

## 37. ADENOSINE 5'-TRIPHOSPHATE (ATP) AS A PROXY FOR BACTERIA NUMBERS IN DEEP-SEA SEDIMENTS AND CORRELATION WITH GEOCHEMICAL PARAMETERS (SITE 994)<sup>1</sup>

Per Kristian Egeberg<sup>2</sup>

### ABSTRACT

Sediment samples were obtained for detailed Adenosine 5'-Triphosphate (ATP) analysis down to 57.8 m below the seafloor (mbsf). The samples were also analyzed for particle-size distribution, calcium carbonate (CaCO<sub>3</sub>), organic carbon, and total nitrogen. The concentrations of ATP ranged between 360 and 7050 pg g<sup>-1</sup> (dry weight sediment), which agree well with a limited number of direct bacteria counts. Principal component analyses show that 63% of the total variance can be accounted for by the first two principal components. The concentration of ATP (bacterial numbers by inference) is virtually independent of the concentration of sedimentary organic carbon, but correlates with CaCO<sub>3</sub> and coarse particles.

### INTRODUCTION

No group of marine organisms has received as much attention as bacteria, and the advances in microbial ecology are indisputable (Epstein and Rossel, 1995). The importance of bacterial activity within surface sediments is now well established. Recent studies have confirmed the presence of activity of bacteria down to several hundred meters below the seafloor (mbsf) in deep-sea sediments (Cragg et al., 1992, 1995, 1996; Parkes et al., 1990). However, little is known about the factors that govern the detailed distribution of bacteria numbers in deep-sea sediments. One reason for this is that few geochemical laboratories possess the equipment and expertise required for enumeration of bacteria by classical microbiological techniques. Hence, there seems to be a demand for a quick and reliable method for estimation of bacterial biomass.

Measurement of Adenosine 5'-Triphosphate (ATP) has been used as a proxy for total living biomass in soil (e.g., Webster et al., 1984; Ciardi and Nannipieri, 1990; Pangburn et al., 1994) and as an estimate of bacteria biomass in coastal marine sediments (Bancroft et al., 1976; Bulleid, 1978), where living cell numbers typically exceed 10<sup>10</sup> per gram (Alongi, 1992; Schallenberg et al., 1989) and the concentration of ATP reach several thousand nanograms per gram of dry sediment (Karl, 1980). However, to the authors knowledge, ATP measurements have not been used previously as a proxy for total bacterial biomass in deep-sea sediments where the total bacterial number may be 1–5 order of magnitude less (Cragg and Parkes, 1994; Cragg, 1994). All living cells contain ATP, and the use of ATP as a proxy for bacteria numbers in sediments is based on the assumption that other benthic organisms have been removed or are absent. Benthic organisms are seldom found below a few decimeters below the sediment-surface, and ATP is rapidly degraded by enzymes on an organism's death (Pangburn et al., 1994); hence only ATP measurements of surficial sediments may be affected by the presence of benthic organisms.

The objectives of this study were to modify existing methods for ATP analysis to enable analysis of deep-sea sediments, and to examine geochemical factors that may govern the detailed distribution of ATP (bacteria numbers by inference).

### ENVIRONMENTAL SETTING

Ocean Drilling Program (ODP) Site 994 (3147.14°N, 75°32.75°W) is located on the southern flank of Blake Ridge at a water depth of 2798 m. The Blake Ridge is a large drift deposit that extends ~400 km in a southeast direction from the continental rise of the passive margin of the southeastern United States (Fig. 1).

The ridge is topographically expressed as a southeast narrowing tongue of sediment with a crest 1200–2500 m above surrounding abyssal plains (Uchupi, 1968).

Sediment samples studied in this work are Pleistocene (lithostratigraphic Units I and II). The units are characterized by alternating decimeter- to meter-thick interbeds of dark greenish gray nannofossil-rich clay and more carbonate-rich greenish gray nannofossil clay. Distinctive features clearly indicative of contour-current deposition were observed in Units I and II. Evidence for long-distance transport of continental slope sediments by the Western Boundary Undercurrent to Site 994 is clearly provided by the a thin red bed of foraminifer-rich terrigenous mud (Paull, Matsumoto, Wallace, et al., 1996).

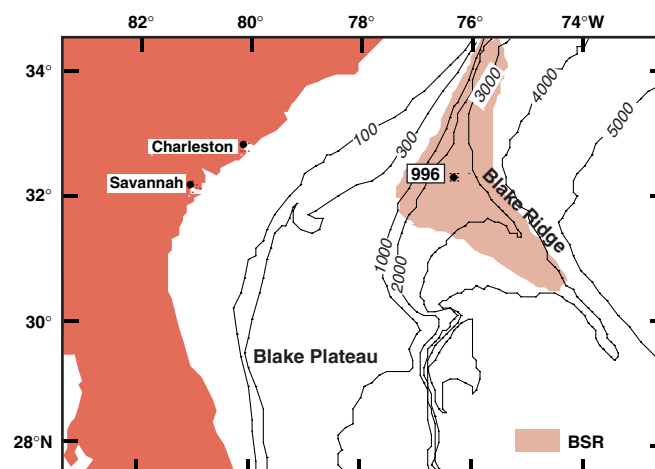


Figure 1. Map showing the location of ODP Site 996. Shaded region characterizes the portion of the Blake Ridge where geophysical evidence indicates the presence of a bottom-simulating reflector (BSR) and, by inference, zones of gas hydrate and underlying free gas (Dillon and Paull, 1983). Depth contours in meters below sea level.

<sup>1</sup>Paull, C.K., Matsumoto, R., Wallace, P.J., and Dillon, W.P. (Eds.), 2000. *Proc. ODP, Sci. Results*, 164: College Station, TX (Ocean Drilling Program).

<sup>2</sup>Agder College, Tordenskjoldst. 65, 4604 Kristiansand, Norway. per.k.egeberg@hia.no

## MATERIALS AND METHODS

### ATP Analysis and Conversion to Bacteria Numbers

Sediment samples for ATP analysis were obtained from the end of core sections immediately after the sections were cut on the ship catwalk. Potentially contaminated sediment was removed first, using a sterile scapula. Then, using a 10-cm<sup>3</sup> plastic cylinder, a minicore sample was taken, sealed in a plastic bag, and dropped into liquid nitrogen. The samples were stored at -20°C and shipped frozen. Samples that had thawed during transport were discarded.

All glassware was cleaned prior to use by soaking for 24 hr in 0.1-M HCl, rinsed in distilled water, and dried at 100°C. All other equipment was stored and cleaned with alcohol. The use of gloves was mandatory. ATP was extracted using the extraction mixture developed by Webster et al. (1984), but omitting Ethylene-dinitrilo-tetraacetate (EDTA) and Zwittergent 3,10. In detail, a 2-cm<sup>3</sup> subcore was taken from the center of the 10-cm<sup>3</sup> minicore by means of a steel tube and extruded into a 15-cm<sup>3</sup> preweighed centrifuge tube. The exact mass of the subcore was determined by weighing. The sample was homogenized with 5 cm<sup>3</sup> of the extraction mixture by means of a hand-held homogenizer. Addition of the strongly acidic reagent mixture led to vigorous CO<sub>2</sub> degassing from the CaCO<sub>3</sub>-rich sediments (see "Results and Discussion" section, this chapter). The centrifuge tubes were placed in an ultrasonic bath for 60 s, an additional volume of 3-cm<sup>3</sup> extraction mixture was added, and the tubes were left covered, but not sealed, for 10 min to degas. The centrifuge tubes were capped and placed in a frame, to prevent further degassing of CO<sub>2</sub> and blowing the caps off. The frame was placed on a rotating wheel, and further extraction continued for 20 min. Following 20 min centrifugation at 4000 rpm, 1 cm<sup>3</sup> of the supernatant was transferred to a test tube containing 1 cm<sup>3</sup> strongly acidic cation exchanger (Dowex C-500), which had been cleansed previously with the extraction mixture. In the original method (Webster et al. 1984) EDTA was added to complex cations that might interfere with the Luciferin-Luciferase system. However, initial tests with the original extraction mixture showed very low luminescence, presumably because the amount of EDTA added was insufficient to sequester the high concentration of Ca<sup>2+</sup> (0.4–1.8 M) in the extracts. Atomic absorption spectroscopic analysis showed that treatment with the cation exchanger removed more than 99% of the Ca<sup>2+</sup>. Hence, EDTA was omitted from the extraction mixture to minimize possible contamination.

The content of ATP in the Ca-free extract was determined based on the reaction with Luciferin-Luciferase (Webster et al., 1984) by the means of a portable Lumac Biocounter M1500, and nucleotide-releasing reagent (NRM) and Luciferin-Luciferase reagent from Lumac. The role of the NRM is similar to the role of Zwittergent 3,10 in the original extraction mixture developed by Webster et al. (1984); hence Zwittergent 3,10 was omitted from the extraction mixture used here. The Lumac reagents have been developed for hygienic tests. During initial tests on sediment samples from Leg 164, these reagents were found to yield to low luminescence for quantitative work. Addition of extra Luciferase prepared by dissolving 5-mg Luciferase (Fluka) in 2-mL deionized water gave satisfactory results. Increasing the concentration of Luciferin had little effect. The acidic Ca-free extract (0.1 cm<sup>3</sup>) was neutralized to pH 7.7 with 0.5 cm<sup>3</sup> of 1 M Hepes (4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid) solution adjusted to pH 8.2 with NaOH(s). The neutralized sample (0.1 cm<sup>3</sup>) was mixed with 0.1 cm<sup>3</sup> NRM, 0.1 cm<sup>3</sup> of the Lumac Luciferin-Luciferase reagent and 0.025 cm<sup>3</sup> of the prepared Luciferase solution in an optical cell and the bioluminescence was measured and integrated for 10 s. Three standard additions of 0.010 cm<sup>3</sup> 2000 pg cm<sup>-3</sup> ATP prepared from Adenosine-5-Triphosphate Disodium Salt hydrate (Fluka) were used to determine the content of ATP in the sample. Recording of the standard-addition curve took about 65 s, during which the bioluminescence decreased by 5%, as determined by repeated analyses of a

sample spiked with 0.030 cm<sup>3</sup> of the ATP standard. Corrections were made for this effect. Repeated (n = 5) analysis of a sample prepared by mixing sediment from several samples (mean ATP 456 pg g<sup>-1</sup>) gave a standard deviation of 54 pg g<sup>-1</sup>. Reagent grade CaCO<sub>3</sub> that had been ignited at 550°C for 6 hr was run as a procedure blank. All measurements were corrected for the blank (51 ± 7 pg g<sup>-1</sup> ATP, n = 6).

Estimates of bacteria cell numbers (cells/gram) from ATP concentration data (pg g<sup>-1</sup>) were obtained by the use of literature data on C/ATP and C/bacteria cell ratios (Karl, 1980; Gerlach, 1978; Findlay et al., 1986). The bacteria cell numbers were further converted to cells per cubic centimeter of wet sediment by means of the porosity data (Table 1) and a mean particle density of 2.71 g cm<sup>-3</sup> (Paull, Matsumoto, Wallace, et al., 1996), for easy comparison with data from direct bacteria enumeration (Wellsbury et al., Chap. 36, this volume).

### Particle Size Distribution

Suspensions of 10 mg sediment in 3 cm<sup>3</sup> 0.5% sodium-pyrophosphate (dispersant) and 1 cm<sup>3</sup> 1% sodium azide (biocide) were left for one week and then dispersed for 60 s in an ultrasonic bath. A subsample was further diluted to an absorbance of 0.7–0.9 with 0.5% sodium-pyrophosphate before the particle-size distribution was determined by means of a HORIBA CAPA-500 centrifugal automatic particle analyzer. Particles in the size range 65–6.5 μm were analyzed in gravitational mode in intervals of 6.5 μm, and particles with diameters less than 6.5 μm were analyzed in centrifugal mode with intervals of 0.5 μm.

### Total Carbon, Nitrogen, and Organic Carbon

Total carbon, nitrogen, and organic carbon were analyzed by the means of a Carlo Erba 1100 elemental analyzer on freeze-dried and homogenized sediments. Inorganic carbon was removed prior to determination of organic carbon by addition of 10-cm<sup>3</sup> 1-M HCl to 0.2 g sediment. The suspensions were centrifuged after 48 hr reaction time, and the particles were washed with 10-cm<sup>3</sup> deionized water, centrifuged, and freeze dried.

### Principal Component Analysis

Calculation of principal components (PCs) were made by means of the software package SIRIUS (Pattern Recognition Systems, High Technology Center in Bergen, N-5007 Bergen, Norway) after normalization of the variables by division by their standard deviation.

## RESULTS AND DISCUSSION

### ATP

The firefly Luciferase assay is affected by pH, ionic strength, metallic ions, and the presence of chromophores in the extracts. Hence, the content of ATP was determined from standard addition curves as recommended by Ciardi and Nannipieri (1990). All standard addition curves had linear regression coefficients higher than 0.995. Although treatment with the cation exchanger and the use of the HEPES buffer reduced some of these interferences, considerable intersample variations in the specific bioluminescence (luminescence/mass of ATP) remained. The slope of the standard addition curves varied by a factor of 2.5 between the extreme cases (Fig. 2A). Despite these variations, the mean relative standard deviation between single point determinations of ATP and determinations of ATP based on the standard addition curves was only 8.6% (maximum relative standard deviation = 28%; Fig. 2B).

The sedimentary concentrations of ATP (360–7050 pg g<sup>-1</sup>, mean 1460, n = 54; Table 1) were in the expected range (see "Introduction"

**Table 1. Porosity, particle diameter, ATP, organic carbon, calcium carbonate, and total nitrogen contents and C/N ratios (mole/mole) of sediments from Holes 994A and 994C.**

Core, section, interval (cm)	Depth (mbsf)	Porosity	Diameter* (µm)	Diameter† (µm)	ATP (pg g <sup>-1</sup> )	Organic carbon (wt%)	CaCO <sub>3</sub> (wt%)	Total nitrogen‡ (wt%)	Total nitrogen** (wt%)	C/N
164-994A-										
1H-1, 138-140	1.38	0.74	0.748	9.87	879	0.30	15.8	0.049	0.053	6.9
1H-2, 0-5	1.55	0.73	0.759	6.88	665	0.84	14.3	0.096	0.105	9.8
1H-2, 138-140	2.88	0.72	0.714	4.53	993	0.92	14.6	0.101	0.112	10.1
1H-3, 138-140	4.38	0.74	0.743	5.85	1128	0.42	18.4	0.067	0.070	7.2
1H-4, 138-140	5.88	0.71	0.746	6.64	356	0.58	19.1	0.080	0.079	8.5
1H-5, 138-140	7.38	0.67	0.838	13.16	2789	0.33	31.6	0.050	0.053	7.4
2H-1, 138-140	9.28	0.64	0.858	6.55	671	0.40	44.2	0.052	0.051	9.0
2H-2, 115-130	10.55	0.60	0.956	7.60	1741	0.28	57.2	0.043	0.044	7.5
2H-2, 138-140	10.78	0.59	0.878	7.82	6568	0.23	48.0	0.040	0.042	6.4
2H-3, 138-140	12.28	0.67	0.931	8.91	597	0.22	42.7	0.037	0.037	7.0
2H-3, 115-130	12.50	0.68	0.888	10.31	1747	0.44	46.4	0.054	0.061	9.0
2H-4, 115-130	13.55	0.66	0.902	13.97	2873	0.94	34.6	0.082	0.096	12.5
2H-4, 138-140	13.78	0.66	0.842	9.69	5452	0.76	28.6	0.085	0.089	10.2
2H-5, 0-5	13.90	0.66	0.824	9.56	1565	0.14	26.5	0.035	0.035	4.7
2H-5, 115-130	15.05	0.68	0.861	5.22	973	0.51	31.6	0.061	0.057	10.2
2H-5, 138-140	15.28	0.69	0.880	6.70	784	0.40	41.1	0.056	0.056	8.3
2H-6, 115-130	16.55	0.72	0.864	9.36	852	1.16	30.1	0.108	0.118	12.1
3H-1, 10-25	17.50	0.71	0.747	5.89	477	0.74	20.8	0.065	0.071	12.7
3H-1, 85-110	18.25	0.70	0.781	6.27	678	0.65	23.1	0.066	0.066	11.5
3H-1, 138-140	18.78	0.69	0.860	6.58	1661	0.62	25.8	0.072	0.068	10.3
3H-2, 0-5	18.90	0.68	0.840	7.10	1498	0.67	25.1	0.065	0.067	11.6
3H-2, 10-25	19.00	0.68	0.803	3.90	836	0.64	24.4	0.069	0.066	11.0
3H-2, 115-130	20.05	0.67	0.823	4.87	1019	0.39	19.8	0.055	0.046	9.1
3H-2, 138-140	20.28	0.67	0.822	5.85	560	0.47	21.6	0.065	0.055	9.1
3H-3, 10-25	20.50	0.66	0.775	4.69	2238	0.60	22.8	0.067	0.065	10.7
3H-3, 45-60	20.85	0.66	0.868	8.45	1363	0.67	23.8	0.071	0.075	10.7
3H-3, 80-90	21.20	0.66	0.802	6.91	5611	0.71	24.7	0.066	0.066	12.6
3H-3, 115-130	21.55	0.66	0.761	5.02	1116	0.46	26.1	0.054	0.055	9.9
3H-3, 138-140	21.78	0.65	0.790	5.12	527	0.75	19.4	0.055	0.055	15.9
3H-4, 0-5	21.90	0.65	0.785	3.99	1035	0.66	20.9	0.066	0.069	11.5
3H-4, 10-25	22.00	0.65	0.793	5.80	248	0.37	17.2	0.054	0.046	8.6
3H-4, 45-60	22.35	0.65	0.781	4.76	391	0.59	18.9	0.052	0.051	13.2
3H-4, 80-95	22.70	0.66	0.898	7.48	364	0.26	28.4	0.027	0.037	9.7
3H-4, 115-130	23.05	0.66	0.873	5.67	268	0.31	26.1	0.032	0.041	9.8
3H-4, 138-140	23.28	0.67	0.749	4.22	377	0.35	23.2	0.045	0.052	8.6
3H-5, 115-130	24.55	0.67	0.809	6.57	7054	0.74	49.0	0.088	0.080	10.3
3H-5, 138-140	24.78	0.67	0.906	7.92	232	0.25	40.1	0.051	0.050	5.8
3H-6, 10-25	25.00	0.67	0.868	5.80	382	0.50	44.2	0.074	0.072	8.1
3H-6, 45-60	25.35	0.67	0.831	10.88	5883	0.53	43.4	0.063	0.064	9.8
3H-6, 80-95	25.70	0.68	0.799	9.80	4570	0.83	31.8	0.105	0.103	9.3
3H-6, 115-130	26.05	0.68	0.746	8.14	484	0.87	32.1	0.103	0.110	9.6
3H-6, 138-140	26.28	0.68	0.754	4.46	353	0.72	37.4	0.090	0.094	9.1
4H-2, 135-140	28.84	0.71	0.832	5.97	4909	0.46	40.5	0.058	0.063	8.8
4H-3, 135-140	30.34	0.70	0.821	6.95	374	0.38	20.2	0.059	0.050	8.1
4H-4, 135-140	31.84	0.67	0.751	3.41	309	0.50	18.4	0.065	0.056	9.7
4H-5, 135-140	33.34	0.64	0.825	10.23	489	0.31	22.9	0.052	0.037	8.3
164-944C-										
5H-1, 145-150	34.35	0.60	0.789	5.98	472	0.36	7.2	0.058	0.047	8.1
164-944A-										
4H-6, 135-140	34.84	0.58	0.927	9.99	241	0.21	10.1	0.053	0.038	5.4
164-944C-										
5H-2, 135-140	35.75	0.58	0.816	5.98	302	0.25	11.1	0.062	0.041	6.0
5H-3, 145-150	37.35	0.61	0.780	7.32	652	0.82	17.5	0.098	0.089	10.3
5H-4, 140-145	38.80	0.66	0.771	3.31	327	1.07	13.9	0.116	0.118	10.6
5H-5, 140-145	40.30	0.68	0.771	4.37	327	0.60	13.1	0.101	0.102	6.9
7H-3, 138-140	56.35	0.70	0.778	3.52	435	1.33	16.6	0.142	0.126	11.6
7H-4, 138-140	57.80	0.70	0.814	6.64	390	1.22	19.1	0.135	0.126	10.9

Notes: \* = number-averaged diameter  $\left( d = \left[ \frac{\sum n_i \cdot d_i^3}{\sum n_i} \right]^{1/3} \right)$ . † = volume-averaged diameter  $\left( d = \frac{\sum n_i \cdot d_i^4}{\sum n_i \cdot d_i^3} \right)$ .  $d_i$  is the mean diameter of size class  $i$  with  $n_i$  number of particles. ‡ = measured on acid-washed sediments and corrected for the loss of CaCO<sub>3</sub>. \*\* = measured directly on freeze-dried sediment.

section, this chapter). The sampled sequence may be divided in two parts based on the ATP results: an upper part (0–30 mbsf) where the concentrations of ATP fluctuated considerably, and a deeper part (30–58 mbsf) with a remarkably constant level of ATP (Fig. 3). The distribution of ATP concentrations in the upper part of the sediment did not show any obvious depth trend.

A rough idea of the number of bacterial cells may be obtained by the use of literature data on C/ATP and C/bacteria cell ratios. Data collected by Karl (1980) show that the C/ATP ratios of microbial cells vary considerably (28–510, Karl [1980], table 3, p. 752). Despite these significant variations, indirect data from several independent field investigations have indicated that the commonly used mean

ratio of 250 is a close approximation of the true in situ C/ATP ratio (Karl, 1980). The minimum and maximum ATP concentrations corresponded to  $10^{-7}$  and  $2 \times 10^{-8}$  cells g<sup>-1</sup> of sediment respectively assuming a conversion factor of  $10^{-14}$  g C cell<sup>-1</sup> (Gerlach, 1978; Findlay et al., 1986). This factor is well within the range of published conversion factors, however it is somewhat conservative and may underestimate bacterial carbon (Schallenberg and Kalf, 1993). Nevertheless, conversion to cells per cubic centimeter resulted in a surprisingly good overall match with the direct bacterial count numbers from Wellsbury et al. (Chap. 36, this volume) (Fig. 4). Much of the variations in the ATP-based bacteria numbers may be attributed to geochemical factors (see “Correlation of ATP and Geochemical Pa-

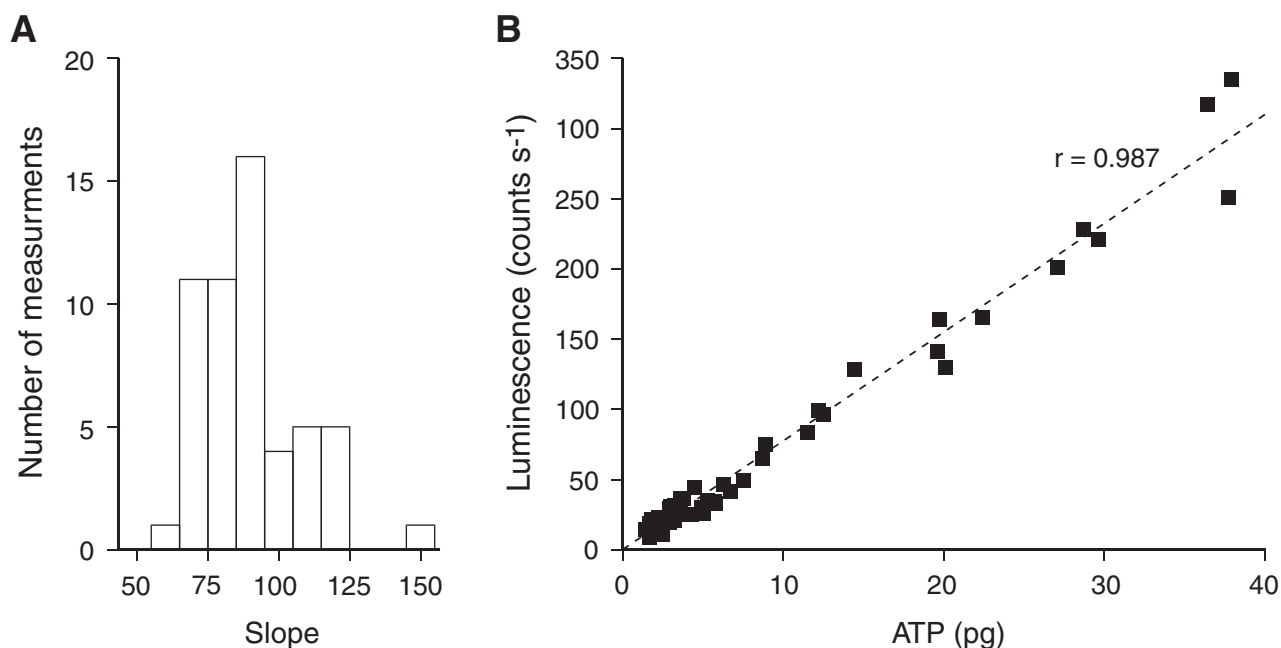


Figure 2. The slopes of the standard-addition curves (A) used for quantification of ATP vary by a factor of 2.5 due to matrix effects. Single-point measurements of luminescence may be used to determine the content of ATP (B) with an average standard deviation of 8.6%, but due to matrix effects the results obtained may differ from the results obtained with the standard-addition method by 50% for samples containing less than 2 pg ATP.

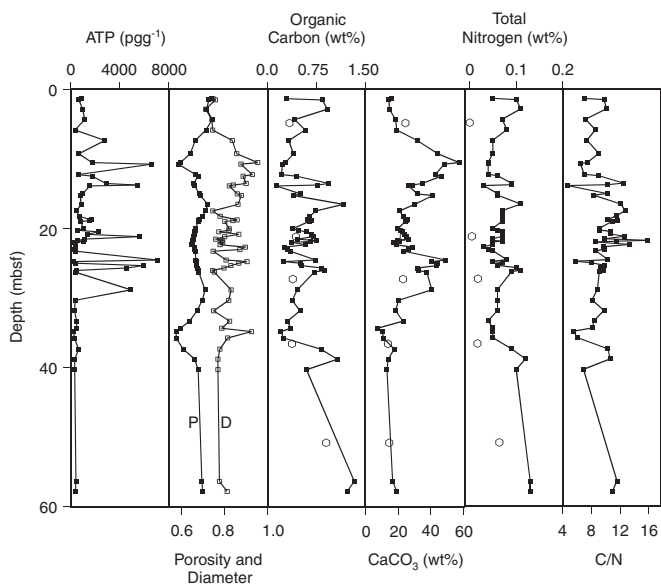


Figure 3. Distribution of ATP ( $\text{pg g}^{-1}$ ), porosity (volume fraction, interpolated from Paull, Matsumoto, Wallace, et al., 1996), number averaged diameter ( $\mu\text{m}$ ), organic carbon (wt%),  $\text{CaCO}_3$  (wt%), total nitrogen (wt%) and C/N ratios in sediments from Site 994. Open circles are results from Paull, Matsumoto, Wallace, et al. (1996).

rameters” section, this chapter); hence the smoother trend of the bacteria numbers based on direct enumeration may be due to the limited numbers of observations.

### Particle-Size Distribution

None of the samples contained particles greater than 65  $\mu\text{m}$ . All samples were characterized by a bimodal particle-size distribution

(Fig. 5), with one peak at 1.8  $\mu\text{m}$  and one peak at 9.8  $\mu\text{m}$ . The positive correlation (see “Correlation of ATP and Geochemical Parameters” section, this chapter) between  $\text{CaCO}_3$  and mean particle diameter suggests that carbonate skeletons contribute to the population of larger particles, whereas the population of smaller particles probably consist mainly of clay minerals. On number basis, the mean particle diameter varied between 0.96 and 0.58  $\mu\text{m}$ , and there is an inverse relation between mean particle diameter and sediment porosity (Fig. 3).

### Total Carbon, Nitrogen, and Organic Carbon

The content of  $\text{CaCO}_3$  and organic C ranged between 7.2%–57.2% and 0.14%–1.33% by weight, respectively (Table 1), and compared favorably with the shipboard determinations (Fig. 3). The analytical program used in this work provides two measurements of total N, one on the bulk sample when analyzing for total C and one on the acid-washed sample when analyzing for organic C. After correcting the latter for the loss of weight caused by dissolution of  $\text{CaCO}_3$  (assuming 100% dissolution of calcium carbonate), the two N determinations yielded very similar results (mean relative standard deviation of 4.5%,  $n = 54$ ; Table 1). The mean concentration of N ranged from 0.035 to 0.126 wt%, whereas the range of shipboard determinations of total N on samples from the same interval is 0.0005–0.064 wt%. The C/N values estimated from the shipboard data (16–790) indicate that the shipboard determinations of N are unreliable for this part of the sediment sequence. The range of C/N values (4.6–16) based on results from this work indicate that the organic matter is of a mixed terrestrial and marine origin. Similar results were noted by Katz (1983) in sediment collected from the Blake Ridge during Deep Sea Drilling Project (DSDP) Leg 76.

### Correlation of ATP and Geochemical Parameters

Principal component analysis is aimed at finding and interpreting hidden, complex, and possibly casually determined, relationships between features in a data set. The method decomposes the variance of a data matrix into uncorrelated (orthogonal, independent) principal

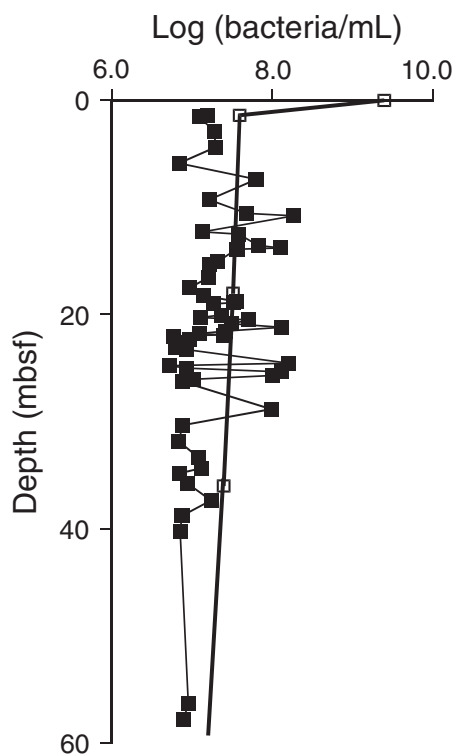


Figure 4. Bacterial numbers derived from the ATP measurements (filled squares) compared with results from direct bacteria enumerations (open squares; Wellsbury et al., Chap. 36, this volume).

components (PCs), which are linear combinations of the original variables. Interpretation of a PC is made possible by examination of the sign and magnitude of the loading in loading plots.

The results (Fig. 6) reveal several interesting features that may help explain factors governing the distribution of ATP (and by inference, bacterial numbers) in the sediments collected from Site 994. The two first PCs explain 63% of the variance, and there is a distinct grouping of the geochemical parameters. The positive correlation of organic carbon, total nitrogen, and porosity on PC1 is expected because most of the nitrogen is organic (adsorbed inorganic nitrogen contribute little), and fine-grained, high-porosity sediments usually contain more organic debris than coarse, low-porosity sediments. The fact that the C/N ratios also correlate with organic carbon suggests that enrichment in organic carbon is caused primarily by terrestrial organic matter (Jasper and Gagosian, 1990; Matson and Brinson, 1990). Distinctive sedimentary features clearly indicative of contour-current deposition of long-distance transported continental slope sediments were observed in lithologic Units I and II (0–160 mbsf; Paull, Matsumoto, Wallace, et al., 1996). Old, reworked terrestrial organic debris is less susceptible to bacterial breakdown, and this is probably why ATP is almost uncorrelated with organic carbon and total nitrogen (Fig. 6). It has been noted previously that organic matter quality may be more important than organic matter quantity (e.g., Parkes et al., 1990), although on a broader scale it is firmly established that organically enriched environments in general contain higher bacterial numbers than organic-poor environments (e.g., Schallenberg and Kalf, 1993).

The largest loading of ATP is found on PC2, which also contains high contributions from  $\text{CaCO}_3$  and particle diameter. One possible interpretation is that the content of  $\text{CaCO}_3$  particles reflects input of marine organic matter that is younger and more available for microbes than terrestrial organic matter, but that the amount of organic carbon associated with the carbonate skeletons is insufficient to im-

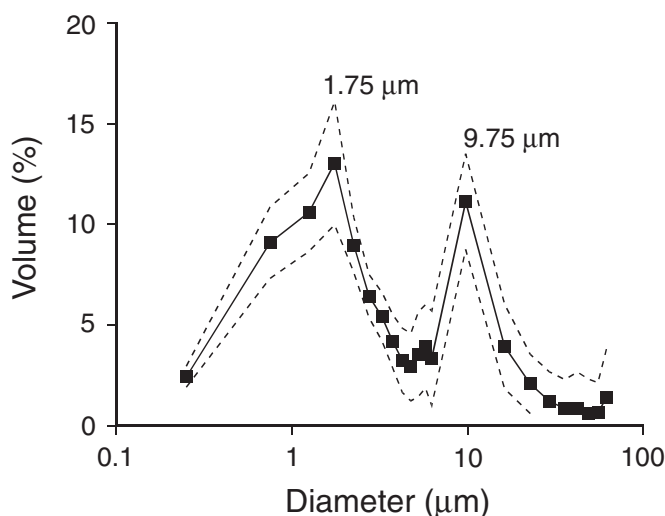


Figure 5. Mean particle-size distribution for the 54 samples (symbols)  $\pm 1$  standard deviation (broken lines). The population of smaller particles is believed to consist primarily of clay minerals, whereas carbonate particles contribute to the population of larger particles.

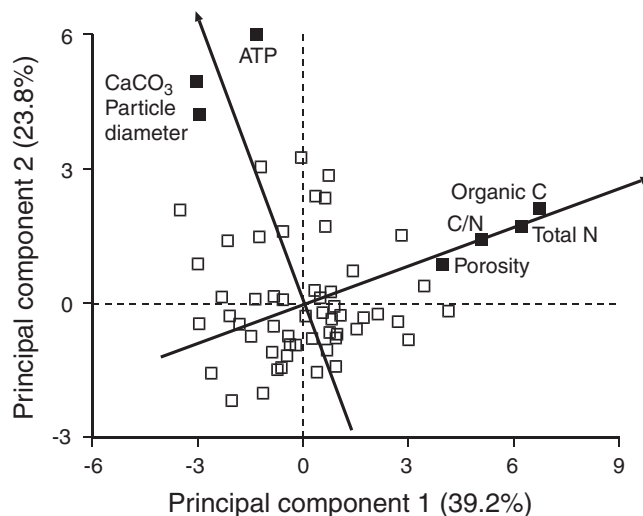


Figure 6. Results of principal component analysis. The positions of the 54 samples and the seven geochemical parameters in the space defined by the two first principal components are indicated by open and filled symbols, respectively. The solid vectors were fitted by eye to illustrate the orthogonality of the two sets of parameters (see "Correlation of ATP and Geochemical Parameters" section, this chapter).

pact the C/N ratio. It may also be speculated that particle size is the primary governing factor. The mean average particle diameter (0.58–0.96  $\mu\text{m}$ ), and hence the size of the pore space, is in the same range as the size of sedimentary bacteria (Schallenberg and Kalf, 1993). Therefore, the number of bacteria may be limited by spatial restrictions. Sediments enriched in coarse carbonate skeletons would promote proliferation of bacteria due to larger pore spaces and the supply of marine organic matter. In shallow-water sediments, bacteria numbers have been found to correlate negatively with mean grain diameter because coarse sediments are depleted in organic matter (Al-Rasheid and Sleight, 1995). However, the mean grain diameter of these sediments were 2–3 orders of magnitude greater than the sediments studied here.

Inclusion of pore-water data on sulfate and methane concentrations did not enhance the amount of variance explained by PC analysis. This should not be surprising because sulfate-reducing bacteria and anaerobic fermentative heterotrophs constitute only a small fraction of total bacteria numbers in deep-sea sediments (e.g., Cragg et al., 1992; Cragg et al., 1996).

## CONCLUSIONS

The conclusions derived in this study are as follows:

1. Classical methods for ATP analysis of soil samples may be modified for analysis of calcareous, deep-sea sediments by removing the large excess of  $\text{Ca}^{2+}$  with a strongly acidic cation exchanger and increasing the amount of Luciferase. The half-life of the luminescence reaction of the 1-M HEPES buffer is sufficiently long to allow recording of standard addition curves.
2. When taking parameters from the literature on C/ATP and C/bacteria cell, the bacteria number derived from the ATP concentration is similar to bacteria numbers obtained by direct enumeration.
3. The two first principal components explain 63% of the total variance when ATP concentration, particle diameter,  $\text{CaCO}_3$ , porosity, organic carbon, total nitrogen, and C/N values are included in a principal component model.
4. ATP is correlated with  $\text{CaCO}_3$  and particle size, and virtually independent of organic carbon, total nitrogen, and C/N values.

## ACKNOWLEDGMENTS

I thank C. Paull, R. Matsumoto, and P. Wallace for organizing the remarkably successful expedition, A. Pimmel and T. Bronk in the JOIDES Resolution chemistry laboratory for their careful work, and P. Wellsbury for letting me use his total bacteria numbers. This work was funded by the Norwegian Research Council.

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**Date of initial receipt: 27 April 1998**

**Date of acceptance: 10 March 1999**

**Ms 164SR-217**