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Activities of Microorganisms in Deep, Unconsolidated Eastern Coastal Plain Sediments

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Abstract

Activities of microorganisms residing in deep Eastern Coastal Plain sediments were examined from more than 100 sediment samples from eight boreholes. Radiolabeled time-course experiments assessing *in situ* microbial activities were initiated at the site within 30 minutes of core recovery. [^{14}C-1-]acetate incorporation into lipids, [methyl-^{3}H-]thymidine incorporation into DNA, [^{14}C-2-]acetate and [^{14}C-U-]glucose mineralization, and 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 sands exhibited orders of magnitude greater activity and culturable microorganisms than the low permeable dense clay zones. Subsurface sediments in association with older groundwater typically exhibited less biomass and activity. Sediments severely contaminated with trichloroethylene exhibited negligible activity and biomass, whereas water-saturated sediments beneath the plume exhibited high levels of activity. Regardless of depth, sediments which contained greater than 20% clay particles exhibited low activities and lower culturable microorganisms.

Time Course Experiments. Sediment aliquots were inoculated for aerobic and anaerobic activity experiments on-site within 30 minutes of core extrusion. All isotope solutions (1-50 μCi) were frozen prior to use, thawed, and transferred with gas-tight syringes (Hamilton Company, Reno, NV). Time-course experiments were performed in duplicate, using sterile polypropylene centrifuge tubes for aerobic isotope incorporation experiments and anaerobic crimp top tubes (Bellco Glass Company, Vineland, NJ) for anaerobic experiments. All incubations were at ambient temperature, which was similar to the *in situ* temperature of 20-25°C.

Acetate incorporation experiments contained 2.0-gram sediment, 5.0 μCi of radiolabeled acetate, and 1.0 ml of sterile distilled water. At t_0 and appropriate time points, duplicate incorporations were inhibited with 3.0 ml of a phosphate-buffered, chloroform-methanol solution. Sulfate reduction experiments utilized 2 μCi of isotopes and time points were inhibited with 0.5 ml of 2.0 M anaerobic, sodium hydroxide. Mineralization time-course experiments also contained 2.0 μCi of isotopes and were inhibited with sodium hydroxide.

Analytical Procedures. In the laboratory, acetate incorporation experiments were thawed and sediments extracted by a modification[6,11] of the single phase, chloroform-methanol method.[2] The lipid extraction was evaporated to dryness and portions were counted by 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 then extrapolated to dpm/day.

Radioactive ^{14}C-carbon dioxide and ^{14}C-methane from mineralization and time course experiments, respectively, were examined by gas chromatography-gas proportional counting.[7] A Packard 417 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. Radioactive sulfide, from sulfate reduction experiments, was recovered after acidification into zinc acetate and counted via scintillation counting.

Results

In all boreholes examined, surface samples exhibited the greatest culturable counts and activities followed by the aquifers.[1,9] Lowest activities and biomass were associated with confining clay zones. Depth did not appear to determine the subsurface biomass or radiotracer incorporation rates.[9] Instead, there were zones deep beneath the surface which exhibited large and active microbial communities. This report focuses on nutrient limitations, sulfate reduction, and the influence of geology and hydrology on microbial biomass and activities.

The results in Table 1 show the effects that nutrient additions on subsurface water-producing sands. Five μCi of carrier-free [^{3}H]-acetate was added to replicate 3-g sediment aliquots which contained 1.0 ml sterile nanopure water. The water contained either no additions, 10 μM or 500 μM of sodium nitrate, sodium phosphate, sodium sulfate, or glucose. Two additional treatments included a supplemental mineral solution or varying the water content to 0.25, 0.5, 1.0 and 2.0

conductivities without regard to depth (Table 5). Sediment lithologies were of two major types of particle distributions, either greater than 20% clays or greater than 70% sands. Sandy samples were considered in two sub groups, unsaturated or with D<10 (which also meant that hydraulic conductivity < 200 µm/sec) or saturated with D>10 and hydraulic conductivity(K) > 200 µm/sec. Nineteen samples contained greater than 20% clays, 17 samples were unsaturated or low permeability sands, and 25 samples were water-producing sands. The data in Table 5 show the relationship between sediment particle size and moisture content with microbial activities and culturable biomass.

Clays consistently exhibited lower activities and biomass than sands regardless of depth. Culturable biomass in sands were similar regardless of permeability. Interestingly, microbial activities between the two sand groups were dramatically different. Unsaturated or low permeability sands exhibited activities similar to the clay samples, while activities from water-producing sands averaged 100 times more activity than low permeability samples. These results suggest that although many sandy sediments may contain similar populations, those with abundant water availability may exhibit far greater activities.

Discussion

Presence and diversity of subsurface microorganisms is well established.[1,3,8-10] Investigators agree that water-producing sands exhibit greater colony forming units than do confining clays. Factors controlling activities and biomass are less understood. This work reports evidence that water may be the single most important factor controlling subsurface microbial activities.

The addition of water or nutrients led to stimulation of microorganisms residing in subsurface sediments within 16 hours of collection. Two of six sediments were stimulated by additions of glucose, suggesting that carbon may not be the most limiting factor. Physical evidence[10] from lithological logs indicated that lignite and even wood was present in several of the subsurface formations, again suggesting that carbon may not be the most limiting nutrient. Mineral solutions were not stimulatory to most sediments, which agreed with the pore water chemistries that trace elements were present in subsurface sediments and pore waters. Nitrate and sulfate, which are alternative electron acceptors for respiration, led to stimulation in a couple formations, but evidence suggests that these electron acceptors are not major participants in subsurface carbon and electron flow in these sediments. Phosphate additions of 10 µM led to stimulation of microbial activities in five of six samples examined. Pore water chemistry data agreed that phosphates were below 1 mg/Kg and a likely limiting nutrient. The only sample not stimulated by phosphate was a dense clay layer in the Pee Dee formation. This formation exhibited few colony forming units and negligible activity.

The only addition which caused high levels of stimulation in all sediments examined was the addition of 2.0 ml of water. Water stimulation of clays was expected since nutrients were likely sedimented or bound in the confining layers. Water stimulation of sands was unexpected. Additions of 0.5 - 1.0 ml of water did

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Table 2. Evaluation of nutrient limitations within subsurface confining layers.

[methyl-^{3}H]Acetate incorporation into lipids x 10^3 per day.

Formation (Depth m)	Nutrient	Stimulation[a]	Nutrient	Stimulation
Ellenton Clay (194 m)	NO_3	-	Glu	+
	PO_4	+++	Min[b]	±
	SO_4	-	H_2O[c]	++
Pee Dee Clay (239 m)	NO_3	-	Glu	-
	PO_4	-	Min	-
	SO_4	-	H_2O	+++

a Stimulation of 1.5 - 3 over control = ±, >3 = +, >6 = ++, and >10 = +++. Treatments were no additions, 10 µM, or 500 µM.

b Min. was a complex mineral solution at conc. used in defined media.

c H_20 was total water added to sediments which was 0.25, 0.5, 1.0 and 2.0 ml, respectively.

Experimental procedures: An inhibited control and pairs of duplicate tubes containing 3-gram sediments and 1.0 aqueous phase (except H_2O tubes) were inhibited after six or 16 hour incubations and radioactive lipids extracted and counted. Aqueous phases contained 5.0 µCi of [methyl ^{3}H] acetate and appropriate additions. Results were compared to the 1.0 ml H_2O experiment. Additions were sodium nitrate, sodium phosphate, sodium sulfate, glucose, and a stock mineral solution.

Table 3. Differential incorporation of [^{14}C]- and [^{3}H]-acetate into lipids of microorganisms from subsurface aquifers

Depth	Acetate incorporation into lipids (log dpm per day)	
(m)	[^{14}C]-acetate	[^{3}H]-acetate
256	4.7	4.2
290	4.7	5.1
406	5.2	3.3
463	3.4	2.2

Table 5. Influence of geological and hydrological properties on microbial biomass and activity.

Sediment characteristic	Colony forming units (log)	Activity (logs) as ^{14}C-acetate incorp. into lipids	Number of samples
> 20% clay	2.1	2.2	19
>70 % sands unsaturated or D<10, (K<200 um/s)	4.2	2.3	17
>70 % sands saturated and D > 10, (K>200 um/s)	4.2	4.3	25

Results from 61 sediment samples from 7 to 500 m depths examined from four core holes over a four year period.

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