 11,500 yBP [25]. For the flow path between the recharge zone and P-28, the oxygen consumption rate was estimated to be 57 nmol kg^{-1} $year^{-1}$. If this rate were assumed to be linear, depletion of oxygen in the groundwater could occur in ~1300 years. Oxygen consumption in groundwater over relatively long time-periods may be a result of either inorganic reactions involving reduced metals or microbial utilization of reduced organic matter.

Point concentrations of CO_2 plotted in Fig. 2 against groundwater age represent microbial respiration as an indicator of subsurface microbial activity. The portion of the flow path between recharge and Aiken is open to equilibration with atmospheric CO_2; while beyond Aiken the groundwater flow paths are closed to atmospheric CO_2. The processes described for the closed system conditions in Fig. 2 represent a simplification of complex interactions between microorganisms likely occurring in the degradation sequence of reduced organic matter. For example, fermenting microorganisms oxidize complex organic matter to organic acids, which were detected in cores from the Middendorf aquifer [25]. Organic acids may be further oxidized by heterotrophs or sulfate- or iron-oxidizing bacteria, all of which have been observed from the Middendorf cores [1, 14, 17, 25]. The CO_2 accumulation rate is a function of the availability of oxygen, sulfate, and ferrous iron as terminal electron acceptors, as well as the concentration of reduced organic matter. Although reduced organic matter, lignite, was present in all of the boreholes, the amount of lignite was much greater in the lower delta plain depositional environment at C-10. Wells P-28 and P-29 have relatively high concentrations of dissolved oxygen, therefore, the variability in the CO_2 accumulation rate (Fig. 2)

Table 4. CO_2 production as an indicator of cellular activity in sedimentary environments[a]

	Sedimentary environments				
	anaerobic[b] digestors	Lake Mendota[c] sediments	Knaack Lake[c] sediments	Water-producing aquifer sands	Confining clays
Colony forming units ml^{-1}	10^8	10^7	10^6	10^6	10^3
Activity per cell from isotopes (pmol CO_2 $year^{-1}$)	100	40	10	6 0.04*	20 0.0006*
Activity per cell by geochemistry (pmol CO_2 $year^{-1}$)	200	80	40	0.00002	<0.00002
Estimated biomass doubling times	days (1–3)	days (5–10)	days (10–30)	centuries (0.3–500)	millennia (2–>200)

[a]Colony forming units were calculated per ml volume rather than g dry weight. Estimates of the turnover rates for ^{14}C-acetate and glucose were uses to calculate cellular activity from isotope experiments where (*) represents where ^3H-acetate values were used. CO_2 accumulation was used for geochemical estimates. Biomass doubling times were estimated assuming 1 g of biomass production per mole of CO_2 evolved and ^3H-acetate values were used for subsurface sediments
[b]Results calculated from Conrad et al. [10]
[c]Phelps and Zeikus [29, 30]; Phelps et al., unpublished data

may be reflective of the lignite encountered along the respective flow paths (note from Fig. 1 that P-28 and P-29 lie on nearly parallel flow paths).

The CO_2 accumulation rate (Table insert, Fig. 2) was calculated by dividing the CO_2 concentration by the groundwater age, representing average rates over the length of the flow path segments. The highest CO_2 accumulation rate occurred between Aiken and P-29, while the lowest occurred between Aiken and P-24. Under closed system conditions examined (Aiken to C-10), the production of CO_2 was due to the oxidation of reduced organic matter, and the CO_2 accumulation rate was estimated to be 11 nmol kg^{-1} $year^{-1}$.

Another means of evaluating microbial activity may be cellular rates of CO_2 accumulation. Table 4 compares radioisotope and geochemical estimates of CO_2 production rates on a cellular basis for several sedimentary environments. Results are expressed as activity per cell, with cellular densities expressed as colony forming units per milliliter rather than colony forming units per gram dry weight. Anaerobic digester sediments [10] and Knaack Lake sediments [29] contained <3% solids; consequently, the colony forming units per volume shown in Table 4 are 2 orders of magnitude lower than colony forming units expressed per gram dry weight [10, 29, 30]. Cellular activities based on radiosiotope time-course experiments and those based on geochemical measurements of end product accumulations were quite similar for anaerobic digestors and lake sediments (Table 4). If 1.0 g of biomass was produced for each mole of CO_2 evolved, then doubling times would range from 1 to 3 days for anaerobic sewage sludge and 1 to 3 weeks for lake sediments. These doubling times seem reasonable based on retention times of anaerobic digestors, microbial activities in Lake Mendota sediments [30], and published reports of bacterial growth rates in freshwater sediments [11, 27]. However, when the same technique was applied to subsurface sediments, radioisotopic

Table 5. Metabolic activities expected from water chemistry analyses versus metabolic activities estimated from radiotracer experiments

Type of metabolism	Activity expected from water chemistry (μmol kg^{-1} year^{-1})	Activity estimated from isotope experiment (μmol kg^{-1} year^{-1})	Possible overestimate factor
Oxygen consumption	<0.05	>50,000	10^6
Soluble carbohydrate consumption	<6[a]	>30,000	10^4
Acetate consumption	<20[a]	>3000	10^3
Sulfate reduction	<0.001	>10	10^4
Biomass doubling time (years)	>5000	<0.1	10^4

[a] These values are based on observed transformation rate constants and pool sizes used for assumptions in the Methods section. Values based on O_2 requirements for oxidation (<50 nM O_2 year^{-1} availability) would be 2–3 orders of magnitude lower

and geochemical estimates of cellular activity differed by orders of magnitude (Table 5). For example, ^{14}C-acetate and ^{14}C-glucose experiments suggested that cellular activities may be >5 pmol CO_2 year^{-1}. ^{3}H-Acetate experiments, which added less carbon, suggested cellular activities were <40 fmol CO_2 year^{-1}. In contrast, the geochemical estimates of CO_2 accumulations suggested cellular activities were 3 orders of magnitude less than predicted from the biological experiments. ^{14}C-Radioisotope experiments suggested biomass doubling times of months. Estimates based on ^{3}H-acetate were years, compared to centuries and millennia for the geochemical-based estimates.

Table 5 compares activities estimated from groundwater chemistry with those estimated from radiosiotope time-course experiments. A possible overestimate factor of the radioisotope experiments is also provided. Based on groundwater age and hydrologic studies, the average groundwater velocity from the recharge to C-10 was ~3 m year^{-1} [35]. Water from the vicinity of C-10 was estimated to be ~11,500 yBP, suggesting an oxygen consumption rate <0.05 μmol kg^{-1} year^{-1} (Fig. 2). Radiotracer time-course experiments suggested that acetate and glucose pools of 0.4 and 20 μg g^{-1} (7 and 100 μM, respectively) were oxidized daily, which could result in >50 mM year^{-1} oxygen consumption. In contrast to the large substrate pools present in the radiotracer time-course experiments, less than 0.4 mg liter^{-1} DOC was detected from subsurface waters [26], a small percentage of which was hydrophilic [26], suggesting that the soluble glucose and acetate pools were <10 μg kg^{-1} (50–150 nM). Assuming a 3-day transformation time, the carbohydrate consumption rate based on water chemistry would be <6 μmol kg^{-1} year^{-1} (Table 5). Efficient aerobic biomass production would require much of the available oxygen per generation time, even though radioisotope time-course experiments suggested that the biomass could reproduce several times per year. Sulfate reduction rates > 1 nmol kg^{-1} year^{-1} would have produced sulfide accumulations, but sulfide was not detected in the groundwaters. Radiotracer estimates of sulfate

reduction rates as low as 0.1 μmol kg^{-1} day^{-1} (Table 2) would have resulted in a 10^4 overestimate of the sulfate reduction in situ. In all types of metabolism examined, microbial activities estimated from water chemistry analyses were 3–6 orders of magnitude lower than activities estimated from radioisotope time-course experiments.

Discussion

The geochemical evidence suggests that microbial metabolism in porous subsurface environments is extremely slow (nano- to micro molar quantities per year). The presence of DO in groundwaters that are thousands of years old and tens of kilometers from groundwater recharge zones attest to slow in situ oxygen consumption by microorganisms. Although laboratory experiments indicate that radioisotopes are rapidly incorporated into microbial lipids and DNA [32, 37], bacterial growth rates of 10^6 cells g^{-1} day^{-1} could remove oxygen from the groundwater within days. Average microbial community doubling times of centuries appear to correspond better with the geochemistry data. Accordingly, the average bacterium may be struggling to maintain DNA integrity with little opportunity for growth, and only a small fraction of the microbial community may be dividing at any time.

Despite their low metabolic activity, subsurface microorganisms can impact the groundwater chemistry by consuming oxygen [16, 25, 32] and producing CO_2 [4, 7, 25]. The CO_2 production modeled by Murphy et al. [25] was 1–107 nmol kg^{-1} year^{-1} (Fig. 2), a range which was similar to values obtained by Chapelle and Lovley [5] in downgradient portions of the same aquifers. Microorganisms also impact groundwater chemistry by reducing carbon to methane [17], reducing iron [21, 25], or producing sulfide [17, 18]. Sulfate reducers and low levels of sulfate reduction were also reported in these sediments by Jones et al. [18]. Even so, sulfate reduction from these deep subsurface sediments was orders of magnitude lower than in typical lake and marine sediments [5, 17, 18, 20, 30].

Radiotracer techniques assessing anaerobic digester sediments, surface soils, and lake sediments often approximate chemical measurements that estimate microbial activities [10, 20, 29, 30]. Subsurface sediments may represent extreme cases where the rates of microbial metabolism are several orders of magnitude slower. ^{3}H-Acetate experiments, which added fewer micromoles of substrate, more closely approximated in situ rates than the ^{14}C-radioisotope studies which greatly increased the substrate pools. Similar to the results shown here, Chapelle and Lovley [5] reported that ^{14}C-acetate and ^{14}C-glucose turnover rate constants were often >70 year^{-1} (<125 h turnover time) from downgradient anaerobic portions of these aquifers. The inappropriateness of ^{14}C-radioisotope studies for estimating in situ rates of microbial metabolism in subsurface environments likely reflects several factors, including their lower specific activity. Other factors contributing to overestimates may include physical procedures such as the mixing of nutrients and organisms which may stimulate microbial activities [11].

Results reported by Chapelle and Lovley [5] also indicated discrepancies of 3–4 orders of magnitude between estimates based on ^{14}C-acetate metabolism and geochemical estimates of CO_2 production in downgradient portions of these aquifers. Our results agreed with their study. In contrast, Thorn and Ventullo estimated

bacterial growth rates in other subsurface sediments to be 10^6 cells g^{-1} day^{-1} [37], growth rates which could remove DO from groundwater within days. Their estimates of microbial growth rates in freshwater lake sediments [37] were similar to those in Table 5, and the [methyl-^{3}H]thymidine incorporation data of Thorn and Ventullo from the subsurface [37] agreed with similar experiments performed with SRS sediments [32], suggesting that radioisotope studies could overestimate in situ activities.

Nutrients transported by groundwater may stimulate microbial activities in subsurface environments, particularly during bioremediation efforts. The lack of oxygen availability could result in anoxic conditions and undesirable accumulations of volatile fatty acids and sulfide. Measuring the effects of nutrient supplements or in situ rates of metabolism during biorestoration could be problematic. If, as in these studies, ^{14}C-isotopes were transformed at accelerated rates, inappropriate conclusions as to in situ toxicant degradation could be drawn.

In summary, estimates of metabolic rates from radiotracer experiments appeared to overestimate in situ metabolic rates of microbial communities in subsurface sediments. Electron donating and electron accepting activities of microorganisms residing in these porous subsurface environments were nano- to micromolar per year in contrast to the milli- to molar per year estimated by classical radioisotope experiments. The overestimation of in situ subsurface microbial activities by laboratory experiments may pose problems for ecological studies as well as for studies evaluating biological restoration of contaminated environments.

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