

46. BACTERIAL BIOMASS AND ACTIVITY IN THE DEEP SEDIMENT LAYERS OF THE JAPAN SEA, HOLE 798B¹

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ABSTRACT

Sediment whole-round cores from a dedicated hole (798B) were obtained for detailed microbiological analysis, down to 518 m below the seafloor (mbsf). These sediments have characteristic bacterial profiles in the top 6 mbsf, with high but rapidly decreasing bacterial populations (total and dividing bacteria, and concentrations of different types of viable heterotrophic bacteria) and potential bacterial activities. Rates of thymidine incorporation into bacterial DNA and anaerobic sulfate reduction are high in the surface sediments and decrease rapidly down to 3 mbsf. Methanogenesis from CO₂/H₂ peaks below the maximum in sulfate reduction and although it decreases markedly down the core, is present at low rates at all but one depth. Consistent with these activities is the removal of pore-water sulfate, methane gas production, and accumulation of reduced sulfide species. Rates of decrease in bacterial populations slow down below 6 mbsf, and there are some distinct increases in bacterial populations and activities that continue over considerable depth intervals. These include a large and significant increase in total heterotrophic bacteria below 375 mbsf, which corresponds to an increase in the total bacterial population, bacterial viability, a small increase in potential rates of sulfate reduction, and the presence of thermogenic methane and other gases. Bacterial distributions seem to be controlled by the availability of terminal electron acceptors (e.g., sulfate), the bioavailability of organic carbon (which may be related to the dark/light bands within the sediment), and biological and geothermal methane production. Significant bacterial populations are present even in the deepest samples (518 mbsf) and hence it seems likely that bacteria may continue to be present and active much deeper than the sediments studied here. These results confirm and extend our previous results of bacterial activity within deep sediments of the Peru Margin from Leg 112, and to our knowledge this is the first comprehensive report of the presence of active bacterial populations from the sediment surface to in excess of 500 mbsf and sediments > 4 m.y. old.

INTRODUCTION

Marine sediments play a major role in the biogeochemical cycling of elements (Jørgensen, 1983). Relative to seawater, organic matter is concentrated 10,000- to 100,000-fold in sediments, and this material is used for energy by bacteria. As a consequence, bacteria drive the chemical cycles within the sediment. Bacteria also have a profound effect on the extent to which organic matter is degraded or preserved, and they contribute their own biomass to the organic matter ultimately preserved within deep sediments, which on maturation may produce oil and gas deposits. The importance of bacterial activity within surface sediments is now well established, as it is intimately involved in cycling of nitrogen (Sørensen, 1978), iron manganese, and sulfur (Sørensen and Jørgensen, 1987), in addition to the diagenesis of organic matter.

Indirect geochemical evidence suggests that microbial activity continues to considerable depths within the sediment (chemical changes in pore water, gas production, modification of organic complexes such as kerogen, concretion formation, and isotopic evidence; Krumbein, 1983). Temperature is unlikely to limit bacterial activity until several kilometers below the surface, as bacteria can grow in temperatures exceeding 100° C (Jannasch and Taylor, 1984) and the thermal gradient of the Earth's crust is approximately 10°–40° C/km. The presence and activity of microorganisms in pristine deep subsurface environments (greater than 50 m below the surface) however, has not been confirmed by appropriate microbiological data (Sinclair and Ghiorse, 1989). Early research was based solely on the enrichment of viable bacteria (Rittenberg, 1940; ZoBell, 1958; Davis, 1967), with the possibilities of deep sediment layers being contaminated by the more

active surface sediments. A few more recent publications have reported the detection or cultivation of bacteria from sediment depths of 200 m (Oremland et al., 1982; Belyaev and Ivanov, 1983; Bianchi, 1986), and low levels of anaerobic activity have been found between 4 and 167 m in marine sediments (Whelan et al., 1985; Tarafa et al., 1987). Extensive investigations of deep aquifers (Erlich and Ghiorse, 1989; Balkwill et al., 1989; Chapelle and Lovely, 1990; Fredrickson et al., 1991) have confirmed the presence of bacteria, and low levels of bacterial activity, in samples over 400 m below the surface. These data reinforce our results from deep marine sediment layers from the Peru Margin, demonstrating significant bacterial populations and activities down to 80 mbsf (Cragg et al., 1990; Parkes et al., 1990). In this study, and those of deep aquifers (Balkwill, 1989), bacterial populations did not decrease continuously with depth, which suggests that significant microbial populations may exist to even greater depths.

We obtained intact core sections from Leg 128 in the Japan Sea from depths in excess of 500 mbsf to determine the presence of bacterial populations and activities in sediments significantly deeper than those previously investigated.

MATERIALS AND METHODS

Shipboard Handling

Sediment samples from Hole 798B, water depth 900 m, were obtained between 28 and 30 August 1989. A total of 22, 25-cm whole-round cores plus one sample of 1 cm³ (Table 1) were removed from the middle of 1.5-m core sections and the core-catcher, respectively. The section to be cut was cleaned and wiped with alcohol and then placed in a specially constructed sterile rig (Fig. 1A) and the core cut with a sterile hacksaw blade under jets of sterile oxygen-free-nitrogen (OFN) filtered through sterile 0.2 µm gas filters to maintain anaerobic conditions. The cut ends were covered with a sterile metal slice and the 25-cm whole-round core removed from the rig. The cut ends were then flamed and capped with sterile (gamma irradiated) core end-caps while gassing with a sterile OFN gassing jet to expel any air. The whole-round cores were then inserted into gas-tight anaerobic bags containing an "Anaerocult-A" sachet (Merck, Eastleigh,

¹ Pisciotta, K. A., Ingle, J. C., Jr., von Breyman, M. T., Barron, J., et al., 1992. *Proc. ODP, Sci. Results*, 127/128, Pt. 1: College Station, TX (Ocean Drilling Program).

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Table 1. Detailed list of Leg 128 samples provided for bacteriological examination.

Sample	Designation	Interval (cm)	Depth (mbsf)
128-798B-			
1.	1H-1,	0.00-0.25	0.00
2.	1H-1,	0.25-0.50	0.25
3.	1H-1,	0.50-0.75	0.50
4.	1H-1,	0.75-1.00	0.75
5.	1H-1,	1.00-1.25	1.00
6.	1H-2,	0.10-0.35	1.60
7.	1H-2,	1.02-1.27	2.52
8.	1H-3,	0.75-1.00	3.75
9.	1H-4,	1.00-1.25	5.50
10.	2H-1,	0.20-0.45	9.60
11.	2H-3,	0.20-0.45	12.60
12.	3H-4,	0.60-0.85	23.50
13.	4H-7,	0.31-0.56	36.71
14.	6H-4,	0.22-0.47	51.02
15.	9H-3,	0.80-1.05	78.70
16.	14H-7,	0.98-1.23	133.18
17.	21X-2,	0.20-0.45	192.70
18.	27X-3,	0.80-1.05	252.80
19.	36X-2,	0.40-0.65	336.50
20.	40X-2,	0.45-0.70	375.15
21.	45X-3,	0.35-0.60	424.85
22.	53X-4,	0.25-0.50	503.45
23.	54X-CC	1 cm ³	517.90

U.K.) to remove all oxygen, and sealed with a modified impulse bag-sealer (Jencons, Leighton Buzzard, U.K.). Samples were temporarily stored in the ship cold room at 4° C.

For Samples 1-5 (Table 1) prior to sealing, a 1-cm³ sample was taken for "fresh" direct bacterial counts, from the lower end of the whole-round core, using a sterile (autoclaved) 5-mL syringe with the needle end removed, and stored in a serum vial containing 9 mL of

filter-sterilized (0.2 µm) 4% formaldehyde in artificial seawater. On Samples 6-22 the 1-cm³ sample was taken from the cut end adjacent to the lower part of the 25-cm whole-round core, after flaming the end.

A 6-cm whole-round core was then removed from the adjacent sediment for extraction of pore-water for chemical analysis. After squeezing in the ships' rig, subsamples were either stored in sealed vials in the freezer, or preserved for sulfate and sulfide analyses (4 mL in 1 mL of 10% zinc acetate) before being refrigerated.

Ship - Laboratory Transportation

The whole-round cores for microbiological analysis were transported from *JOIDES Resolution* by Cragg and Parkes on 3 September 1989 aboard a Japanese tugboat, *Nippon Maru*. They were stored in a commercial refrigerator at 5° C on board for the 2.5-day journey to Moji, Japan, where they were packed into a large, insulated, pre-chilled (dry ice) aluminum trunk with "Igloo" freezer packs. The trunk was customs-sealed and transported by air to the laboratory. On arrival (7 September) the trunk contents were transferred to a cold room at 4° C. During transportation the samples had remained cold.

The samples of frozen sediment and the frozen pore-water samples remained on board the *JOIDES Resolution* and were forwarded to our laboratory (packed in dry ice) from ODP in April 1990.

Laboratory Handling

Whole-Round Cores

Initial Handling

The sealed whole-round cores were opened in the laboratory on 19 September 1989 (3 weeks after they had been taken). All primary handling and subsequent subsampling was conducted at 16° C under sterile nitrogen jets and aseptic techniques in a laminar flow cabinet. The 25-cm whole-round cores were divided into sequential 5 × 5-cm sections. For the upper samples this was done by uncapping both ends of

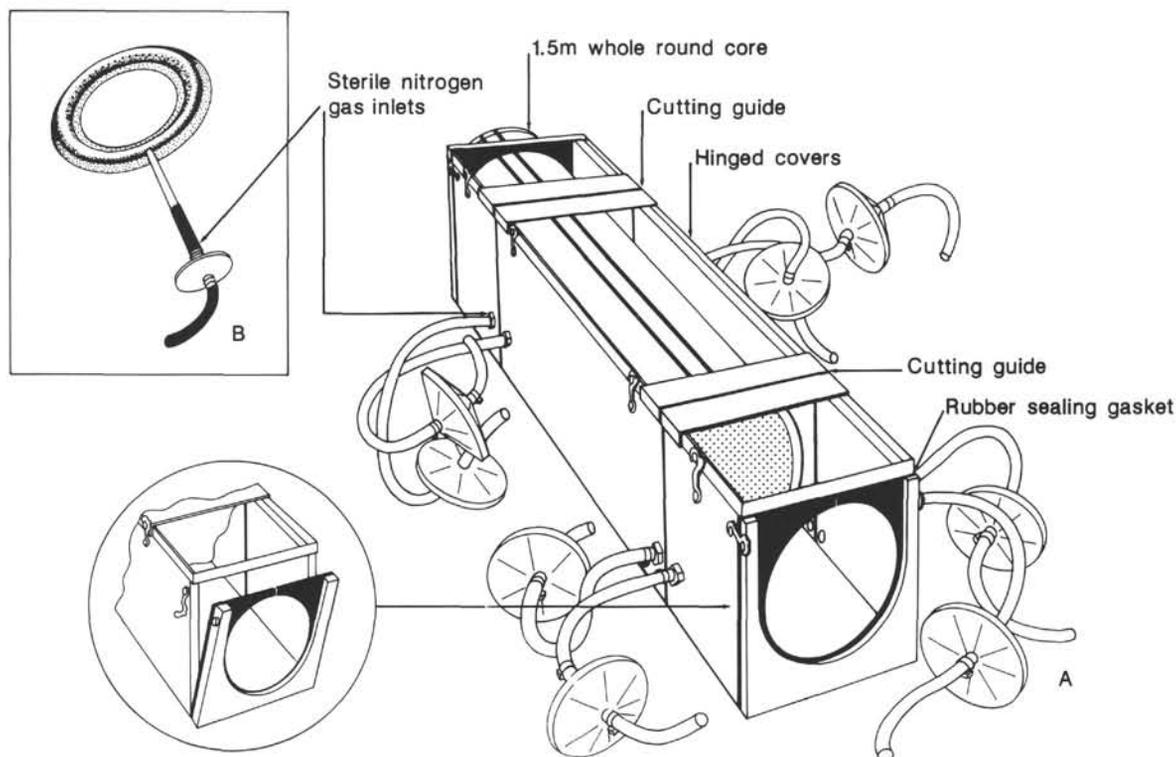


Figure 1. A. Cutting rig designed to remove sections of core under sterile and anaerobic conditions. B. Gassing jet designed to provide a constant stream of oxygen-free nitrogen over sections of whole-round core during subcoring.

the core, and briefly flaming them before extruding 5-cm sediment sections into sterile polycarbonate sleeves using a sterile stepped plunger inserted into the base of the whole-round core. Each 5-cm section was removed by slicing through the core with a sterile slice. For the middle and lower samples, where the sediment was more compacted, the plunger system of core extraction proved inadequate, and 5-cm whole-round cores were cut using the core-cutting apparatus described above.

Subsampling

Each 5-cm whole-round core was assigned to a particular activity measurement, together with the relevant most-probable-number (MPN) viable count of specific bacterial types, direct counts, and some additional measurements (Table 2). Within each 5-cm whole-round core, subcores were taken using sterile (autoclaved) 5-mL plastic syringes (Beckman-Dickinson, Oxford, U.K.) from which the leuc end had been removed. Volumes taken were $10 \times 5 \text{ cm}^3$ (activity measurements), $2 \times 5 \text{ cm}^3$ (MPN and direct counts), $2 \times 5 \text{ cm}^3$ (porosity) and $2 \times 5 \text{ cm}^3$ (pore-water analysis). During subcoring a flow of sterile OFN was maintained across the surface of the whole-round core from a gassing rig consisting of a copper ring with inwardly facing jets mounted on a circular flange that rested on the polycarbonate sleeve-edge (Fig. 1B). Sediment adjacent to the core liner was not sampled. Syringe subcores were sealed with sterile butyl rubber "Suba Seals" (Wm. Freeman and Co. Ltd., Barnsley, U.K.) and either (1) stored for a few hours (MPN), or overnight (activity measurement) in anaerobic jars at 16°C , (2) centrifuged for chemical pore-water analysis, or (3) dealt with immediately (porosity). Sediment subcores were replaced by sterile perspex pegs of the same size to enable the remaining sediment to be sampled intact. Sterile disposable gloves were used during handling aboard ship and in the laboratory.

Potential Activity Measurements

Sample Handling

Three isotopes were separately injected into the syringe subcores using a glass microsyringe and special rig that allowed steady injection of isotope along the center line of the subcore. Each group of 10 subcores was divided into 1 time-zero control and 3×3 incubation periods. Those requiring incubation were sealed in anaerobic bags as described above, and incubated at 16°C mean downhole temperature for various periods (Table 3). Incubation was terminated by placing the bags into a freezer at -20°C where they were stored until analyzed. Subcores for time-zero control were pre-chilled (4°C), injected, and immediately frozen (-20°C), and then sealed in anaerobic bags and stored frozen.

Isotope Preparation

All isotopes were obtained from Amersham International (Amersham, U.K.). They were diluted with autoclaved distilled water and membrane filter sterilized ($0.2 \mu\text{m}$) into a number of glass vials. Both ^{14}C sodium bicarbonate and ^3H -thymidine were stored at 4°C , and the ^{35}S -sulfate was stored at -20°C . The ^3H -methyl thymidine was supplied as a sterile solution containing 2% ethanol. This solution was hand warmed and the ethanol blown off using a sterile OFN gassing jet prior to use. Injections into the syringe cores were: $^{35}\text{SO}_4$ $7.2 \mu\text{L} = 2.28 \mu\text{Ci}$ (sulfate reduction); H^{14}CO_3 $7.2 \mu\text{L} = 3.77 \mu\text{Ci}$ (methanogenesis and acetogenesis); ^3H -methyl thymidine $32 \mu\text{L} = 14.7 \mu\text{Ci}$ (thymidine incorporation).

Measurement of Activity

Rates of sulfate reduction were determined from the proportion of ^{35}S -labeled sulfide produced from the ^{35}S -sulfate injected. Sulfide

Table 2. Breakdown of microbiological measurements made on each of the 5-cm core sections obtained from a 25-cm whole-round core.

Core section (cm)	Activity measurement	Bacterial count	Other
1-5	Sulfate reduction	MPN SO_4^{2-} reducers (acetate and lactate) Direct count	Porosity SO_4^{2-} & S^- analysis
5-10	Methane production	MPN CH_4 producers Direct count	Porosity
10-15	Acetate production	MPN acetogens	Porosity
15-20		MPN NO_3^- reducers MPN NH_4^+ producers (aerobic and anaerobic) MPN Heterotrophs Direct count	Porosity
20-25	Thymidine incorporation	Direct count	Porosity

distillation and radioactive counting was as described by Parkes and Buckingham (1986) and both acid-volatile-sulfide (AVS) and pyrite plus sulfur (PVS) fractions determined. Methanogenic activity was calculated from the amount of $^{14}\text{CH}_4$ produced. This was oxidized to $^{14}\text{CO}_2$ and counted by liquid scintillation as described in Cragg et al. (1990). Estimates of thymidine incorporation were made following an extraction procedure based on the protocols of Karl (1982), Craven and Karl (1984), and Carmen et al. (1988) (Fig. 2). Daily activity rates were independently calculated for each of the incubation periods after subtraction of any DPM counts obtained from the respective time-zero incubations (sulfate reduction mean DPM = 314; methanogenesis mean DPM = 32; thymidine incorporation mean DPM = 1956). Rates of sulfate reduction and methane production were calculated from the activity rates, the concentrations of pore-water sulfate and alkalinity respectively, and sediment porosity (Jørgensen, 1978).

Counts of Viable Bacteria

An MPN technique (Colwell, 1979) was used to estimate numbers of viable bacteria. This involved between 5 and 10 dilution levels starting with the sediment and descending serially by either triplicate or quadruplicate 1:5 dilutions.

Preparation of MPN Vials

All MPN enrichments were performed in 7 mL hypovials sealed with butyl rubber septa and aluminum caps (Pierce and Warriner, Chester, U.K.). Hypovials for the serial dilutions were 30 mL in size and contained 20 mL of medium. A full description of vial preparation is given by Cragg et al., (1990).

Dispensing System

The medium-dispensing system consisted of a 30-mL plastic syringe joined via a leuc-lock to a plastic three-way stopcock which was in turn connected to a second three-way stopcock (Gallenkamp, Loughborough, U.K.) and then to a syringe needle. The upper stopcock side-arm was connected to a pressurized medium-container and the lower stopcock side-arm, via a sterile $0.2 \mu\text{m}$ gas filter, to a low-pressure gas supply (either $\text{N}_2(80\%)/\text{CO}_2(20\%)$ or $\text{H}_2(80\%)/\text{CO}_2(20\%)$, depending on media type). By controlling medium and gas flow with the stopcocks, evacuated 7-mL vials were first filled

Table 3. Incubation periods (days) for potential activity measurements, zero and three incubation times for each sample. The samples were divided into four groups based on depth and anticipated activity rates.

Sample	Depth (mbsf)	Length of incubation period (days)				Thymidine incorporation
		Sulfate reduction	Methanogen	Acetogen		
1	0.00	0.00	0.00	0.00	0.00	
2	0.25	0.50	0.50	0.50	0.17	
3	0.50	1.00	1.03	1.00	0.33	
4	0.75	1.50	1.50	1.50	0.50	
5	1.00					
6	1.60					
7	2.52	0.00	0.00	0.00	0.00	
8	3.75	1.00	1.00	1.00	0.50	
9	5.50	5.00	5.00	5.00	1.00	
10	9.60	12.00	12.00	12.00	1.50	
11	12.60					
12	23.50	0.00	0.00	0.00	0.00	
13	36.71	3.00	3.00	3.00	1.01	
14	51.02	15.00	15.00	15.00	2.00	
15	78.70	24.00	24.00	24.00	3.00	
16	133.18					
17	192.70	0.00	0.00	0.00	0.00	
18	252.80	5.00	5.00	5.00	1.06	
19	336.50	20.00	20.00	20.00	3.19	
20	375.15	35.13	35.22	35.18	5.21	
21	424.85					
22	503.45		not measured			
23	517.90		not measured			

with 5 mL of sterile media and then pressured to slightly above ambient with gas. The tops of the septa were smeared with a silicone rubber sealant to improve the physical integrity of the seal and hence help prevent leakage through the pierced septum. Those used for detecting lactate-utilizing sulfate-reducing bacteria (SRB) were not smeared with sealant because the acetate present in the sealant might have diffused into the media.

Preparation of Media

All media contained NaCl and MgCl₂ in quantities approximating *in-situ* salinities of Hole 798B (32 and 5 g/dm³ respectively). A detailed description for the preparation of media to detect acetate and lactate SRB, methanogens, and nitrate respirers, together with the diluent medium is given in Cragg et al., (1990). The medium described for viable heterotrophs was further modified by the addition of two extra carbon sources; chitin and cellulose, both at 0.5 g/dm³ added to the hypovials prior to sterilization.

Acetogens were enriched in Widdel's marine media (Pfennig et al., 1981) as modified in Cragg et al. (1990) for enrichment of methanogens, with the further modifications of omitting cysteine, but adding casamino acids (0.1 g/dm³) and a pH indicator—Bromocresol green (0.15 g/dm³) prior to autoclaving, and the omission of both methylamine and acetate after autoclaving.

Ammonifying bacteria were enriched using Crossley Milk Medium (Oxoid, Basingstoke, U.K.) supplemented with 3% wt/vol NaCl. Aerobic ammonifying bacteria were differentiated from anaerobic ammonifiers by incubating a duplicate set of samples in screw cap vials lined with a thin silicone rubber septum which allowed gas exchange.

All media were dispensed some weeks prior to inoculation and all vials that were not reduced (pink color due to resazurin) were discarded. Resazurin was not present in the medium for enriching ammonifiers. Any potential bacterial contamination in the media would have developed during this period, however none was observed.

Inoculation and Incubation Procedures

Initial manipulations were performed in an anaerobic cabinet that had been sterilized by swabbing with an antiseptic—Hibitane (ICI, Macclesfield, U.K.) at 0.5% in 70% ethanol, followed by 1 hr under ionizing UV light. One 5-mL syringe subcore was ejected into the first diluent vial, which was mixed and successively diluted (5 mL into 20 mL). For the zero dilution level a 1-mL sample of sediment was extruded directly into one 7-mL hypovial. The dilution vials were removed from the anaerobic cabinet and the contents from each dilution level dispensed, using sterile 20-mL syringes preflushed with OFN, in 1-mL aliquots into the 7-mL hypovials. All vials, apart from those used to enrich lactate-utilizing SRB were again smeared with silicone rubber sealant. Incubation was at 16° C and vials were checked weekly for growth.

Presumptive growth was detected by media blackening for SRB, acid and/or gas production for nitrogen reducers, direct microscopic counting for viable heterotrophs, headspace methane for methanogens and indicator color change for acetogens and both groups of ammonifiers. Viable counts and confidence limits were calculated according to Hurley and Roscoe (1983), and any vials that became oxidized after inoculation were not included in the MPN calculations.

Detection of Methane

Headspace gases were analyzed by gas chromatography (Parkes and Taylor, 1985). Vials were repressurized to 2 bars with H₂/CO₂ and smeared with sealant before incubation was resumed.

Detection of Nitrate Reduction

Using subsets of the nitrate reducer vials, the removal of nitrate and the presence/absence of nitrite was assessed with a nitrate reduction test (Cruikshank et al., 1975), and the presence/absence of

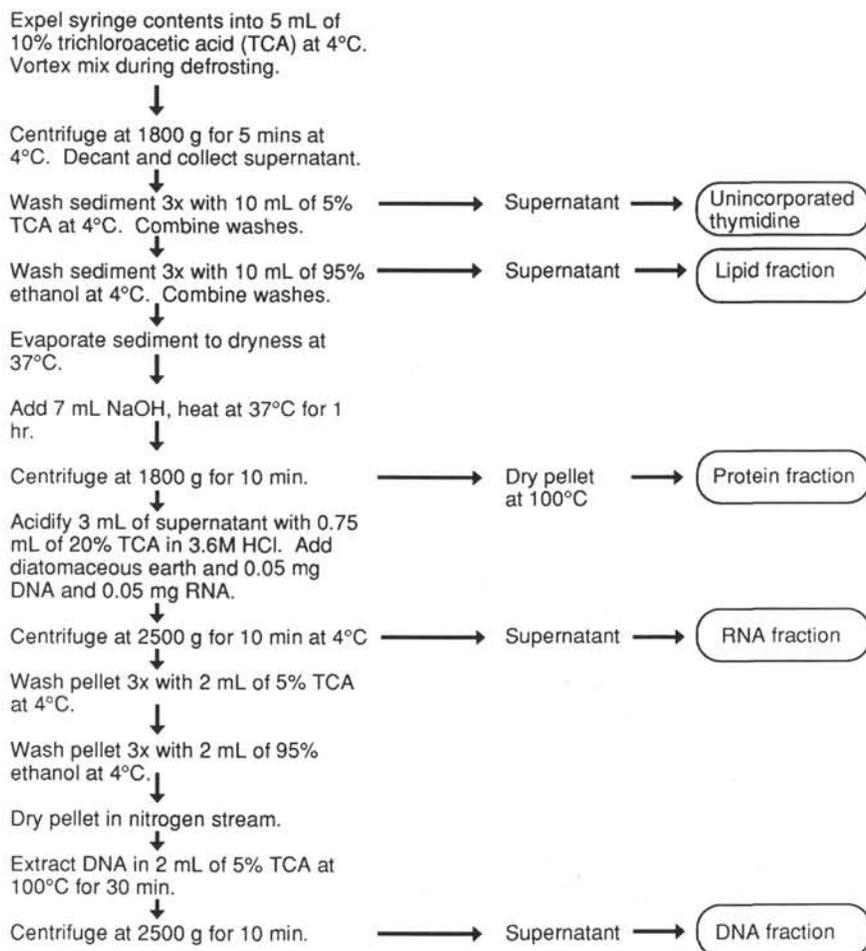


Figure 2. Protocol for the extraction of ^3H -labeled DNA from bacteria in sediments. Based on the methods of Karl (1982), Craven and Karl (1984), and Carmen et al. (1988).

ammonium was determined with an indophenol blue colorimetric test (Golterman et al., 1978).

Direct Microscopic Observation

Direct counts were made of bacteria from each sediment depth using the Acridine Orange technique following the general recommendations of Fry (1988). A full description is given in Cragg et al. (1990). Using this method a count was made of both total bacterial cells and those involved in cell division. The latter were either cells with a clear invagination, or pairs of cells of identical morphology, and these were counted as one and two cells, respectively, in the total bacterial count.

Chemical Analyses

Pore-water sulfate was determined by means of ion chromatography (Dionex, Sunnyvale, CA, U.S.A.) using an AS4A separator column, anion micromembrane suppressor, and a bicarbonate eluent, on both pore waters obtained aboard ship and in the laboratory. Sulfide, AVS, and PVS concentrations were determined colorimetrically (Cline, 1969).

Organic carbon values were obtained from shipboard data (Ingle, Suyehiro, von Breymann, et al., 1990). As our sampling procedure removed entire core sections there were no coincident data, and an average of all adjacent (± 2.5 m around our sample) organic carbon concentrations was calculated.

RESULTS

It was evident that the special procedures adopted during shooting of the first core of Hole 798B (Ingle, Suyehiro, von Breymann, et al., 1990) had successfully recovered much of the sediment close to the sediment/water interface, as intended. Best estimates, obtained from pollen analysis (L. Heusser, pers. comm., 1991), suggest that a maximum of only 60 cm was missing from the top of the core. Although this is important microbiologically, for clarity, the official ODP depths have been used throughout this paper.

Direct Counts

Bacteria are present at all depths to 517.90 mbsf (Fig. 3). There is an initial rapid decline in numbers from 1.37×10^9 cells/cm³ at 0.025 mbsf to 1.9×10^8 cells/cm³ at 0.075 mbsf (seven-fold decrease). This is followed by a more gradual logarithmic decline to 1.11×10^7 cells/cm³ at 517.90 mbsf representing an overall 120-fold decrease. Against this background of overall decrease in bacterial numbers there are some distinct increases that continue over considerable depth intervals, for example at 10, 50, and 200 mbsf. The detection limit using this procedure was 10^5 cells/cm³ and the minimum count recorded was 8.10×10^5 cells/cm³ at 503.67 mbsf, an almost 1700-fold decrease from the near-surface count. Numbers of dividing cells declined at a similar rate to the total bacterial count, with a rapid decrease from 1.15×10^8 cells/cm³ at 0.025 mbsf to 1.19×10^7 cells/cm³ at 0.075 mbsf, a 10-fold decrease (Fig. 4). Below 2 mbsf the decline

was more gradual, to approximately 3×10^6 cells/cm³ around 500 mbsf (in samples where dividing cells were observed), representing a more than a 40-fold decrease over near-surface counts. The number of samples that contained no detectable dividing cells increased with depth. These data have been assigned the detection limit of 10^5 cells/cm³. Over the complete depth range dividing cells represented, on average, 4.8% (range 0.5%–12.3%) of the direct count (Figs. 3 and 4). Direct counts and dividing cell counts, on fresh samples taken aboard ship, showed no overall significant difference ($p = 0.11$ and $p = 0.40$, respectively; $n = 21$) to stored samples taken during laboratory handling. However, as all other data were obtained from stored sediment samples, only those data are presented here.

Viable Counts (Table 4 and Fig. 5)

Aerobic ammonifying bacteria are present in low numbers, 3.19×10^2 bacteria/cm³ at 0.175 mbsf (Fig. 5A), and decline rapidly to zero by 3.92 mbsf. Occurrence below this depth is limited, with minor rises between 3.92 and 23.67 mbsf, 36.8 and 133.3 mbsf, and at 503.6 mbsf (3 bacteria/cm³). Anaerobic ammonifying bacteria are more numerous, with 2.72×10^4 bacteria/cm³ at 0.175 mbsf (Fig. 5D), and although numbers decline in a similarly rapid way to the aerobic ammonifiers, the bacteria are present in all samples except at 133.33 mbsf, with small rises at 78.9 and 192.9 mbsf. Between 336.7 and 503.6 mbsf there is a significant ($p < 0.05$), increase in numbers to 117 bacteria/cm³.

Nitrate-reducing bacteria were present at all depths (Fig. 5B). Numbers are constant at approximately 150 bacteria/cm³ down to 1.77 mbsf and then, after a decline, remain constant at approximately 11 bacteria/cm³. After this there was a significant ($p < 0.05$) increase to 31 bacteria/cm³ at 503.6 mbsf. As the presence of nitrate-reducing bacteria is only presumptive, a subset of the MPN vials were chemically tested for

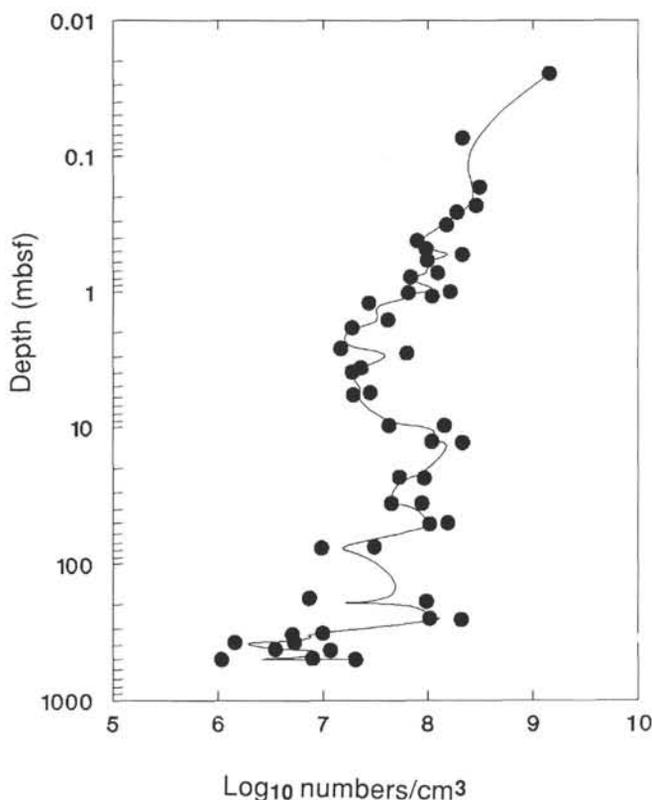


Figure 3. Depth distribution of total bacteria using the Acridine Orange Direct Count (AODC) technique to 517.90 mbsf (line is computer-generated best-fit for the data).

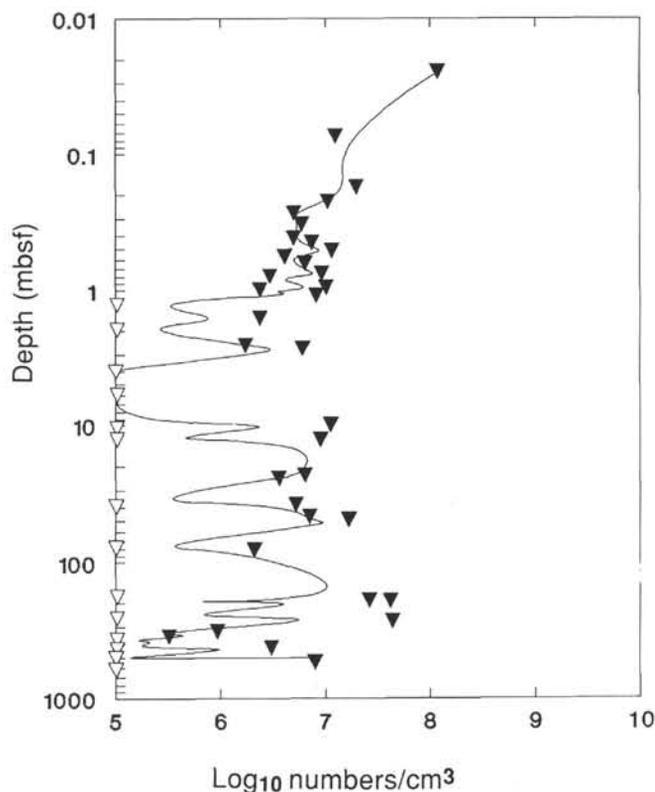


Figure 4. Depth distribution of dividing and divided bacterial cells using the AODC technique to 517.90 mbsf. Open symbols indicate zero counts that have been given the nominal value of the detection limit of 10^5 cells/cm³ (line is computer-generated best-fit for the data).

reduced nitrogen species. The removal of nitrate, together with the presence of nitrite or ammonium, confirmed that active nitrate reduction is indeed taking place. The absence of all three nitrogen compounds in positive MPN vials indicates that nitrate was reduced to nitrogen gas. As these chemical tests were conducted on a subset of the vials it is not possible to give an accurate MPN for the different types of nitrate-metabolizing bacteria. However, bacteria that reduce nitrate to ammonium were not detected below 1.77 mbsf.

SRB that separately utilize acetate and lactate showed very similar profiles (Table 4) and are presented here as combined data (Fig. 5C). The highest counts of 3.4×10^4 bacteria/cm³ occurred at 0.125 mbsf and there was a gradual decline to 1.1×10^3 bacteria/cm³ at 1.72 mbsf before a rapid decline to zero. An increase in numbers of both acetate-, and lactate-utilizing SRB was present between 3.77 and 23.5 mbsf, and a single positive vial from the acetate-utilizing bacteria occurred at 78.7 mbsf (Table 4).

The presence of acetogens was indicated by a pH change and therefore results are presumptive. They are present in low numbers at all depths (Fig. 5E). Near the surface (0.125–2.64 mbsf) numbers averaged 360 bacteria/cm³, with a peak of 1.1×10^3 bacteria/cm³ at 1.72 mbsf (Table 4). Below 2.64 mbsf counts averaged 40 bacteria/cm³ with small, insignificant decreases at 36.8 and an increase toward the bottom of the core between 336.6 and 503.6 mbsf.

Viable anaerobic heterotrophs occurred in the greatest numbers of all groups counted, with an average of 5.3×10^4 bacteria/cm³ between 0.175 and 9.77 mbsf, and two significant ($p < 0.05$) increases to 4.2×10^5 and 2.9×10^4 bacteria/cm³ at 0.675 and 9.77 mbsf, respectively (Fig. 5F). Counts then declined to near zero with a significant ($p < 0.05$) peak of 114 bacteria/cm³ at 78.9 mbsf and a progressive, and very highly significant ($p < 0.01$) increase from 336.7 to 503.5 mbsf, peaking at 1.26×10^4 bacteria/cm³.

Table 4. Distribution of bacterial populations in sediment to 503.57 mbsf (\log_{10} numbers/cm³).

Sample Depth (mbsf)	(a) Aerobic ammonifiers	(b) Anaerobic ammonifiers	(c) Nitrate reducers	(d) Sulfate reducers (acetate)	(e) Sulfate reducers (lactate)	(f) Acetogens	(g) Heterotrophs	(h) Total anaerobic	(i) Mean AODC cells/cm ³	(j) Anaerobic viability (% of AODC)
0.00–0.25	319	27,200	184	27,600	6400	263	49,600	111,247	272,521,016	0.0408
0.25–0.50	57	4500	158	756	1275	700	1950	9339	113,240,036	0.00825
0.50–0.75	59	17,000	188	273	4225	117	416,000	437,803	77,019,377	0.568
0.75–1.00	18	1125	188	45	120	117	3150	4745	78,749,898	0.00603
1.00–1.25	4	1525	56	14	80	79	19,200	20,954	51,880,004	0.0404
1.60–1.85	5	350	117	925	188	1125	4150	6855	30,549,211	0.0224
2.52–2.77	1	10	13	0	1	117	1962	2103	46,131,757	0.00456
3.75–4.00	0	1	13	0	0	53	3750	3823	19,319,683	0.0198
5.50–5.75	1	2	13	1	0	59	184	259	30,338,912	0.000854
9.60–9.85	NR	NR	7	1	3	59	28,600	28,670	83,945,999	0.0342
12.60–12.85	2	1	10	1	1	59	1	73	131,219,990	0.0000556
23.50–23.75	0	1	10	0	0	59	1	71	39,627,803	0.000179
36.71–36.96	0	1	10	0	0	12	1	24	52,966,344	0.0000453
51.02–51.27	1	1	13	0	0	59	3	76	105,196,187	0.0000722
78.70–78.95	1	7	13	1	0	59	114	194	15,381,546	0.00126
133.18–133.43	0	0	10	0	0	59	1	70	4,720,630	0.00148
192.70–192.95	0	5	5	0	0	31	3	44	22,438,819	0.000196
252.80–253.05	0	1	10	0	0	20	3	34	50,699,071	0.0000671
336.50–336.75	0	1	10	0	0	20	1	32	5,834,451	0.000548
375.15–375.40	0	2	2	0	0	31	18	71	2,137,962	0.00332
424.85–425.10	0	31	3	0	0	59	12,600	12,693	4,446,313	0.285
503.45–503.70	3	117	31	0	0	31	12,600	12,779	6,823,387	0.187

Data are not presented here for counts of methanogenic bacteria, as they have not yet grown sufficiently for their numbers to be calculated. In previous work (Cragg et al., 1990), methanogens have taken more than 18 months to enrich, with two subcultures, and that period has not yet elapsed since these samples were obtained. Preliminary data (headspace methane analysis) indicate the presence of methanogens between 0.825 and 2.59 mbsf.

Compared to all other MPN results the nitrate-reducers and the acetogens showed much reduced changes in bacterial numbers with depth. This suggests that the growth medium employed was probably not sufficiently selective for the intended populations and should be improved in the future.

The different groups of anaerobic bacteria identified by MPN enrichment can be added together to give some estimate of the total culturable anaerobic population, although the different types are not totally exclusive (e.g., nitrate and sulfate reducers are specialized heterotrophs, Table 4). The total anaerobic culturable population, expressed as a percentage of the direct count, provides a measure of the viability of the anaerobic bacteria. Viabilities are low, (0.000045%–0.57%), and cover a wide range (12,600-fold). Highest values occurred in the top 10 m of the core and toward the base at 425 and 503.5 mbsf (Table 4).

Potential Activities

Rates of activity obtained in this study should be regarded as potential because we are, as yet, unable to assess the effects of storage (although this should be minimal, as samples were handled 3 weeks after collection) and pressure reduction effects encountered during core retrieval. Incubations were done at the average *in-situ* temperature but were not conducted at *in-situ* pressures.

[³H]-thymidine incorporation into bacterial DNA occurs primarily in the upper section of the core (Fig. 6). Maximum rate was 0.48 pmol/cm³/d at 0.225 mbsf, declining logarithmically to zero at 5.72 mbsf. Trace levels of activity (0.0004 and 0.0001 pmol/cm³/d) were observed at 23.72 and 78.92 mbsf, respectively.

Sulfate reduction is also concentrated in the upper 5–6 m of the core (Fig. 7A), with a maximum rate of 24.77 nmol/cm³/d at the shallowest depth (0.125 mbsf), declining rapidly to 3.3 nmol/cm³/d at 0.775 mbsf and then more gradually to 0.06 nmol/cm³/d at 5.52 mbsf. The maximum rate of sulfate reduction is low but comparable to pre-

viously published rates for more productive sites such as inshore marine sites (0.2–2800 nmol/cm³/d; Senior et al., 1982; Parkes and Taylor, 1985; Parkes and Buckingham, 1986), and also with rates from continental shelf surface sediments (0.0001–46 nmol/cm³/d; Sorokin, 1962; Tsou et al., 1973; Goldhaber and Kaplan, 1975; Jørgensen, 1983; Parkes and Taylor, 1985; Edenborn et al., 1987; Cragg et al., 1990). Sulfate reduction is present at all depths to 424.9 mbsf (Fig. 7B), although the rates are very low; between 12.62 and 424.9 mbsf the average rate is only 1.7 pmol/cm³/d. However between these depths there was a small increase in sulfate reduction rates at 78.72 mbsf (3 pmol/cm³/d) and again toward the bottom of the core (Fig. 7B). Pore-water sulfate has a similar profile to that of sulfate reduction and declines rapidly from 18.55 mmol/dm³ at 0.125 mbsf to 0.78 mmol/dm³ by 9.62 mbsf (Fig. 7A). Although sulfate is found in all samples below 9.62 mbsf, the maximum concentration is only 0.92 mmol/dm³ (78.72 mbsf).

The depth profile of sulfate reduction rates is significantly correlated ($r = -0.755$; $p < 0.002$, $n = 21$) with changes in the total pool of reduced sulfide. The relationship between sulfate reduction and each of the two pools of sulfide (AVS and PVS), however, is quite different. Over the first meter, rates of sulfate reduction significantly correlated ($r = 0.991$; $p < 0.001$, $n = 4$) with a similar decline in the concentration of AVS (Fig. 8). At the same time a significant negative correlation ($r = -0.925$; $p < 0.05$, $n = 4$) was observed between the sulfate reduction rate and PVS concentration (Fig. 8). Maximum concentrations of both AVS and PVS are, however, much deeper in the sediment than sulfate reduction rates; AVS, 2.53 mmol S/dm³ at 12.62 mbsf, and PVS, 33.30 mmol S/dm³ at 36.73 mbsf, and there are considerable concentration changes with sediment depth. Most noticeably there are increases in both AVS and PVS at about 6 mbsf, between 36.7 and 131.7 mbsf, and toward the bottom of the core (Fig. 8). The average concentration of PVS (21.29 mmol S/dm³) is very much higher than that of AVS (0.97 mmol S/dm³).

Methanogenesis from CO₂/H₂ occurs principally in the top 1 m of the core with a mean rate of 0.55 nmol/cm³/d (Fig. 9). Maximum rates averaged 0.61 nmol/cm³/d between 0.325 and 0.825 mbsf. This is comparable to rates of 0.01–20 nmol/cm³/d found in the Baltic Sea (Lein et al., 1981), 0.2–1.0 nmol/cm³/d and 0.5–1.8 nmol/cm³/d in clean and organically enriched sea-loch sites (Parkes and Taylor, 1985), and 0.18 nmol/cm³/d on the Peru margin (Cragg et al., 1990). Below 1.65 mbsf, methanogenesis rates were recorded at all depths to 424.9 mbsf, except 252.8 mbsf. Small increases of 0.124 and

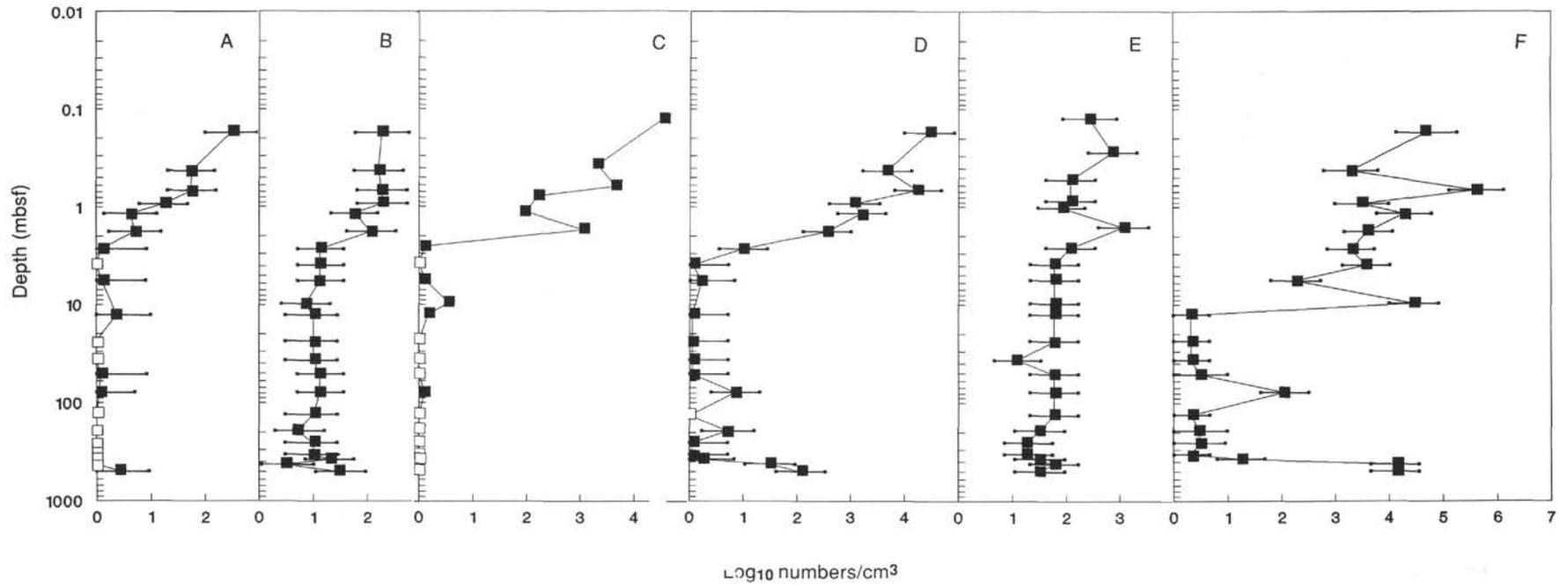


Figure 5. Depth distribution of the viable bacterial population to 517.90 mbsf, obtained using a most-probable-number technique. **A.** Aerobic ammonifiers. **B.** Nitrate reducers. **C.** Sulfate-reducers (acetate and lactate utilizing combined). **D.** Anaerobic ammonifiers. **E.** Acetogens. **F.** Heterotrophs (horizontal bars are 95% confidence limits, and open symbols indicate a zero count).

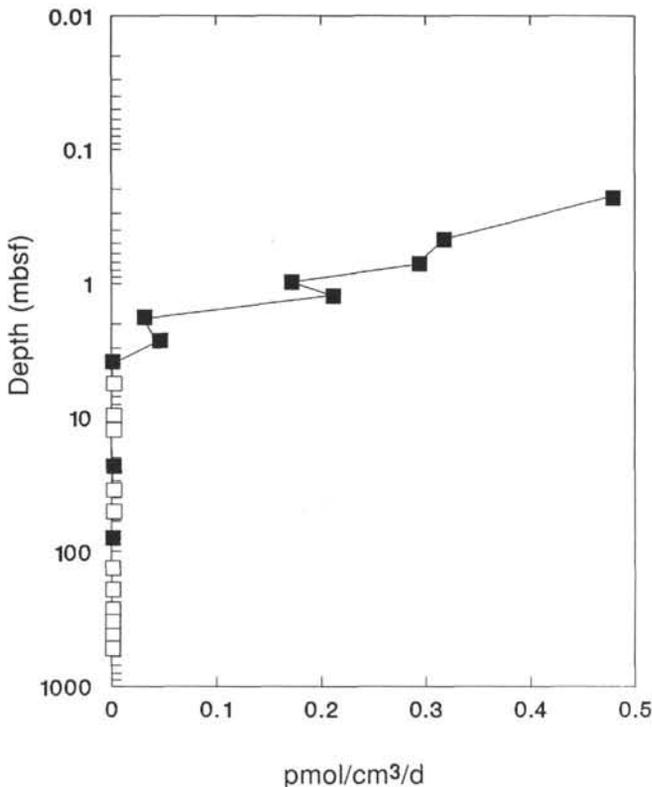


Figure 6. Depth distribution of rates of [^3H] - thymidine incorporation into bacterial DNA ($\text{pmol}/\text{cm}^3/\text{d}$), to 425 mbsf. Open symbols indicate no incorporation detected.

$0.094 \text{ nmol}/\text{cm}^3/\text{d}$ were observed at two depths, 2.59 and 78.77 mbsf, respectively. Pore-water methane concentrations are negligible to 5.82 mbsf and then increase sharply to $0.93 \mu\text{mol}/\text{cm}^3$ before declining to a mean of $0.26 \mu\text{mol}/\text{cm}^3$ down to 375.4 mbsf (Fig. 9). The large increase in methane concentrations below 375.4 mbsf, to $1.76 \mu\text{mol}/\text{cm}^3$, is due to thermogenic gas production (Ingle, Suyehiro, von Breyman, et al., 1990).

DISCUSSION

Direct microscopic determination of intact bacterial cells clearly shows that significant populations of bacteria were present in all samples analyzed (Fig. 3). In addition, a portion of this population was dividing even in the deepest sample, 518 mbsf (Fig. 4). Despite the overall logarithmic decrease in bacteria with increasing sediment depth, there are several discrete increases in bacterial numbers, which continue over considerable depth intervals (Fig. 3). These increases are at 10, 50, and 200 mbsf, and there is also an indication of an increase in bacteria toward the bottom of the core. This situation is very different from the generally accepted view that the bulk of bacterial changes within sediments occur within the top 10 cm, and that below 1 m, changes in bacterial populations are extremely limited (Rheinheimer, 1985). These increases in bacterial concentrations are seen more clearly when the data are averaged over each 25-cm whole-round core section (Fig. 10), and a close positive relationship between total bacteria and numbers of dividing cells is also apparent.

Although the acridine orange epifluorescence technique does not discriminate between living, dormant, or possibly recently dead cells, the presence of dividing cells is an index of growth and hence viability (Hagström et al., 1979). The frequency of dividing cells can be used to estimate bacterial growth rates in pelagic environments, but its

application in sediments is more problematical as divided cells may remain attached or in close proximity for long periods of time after division, thus making accurate estimation of growth difficult (Newell and Fallon, 1982; Fallon et al., 1983). Growth studies on stimulated population of sediment bacteria (Getliff, 1991), including samples from this core, (Getliff et al., this volume) have, however, clearly demonstrated that increases in numbers of dividing cells coincide with midlogarithmic growth phase, and that subsequently numbers of dividing cells rapidly decrease. These data, coupled with the clear increases in numbers of dividing cells at specific depth intervals, strongly suggest that bacteria are actually growing in the sediment rather than just surviving, as the mere survival of dividing cells from the more active surface layers cannot explain the observed profile (Fig. 4). Similarly, the increases in bacterial numbers in deeper layers cannot be explained by the mixing of surface sediment (with high bacterial concentrations) into deeper sediment layers as a result of smearing along the core liner during coring, as the sediment layers immediately above actually have lower bacterial populations (Figs. 3 and 10). Although massive mixing of sediment during coring could produce an artificial bacterial profile, it is evident from the good core recovery (99%) and from all shipboard measurements that coring disturbance was minimal and individual horizons excellently preserved (Ingle, Suyehiro, von Breyman, et al., 1990). In addition, the analysis of bacterial concentrations horizontally across a sectioned whole-round core showed no significant differences in bacterial numbers between sediment adjacent to the core liner and that toward the center of the core (unpubl. data). As a further precaution, however, samples for bacterial counts were only taken from the center of a whole-round core.

The profile of bacteria with depth appears to fall into three broad sections: (1) a top section (0–6 m) where bacterial numbers are decreasing rapidly, (2) a middle section (6–375 m) where the rate of decrease is less and there are some distinct increases in bacteria, and (3) a bottom section (375–518 m) where bacterial numbers consistently increase (Fig. 10; this figure includes direct counts for the deepest sample, not included in Figure 3 as this was a "fresh" sample, see Results). Numbers of dividing cells exhibit a similar profile, but this is complicated by the absence of dividing cells in some samples below 1 m. After a rapid decrease in dividing cells over the topmost 2 m, they are either below the detection limit or continue to be present as a constant proportion (average 4.8%), of the total bacterial population (Figs. 4 and 10). In this section there were therefore distinct periods of bacterial growth (10, 50, 200, and below 375 mbsf) that correspond with increases in the total bacterial population (Figs. 3, 4, and 10). Between 375 and 518 mbsf, although numbers of dividing cells remain relatively constant at $2.5 \times 10^5/\text{cm}^3$, they represent an elevated proportion of the total population (average 6.25%). Percentage viabilities also increase over this depth range (Table 4).

Despite the overall decrease in the bacterial population with sediment depth, the populations in the deeper layers are still very significant below 300 mbsf, representing, on average, 5% of the near-surface population (average for the top 25 cm; Table 4) and even for the deepest sample at 518 mbsf, 4% of the near-surface population. These values are slightly higher than those found previously for deep sediment layers from the Peru Margin (1% at 80 mbsf, Leg 112; Cragg, et al., 1990). However, the average surface bacterial population (0–25 cm) in Peru sediments ($1.37 \times 10^9/\text{cm}^3$) is significantly larger than that reported here, ($5.29 \times 10^8/\text{cm}^3$). Such significant bacterial populations must have profound effects on continued diagenetic reactions within these sediments, as a source of energy would be required to maintain these populations.

Viable Bacterial Populations

Bacteria were cultured, in various media, from all depths sampled (Figs. 5A–5F). Depth profiles of viable bacteria generally have a distribution similar to the total bacterial population (Fig. 3), with a

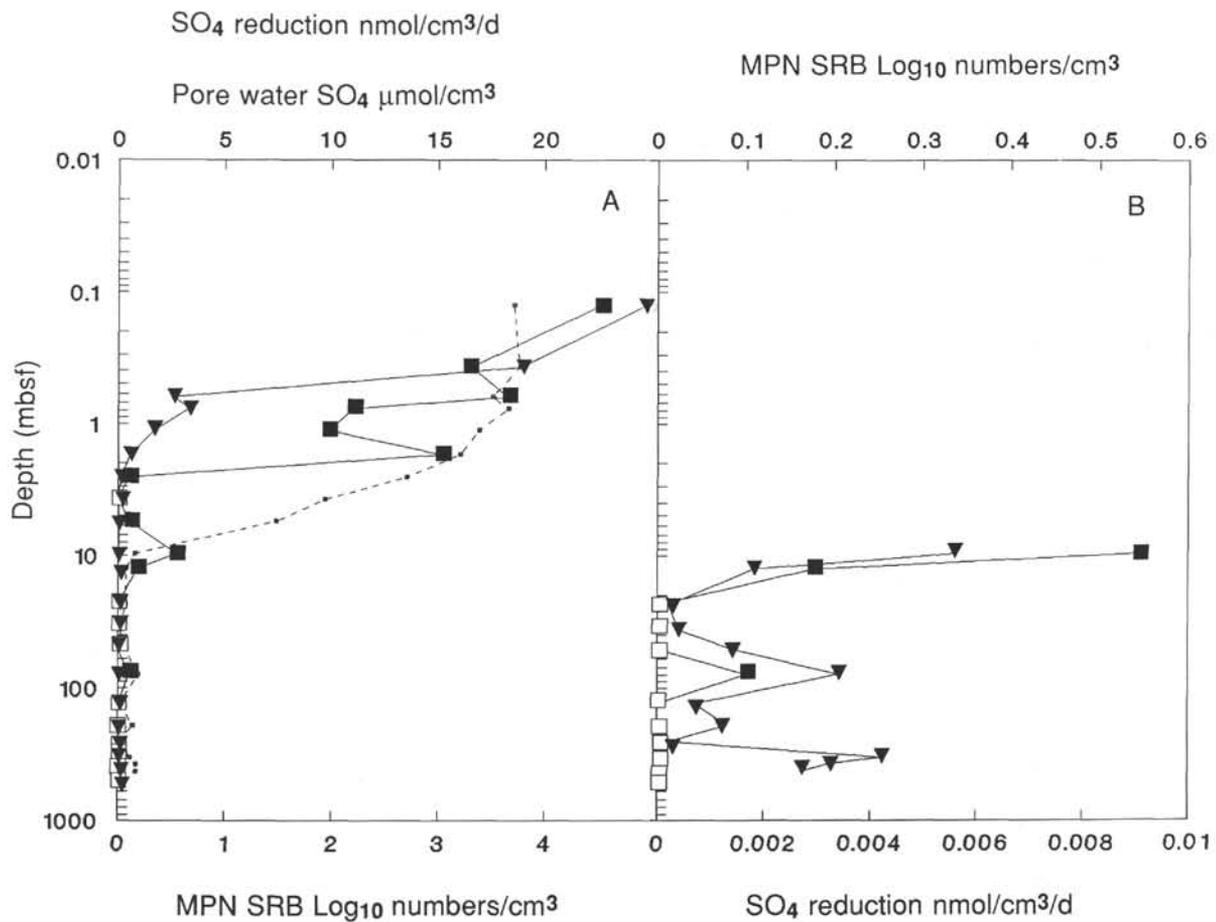


Figure 7. Depth distribution of pore-water sulfate (■ - - - ■), $\mu\text{mol}/\text{cm}^3$, potential sulfate reduction activity (\blacktriangledown — \blacktriangledown), $\text{nmol}/\text{cm}^3/\text{d}$, and MPN viable count of sulfate-reducing bacteria (■—■), \log count/ cm^3 . (A) to 517.90 mbsf and (B) between 9.60 and 517.90 mbsf with expanded scales (pore-water sulfate not included).

rapid decrease over the top 6 m. There was a trend of bacterial succession with increasing sediment depth, based on the use of the expected sequence of respiratory terminal electron acceptors: oxygen, nitrate, sulfate, carbon dioxide (Parkes and Senior, 1988). This is reflected in the depth at which the initial high-surface populations reached zero or minimum values. These populations are aerobic ammonifiers (3 mbsf, Fig. 5A), nitrate-reducing bacteria (3 mbsf, Fig. 5B), sulfate-reducing bacteria (3 mbsf, but with significant increases deeper down, Fig. 5C), anaerobic ammonifiers (4 mbsf, Fig. 5D), acetogens (4 mbsf, Fig. 5E); and finally, anaerobic heterotrophic bacteria that do not require an external respiratory electron acceptor, 6 mbsf (Fig. 5F). In the middle section (6–375 mbsf), as for the total bacterial population, numbers of viable bacteria are more constant except for a few marked increases at specific depths. For viable counts these are at 10 mbsf (aerobic ammonifiers, sulfate-reducing bacteria, and anaerobic heterotrophic bacteria), 80 mbsf (aerobic and anaerobic ammonifiers, sulfate-reducing bacteria, and anaerobic heterotrophic bacteria), and below 375 mbsf (aerobic and anaerobic ammonifiers, nitrate-reducing bacteria, acetogens, and anaerobic heterotrophic bacteria).

Viable counts must be interpreted with caution as, in addition to the potential for contamination, they represent such a small proportion of the total bacterial population (Table 4), and the bacteria selected in rich media may not be representative of those actually active *in situ* (Parkes and Taylor, 1985). However the strong positive correlation ($p < 0.002$, $n = 15$) between numbers of viable sulfate-reducing bacteria and both sulfate reduction rates and the pore-water sulfate concentration (Figs. 7A and 7B), demonstrates that this MPN is a realistic index

of the presence of specific types of bacteria within the sediment. This demonstration, together with our previous data showing positive relationships between MPN viable counts and activity within deep sediment layers (Parkes et al., 1990), suggests that all the MPN series probably enriched a small, but meaningful, subset of the natural bacterial population, and that contamination was not a problem.

The general similarity of the MPN profiles is not surprising, as they are all different types of heterotrophic bacteria. Although bacteria able to utilize a complete range of electron acceptors (O_2 to CO_2) were isolated, apart from the strict anaerobes, their *in-situ* metabolism is likely to be quite different, or they may be dormant. For example, the common capacity of facultative anaerobic heterotrophic bacteria to reduce nitrate would explain the presence of nitrate-reducing bacteria within a sediment that is unlikely to contain nitrate except very close to the sediment surface (as sulfate is very rapidly removed below 1 mbsf, Fig. 7A). The increase in five of the six different viable counts after 375 mbsf (Figs. 5A–5F) was very surprising, but it is extremely unlikely that this was caused by contamination during coring as the sediment above had, generally, much lower bacterial populations. Also, the increase in the total viable anaerobic population corresponds to an increase in the total bacterial population (Fig. 10), and the average viability of the deepest layers (375–518 mbsf) was considerably higher than the middle section, and similar to the average viability of the top 6 m of sediment (Table 4). Overall viabilities of anaerobic bacteria are approximately a factor of 10 times higher than those observed in deep sediment layers in Peru (0.0000087%–0.03% over the top 80 mbsf; Cragg, et al., 1990, and Table 4). This may

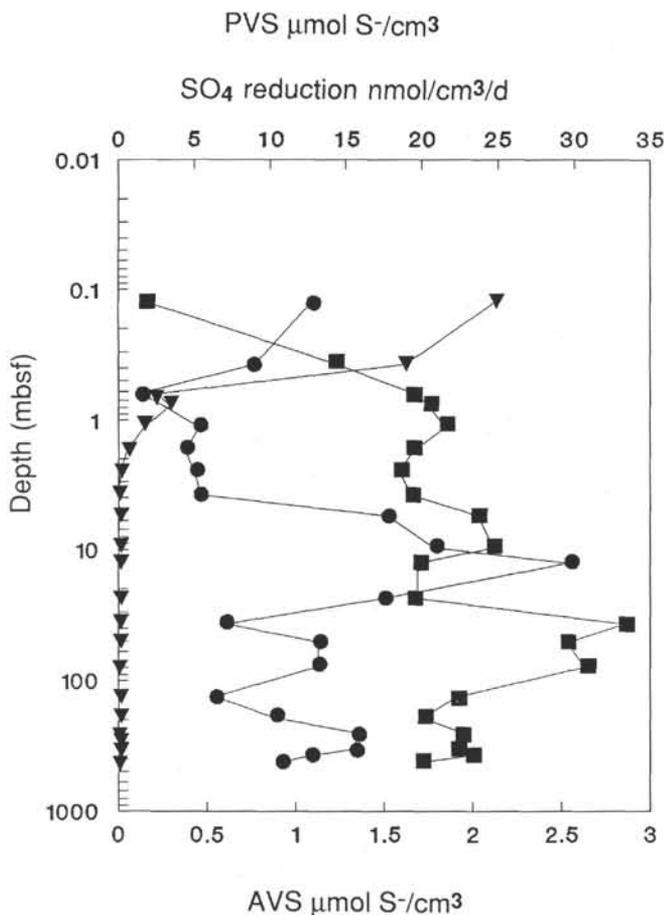


Figure 8. Depth distribution of concentrations of acid-volatile sulfide (AVS: ●—●) and pyrite-plus-sulfur (PVS: ■—■), $\mu\text{mol S}/\text{dm}^3$, and potential rates of sulfate reduction (\blacktriangledown — \blacktriangledown) $\text{nmol}/\text{cm}^3/\text{d}$, to 517.90 mbsf.

reflect real differences in the bacterial populations at the two sites, however there are some experimental differences between these studies. (Storage time was reduced for Japan [Japan sediments—3 weeks, Peru sediments—2 months], and there were improvements in storage conditions, handling, and media).

Rates of Bacterial Activity

Bacterial activity within intact subcores has profiles similar to those of the bacterial populations, high activity within surface sediments decreasing rapidly down to 3 mbsf (Figs. 11A and 11B). Anaerobic sulfate reduction correlated significantly with both the pore-water sulfate profile (+ve, $p < 0.05$; Fig. 7A), and the total pool of reduced sulfide (–ve, $p < 0.002$; Fig. 8). The negative correlation between sulfate reduction and the total reduced sulfide pool is due to reoxidation of the bulk of the sulfide near the sediment surface (Jørgensen, 1982), where rates of sulfate reduction are maximum (Fig. 7A), and the sediment is “open” due to bioturbation and physical disturbance. Metal sulfides build up below this zone but rates of sulfate reduction are then relatively low. Sulfate reduction is present at all depths and resulted in the accumulation of significant concentrations of reduced sulfide species (Fig. 8). The end products of active sulfate reduction varied considerably down the core. Initially AVS is the dominant product although this rapidly decreases and PVS production increases, its rate of production following closely the removal of pore-water sulfate (Figs. 12A and 12B). Changes in the total pool of both AVS and PVS near the sediment surface are similar to those

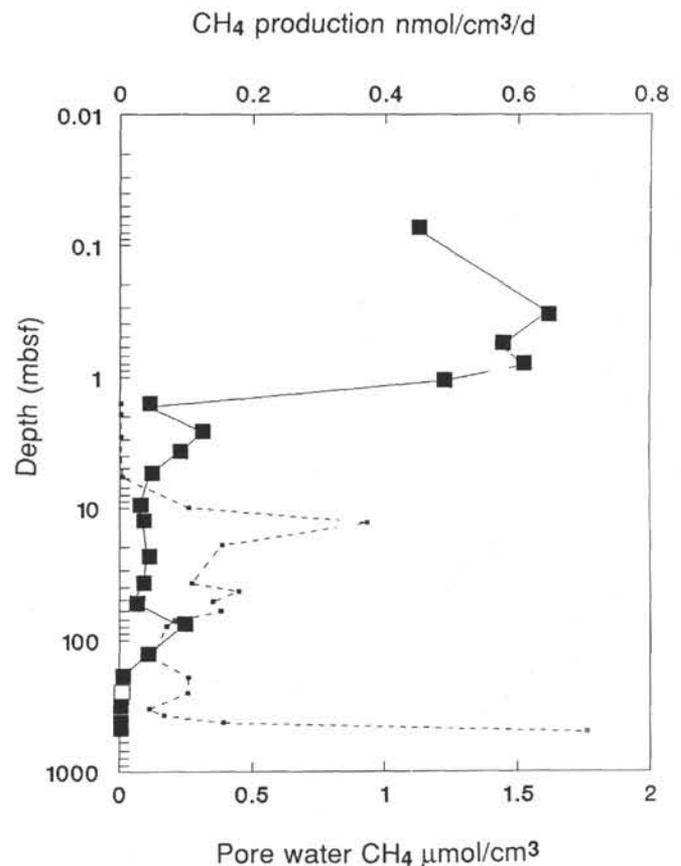


Figure 9. Depth distribution of sediment methane concentrations (●—●—●) $\mu\text{mol}/\text{cm}^3$ and potential rates of methane production (■—■) $\text{nmol}/\text{cm}^3/\text{d}$ to 517.90 mbsf. Open symbols indicate zero-value data.

observed for the above sulfate reduction rates; AVS is high near the sediment surface and then decreases with depth, while PVS has the opposite trend with depth (Fig. 8). At approximately 6 and 80 mbsf and toward the bottom of the core, both AVS and PVS concentrations increase, corresponding with increases in the rate of sulfate reduction (Figs. 7B, 8, and 13).

Methanogenesis from CO_2/H_2 , although high near the sediment surface, increases with depth to reach highest rates immediately beneath the maximum rate of sulfate reduction (Fig. 11A). Despite reasonable rates of methanogenesis, ($0.55 \text{ nmol}/\text{cm}^3/\text{d}$), no methane gas is present in the upper sediment (0–5 mbsf; Fig. 9). The methane produced must have, therefore, diffused out of the sediment, or have been consumed within the sediment by bacterial activity. Methanogenesis subsequently decreases rapidly, and after 1.65 mbsf only low rates were detected. Below 5.6 mbsf, however, these low rates were accompanied by the presence of methane gas (Fig. 13). Subsequent increases in methanogenesis appear to be loosely associated with increases in methane gas concentrations higher in the sediment. The marked increase in methanogenesis at about 80 mbsf, for example, produced a small increase in methane gas concentration immediately above it (Figs. 9 and 13). With only one exception, low rates of methanogenesis were present at all depths.

Rates of thymidine incorporation into DNA have been used as a quantitative measure of bacterial growth (Moriarty, 1990). Within sediments, however, thymidine can be rapidly metabolized as an energy source in addition to direct assimilation into DNA (Staley and Konopka, 1985; Hollibaugh, 1988; Brittain and Karl, 1990), which makes accurate estimation of growth rates difficult. Hence thymidine

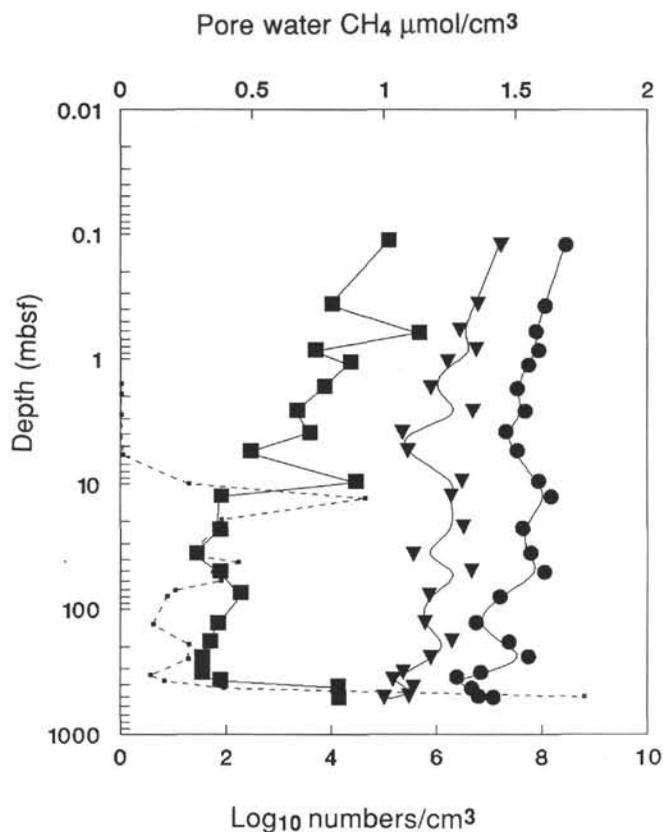


Figure 10. Depth distribution of bacterial populations, total bacteria (●—●), numbers of dividing and divided cells (▼—▼), both averaged for 25-cm depth sections (zero data for dividing cells assigned the value of the detection limit, $10^5/\text{cm}^3$), total anaerobic viable count (■—■) bacteria/cm³, and methane (■—■) μmol/cm³ to 517.90 mbsf. Data for total bacteria and dividing cells are presented with a computer-generated curve of best fit.

incorporation rates are used here only as an index of bacterial growth. The depth profile of thymidine incorporation was very similar to those of other activity measurements, being high near the sediment surface and decreasing rapidly to low rates by 3 mbsf (Fig. 11A). This correlates with the distribution of numbers of dividing bacteria ($r = 0.63$; $p < 0.02$, $n = 15$, 0–80 mbsf).

General

Even in the deepest sediment layers where rates of bacterial activity are very low, there is a good agreement between the profiles of the totally independent measurements of activity (Fig. 11B). For example, the only two positive rates of thymidine incorporation below 5.72 mbsf correspond with either increases in methanogenesis (23.7 mbsf) or methanogenesis and sulfate-reduction (78.9 mbsf). Changes in bacterial activity with sediment depth are reflected in a similar distribution of viable total anaerobic heterotrophic bacteria (Figs. 7, 11A, and 11B), although the increase in the viable bacterial population below 375 mbsf is particularly pronounced compared to increases in activities. This may reflect the lack of an appropriate activity measurement for these deep bacteria.

Sulfate reduction is quantitatively more important than methanogenesis in the top 10 m of sediment (average rate for sulfate reduction and methane production was 5.2 and 0.31 nmol/cm³/d respectively). These results, therefore depict the standard profile within marine sediments with sulfate-reducing bacteria out-competing methanogenic bacteria for common growth substrates. Thus sulfate reduction is both closer to the supply

of organic matter at the sediment surface and quantitatively dominant over methanogenesis. In addition to this competitive relationship there seems, however, to be a more subtle positive interaction.

Threshold concentrations of sulfate at which sulfate reduction can no longer effectively compete against methanogenesis are between 5 and 10 mmol/dm³ for surface sediments (Westrich, 1983). Hence as sulfate reduction decreases from around 18 mmol/dm³ sulfate in these sediments (Fig. 7), some other factor must be limiting, probably organic carbon. The increase in sulfate reduction at 6 mbsf coincides exactly with the first appearance of dissolved methane (Fig. 13), and this small secondary peak in sulfate reduction may be due to the use of methane as an organic substrate. Secondary peaks in sulfate reduction associated with anaerobic methane oxidation have been demonstrated previously (Iversen and Jørgensen, 1985), and anaerobic methane oxidation was also thought to be important in determining the bacterial profiles in the subsurface sediments from Peru (Parkes et al., 1990). The subsequent decrease and continued low levels of sulfate reduction is probably the result of sulfate limitation, as the sulfate concentration decreases rapidly to less than 1 mmol/dm³ by 10 mbsf, and hence methane gas concentrations increase due to continued methanogenesis (Fig. 13).

The increase in the total bacterial population, total anaerobic heterotrophs, bacterial viability (Table 4) and the small peak in sulfate reduction rates (Fig. 7B) below 375 mbsf, all coincide with a large increase in methane (Fig. 9) and other hydrocarbon gases (up to C₅) from geothermal sources (Ingle, Suyehiro, von Breyman, et al., 1990). Although there is also an increase in organic carbon toward the bottom of the core, this increase does not occur until 475 mbsf (Ingle, Suyehiro, von Breyman, et al., 1990), some 50 m below the first increase in heterotrophic bacteria (Figs. 10 and 14). It seems highly likely that the increase in bacterial populations and activities is a response to the rise in methane concentrations, or some other component of the thermogenic gas, as no other chemical change was detected within this depth horizon. Higher in the core there is also an association of elevated bacterial populations with increases in methane gas, which is presumably biogenic in origin (Fig. 10). For example, between 12.6 and 503 mbsf there is a significant ($p < 0.05$, $n = 12$) positive relationship between numbers of total anaerobic viable bacteria and methane gas concentrations. It is unclear what controls changes in rates of methanogenesis below its subsurface maximum, and hence methane concentrations, as there is no significant relationship between methanogenesis and organic carbon (0–425 mbsf, Fig. 14). The bioavailability of the organic carbon, however, may be much more important than its absolute concentration (Parkes et al., 1990). Cyclical palaeoceanographic productivity and bottom-water oxygen changes in the Sea of Japan thought to be intimately related to the cyclical light and dark bands within this sediment (Ingle, Suyehiro, von Breyman, et al., 1990; and others, this volume), are likely to have resulted in fluctuations in the bioavailability of the organic carbon deposited and hence in both the size and persistence of bacterial populations that subsequently developed. Indeed the distribution of dividing cells between light and dark bands (K. Folmi, pers. comm., 1991) is significant ($p < 0.02$), with low numbers of dividing cells being strongly associated with light bands.

SUMMARY

The sediments studied have characteristic bacterial profiles in the top 6 mbsf, with high, but rapidly decreasing numbers of total and dividing bacteria, and populations of different types of viable heterotrophic bacteria, which are highly correlated with potential rates of bacterial activity. Rates of anaerobic sulfate reduction, and [³H]-thymidine incorporation into bacterial DNA are high in the surface sediments and decrease rapidly down to 3 mbsf. Sulfate reduction is quantitatively more important than methanogenesis from CO₂/H₂, which peaks below the maximum in sulfate reduction. Consistent with

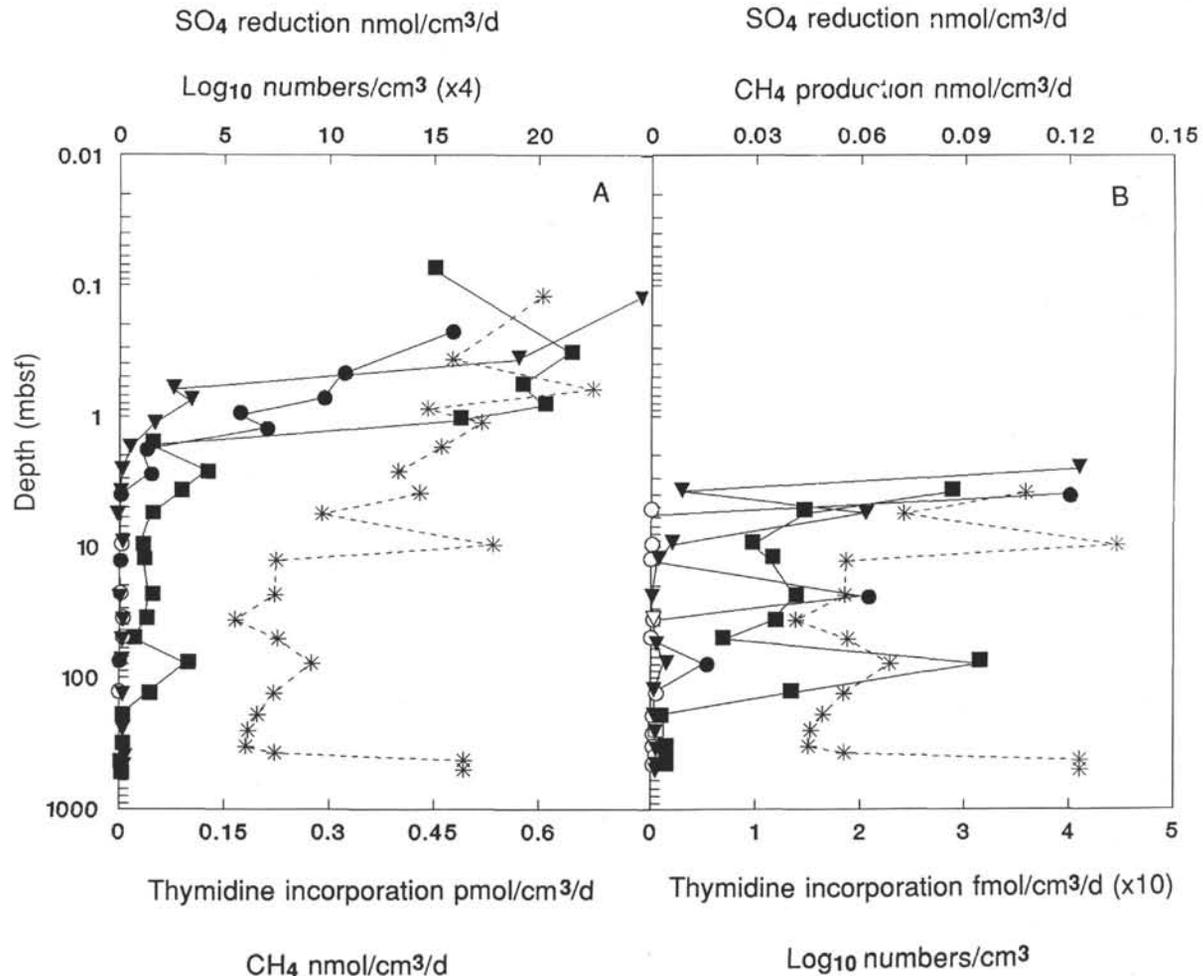


Figure 11. Similarity of the distribution of bacterial activity and viable bacterial populations with sediment depth, sulfate reduction (∇ — ∇) $\text{nmol}/\text{cm}^3/\text{d}$, methanogenesis (\blacksquare — \blacksquare) $\text{nmol}/\text{cm}^3/\text{d}$, thymidine incorporation into DNA (\bullet — \bullet) $\text{pmol}/\text{cm}^3/\text{d}$, and log total anaerobic viable count ($*$ — $*$) $\text{bacteria}/\text{cm}^3 \times 4$ (A) to 517.90 mbsf and (B) between 9.60 and 517.90 mbsf with expanded scales (thymidine incorporation — $\text{fmol}/\text{cm}^3/\text{d} \times 10$; log total anaerobic viable count — $\text{bacteria}/\text{cm}^3$). Open symbols indicate zero-value data.

these activities is the removal of pore-water sulfate, methane gas production and accumulation of reduced sulfide species. Below 6 mbsf the rate of decrease in bacterial populations declines and there is also some distinct increases in both bacterial populations and bacterial activities that continue over considerable depth intervals (Figs. 10 and 11). Most dramatic is the large and significant increase in total heterotrophic bacteria below 375 mbsf, which corresponds to an increase in the total bacterial population, bacterial viability, a small increase in potential rates of sulfate reduction, and the presence of thermogenic methane and other hydrocarbon gases. Bacterial distributions seem to be controlled by the availability of terminal electron acceptors (e.g., sulfate), the bioavailability of organic carbon, which may be related to the dark/light cycles within the sediment, and methane gas, both biological and geothermal. Significant bacterial populations are present even in the deepest samples (518 mbsf, approximate age 4.3 Ma; Ingle, Suye-hiro, von Breyman, et al., 1990) and must have a profound effect on continued diagenetic reactions within the sediment, as a source of energy would be required to maintain these populations. The presence of thermogenic gases may provide an appropriate energy source and hence bacteria may continue to be present and active much deeper than the sediments studied here. These results confirm and extend our previous results of bacterial activity within

the deep sediments of the Peru Margin from Leg 112 (Cragg et al., 1990) and to our knowledge this is the first comprehensive report of the presence of active bacterial populations from sediment surface to depths in excess of 500 mbsf and over 4 m.y. old.

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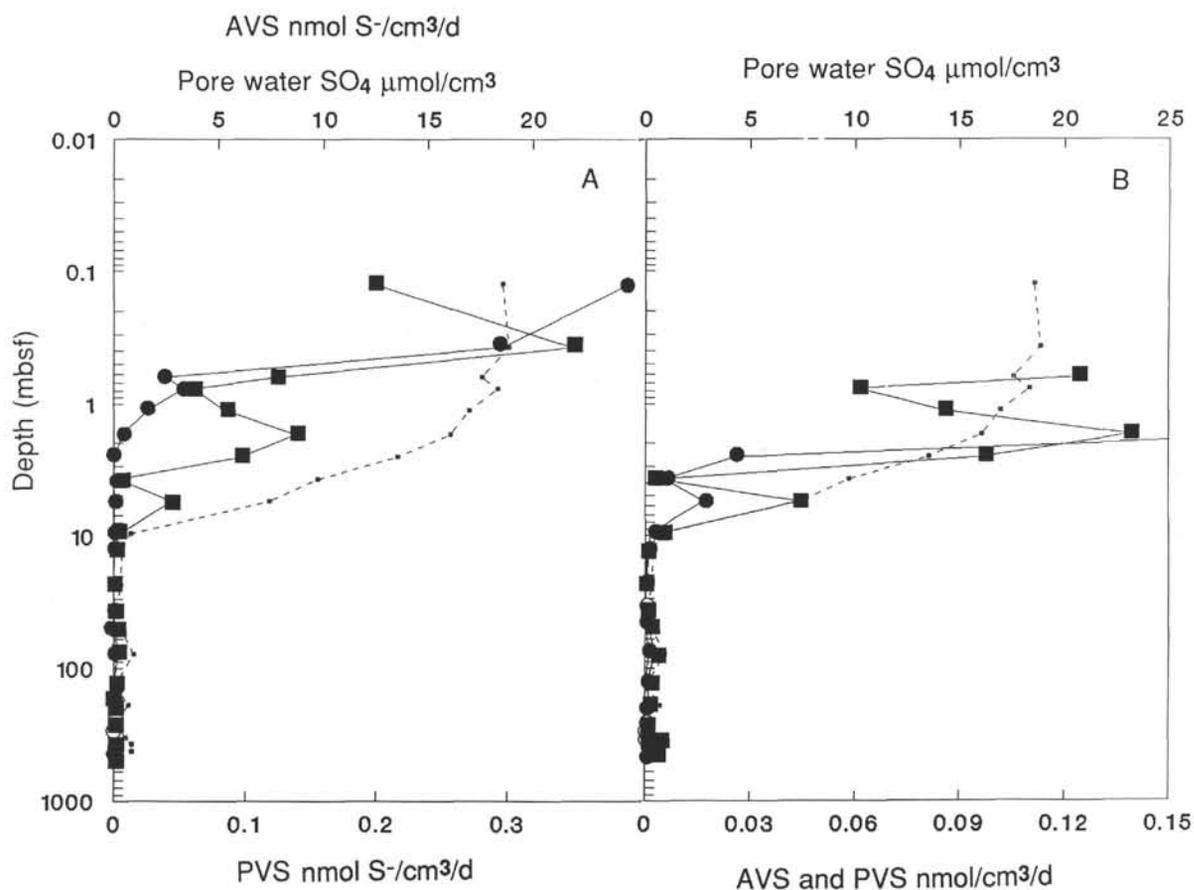


Figure 12. Relationship between different end products of sulfate reduction and removal of sulfate with sediment depth, sulfate reduction to AVS (● — ●) and PVS (■ — ■), $\text{nmol}/\text{cm}^3/\text{d}$, and pore-water sulfate concentrations (• - - - •) $\mu\text{mol}/\text{cm}^3$ (A) to 517.90 mbsf and (B) between 0.625 and 517.90 mbsf with expanded scales. Open symbols indicate zero-value data.

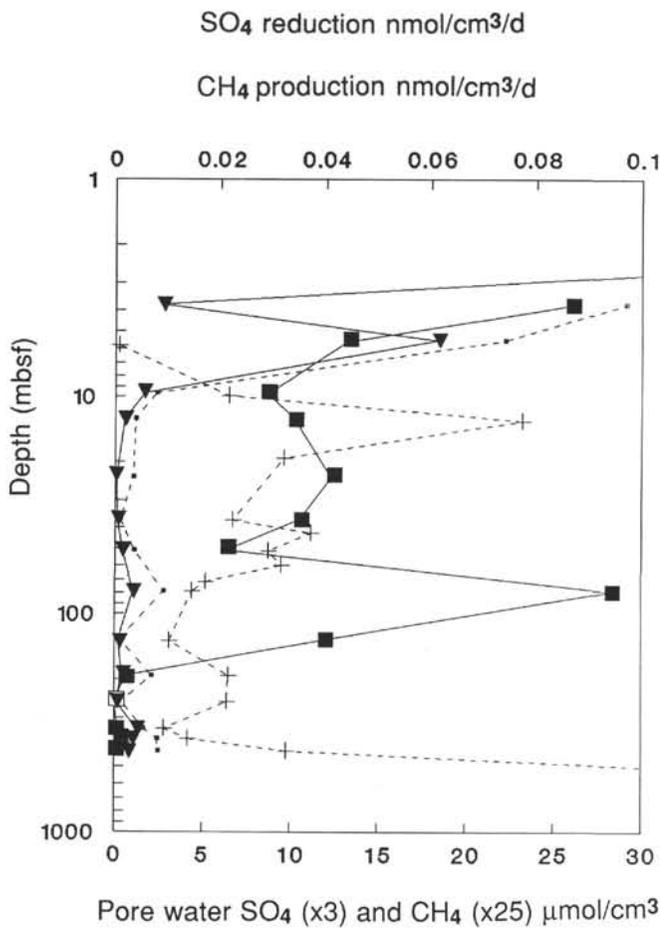


Figure 13. Relationship between potential rates of anaerobic bacterial activity and associated chemical changes within the sediment, sulfate reduction (∇ — ∇) $\text{nmol}/\text{cm}^3/\text{d}$, methanogenesis (\blacksquare — \blacksquare) $\text{nmol}/\text{cm}^3/\text{d}$, pore-water concentrations of sulfate (\bullet — \bullet) $\mu\text{mol}/\text{cm}^3 \times 3$, and methane ($+$ — $+$) $\mu\text{mol}/\text{cm}^3 \times 25$, between 3.77 and 517.90 mbsf. Open symbols indicate zero-value data.

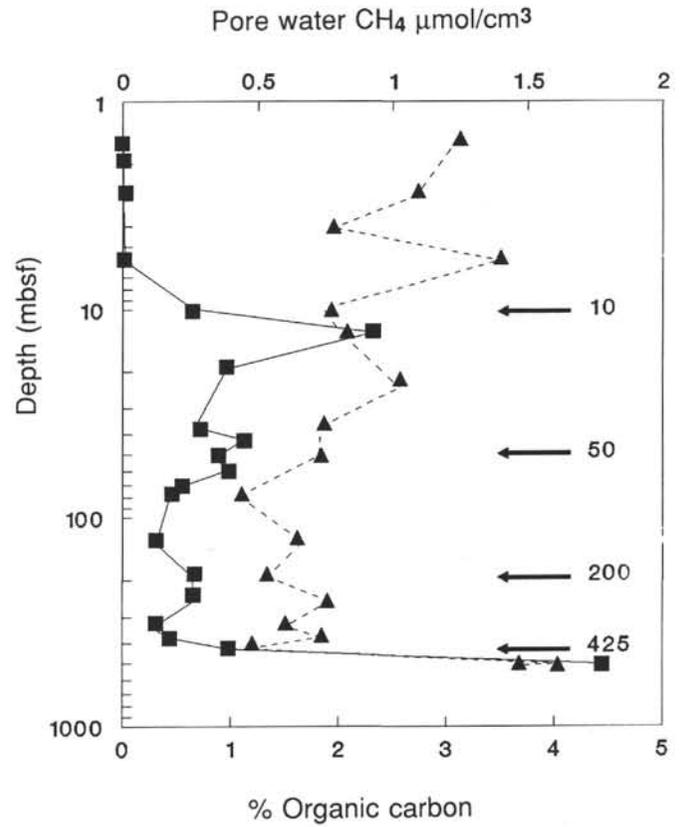


Figure 14. Relationship between increases in bacterial populations, methane and organic carbon with sediment depth, methane (\blacksquare — \blacksquare) $\mu\text{mol}/\text{cm}^3$, and percentage organic carbon (\blacktriangle — \blacktriangle) derived from shipboard data — Ingle, Suyehiro, von Breyman, et al. (1990). Arrowed numbers on the right of the graph indicate depths where bacterial populations increase.