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Effect of Treated-Sewage Contamination upon Bacterial Energy Charge, Adenine Nucleotides, and DNA Content in a Sandy Aquifer on Cape Cod

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Changes in adenylate energy charge (EC_A) and in total adenine nucleotides (A_T) and DNA content (both normalized to the abundance of free-living, groundwater bacteria) in response to carbon loading were determined for a laboratory-grown culture and for a contaminated aquifer. The latter study involved a 3-km-long transect through a contaminant plume resulting from continued on-land discharge of secondary sewage to a shallow, sandy aquifer on Cape Cod, Mass. With the exception of the most contaminated groundwater immediately downgradient from the contaminant source, DNA and adenylate levels correlated strongly with bacterial abundance and decreased exponentially with increasing distance downgradient. EC_As (0.53 to 0.60) and the ratios of ATP to DNA (0.001 to 0.003) were consistently low, suggesting that the unattached bacteria in this groundwater study are metabolically stressed, despite any eutrophication that might have occurred. Elevated EC_As (up to 0.74) were observed in glucose-amended groundwater, confirming that the metabolic state of this microbial community could be altered. In general, per-bacterium DNA and ATP contents were approximately twofold higher in the plume than in surrounding groundwater, although EC_A and per-bacterium levels of A_T differed little in the plume and the surrounding uncontaminated groundwater. However, per-bacterium levels of DNA and A_T varied six- and threefold, respectively, during a 6-h period of decreasing growth rate for an unidentified pseudomonad isolated from contaminated groundwater and grown in batch culture. These data suggest that the DNA content of groundwater bacteria may be more sensitive than their A_T to the degree of carbon loading, which may have significant ramifications in the use of nucleic acids and adenine nucleotides for estimating the metabolic status of bacterial communities within more highly contaminated aquifers.

Increasing attention is focusing on bacterial adenylates and nucleic acids in contaminated groundwater environments. This is due, in part, to the role of bacterial growth and activity in the fate of organic aquifer contaminants. Adenylate energy charge (EC_A), which is defined as (ATP + 1/2 ADP)/(ATP + ADP + AMP) (17), and ratios of ATP to DNA (13) have been used for comparative purposes to help assess the metabolic status of bacterial populations in surface water environments and laboratory cultures. Although the ATP contents in groundwater habitats have been reported (14, 35), little is known about intracellular ratios of AMP, ADP, and ATP in subsurface environments and how these ratios are affected by the presence of organic contaminants. Also, it is not known how subsurface contamination might affect the ratio of bacterial ATP to DNA.

The purpose of our investigation was to examine how contaminants deriving from the input of treated sewage affect EC_A, the ratio of ATP to DNA, and levels of total adenine nucleotides (A_T) and DNA (both normalized to bacterial abundance) for a community of free-living bacteria within a carbon-limited, sandy aquifer. The study involved an analysis of changes in per-bacterium levels and ratios of AMP, ADP, ATP, and DNA for groundwater samples collected from wells located along the length of a 4-km contaminant plume. Values obtained from groundwater samples were compared with those reported for other aquatic habitats and for laboratory cultures. Alterations in EC_As and in bacterial DNA and adenine nucleotide contents in response to amendments of organic nutrients were also assessed in the laboratory. Our findings suggest that A_T, EC_A, and the ratio of ATP to DNA may be useful in studies involving microbial activity in contaminated aquifers, although changes in the ratios and per-cell levels of these nucleic acids and adenine nucleotides within the carbon-limited zones of the contaminant plume were slight.

MATERIALS AND METHODS

Study site. The study site was located on Cape Cod, Mass., where there is an unconfined, sand-and-gravel aquifer (Fig. 1). Disposal of secondarily treated sewage onto rapid-infiltration beds from a sewage treatment plant since 1936 has resulted in an oxygen-depleted plume of contaminated groundwater more than 4,000 m long, 1,000 m wide, and 23 m thick. The contaminated portion of the aquifer is the site of an ongoing multidisciplinary research program administered by the U.S. Geological Survey. Detailed descriptions of the hydrology and geochemistry at this site have been published previously (2, 11, 21, 33).

Sample collection. Groundwater for bacterial enumeration and adenylate and DNA analyses was collected along a longitudinal transect through the suboxic core of the contaminant plume (Fig. 1) in July and October 1988. Sampling wells were constructed of either 5- or 3.2-cm-diameter polyvinyl chloride pipe, each with a 0.6- to 0.9-m-long slotted screen. Prior to sample collection, wells were evacuated until specific conductance and temperature had stabilized. Samples were collected from the 5-cm-diameter wells with a submersible stainless steel pump (model SP81; Keck

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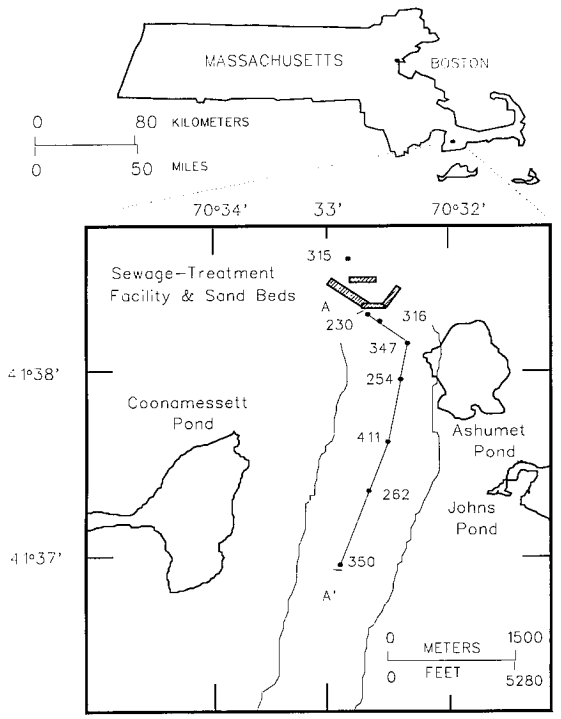

FIG. 1. Location of study site. The sampled transect through the contaminant plume is indicated by the line from A to A', plume boundaries are denoted by the irregular lines on either side of the transect line, and observation wells are denoted by numbered dots.

Geophysical Instruments, Inc., Okemos, Mich.) connected to Teflon tubing. Samples from the 3.2-cm-diameter well were taken with a suction lift centrifugal pump. Samples used for bacterial enumeration were collected in acid-washed 100-ml polyethylene bottles, preserved with formalin (2 to 3% final concentration), and stored in the dark at 4°C. Samples for adenylate and DNA analyses were placed in acid-washed, 20-liter polyethylene carboys and 0.5-liter polypropylene bottles, respectively. Replicate samples were obtained from each location and kept on ice until processed.

DNA analyses. Samples for DNA analyses were filtered (<390 mm Hg [ca. 5,200 kPa]) by using a multiport sampling manifold with polycarbonate membrane filters (25-mm diameter, 0.2-μm pore size; Nuclepore Corp., Pleasanton, Calif.). It was assumed that the microbial community collected in this manner consisted almost exclusively of bacteria, since bacteriophages would pass through the filter and the aquifer protozoa appear to be largely surface associated (19a). Filters were placed in sterilized borosilicate scintillation vials with 2.0 ml of STE (10 mM Tris, 100 mM NaCl, 1 mM EDTA [pH 7.5]) buffer and kept at −20°C until analysis. In most cases, analyses were completed within 24 h. Analysis for microbial DNA was in accordance with previously reported methods for fluorometrically determining DNA content by using Hoechst 33258 fluorochrome (7, 26). The following modifications were made: 1 mg of lysozyme (Sigma Chemical, St. Louis, Mo.) · ml^{-1} in a pH-buffered (pH 7.5) solution (10 mM Tris HCl, 67.5 mM EDTA) was used to lyse the bacteria (2 h, 25°C); the suspension was then subjected to treatment with 0.4% Triton X-100 (1 to 2 h, 1 to 2°C), probe sonication (15 to 30 s, 55 W), dilution with 8 ml of STE buffer, and centrifugation (10 min, 12,000 × *g*, 3°C) Centrifuge tubes were placed on ice, and 2.0-ml replicates were removed for fluorometric DNA determination (26). The extent of bacterial lysis was assessed by fluorescence microscopy. DNA recovery was assessed by internal standards (calf thymus I DNA; Sigma Chemical). Corrections were made for differences in G-C contents of the groundwater bacteria (estimated at 50%) and of the calf thymus standard (43%), for nonspecific fluorescence levels after treatment with DNase, and for DNA sorption onto polycarbonate membrane filters.

Adenylate analyses. The adenylate assay employed was essentially the same as that reported by Walker et al. (34) with minor modifications. Groundwater samples (2 to 15 liters) were filtered with polysulfone filters (142-mm diameter, 0.2-μm pore size; GA-8S; Gelman Sciences, Inc., Ann Arbor, Mich.). Large volumes (3 to 15 liters) of groundwater had to be filtered to obtain enough microbial biomass for a reproducible analytical signal. Because a deleterious effect attributed to vacuum filtration (15, 19, 32) had been previously reported, we examined the relationship between filtration and EC_A for this site. EC_A was measured for eight different volumes (3 to 16 liters) of groundwater collected from site 230. EC_As were very similar (0.60 ± 0.03) for all eight samples and showed no relationship to the volume filtered.

Filters were placed in petri dishes and extracted with 10 ml of ice-cold 0.15 M H_3PO_4. Extracts were then transferred to scintillation vials, adjusted to pH 3.5 with NaOH, and frozen. Selected filter blanks and extracts were spiked with a mixed internal standard (1 mM [each] AMP, ADP, and ATP) to monitor individual compound recovery (90% ± 16%, 91% ± 6%, and 94% ± 6%, respectively [n = 15]). The extracts were lyophilized, derivatized (2 ml of 0.5 M chloroacetaldehyde for 3 h at 60°C), lyophilized again, and dissolved in high-performance liquid chromatography (HPLC)-grade water prior to analysis. Analysis of the 1-N^6-etheno derivatives was done on a Perkin Elmer liquid chromatograph equipped with a Whatman Partisil 10 (C-18) 25-cm analytical column. The mobile phase was 8% methanol–92% phosphate buffer (0.15 M; pH 6.0). Detection was accomplished with a Perkin Elmer LS-4 fluorescence spectrometer operating at 290-nm excitation and 440-nm emission wavelengths. Compounds were identified by comparison of the retention times with authentic derivatized standards (Sigma Chemical). The values obtained for DNA content and A_T were normalized to bacterial abundances, which were determined by the acridine orange direct count method of Hobbie et al. (12).

Glucose addition experiment. The effect of glucose addition on the EC_A was examined in a 48-h incubation experiment. Samples (20 liters) of the contaminant plume were taken in polyethylene Cubitainers from a well 25 m downgradient of the sewage treatment plant (site 230). Half of the samples were amended with 10 mM glucose in order to simulate the effects of high organic loading that may occur as a result of substantive contamination of an aquifer. This also served as a check on the degree to which the EC_A of groundwater bacteria could be artificially altered, thereby ensuring the validity of the method. The samples were incubated at ambient air temperature (18°C) for 48 h. Subsamples were taken at 0, 24, and 48 h and processed for adenylate content.

Growth experiments. Batch experiments were performed to examine variations in DNA content, A_T, and EC_A with growth rate, using an unidentified groundwater isolate from a well-water sample collected 0.45 km downgradient from the sewage treatment plant. Cultures were grown in nutrient

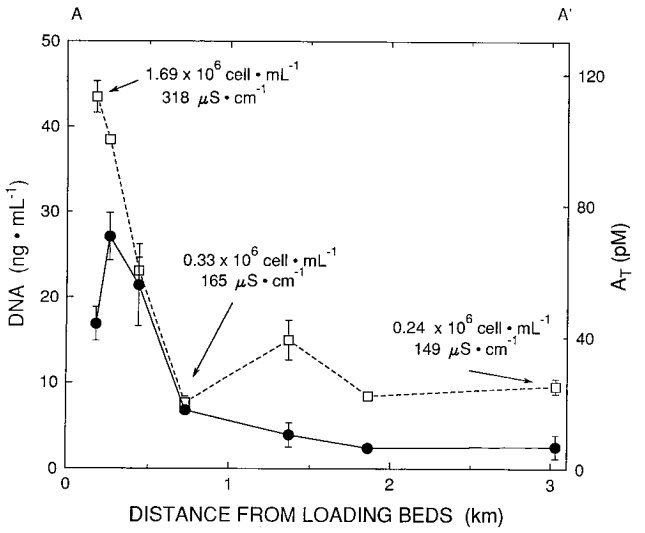

FIG. 2. Bacterial DNA content (●) and A_T (□) in groundwater as a function of distance downgradient from the treated-sewage discharge area. Values are the means for at least three replicate samples ± 1 standard error about the mean. In-graph numbers are the abundances of free-living bacteria and the specific conductivities of the groundwater.

medium (2.5 g of peptone and 1.5 g of beef extract · liter^{-1} [pH 6.8]). Samples for DNA and A_T analyses were taken at the end of log-phase growth (approximately 15 h after inoculation). Additional samples were taken at 17, 18, 19, 22, and 42 h after inoculation, during the period of declining growth rate. Samples for DNA analyses (3 ml) were filtered with polycarbonate membrane filters, purified by hydroxyapatite liquid chromatography (3), and analyzed by the fluorometric procedure described above. Samples for adenylate analyses (5 ml) were filtered with membrane filters (47-mm diameter, 0.2-μm pore size; GA-8S; Gelman Sciences), extracted, and analyzed by liquid chromatography as described above.

RESULTS

Field studies. In general, population density, A_T, and DNA content for the free-living bacterial community decreased with increasing distance downgradient from the sewage treatment plant (Fig. 2). Although A_T for bacteria along the sampled transect through the contaminant plume ranged from 2.48×10^{-20} to 7.89×10^{-20} mol · cell^{-1}, no statistical difference between average values for contaminated and uncontaminated groundwater was found. However, per-bacterium levels of ATP and DNA appeared to be almost twice as high in the contaminant plume as in an uncontaminated zone in the aquifer, but only one sample was collected for determination of the average bacterial ATP content for the free-living bacteria in the uncontaminated aquifer. The highest bacterial DNA content was found 0.73 km downgradient (20.7 fg of DNA · cell^{-1}), but values as low as 6.46 fg of DNA · cell^{-1} were observed elsewhere in the plume. The EC_A was low (0.56 to 0.60) and uniform throughout the contaminant plume transect; no significant differences from those values observed in uncontaminated groundwater were found (Table 1). Similarly, ratios of ATP to DNA (calculated on a weight-per-weight basis) in contaminated and uncontaminated zones of the aquifer were relatively low (0.001 to 0.003).

TABLE 1. EC_A, per-bacterium quantities of DNA, A_T, and ATP content estimated for contaminated and pristine groundwater at the Cape Cod, Mass., study site

Groundwater type[a]	EC_A	DNA content (fg · cell^{-1})	A_T (10^{-20} mol · cell^{-1})	ATP content (10^{-20} mol · cell^{-1})
Uncontaminated	0.56[b]	5.79[c]	6.82[b]	1.64[c]
Contaminated[d]	0.58 ± 0.01	10.3 ± 1.84	6.02 ± 1.04	2.70 ± 0.45

[a] Uncontaminated: specific conductivity, <80 μS; DOC, <0.2 mg/liter; dissolved oxygen concentration, >3 μM. Contaminated: specific conductivity, >80 μS; DOC, 1 to 4 mg/liter; dissolved oxygen concentration, <3 μM.
[b] Values from one site outside contaminant plume.
[c] Average from two sites outside contaminant plume.
[d] All values are averages of measurements ± standard error from seven sites located 0.1 to 3.03 km from the loading beds.

The degrees of association between bacterial numbers, DNA content, ATP content, and A_T for the contaminant plume are given in Table 2. In general, levels of ATP, A_T, and DNA correlated strongly with the abundances of free-living bacteria. The strongest correlation was observed between DNA content and bacterial levels ($r = 0.90$; $P < 0.001$; $n = 9$). Except in the highly contaminated groundwater immediately downgradient from the loading beds, the patterns of spatial variability of bacteria, A_T, and DNA content along the longitudinal transect through the oxygen-depleted core of the plume were similar. Although the maximal A_T (113 pM) and DNA content (27.1 ng · ml^{-1}) were not found in the same sample, significant correlations between ATP content, DNA content, and A_T along the transect through the plume were observed. The weakest correlation observed was between levels of DNA and ATP ($r = 0.75$; $P < 0.05$; $n = 7$).

Laboratory studies. The results of an experiment designed to assess the responses in physiological status of free-living groundwater bacteria to substantive organic loading are depicted in Fig. 3. During a 48-h experiment, the EC_A for bacteria collected from the contaminant plume and amended with glucose exhibited a 20% increase, while the EC_A for bacteria in unamended samples increased only 6% over the same interval. The highest observed EC_A in glucose-amended groundwater was 0.74. In the glucose-amended samples, the increase in EC_A was accompanied by a fivefold increase in A_T; biomass changes over 48 h in unamended samples were less than twofold. In all cases, the volumes of samples filtered in the amendment experiment were comparable to those filtered in the field (transect) study.

Variations in the average adenylate and DNA contents of

TABLE 2. Correlation coefficient (r) and level of significance for linear regressions between abundance of free-living bacteria, DNA, ATP, and A_T contents for well-water samples collected along a 3-km transect through the contaminant plume at the Cape Cod, Mass., study site

Parameter	Correlation coefficient (level of significance) for:		
	A_T	DNA content	ATP content
Bacterial abundance	0.79 (<0.03)	0.90 (<0.001)	0.76 (<0.05)
ATP content	0.89 (<0.007)	0.75 (<0.05)	
DNA content	0.82 (<0.02)		

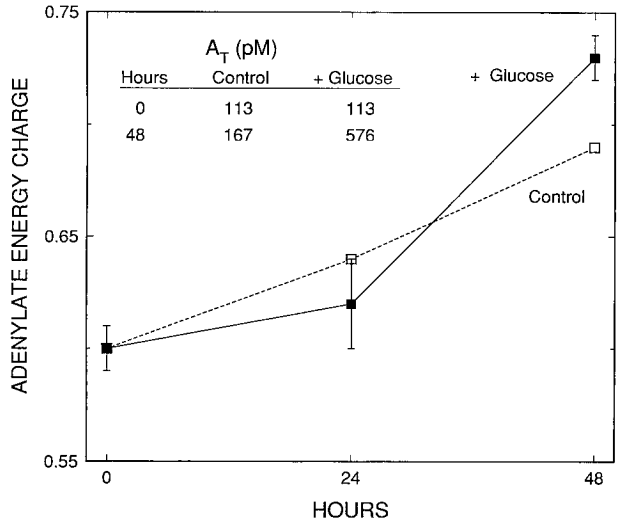

FIG. 3. Temporal variations in EC_A for groundwater samples collected from the contaminant plume on Cape Cod, Mass., and incubated with and without 10 mM (final concentration) glucose. Values are the means for three replicates ± 1 standard error. The tabular inset shows corresponding temporal changes in A_T.

a laboratory-grown groundwater isolate during late log and early stationary phases of a batch growth experiment are depicted as a function of time in Fig. 4. Per-bacterium levels of DNA and A_T varied six- and threefold, respectively, during a 6-h, 10-fold decline in growth rate; bacterial DNA content decreased from 11.3 to 2.02 fg · $cell^{-1}$, and A_T decreased from 153 × 10^{-20} mol · $cell^{-1}$ to 56 × 10^{-20} mol · $cell^{-1}$. The pseudomonads, isolated from an oligotrophic groundwater population, required about 10 h after inoculation to attain a 10-fold increase in abundance in nutrient-rich medium. There was a strong correlation between bacterial growth rate and bacterial A_T ($r = 0.90$ at $P < 0.02$) and between growth rate and per-bacterium levels of DNA ($r = 0.95$ at $P < 0.005$). In a similar experiment, the EC_A decreased from 0.81 to 0.75 from exponential to stationary phase.

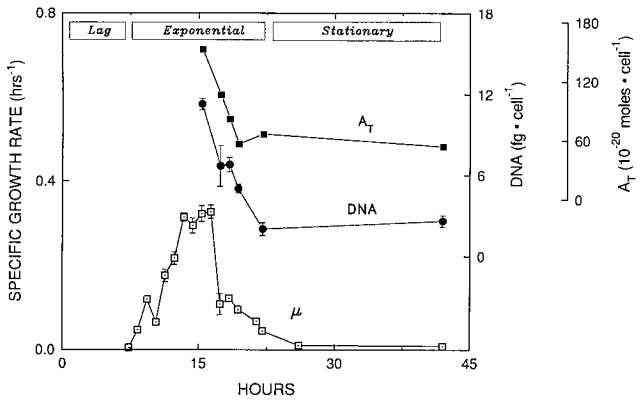

FIG. 4. Temporal changes in average bacterial growth rate (μ), DNA content, and A_T for a pseudomonad isolate during a batch growth experiment involving high-nutrient media. The isolate was obtained from contaminated groundwater on Cape Cod, Mass. Measurements are the means for triplicate samples ± 1 standard error.

DISCUSSION

EC_A. EC_As of ~0.8 to 0.9 are indicative of active microbial growth, whereas values of ~0.6 and ~0.5 are more indicative of stationary phase and senescence, respectively (1, 4). In spite of organic contaminant loading caused by continued on-land discharge of treated sewage, EC_As for groundwater bacteria sampled throughout the transect of the contaminant plume were uniformly low and showed no relationship to the distance from the contaminant source. Also, there appeared to be no difference between EC_As within the contaminant plume and in uncontaminated groundwater (Table 1). EC_As from seven locations in the contaminant plume were similar (0.58 ± 0.01 [Table 1]) and comparable to those reported for cultures that are either undergoing carbon starvation or in stationary phase (4). This suggests that free-living bacteria throughout the contaminant plume exist under conditions of metabolic stress. However, as noted by Karl (17), an EC_A for an environmental sample is a mass-weighted mean of the total microbial community, and environmental EC_As indicative of metabolic stress could result from the presence of stressed or senescent populations along with active populations.

EC_As that suggest metabolic stress and even significant loss of viability have been reported in several studies in which adenine nucleotides were extracted from environmental samples. Souza-Lima and Romano (31) reported EC_As as low as 0.48 for microplankton from the surface microlayer of the ocean. Riemann and Wium-Andersen (27) found that EC_As remained between 0.6 and 0.7 during the development and crash of a spring diatom bloom in a eutrophic lake. Low EC_As (0.22 to 0.32) which did not vary significantly with the season have been reported for streambed sediments (16), and values ranging from 0.19 to 0.49 were found in depth profiles of continental shelf sediments (5). Kinniment and Wimpenny (20) found that EC_As in laboratory-grown biofilms never exceeded 0.6 and were as low as 0.22. It is unknown what the normal or expected EC_A would be for a given environment, but the EC_As we report here for free-living bacteria in a contaminated sand-and-gravel aquifer, while indicative of metabolic stress, are certainly within the range of values that have been found in other environments and may be representative of nutrient-limited groundwater environments.

Our data suggest that the low EC_As we observed for the aquifer are not a procedural artifact. Filtration-induced stress leading to disproportionate decreases in intracellular ATP levels relative to A_T (19) appears to be minimal at the Cape Cod site, since increasing the volume filtered from 3 to 16 liters had no effect upon the EC_A. There is a possibility that such an effect can occur for sample volumes smaller than the 3 liters needed to ensure high enough concentrations of adenylates to accurately determine EC_A. However, this effect was not observed for phytoplankton with growth rates typical of oligotrophic open oceans (32).

As an additional check, the sensitivity of EC_A in groundwater samples to substantive changes in available nutrient levels was also assessed (Fig. 3). Glucose-amended samples exhibited a relatively large increase in EC_A (20%) and a fivefold increase in biomass as measured by A_T. However, the EC_A increased only after 24 h, indicating that microbial populations from this site adapted slowly to changes in organic loading and carbon type. Lag periods of at least 5 to 10 h before uptake of added thymidine were reported by Harvey and George (9) for groundwater samples collected from the contaminant plume, and several-hour lag times are

TABLE 3. Comparison of DNA content, ATP content, and ATP/DNA ratios for Cape Cod groundwater, other aquatic environments, and pure culture studies

Sample source or isolate	AODC[a] ($10^6 \cdot ml^{-1}$)	DNA content ($fg \cdot cell^{-1}$)	ATP content (10^{-20} $mol \cdot cell^{-1}$)	ATP/DNA ratio	Reference[b]
Subsurface					
Cape Cod	0.5–4.0	2.0–21	1.1–4.2	0.001–0.003	This study
California	0.4–0.5	2.8–15	NA[c]	NA	23
Denmark	NA	NA	4.3–108	0.002–0.055[d]	14
Oklahoma	1.2–49[e]	NA	0.03–35	<0.001–0.018[d]	35
Potomac River	1.65	10.0[f]	NA	NA	24
Atlantic Ocean	0.8–1.3	5.7	NA	NA	25
Isolates					
Soil isolates	NA	NA	26–66	0.001–0.033[d]	22
Vibrio proteolytica	250–2,500	5.0–10.5	389–507[g]	0.18–0.26	13
Pseudomonad	500–1,300	2.0–11.3	75–106	0.06–0.17	This study

[a] AODC, acridine orange direct count.
[b] Source of data for each sample.
[c] NA, not available.
[d] AODC converted to DNA concentration (conversion factor of 10 fg of DNA · $cell^{-1}$).
[e] Number of cells (10^6) per gram of sediment.
[f] Estimated value.
[g] ATP content per cell converted to ATP/DNA ratio (10 fg of DNA · $cell^{-1}$)

frequently noted for carbon amendments during denitrification activity assays with groundwater and aquifer sediments (28). Therefore, it appears that lags which precede uptake of added organic compounds may be a common feature for bacterial communities within the plume. The modest increases in EC_A for unamended samples may have resulted from increased temperature and dissolved oxygen concentration relative to in situ conditions (10 to 14°C; <0.5 μM O_2) (8). All samples were incubated at room temperature without provisions to maintain in situ levels of O_2. Results of the glucose amendment experiment demonstrated that EC_As greater than 0.6 could be obtained for groundwater bacteria from the contaminant plume, even when the bacteria were concentrated by filtration from large sample volumes (up to 9 liters).

ATP/DNA ratios. Ratios of ATP to DNA can also yield important information relating to the metabolic status of a bacterial population. Because the amount of ATP per cell has been shown to increase to a maximum during log-phase growth for some organisms, while the amount of DNA per cell appears to remain relatively constant, Jeffrey and Paul (13) proposed that the ATP/DNA ratio could be used as an index of metabolic activity. Using data from studies in which both ATP content and bacterial abundance were measured, as well as an established factor of conversion between bacterial abundance and DNA content of 10 fg of DNA · $cell^{-1}$ (26), we calculated ATP/DNA ratios for several different aquatic habitats (Table 3). In general, the ratios calculated in this manner for environmental samples were substantially lower than those reported for both free-living and attached cells of a laboratory-grown estuarine isolate (13) and for our groundwater isolate grown in batch culture (Table 3). In contrast, the calculated ATP/DNA ratios for the other groundwater habitats agreed reasonably well with what we observed for the indigenous bacterial populations within the contaminant plume (0.001 to 0.003). However, the fact that a groundwater bacterium's DNA content may not be constant in situations of organic loading (Fig. 4) may need to be taken into consideration in applying ATP/DNA ratios to groundwater environments that are more highly contaminated.

The low metabolic state suggested by EC_As and ATP/DNA ratios for groundwater bacteria is consistent with previous results involving uptake of radiolabeled organic compounds (9, 10, 29). This suggests that microbial activity in the contaminant plume is low relative to activity in many surface water habitats, in spite of the elevated levels (up to 4 mg/liter) of dissolved organic carbon (DOC) within the plume. Although Harvey and Barber (8) have observed that about two-thirds of the spatial variability in the abundance of free-living bacteria in the plume may be statistically explained simply by the spatial distribution of DOC, much of the DOC consists of highly refractory compounds (2, 33). Rates of denitrification, a predominant electron-accepting process within the plume, are also low (30) and limited by carbon availability (28). Furthermore, the average generation time for free-living bacteria in much of the contaminant plume appears to be on the order of several days to weeks (9), and heterotrophic activity (measured by glucose uptake) is low (10, 29).

DNA, ATP, and A_T. Per-bacterium levels of ATP in contaminated and uncontaminated waters were not statistically different ($t = 0.83$ at $P < 0.22$), although the average bacterial ATP content in the contaminant plume appeared to be higher than that for a groundwater sample taken from an uncontaminated zone in the aquifer (Table 1). Intracellular ATP levels have been reported to vary with metabolic state, growth rate, and nutrient limitation (13). It is not entirely clear how growth rate and cellular ATP levels are related, although there is evidence that per-bacterium levels of ATP vary between 2 and 6 μmol/mg (dry weight) in exponentially growing cultures (17), regardless of nutrient conditions. The average bacterial ATP level for the seven sites in the contaminant plume ($[13.70 \pm 6.04] \times 10^{-18}$ g of ATP · $cell^{-1}$) was within ranges reported for sediment-associated bacteria in contaminated and uncontaminated aquifers (35) but was much lower than levels determined for bacteria in a contaminated Danish aquifer (14) and for soil isolates

(22). The Danish aquifer samples, which were obtained from shallower zones, contained higher concentrations of dissolved oxygen and organic carbon than did the Cape Cod samples. Also, ATP was measured by two different techniques: a luciferin-luciferase assay was used for the Danish samples, whereas an HPLC-fluorometric assay was used in this study. Davis and White (6) found that the energy charges and ATP contents of pure cultures and microfouling communities were sensitive to environmental changes, particularly to changes in dissolved oxygen concentration. Therefore, differences in environmental conditions may help account for the apparent differences in average bacterial ATP contents of the Danish and Cape Cod aquifers.

With the exception of wells closest to the loading beds, A_T and DNA levels in the contaminant plume closely follow bacterial abundance (Fig. 2). There is an inverse relationship between abundance of free-living bacteria and distance from the contaminant source (10), which appears to be related to spatial changes in concentrations of non-alkylbenzene sulfonate surfactant DOC (8). It is likely that the decreases in groundwater levels of DNA, ATP, and A_T with downgradient distance are simply a reflection of decreases in bacterial abundance along the same transect. There have been a number of reports in which levels of DNA (23, 26) and ATP (18) have been related to microbial biomass. There is considerably less information concerning relationships between microbial biomass and A_T, although such a relationship might be expected.

Although the average bacterial DNA and A_T contents were relatively constant throughout the plume, these values were both subject to change in the laboratory. In our batch growth experiment involving a groundwater isolate, there were respective six- and threefold declines in the average bacterial DNA content and A_T during a 6-h period of declining growth rate (Fig. 4). However, growth conditions in the batch experiment were very different from those in situ; growth rates in the laboratory study were nearly an order of magnitude greater than the highest values found for groundwater bacteria by Harvey and George (9) (0.35 h^{-1} versus 0.047 h^{-1}). Nevertheless, given the substantial changes in DNA content exhibited by our groundwater isolate, it may be incorrect to assume that the average DNA content of groundwater bacteria should be constant. There was a twofold difference in the per-bacterium DNA contents of samples collected within and outside the plume. For aquifers more contaminated than the one we studied in Cape Cod, bacterial DNA content may vary substantially.

In summary, nucleic acid and adenylate indicators of metabolic activity (EC_A and ATP/DNA ratio) that have been used in surface water studies appear to be useful tools for assessing the metabolic status of microbial communities in contaminated aquifers. For the contaminant plume at Cape Cod, these indicators suggest that the metabolic potential and activity of the free-living bacteria are very low, in spite of the presence of organic contaminants derived from the sewage treatment plant. The use of the ATP/DNA ratio as an indicator of metabolic status assumes that the average bacterial DNA content is relatively constant. However, our findings that the average bacterial DNA content in groundwater bacteria can change significantly in response to alterations in nutrient conditions suggest that care should be taken when the ATP/DNA ratio is used in comparative assessments of community metabolic status of chemically different environments. More research is needed to assess the metabolic potential of groundwater microbial communities and the ability of those communities to respond to changes in nutrient conditions as a result of aquifer contamination.

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