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Geomicrobiology Journal, Volume 7, pp. 79–91
Printed in the UK.

0149-0451/89 $3.00 + .00

Microbial Activities in Deep Subsurface Environments

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Abstract *Activities of microorganisms residing in terrestrial deep subsurface sediments were examined in 46 sediment samples from three boreholes. Radiolabeled time course experiments assessing in situ microbial activities were initiated within 30 min of core recovery. [1-C^{14}]Acetate incorporation into lipids, [methyl-^{3}H]thymidine incorporation into DNA, [2-^{14}C]acetate, and [U-^{14}C]glucose mineralization in addition to microbial enrichment and enumeration studies were examined in surface and subsurface sediments. Surface soils contained the greatest biomass and activities, followed by the shallow aquifer zones. Water-saturated subsurface sands exhibited three to four orders of magnitude greater activity and culturable microorganisms than the dense clay zones, which had low permeability. Regardless of depth, sediments that contained more than 20% clays exhibited the lowest activities and culturable microorganisms.*

Early twentieth century microbiological studies did not detect large microbial populations in subsurface soils (Waksman 1916, 1932; Starc 1942) and for decades it was generally accepted that the role of microorganisms in the terrestrial deep subsurface was insignificant (Alexander 1977). Evidence of deep subsurface microbial populations appeared in the literature (Buswell and Larson 1937; ZoBell 1947; Dockins et al. 1980), but little attention was focused on the presence or significance of subsurface microorganisms. A major obstacle was overcome when sampling techniques were developed that allowed aseptic recovery of sediments (Wilson et al. 1983). Recent studies incorporating these techniques have established that aquifers and subsurface sediments contain substantial microbial populations (Wilson et al. 1983; White et al. 1983; Ghiorse and Balkwill 1983; Chapelle et al. 1987; Kölbel-Boelke et al. 1988; Fliermans et al. 1988; Thorn and Ventullo 1988; Boone and Balkwill 1988).

Received June 1, 1988; accepted November 14, 1988

Although little is known about the activities of microorganisms inhabiting subsurface sediments, recent studies have shown that a variety of microbial communities are present in subsurface terrestrial environments. Heterotrophs from shallow aquifers were investigated by Ghiorse and Balkwill (1983), and sulfate-reducing anaerobes were detected in subsurface aquifers by Dockins et al. (1980). Chapelle et al. (1987) have suggested that subsurface microorganisms may impact groundwater quality through the production of carbon dioxide.

Microbiology of groundwaters and subsurface sediments have received increased attention due to severe groundwater contamination and impending loss of substantial portions of our subsurface potable water resources (Pye and Patrick 1983; Westerick et al. 1984). Predominant contaminants include short-chained halogenated hydrocarbons associated with defense, dry cleaning, and metal finishing industries. Classical remediation technologies may not be suitable for reclaiming contaminated aquifers and vadose zones, since subsurface contaminants are difficult to quantify, unlikely to volatilize, and often unmanageable (Schwarzenbach and Giger 1985). Consequently, reclamation of some subsurface habitats may necessitate on-site biological remediation. Examination of the ecology, physiological potentials, in situ carbon and electron flow, growth, and nutrient status are prerequisites to satisfactory biological remediation of subsurface environments (Wobber 1986).

This report centers on the examination of subsurface microbial activities occurring within minutes of sediment recovery. Radioisotope time-course experiments measured anabolism of microbial lipids and DNA, and catabolic mineralization of acetate and glucose in surface soil and 46 subsurface sediment samples. The radioisotope experiments distinguished microbial activity between lithologic formations within a given borehole or between boreholes and were effective in evaluating activities spanning five orders of magnitude.

Materials and Methods

Description of Experimental Site

The Savannah River Plant (SRP) is a 768-km^2 limited-access facility operated for the Department of Energy by E. I. duPont de Nemours and Company. The SRP is located approximately 32 km southeast of the geological Fall Line separating the Piedmont from the Coastal Plain. The site is located within the Upper Atlantic Coastal Plain on the Aiken Plateau adjacent to the Savannah River (Fig. 1). Unconsolidated sediments extend to depths of about 300 m and are underlain by crystalline metamorphic rock or consolidated mudstone. The sampled geologic formations beneath the SRP are shown in Figure 1. Sandy aquifer zones are dispersed between clay and silt formations. Two major confining clays retarding vertical water flow are located in the Pee Dee and Middendorf formations. For more information on this site see Sargent and Fliermans (1989).

Sample Procurement

Professional Service Industries, Inc. (PSI) was contracted to drill and develop the boreholes. One week prior to the microbiological sampling, a continuously cored hole was placed 10 m from the microbiology borehole site. The preliminary hole was used to ascertain the lithology at each site, to familiarize the sampling crews with the types of

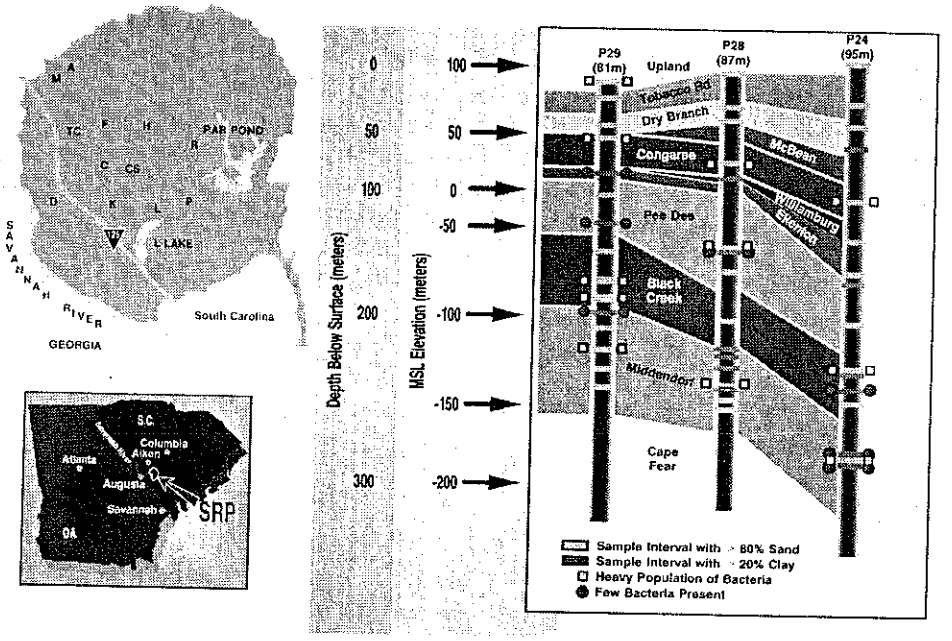

Figure 1. Location of deep core sampling sites and stratigraphic positions of subsurface formations at the Savannah River Plant. Boreholes P29, P28, and P24 were located in areas TC, F, and P, respectively. The distance between boreholes P28 and P24 is approximately 12 km (see Sargent and Fliermans, 1989, for further details).

sediments, and to determine the exact locations for sampling. Undisturbed microbiology sediment samples were collected only from strata that were consistent through a depth of 4 m so that adequate sediments were available for analyses.

The microbiology boreholes were completed in 5 to 8 days with drill crews working 24 hours a day. Between sample depths, the borehole was drilled with a rotary bit and continuously flushed with recirculated sodium bentonite viscosifying drilling fluid (Quikgel, NL Bariod/NL Industries, Houston, TX) to maintain an open borehole. Sediments were collected in core liners that had been steam cleaned or autoclaved prior to use. The sampler was lowered into the borehole and the sample collected in front of the recirculating drilling fluids to prevent sample contamination. Four different sampling tools were used, depending on the type of sediments to be cored. Detailed descriptions of sampling techniques, use of conservative drilling fluid tracers, and quality assurance protocols are published elsewhere (Phelps et al., in press).

Sample Handling in the Field

Undisturbed sediment cores were removed from the sampler and the core liner was immediately carried into the Mobile Microbial Ecology Laboratory (MMEL). Sediments were removed from the core liners using an extruding device (Model P-107, Soiltest, Evanston, IL). Sediments exiting the core liners automatically entered a N_2-flushed glove bag constructed from polyethylene and slotted for gloves, core extruder, and sample removal port. In latter experiments an anaerobic chamber designed by Coy Laboratory Products Inc. (Ann Arbor, MI) was used. All sample manipulations occurred in the glove

bag which was alcohol scrubbed between samples and dismantled daily for thorough cleaning and disinfection. Using alcohol-flamed-sterilized tools, approximately half of the outermost portions of the sediment core was pared away before processing. After paring, the aseptic sample was placed into a flame-sterilized pan, quartered, mixed, and disbursed into sterile Whirl-pak bags. Bags were weighed, placed into quart canning jars, flushed with N_2, sealed, and removed from the glove bag through the airlock. The entire operation was completed within 30 min from the time of collection. Subsamples were immediately distributed for pore-water chemistry measurements and initiation of on-site activity experiments, frozen for lipid analyses, prepared for express shipment to other investigators, and archived.

Gases, Chemicals, and Isotopes

Nitrogen and N_2:CO_2 (90:10%) gases were greater than 99.9% pure. In the laboratory, all gases were passed through copper-filled Vycor furnaces (Sargent-Welch Scientific Co., Skokie, IL) to remove traces of oxygen. All chemicals used were of reagent grade and were obtained from Millinckrodt (Paris, KY) or Sigma Chemical Co. (St. Louis, MO). Glass-distilled solvents and reagents were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). [1-^{14}C]Acetate (56.0 mCi/mmol) and [*methyl*-^{3}H]thymidine (76.0 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, MA). [2-^{14}C]Acetate (56.0 mCi/mmol) and [U-^{14}C]glucose (2.8 mCi/mmol) were obtained from Amersham Corp. (Arlington Heights, IL). All isotope solutions (1–50 μCi) were frozen prior to use, thawed, and transferred with gas-tight syringes (Hamilton Co., Reno, NV).

Analytical Procedures

Sediment samples were inoculated for on-site aerobic and anaerobic activity experiments within 30 min of core extrusion. All isotope incorporation experiments were measured over time and were performed in duplicate using sterile polypropylene centrifuge tubes for aerobic incorporations and crimp top tubes (Bellco Glass Co., Vineland, NJ) for aerobic mineralization studies and all anaerobic experiments. Anaerobic experiments were performed using a N_2:CO_2 (90:10%) gas phase. All incubations were at ambient temperature which was similar to the in situ temperatures of 21 to 24°C.

Time-course experiments consisted of incubation periods with time points of 0, 0.5, 2, 8, 24, and 96 h. Rates of incorporation or mineralization were generally linear within three or more points. Experiments for acetate incorporation into lipids contained 2.0 g sediment, 5.0 μCi of [1-^{14}C]acetate in 1.0 mL sterile distilled water. Aerobic experiments utilized polypropylene tubes, while crimp-top tubes were used for anaerobic time-course experiments. Incorporation was inhibited with 3.0 mL of a phosphate-buffered chloroform–methanol solution. In the laboratory, acetate incorporation experiments were thawed and lipids were extracted from sediments by a modification (White et al. 1979) of the single-phase chloroform–methanol method (Bligh and Dyer 1959). The lipid extracts were evaporated to dryness and resuspended in 2.0 mL chloroform, and aliquots were counted by liquid scintillation counting to determine the amount of radioactivity incorporated into microbial lipids. The earliest time points yielding measurable results were used to calculate a linear rate which was extrapolated to dpm/day. Data were corrected for background and incorporation using the inhibited controls.

Thymidine incorporation tubes contained 1.0 g sediment, 30 μCi of [*methyl*-^{3}H]thymidine plus 2 nmol of nonradioactive thymidine in 1.0 mL of sterile distilled water. Incorporation was inhibited by the addition of 3.0 mL 80% ethanol and samples were frozen at −20°C. Thymidine incorporation experiments were processed by thawing the reaction mixture and lysing the indigenous microorganisms with 2 mL 0.3 M sodium hydroxide containing 1% sodium dodecyl sulfate, 10 mM thymidine, and 1% humic acid. After heating at 110°C for 4 h, the supernatants were collected and dialyzed as previously described (Moriarty and Pollard 1982). Radioactivity incorporated into macromolecules, i.e., DNA, was determined using liquid scintillation counting. Control experiments were conducted on 12 tubes to ensure that dialysis was sufficient and to verify that 60–90% of the radioactivity retained in the dialysis tubing (molecular weight cutoff of 3500) was sensitive to DNAase.

Mineralization experiments were performed using 2.0 g sediment, 2.0 μCi of carrier free [2-^{14}C]acetate or [UL-^{14}C]glucose in 1.0 mL sterile water in 25 mL crimp-top tubes. Mineralization was inhibited with 0.5 mL of 2.0 M sodium hydroxide. One hour before analysis tubes were acidified with 0.5 mL of 6 M hydrochloric acid, and the $^{14}CO_2$ and $^{14}CH_4$ evolved during mineralization and time-course experiments were examined by gas chromatography–gas proportional counting as described by Nelson and Zeikus (1974).

Pore-Water Chemistry

The inorganic composition of pore waters, including major cations, anions, ammonia, sulfur species trace metals, pH, and Eh, were determined on aliquots of pore waters expressed by pressure after a 3-day incubation while saturated with water. Expressed aliquots of pore waters were collected for chemical analyses until conservative tracers added at the initiation of the incubation were detected. Typically less than 5.0 mL of pore water was collected per 400 g of sediment. Moisture content of the sediments was determined prior to water saturation and incubation and again after the 3-day incubation. Pore-water analyses of anions and cations were performed using ion chromatography (Fredrickson et al. 1989).

Enumeration and Enrichment Studies

Five-tube most-probable-number (MPN) dilution series were established aerobically in screw-capped tubes or anaerobically in crimp-top tubes for each sample using medium containing 1.0 g/L each of trypticase, yeast extract, and glucose (TYEG) as energy sources. Single-tube aerobic dilution series were also established using 0.01 g/L TYEG, 0.01 g/L yeast extract plus glucose, and 0.5 g/L yeast extract plus 10 mmol/L methanol. All media contained trace minerals including selenium and molybdate as well as a dilute vitamin mixture and a carbonate or a phosphate buffer (Lynd et al. 1982). Inoculated tubes were incubated 3 months at ambient temperatures, which were similar to the subsurface temperatures of 21 to 24°C.

Results and Discussion

In previous studies at the Savannah River Plant, our laboratory examined microbial activities and biomass in subsurface sediments contaminated with trichloroethylene (Fliermans

et al. 1988). Radioisotope activity measurements determined microbial activities over a range of five orders of magnitude. In this study, we examined microbial activities and culturable microorganisms in uncontaminated deep subsurface sediments from three additional boreholes at the Savannah River Plant.

Data for microbial activities and MPNs from the sediments of borehole P24 are shown in Table 1. Significant anabolic activity and culturable microorganisms were detected in most samples down to a depth of 260 m below the surface. Activity measurements based on radiolabel incorporations were calculated from linear rates of isotope accumulation from duplicate tubes at five or more time points. Acetate incorporation activities (Table 1) varied more than three orders of magnitude between confining clay layers (e.g., Middendorf at 239 m) and aquifers (e.g., Congaree at 92 m). Three sampling depths (Tobacco Road at 34 m; Pee Dee at 146 m; Middendorf at 239 m) exhibited no acetate incorporation during time-course experiments.

Thymidine measures were expressed as − to + + + + based upon the magnitude of activity above background which averaged 900 dpm/day. Thymidine incorporation data showed large deviations between replicates but were useful as a qualitative measure of microbial activity. Thymidine incorporation experiments in some samples agreed with acetate activities in that the samples that did not incorporate acetate (Tobacco Road, 34 m; Pee Dee, 146 m; Middendorf, 239 m) incorporated little thymidine. Similarly, those samples that incorporated the greatest amount of acetate (e.g., Congaree, 92 m; Midden-

Table 1

Microbial Activities and Most Probable Number (MPN) Estimates of Aerobic Heterotrophic Microbial Population Densities from Deep Subsurface Sediments of Borehole P24

Depth (m)	[^{14}C]Acetate into Lipids[a] (dpm/day)	[^{3}H]Thymidine into DNA[b] (dpm/day)	MPN (log/gdw)
0.0	3.00×10^7	+ + + +	7
34	0.0	−	7
45	2.45×10^4	−	4
58	3.00×10^3	−	6
92	6.26×10^5	+ + +	7
119	1.20×10^3	+ + +	4
140	9.30×10^3	+ +	2
146	0.0	−	2
182	5.00×10^3	+ +	3
202	5.18×10^4	+ + +	3
205	3.50×10^2	+ + +	2
239	0.0	+	3
247	4.02×10^4	+ +	4
257	4.30×10^2	+ +	4
261	1.02×10^5	+ + +	5
264	2.54×10^2	nd[c]	4

[a] Data recorded as dpm/day calculated from linear rates of isotope accumulation during time-course experiments.

[b] Linear rates less than 10^3/day, −; $<10^4$, +; $<10^5$, + +; $<10^6$, + + +; $>10^6$, + + + +.

[c] nd, not determined.

dorf, 261 m) were also high for thymidine activity. Sandy sediment materials from the Congaree at a depth of 92 m gave the maximum subsurface acetate and thymidine incorporation rates as well as the largest MPN densities, while Pee Dee confining clay sediments at 146 m exhibited the lowest activities and MPN. In all boreholes examined, surface soil samples exhibited the greatest MPN and radiotracer incorporation activities followed by the shallow Congaree formation sediments (P24, 92 m in Table 1; P28, 59 m, and P29, 39 m not illustrated). Lowest activities and MPN densities were always associated with confining clay zones such as the Pee Dee and Middendorf clays at 146 and 239 m respectively in corehole P24. Depth per se did not appear to correlate with the subsurface biomass or radiotracer incorporation rates; some of the deepest zones beneath the surface exhibited large and active microbial communities (Fig. 1, Table 1).

Time-course experiments to test for mineralization of acetate and glucose were performed under both aerobic and anaerobic conditions with samples from selected depths (Table 2). Anaerobic mineralization activity depended on the availability of alternative electron acceptors in the sediments; it was never significantly greater than the aerobic activity. Radioactive methane was never detected. These findings agreed with chemical analyses of pore water which suggested that these subsurface environments were aerobic or microaerobic and that methanogenesis was not a significant environmental process (Sargent and Fliermans 1989). Similar to the radiolabeled incorporation results observed in aquifer sediments, high rates of mineralization were observed in P24 samples obtained at 59, 112, 182, and 261 m (Table 2). Confining clay layers (71, 146, and 205 m) exhibited the lowest mineralization rates. Considerable mineralization of glucose and acetate were observed in the deepest samples (264 m) from the Middendorf formation. Scarcity of pore waters for pool size determinations and low specific activity of radiotracers impeded efforts to assess and to compare acetate turnover rates between subsurface formations.

Table 2

Aerobic and Anaerobic Mineralization of [^{14}C]Acetate and [^{14}C]Glucose to $^{14}CO_2$ in Subsurface Sediments from Borehole P24

Depth (m)	Aerobic (dpm/day × 10^3)		Anaerobic (dpm/day × 10^3)	
	Glucose	Acetate	Glucose	Acetate
45	291 ± 165[a]	678 ± 60	nd[c]	nd
58	66 ± 36	70 ± 41	67 ± 29	51 ± 4
59[b]	720 ± 72	165 ± 54	nd	nd
71[b]	17 ± 12	9 ± 8	38 ± 29	11 ± 4
112[b]	792	720	nd	nd
146	92 ± 25	18 ± 6	nd	nd
182	720 ± 10	436 ± 99	nd	nd
205	49 ± 33	12 ± 8	35 ± 2	7 ± 3
247	120 ± 62	660 ± 220	61 ± 43	16 ± 3
257	203 ± 72	52 ± 47	17 ± 2	12 ± 3
261	864 ± 192	1065 ± 288	132 ± 51	1036 ± 4

[a] Standard deviation of the mean.

[b] Data are from borehole P28 and correspond to sediment lithologies at depths of 92, 119, and 146 m, respectively, in P24.

[c] nd, not determined.

As shown in Table 3 three formations, the Ellenton silt with >20% silt and clay, the Pee Dee variegated clays, and the Middendorf middle clays with >50% clays, consistently exhibited less activity and less culturable biomass. Only one (P29, 112 m) of nine samples from these formations displayed acetate incorporation rates greater than 1000 dpm/day. Previous studies that examined the Ellenton silt and Pee Dee clay corroborated these findings (Phelps et al. 1987). The result from the P29 Pee Dee sample at 112 m may have resulted from process contamination since it required three persons 3 h to extrude and process this dense asnd compacted clay. Although the core was greatly manipulated the thymidine incorporation values were more variable, yet low for the confining clay zones.

Culturable microorganisms were three to five orders of magnitude less in the clay confining layers than in shallow aquifers or surface soils. Other investigators reported substantially lower numbers of culturable bacteria in confining formations than those reported here; in some cases nonculturable bacteria were detected (see other papers in this issue). Several factors may account for these differences. First, our MPN values were derived from serial dilution enrichments performed in the field only after priority on-site functions were completed. In some cases, the MPN dilutions were initiated after a 4-week delay. Such delays likely allowed growth of some indigenous populations and caused the death of others. Consequently, our MPN results must be viewed with these possibilities in mind. Second, subsample patchiness of moist and dry sands, silts, or clays could account for some of the differences between different laboratories. Finally, our enrichments were set up with one gram of sediment as the inoculum and subsequent dilutions, whereas some investigator's started with 10-g samples diluted 10- or 100-fold for their enumerations. Nonetheless, like other investigators (this issue) we found that samples with the lowest culturable microorganisms and activities were from sediments with the highest clay content.

Other than surface soil, the sandy water-bearing formations contained the highest numbers of culturable microorganisms and the greatest activities, as shown in Table 4. Surface soils contained microbial populations of 10^7/gdw, whereas sediments from sandy subsurface aquifers contained culturable bacteria at 10^5 to 10^7/gdw. Surface soils at each site exhibited radiolabel incorporation rates greater than 10^6 dpm/day for both acetate and thymidine. On average, sediments from the Congaree aquifer displayed the highest activities of the subsurface formations, although they were an order of magnitude less than the surface soils at each site. These data agreed with previous studies of Congaree sediments (Phelps et al. 1987). Measured activities were generally an order of magnitude less (except for P28, 59 m) than surface soils, yet greater than the deeper aquifers in the Black Creek and Middendorf formations. Culturable microorganisms and significant microbial activities were detected in 200-m deep sediments from the Middendorf formation. All Middendorf samples examined in this study were aerobic to microaerobic based upon ionic speciation of sediment materials and dissolved oxygen in waters from developed wells (T. L. Hazen et al., unpublished results). It was not clear whether the microbial activities observed in the Middendorf aquifer were sufficient to remove oxygen, but it appeared that these waters had not been recharged with respect to oxygen for considerable periods of time (H. Bledsoe, personal communication). The detection of isotope incorporation and mineralization activities within minutes of sediment recovery suggests that microorganisms residing in the deep subsurface were metabolically active in situ. However, sampling and processing may result in an increased rate of isotope utilization over time (White et al. 1983). In fact, the rate of acetate incorporation into lipids increased more than threefold within 24 h in four of eight Middendorf aquifer samples (data

Table 3
Microbial Activities and MPN Estimates of Aerobic Heterotrophs in Subsurface Confining Layers

Formation	Borehole	Depth (m)	^{14}C Lipids (dpm × 10^3/day)	[^{3}H]DNA (dpm × 10^3/day)	MPN (log/gdw)
Ellenton silt	P24	119	1	207	4
Ellenton silt	P28	72	1	180	3
Ellenton silt	P29	70	0	67	3
Pee Dee clay	P24	146	0	0	2
Pee Dee clay	P28	115	0	88	3
Pee Dee clay	P29	112	34	55	2
Middendorf middle clays	P24	239	0	7	3
	P28	184	1	0	4
	P29	183	0	18	2

Table 4
Microbial Activities and MPNs in Water-Producing Formations

Formation	Borehole	Depth (m)	^{14}C Lipids (dpm × 10^3/day)	[^{3}H]DNA (dpm × 10^3/day)	MPN (log/gdw)
Surface soil	P24	0	3000	4665	7
Surface soil	P28	0	1128	2920	7
Surface soil	P29	0	2602	3375	6
Congaree sand	P24	92	626	281	7
Congaree sand	P28	59	167	23	6
Congaree sand	P29	39	606	625	6
Black Creek sand	P24	202	52	73	5
Black Creek sand	P28	164	192	140	3
Black Creek sand	P29	152	199	0	5
Middendorf sand	P24	261	102	33	5
Middendorf sand	P28	217	104	248	5
Middendorf sand	P29	201	12	24	4

not shown). Thus, the observed activity of deep subsurface microorganisms may be an overestimate of the in situ activity. It is notable that water-bearing sediments from greater than 200 m exhibited more culturable microorganisms and greater activities than silt and clay layers much closer to the surface (Tables 3 and 4), which highlights the point that depth per se does not limit the quantity and activity of subsurface microorganisms.

Initial evidence suggested that moisture content and sediment texture were major determinants of subsurface microbial populations. Independent of depth, sediments were separated into groups based on sediment texture and qualitative measures of moisture availability. For sediment textures, two major groups of particle distributions were identified: either greater than 20% clays or greater than 70% sands. Moisture content of sandy samples was a qualitative determination. Samples were considered greater than 70% saturated with water if they were recorded as being saturated and water producing at the time of recovery or if the initial sediment moisture content was 70% of the saturated moisture content used for pore-water extraction procedures. As shown in Figure 2, 11 samples contained greater than 20% clays, 14 were unsaturated sands, while 20 were judged as being more than 70% saturated. The data in Figure 2 show the relation between sediment texture and moisture content with respect to microbial activities and MPN data. Regardless of depth, sediments containing greater than 20% clay averaged less than 100 dpm/day [^{14}C]acetate incorporation into lipids and MPN values of 10^3/gdw. Saturated and unsaturated sandy sediments averaged approximately 10^5 bacteria per gram but saturated sands averaged ten times greater acetate incorporation into lipids. There were six samples that showed no incorporation of [^{14}C]acetate into lipids, four of which were clays and none of which were saturated sands. There were 12 samples that incorporated acetate at

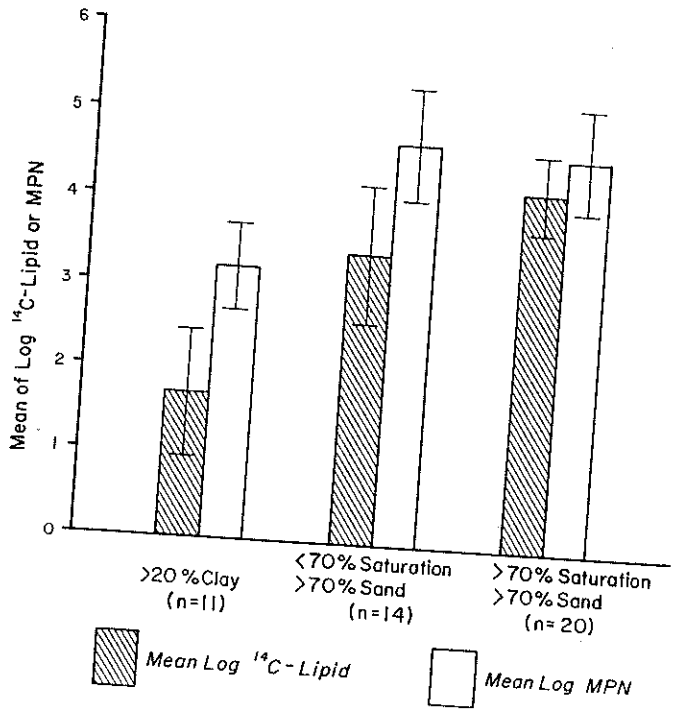

Figure 2. Influence of particle size and moisture content on [^{14}C]acetate incorporation into lipids and log MPN of aerobic heterotrophs per gram dry weight of sediment.

rates greater than 10^5 dpm/day; 10 of these were saturated sands, none were clays. Culturable biomass followed similar trends, in that clays consistently exhibited lower biomass than sands regardless of depth. These results suggest that although many sandy sediments may contain similar microbial populations, those with the greatest available water content may exhibit greater activities. In contrast, sediments containing greater than 20% of fine clay particles may reveal less culturable biomass and activity.

Previous subsurface studies have reported the presence of microorganisms (Ghiorse and Wilson 1988; Phelps et al. 1987; Dockins et al. 1980; Ghiorse and Balkwill 1983), and recently geochemists have emphasized that microorganisms may alter groundwater chemistry (Chapelle et al. 1987). Harvey et al. (1987) demonstrated that microorganisms can be transported long distances through sandy subsurface materials, thus providing an explanation for the source of inocula. The present work and that of other investigators reported in this issue have demonstrated an abundant microflora deep beneath the surface at Savannah River Plant, and that subsurface heterotrophic microorganisms possess the ability to mineralize acetate and glucose producing carbon dioxide which is consistent with the hypothesis of Chapelle et al. (1987). Furthermore, the heterotrophic microorganisms from aquifer sediments were able to assimilate acetate into lipid fatty acids or thymidine into DNA within minutes or hours of sample retrieval. Thus, the deep aquifer sediment microbiota appear poised to metabolize, incorporate, and mineralize nutrients in their ecological habitats.

Acknowledgments

The information contained in the article was developed during the course of work under Contract DE-AC09-76SR00001 with the U.S. Department of Energy.

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