25. EVIDENCE OF MICROBIOLOGICAL ACTIVITY IN LEG 95 (NEW JERSEY TRANSECT) SEDIMENTS[1]

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ABSTRACT

Radioisotope tracer experiments were carried out on sediments recovered from DSDP Leg 95 Holes 612 and 613 to test for viable microorganisms having geochemical significance. Positive indications of anaerobic bacterial activity (sulfate reduction, acetate oxidation, and methanogenesis) were found in sediments recovered from maximum sub-bottom depths of 46.1 m at Site 612 and 134.6 m at Site 613. Further work is needed to identify the organisms responsible for geochemical processes in these sediments.

INTRODUCTION

Sediments recovered during DSDP Leg 95 (New Jersey Transect) were incubated with radiolabeled substrates under anaerobic conditions in order to test whether anaerobic microorganisms might be capable of carrying out specific biogeochemical processes. These experiments were carried out to determine if *in situ* microbiological activity occurring at depth might be responsible for the presence of methane and the depletion of sulfate often observed in rapidly deposited DSDP sediments rich in organic matter (Rice and Claypool, 1981; Whelan et al., in press). The rationale and methodology have been described in detail (Whelan et al., in press) for similar experiments carried out during DSDP Leg 96 (the Mississippi Fan). Whole-round sediment cores were subsampled as the cores were brought on deck, and the samples were treated with radiolabeled substrates (^{14}C methylamine in one set of experiments and 2-^{14}C acetate plus ^{35}S sulfate in another). The samples were incubated under anaerobic conditions and the evolution of radiolabeled products was followed over an 8- to 21-day period. The formation and increase in radiolabeled products during an incubation time series would indicate that microbiological processes were occurring in these sediments. The results reported here are preliminary; the experiments were carried out primarily to determine if any anaerobic microbial activity was present in these sediments. There was no opportunity in this initial work to analyze replicate samples (insufficient core material was available) or to optimize the time/temperature/substrate concentrations, all of which must be done in future work.

EXPERIMENTAL PROCEDURES

The DSDP Leg 95 sediment samples tested for microbiological activity are summarized in Table 1. Those labeled H were recovered with the hydraulic piston corer, which is capable of obtaining a relatively undisturbed sample. The samples labeled X were taken with the extended core barrel and in general also represent relatively undisturbed sediment recovered from more lithified sections. In contrast, rotary cores (indicated by R) can be highly disturbed, particularly in surface sections. Intervals from which cores were obtained without continuously coring are indicated by W (wash cores). The R and W core samples for this study are generally very disturbed and are the sections most likely to contain surface sediments carried into deeper sections by the drilling process. In contrast, fine bedding and other sedimentological features are usually preserved in the H and X cores, and surface caving and contamination are expected to be minimal.

Details of the procedure for the microbiological experiments are described in detail by Whelan et al. (in press). After the core arrived on deck and was cut into sections, subsamples were taken for the microbiological experiments. Adjoining sediment sections were subcored by inserting plastic syringes (20 cm^3, hub end removed) parallel to the core liner. In this way, 20-cm^3 subsamples were obtained from the top and bottom of adjoining 1.5-m core sections. Each sample depth given in this report (Table 1, Figs. 1–3, and text) corresponds to the depth of the cut between the two sampled sections. Depths for samples obtained from wash cores are given as the depth range of the wash core. Care was taken in placing the sampling syringes near the center of the core section, so as not to sample sediments that were in contact with the core liner. The sediment sample ($\sim$20 cm^3) was placed in a round-bottom flask (volume $\sim$50 cm^3) that was continuously flushed with gas. The flushing gas was high-purity nitrogen passed through an indicating oxygen trap (J & W Scientific). The flask was sealed with a rubber stopper and further flushed with oxygen-free nitrogen for 10 min. by inserting the gassing and venting syringe needles through the rubber-stoppered sample flasks. To avoid contamination by non-indigenous flora, all materials were sterilized by autoclaving before use, and aseptic techniques were used for subsequent manipulations. Each sample flask then received either ^{14}C methylamine (0.5 ml, 5 μCi, 46 mCi/mmol) or 2-^{14}C acetate (0.5 ml, 5 μCi 55 mCi/mmol), together with ^{35}S sulfate (0.5 ml, 5 μCi, carrier-free ^{35}S sulfuric acid), all radiolabeled substrates purchased from New England Nuclear Co.). The flasks were incubated at room temperature ($\sim$15°C) for periods up to 21 days. During those periods, bacterial activities in individual flasks were arrested by addition of 10 *N* sodium hydroxide (2 ml), followed by storage at −20°C. This "killing" procedure did not produce radiolabeled products ($^{14}CO_2$ and $^{14}CH_4$ in the case of methylamine and $^{14}CO_2$, $^{14}CH_4$, and $^{35}SH_2$ in the case of sulfate and acetate), in the absence of microbiological activity. This was confirmed by shore-based experiments using San Francisco Bay surface mud (Whelan et al., in

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Table 1. Sub-bottom depth, age, and sediment description of core subsamples used in radiolabeling experiments.

Sample (core and section numbers)	Sub-bottom Depth (m)	Age	Sediment description
Site 612			
1H-2 to 3	3.0	Pleistocene	Unbedded gray mud with pyrite
2H-3 to 4	9.3	Pleistocene	Unbedded gray mud with pyrite
3H-3 to 4	18.9	Pleistocene	Unbedded gray mud with pyrite
4H-3 to 4	28.5	Pleistocene	Unbedded gray mud with pyrite
5H-1 to 2	35.1	Pleistocene	Unbedded gray mud with pyrite
6H-1 to 2	42.1	late Pliocene	Glauconitic dark gray mud
7H-1 to 2	46.1	late Pliocene	Olive gray mud with glauconitic sand
Site 613			
2X-3 to 4	24.3	middle-late Pleistocene	Alternating diatom mud/sand
4X-1 to 2	59.7	middle-late Pleistocene	Glauconitic quartz sand
5W-3 to 4	67.8–115.8	middle-late Pleistocene	Green-gray nannofossil mud
6X-3 to 4	120.5	middle-late Pleistocene	Gassy gray mud
7X-6 to 7	134.6	middle-late Pleistocene	Gray mud/glauconitic sand
8X-3 to 4	139.6	middle-late Pleistocene	Green-gray mud
Hole 603D			
1R-3 to 4	203.0	late early Pliocene	Silty nannofossil mud
Hole 603F			
1W-2 to 3	0–32.6	Pleistocene	Gray nannofossil mud

press). To obtain a time series, three (Holes 613 and 603D) or four (Holes 612 and 603F) flasks were prepared for each sediment section analyzed in this study. The sample flasks received a radiolabeled "treatment," and individual flasks were "sacrificed" at selected times. The criterion for positive identification of microbial activity, therefore, was an increase of radioactive products evolved over the time course of the incubation.

After incubation, the sample flasks were kept frozen for shore-based analyses of radiolabeled products. Radiolabeled products were extracted, trapped, and subsequently counted using scintillation techniques. Briefly, the $^{14}CH_4$ was swept out of the flask, oxidized, and the resulting $^{14}CO_2$ trapped and counted (Whelan et al., in press). Measurements of $^{14}CO_2$ and $^{35}SH_2$ were carried out by sample acidification, gas flushing, and trapping of the resulting gas in 1 *N* sodium hydroxide. An aliquot was counted to give the combined amount for $^{14}CO_2$ and $^{35}SH_2$ (Smith and Klug, 1981a, b). Barium chloride was added to a second subsample to precipitate the ^{14}C carbonate, and the residual solution was counted for $^{35}SH_2$. $^{14}CO_2$ values were calculated by difference.

Pore-water acetate was measured by the gas chromatographic method of Christensen and Blackburn (1982).

RESULTS AND DISCUSSION

Site 612

Site 612 is in a midslope position (1404 m water depth) and is part of a series of holes that make up the New Jersey Transect. The section from 0 to 35 m sub-bottom depth consists of Pleistocene gray nannofossil mud; the samples from 42.1 and 46.1 m sub-bottom were recovered from Pleistocene/Pliocene sediments consisting of both gray nannofossil muds and glauconitic sands. The cores showed no gas pockets as they were brought on deck.

Results of microbiological experiments for Site 612 are shown in Figure 1. For the sulfate and acetate experiments on samples from 3.0 and 35.1 m, $^{14}CO_2$ and $^{35}SH_2$ clearly increased during the radiolabeling experiment. No increase in $^{14}CH_4$ (compared with background levels, indicated by x's in Fig. 1B) was observed in the experiment on the sample from 3.0 m. In the experiment on material from 35.1 m, however, an increase was observed in all three radiolabeled products, but only at the end of the 21-day experiment. Production of $^{14}CH_4$ (from ^{14}C acetate) was ~10,000-fold less than $^{14}CO_2$ and $^{35}SH_2$ evolution. This slow formation rate of radiolabeled products suggests that other experiments carried out during Legs 95 and 96 may not have been incubated long enough to measure activity. The longest incubation in either this or the Leg 96 work was with samples from Site 612. The slow development of radiolabeled products is consistent with results for "cold" (unlabeled) methane experiments on sediment recovered from another nongassy hole, at Site 622 on Leg 96 in the Mississippi Fan (Whelan et al., in press). In those experiments, significant gas evolution was observed only after 13 days, and increased for up to 50 days when the experiment was terminated. For comparison, San Francisco Bay surface mud, which has a high level of anaerobic microbiological activity, developed levels of $^{14}CH_4$ and $^{14}CO_2$ about 2 to 4 orders of magnitude higher than at Site 622, and this occurred within a much shorter time (4 days), after which the activities leveled off (Whelan et al., in press).

The incubation of material from 46.1 m exhibited a clear increase of $^{14}CO_2$, but not of $^{35}SH_2$. Radiolabeled methane shows a puzzling pattern also observed in some of the Leg 96 results—a peak at 7 days followed by a decrease and leveling off at 14 and 21 days. All these values are significantly higher than background level, and indicate microbiological production of ^{14}C methane in all samples after time zero. It is possible that the variations are caused by spatial heterogeneity within the core.

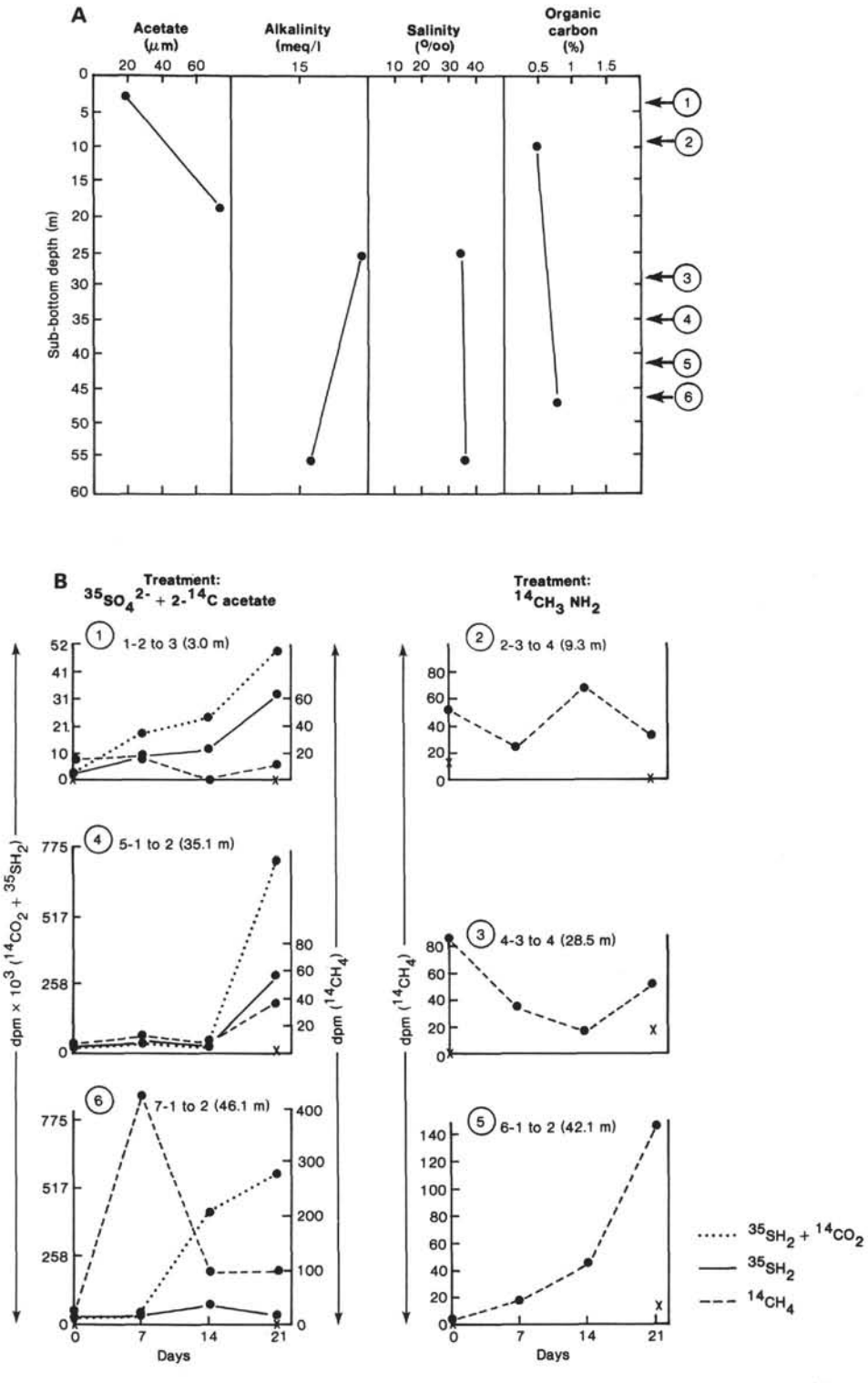

Figure 1. Site 612. A. Summary of pore-water data. B. Results of treatment of sediment samples with radiolabeled tracers. Circled numbers (1–6) in each of the treatment graphs indicate a particular time-series experiment and correspond to the circled numbers to the right of the pore-water data. These help "locate" each set of experiments at the appropriate depth in the pore-water profiles. Numbers next to the circled numbers in the treatments identify the cores and sections from which the samples were taken. The number in parentheses that follows the sample identifier is the sub-bottom depth of the sample. dpm = disintegration per minute. In the treatment graphs, X indicates background level for the analytical procedure. "Days" indicates incubation times.

In this experimental design, each time-point represents an individual subsample rather than a true time series for a single sample.

Experiments on the production of ^{14}C methane from ^{14}C methylamine showed a clear increase with time for the sample recovered from the glauconitic sediment at 42.1 m. As with ^{14}C acetate, however, levels of $^{14}CH_4$ were extremely low. The results for the samples from 9.3 and 28.5 m did not show a steady increase of $^{14}CH_4$ with time, although all these samples had activity levels distinctly above background. In the absence of increased counts with time, it is difficult to argue that bacterial conversion of ^{14}C methylamine to $^{14}CH_4$ is responsible for the slight increase in $^{14}CH_4$ observed in these experiments.

Acetate was detected (~20–70 μM; Fig. 1) in two pore-water samples from Site 612, indicating that this substrate may have been available to any organisms living at depth in the sediment. Pore-water sulfate and methylamine concentrations were not determined for this site. Two sediment organic carbon measurements obtained from this interval fall between 0.5 and 0.9%. No core gas pocket methane was found at this site.

Site 613

Miocene to upper Pleistocene sediments recovered at Site 613 consist primarily of green-gray diatomaceous and calcareous sediments; two intervals (at 59.7 and 134.6 m) contain glauconitic sands (Table 1). Site 613 is at a water depth of 2323 m, approximately 8 km seaward of the toe of the New Jersey continental rise wedge. Upper Eocene through middle Miocene sediments are missing at this site. All Site 613 sediments examined in this work, except for the bottom two samples, at 134.6 and 139.6 m, were recovered from gassy cores. Isotope data (methane $\delta^{13}C$ and deuterium) show the gas to be predominantly biogenic methane produced by the metabolic reduction of carbon dioxide with hydrogen, as is generally observed for most biogenic methane in marine sediments (Whiticar and Faber, this volume).

Results of microbiological radiolabeling experiments on Site 613 samples, using acetate and sulfate substrates, are shown in Figure 2. All samples showed significant $^{14}CO_2$ evolution during the 8-day experiment; the highest amounts were produced in the two shallowest samples (from 24.3 and 59.7 m). The wash core (613-5 [67.8–115.8 m sub-bottom]) may be contaminated with oxygen and shallower material introduced by the drilling process, and had much lower activity. An increase of $^{14}CO_2$ with time was evident in the sample from 134.6 m. Results are not as clear for the samples from 120.5 and 139.6 m. Although activities for both of these samples are above background levels, $^{14}CO_2$ did not increase with time.

Unlike the Site 612 samples, the Site 613 samples showed little if any sulfate reduction, even though pore-water sulfate levels appear to be adequate for such activity (about 12 mM at 60 m). In addition, no ^{14}C methane was produced in the ^{14}C acetate/^{35}S sulfate experiments for these sediments. Thus, little if any anaerobic microbiological sulfate reduction or methane production (from acetate) was observed in sediments from this site.

The results of methanogenesis experiments with ^{14}C methylamine are shown on the right of Figure 2B. Results for the three deepest samples show no methane production for this substrate. Results for the shallower samples were not as clear. Samples had greater than background activity, but $^{14}CH_4$ did not increase with time. Thus, it appears that these low activities may represent either sample heterogeneity or experimental artifacts. It is also possible that the experiments were terminated before a clear trend of methane generation could be established. The results for the shallowest sample, from 24.3 m, are puzzling: there was an obvious decrease of ^{14}C methane over the course of the experiment. Further experiments are required to clarify these results.

Pore-water acetate was present at much lower levels (about 10 μM) than at Site 612 (Fig. 2A). Pore-water sulfate levels were also low (< 10 mM) in deeper sections of the hole. The absence of core gas-pocket methane from 120 to 150 m sub-bottom (Fig. 2A) is consistent with the microbiological experiments in suggesting that microorganisms capable of carrying out methane production are inactive in this interval.

Holes 603D and 603F

The two samples from Holes 603D and 603F were the least satisfactory used in this study. Both are silty nannofossil mud obtained from very disturbed wash and rotary cores (intervals shown in Table 1). The depth intervals from which the samples were obtained (as shown in Fig. 3) cannot be accurately determined. In addition, because of the "washing-down" process, these cores may be contaminated with surficial sediments. However, the samples show very high levels of $^{14}CO_2$ production, probably resulting from aerobic and/or anaerobic acetate oxidation. Elevated levels of ^{35}S hydrogen sulfide and of ^{14}C methane were also observed, the latter only at the end of the experiments utilizing either ^{14}C acetate or ^{14}C methylamine. Thus, bacterial activities were associated with these sediments, and $^{14}CO_2$ production from ^{14}C acetate was 4 to 5 times more active than at Sites 612 and 613. This may have been due to contamination with surficial sediments.

CONCLUSIONS

Radioisotope tracer experiments indicate that bacterial activity was present in sediments recovered from maximum depths of 46.1 m at Site 612 and 134.6 m at Site 613. Acetate oxidation was the most evident activity, and sulfate reduction was the next most evident. These initial results, together with geochemical data from other gassy DSDP sites, indicate the need for further shipboard work to identify and characterize microbial processes occurring at depth in marine sediments and the organisms that mediate these activities. Methanogenic activity from ^{14}C acetate or ^{14}C methylamine was either absent or, in "positive" samples, showed low activity (< 300 dpm/sample). Incubation times used in this work may not have been long enough to detect methanogenic

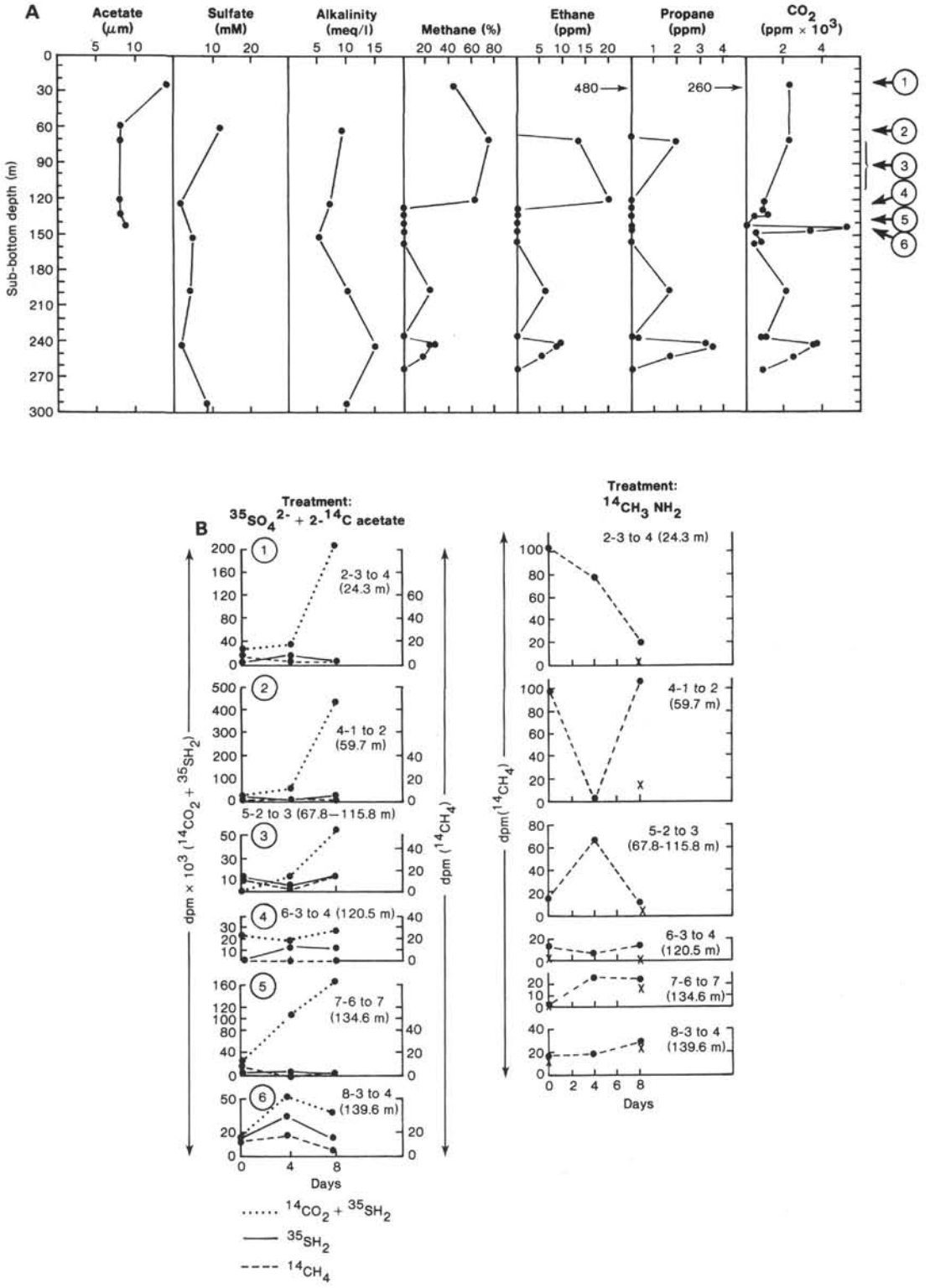

Figure 2. Site 613. A. Summary of pore-water and core gas data. Gas concentrations (methane, ethane, and propane) are for gas pockets which formed as the cores were brought on deck. B. Results of treatment of sediment samples with radiolabeled tracers. Legend and format are the same as for Figure 1.

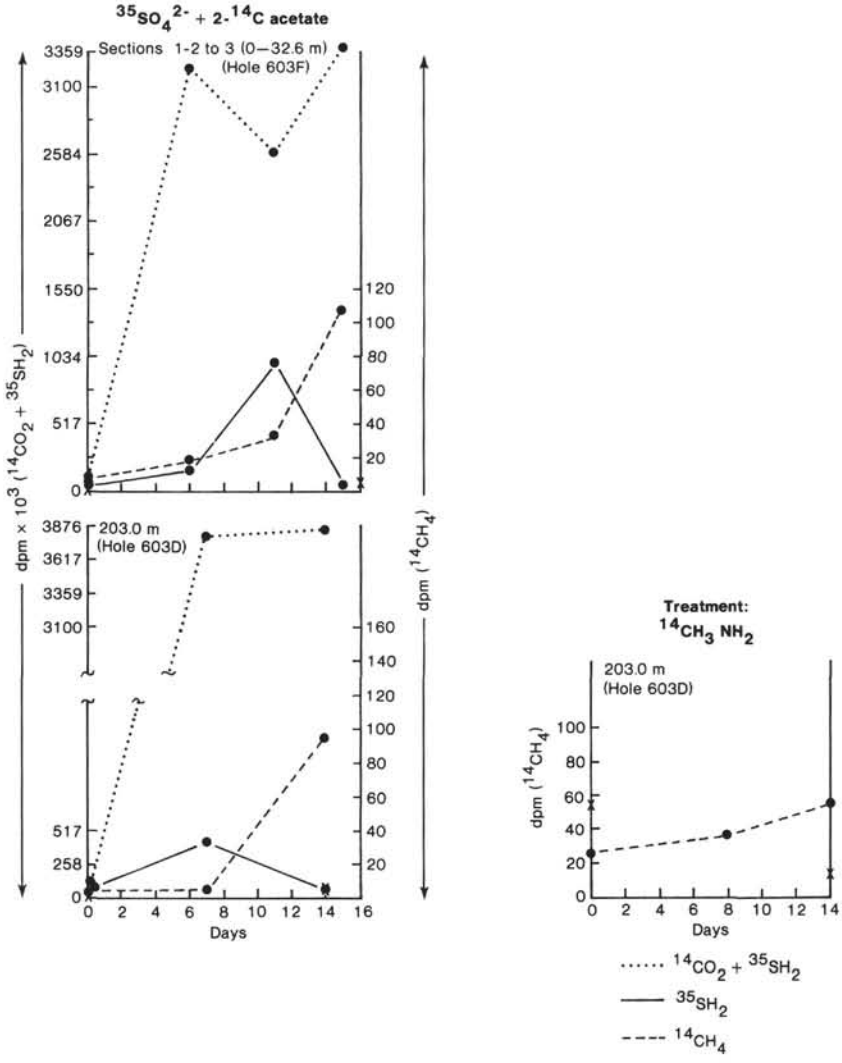

Figure 3. Site 603. Summary of treatment of sediment samples with radiolabeled tracers. Sample depths are sub-bottom depths.

activity. It is also possible, however, that methanogenesis may occur in these sediments by biochemical pathways not tested here (e.g., H_2 reduction of CO_2; Whiticar and Faber, this volume). Future experiments should therefore, be conducted with $^{14}CO_2$ and hydrogen and other possible methane precursors (e.g., formate, methanol, trimethylamine).

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