APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1985, p. 1464–1473
0099-2240/85/121464-10$02.00/0
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Vol. 50, No. 6

Influence of Deep Ocean Sewage Outfalls on the Microbial Activity of the Surrounding Sediment

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Received 9 May 1985/Accepted 6 September 1985

The microbial activity near two deep ocean sewage outfalls off the coast of the island of Oahu, Hawaii, was characterized. Water samples and sediment samples to a depth of 4.5 cm were analyzed from an area of approximately 4.5×10^4 m^2 surrounding the outfalls. Although the effluent water at both sites exhibited heterotrophic activity that was 2 orders of magnitude greater than water from a control site, ambient water samples taken within 1 m of the discharge ports exhibited activity only twice that of the control water. The heterotrophic activity of the outfall sediment was only elevated above that of the control site for surface samples collected within 10 m of the outfall. Likewise, the rates of microbial nucleic acid synthesis and carbon production in the sediment were only elevated immediately adjacent to the outfalls. Total microbial biomass, as determined by the ATP content of the sediment, varied spatially but was generally elevated at the outfall sites. The specific growth rates calculated for the sediment microbial populations, however, were not greater at the outfall sites. At one site the rocks surrounding the diffuser pipe were covered with copious amounts of slime that appeared to be composed entirely of microbial cells and filaments. This microbial mat was extremely active with respect to heterotrophic activity and biomass production. Overall, it appears that the impact of the sewage discharge on the ambient seawater microbiota is slight and that the effect on the sediment microbiota is confined to an area immediately adjacent to the diffuser ports. In the sand itself, the effect is limited to the upper 2 cm at most.

Many large urban centers produce sewage effluent in excess of 100×10^6 liters per day. The treatment and disposal of these large volumes of liquid waste pose great problems near large urban centers where land for this purpose is scarce. One solution which is being used today and which is gaining in popularity is the disposal of raw or partially treated sewage through deep (greater than 50 m) ocean outfalls. The effect of these outfalls on the marine ecosystem is assumed to be small but remains unknown. Although the technology and microbiology of sewage treatment have been the subjects of numerous studies and are fairly well understood (4, 12, 16, 21), the environmental microbiology of sewage effluent discharge (especially marine) has not been well documented. Without apparent evidence or data, Dart and Stretton (4) have stated, "When sewage is discharged into the open sea from long outfalls, the overall biological productivity of the water is raised and the diversity and amount of bottom fauna is changed in the vicinity of the outfall. There are no widespread effects. However, if the opportunity for interchange with unpolluted water is limited severe effects can arise."

Most of the interest in the environmental microbiology of sewage discharge has been concerned with two areas: (i) the identification and survival of indicator organisms (primarily coliforms) (6, 15, 19), and (ii) the degradation of specific components of industrial wastewater (20). The effects (both positive and negative) of sewage effluent on the natural microbial communities at the outfall site have been largely ignored. It was the purpose of this study to examine the microbiology of the sediment and water surrounding two deep ocean sewage outfalls off the coast of the island of Oahu, Hawaii. Specifically, we were interested in conducting a spatial study of the surrounding sediment to measure the effect of the sewage outfall on the heterotrophic activity, biomass, biomass production, and specific growth rates of the sediment microflora.

MATERIALS AND METHODS

Sampling sites. Water and sediment samples were collected from two outfall sites: Sand Island (SI) and Barber's Point (BP). Both outfalls are located off the south coast of Oahu, Hawaii, and receive sewage from the greater Honolulu area. The sewage is mainly residential, although a small portion is derived from light industry (mainly food processing). The SI outfall receives effluent from an advanced primary sewage treatment plant that uses chlorination, screening, flotation, and settling to treat the sewage before discharge. The solid materials removed are incinerated. The plant is designed to process 3.1×10^8 liters per day and is currently operating at 80 to 85% of its maximum capacity. The submarine outfall consists of a 3,816-m-long diffuser pipe of decreasing diameter (213 to 122 cm). The pipe is approximately 70 m below the water surface, resting on a bed of rocks and sand; in some places it is up to 1 m above the ocean floor. Near the distal end and located on both sides of the diffuser pipe are 282 ports. The diffuser ports decrease in diameter from 9 to 7.5 cm toward the end of the pipe. The BP outfall is approximately 20 km west of the SI outfall and is similar to it in physical design but it is presently discharging less sewage (6.5×10^7 liters per day) which receives no treatment other than chlorination. A control site was chosen approximately 10 km east of the SI outfall. This site has no apparent influence from the outfalls and is sufficiently far offshore that it is unaffected by island freshwater runoff. Water depth at the control site is approximately 70 m.

Sampling. The sampling sites were observed and sediment and water samples were taken using the *Makali'i*, a two-person submersible operated by the Hawaii Undersea Re-

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search Laboratory. Water samples were obtained with Niskin samplers attached to the submersible's mechanical arm. For effluent samples, an open Niskin sampler was positioned as close to a diffuser port as possible so that effluent streamed freely through it. The force of the effluent was so strong that only a moment was needed to flush out the ambient seawater before closing the sampler. Surface sediment samples were taken with a plastic tube fashioned into a scoop. A spring lid was closed underwater to prevent sediment loss during transport to the surface. Sediment cores were taken with a small Shipek corer deployed from the support ship after the submersible marked the approximate sampling site with a marker buoy.

All samples were transported to the laboratory as soon as possible (within 8 h) in insulated containers to maintain the in situ temperature of 24°C. Scoop sediment and water samples were processed immediately upon arrival in the laboratory. Intact cores were processed immediately or stored in a refrigerated room. Pilot experiments indicated that there was no significant change in ATP biomass or heterotrophic activity in cores stored at 0 to 4°C for up to 12 h; consequently, intact cores were processed last, up to 10 h after arrival in the laboratory.

Water samples were used directly without further processing. Rock slime was recovered by collecting representative rocks with the submersible's arm. On the surface, the slime mat was removed from the rocks and homogenized in filtered seawater with a Teflon and glass tissue homogenizer. Subcores were taken from the box cores with plastic tubes inserted into the sediment. The sediment was then extruded from the tube and divided into various horizons. Each horizon was then thoroughly mixed with a spatula to provide a homogeneous sample.

ATP determinations. The ATP content of water samples was determined by filtering 10 to 500 ml of water through Whatman GF/F glass fiber filters. The filters were then placed into 5 ml of boiling 60 mM phosphate buffer (pH 7.4) for 5 min. The ATP content of slime homogenates was determined by directly injecting 1 ml of homogenate into 5 ml of boiling phosphate buffer. For sediment samples, 1 cm^3 of wet sediment was added with immediate and thorough mixing to 15 ml of cold H_3PO_4 (1.47 M) containing 0.5% Triton X-100. After 30 min the suspensions were centrifuged, and 10 ml of the supernatant was removed. A 0.5-ml sample of a charcoal slurry (100 mg/ml in distilled water) was added to the supernatant; after 15 min, the suspension was centrifuged. The pellet was washed twice with 10 ml of 0.01 M HCl, and then the adsorbed ATP was eluted from the charcoal pellet with 10 ml of a solution containing 50% ethanol and 10% NH_4OH with intermittent shaking for 30 min. After elution, the suspension was centrifuged, and the supernatant was filtered through two back-to-back Whatman GF/F filters. A 7.5-ml sample of the filtrate was evaporated to dryness in vacuo; the residue was rehydrated in 1 ml of freshly prepared 20 mM Tris buffer (pH 7.8). ATP was then determined for both the phosphate buffer extracts (aqueous samples) and for the Tris buffer extracts (sediment samples) by the method of Karl and Holm-Hansen (10). To correct for the apparent and real losses of extractable ATP, internal standards were added to representative sediment samples and processed in an identical manner.

GTP-UTP determinations. Quantitative measurements of the combined concentrations of GTP and UTP were made on subsamples taken from the ATP extract after hexokinase–glucose-6-phosphate dehydrogenase treatment (8). GTP was used as the primary standard. GTP and UTP were not further separated since our sample extracts contained an unknown substance(s) which inhibited the UDP-glucose pyrophosphorylase reaction.

Heterotrophic activity measurements. Relative heterotrophic activity was measured by a modification of the single concentration of substrate method of Griffiths et al. (5) with [U-^{14}C]glutamic acid (specific activity, 294 mCi/mmol). Approximately 250 mg of wet sediment was added to a serum bottle, and 5 ml of filter-sterilized bottom water amended with 0.15 μCi of glutamic acid was added. Incubations were carried out in triplicate for 45 min at room temperature before the reaction was terminated by the injection of 0.5 ml of 1 M HCl. Parallel samples injected with 0.5 ml of 1 M HCl at time zero served as controls. After the collection of the $^{14}CO_2$ on filter paper wicks saturated with β-phenethylamine (20 min), the bottles were placed on ice, and 19.5 ml of cold 2 M HCl was added to precipitate the macromolecules. After 1 h, duplicate 10-ml portions of the suspension were filtered onto Millipore HA membrane filters and washed twice with 5 ml of 1 M HCl. The filters were dried overnight at 60°C and then oxidized in a Oxymat model JA101 sample oxidizer in preparation for liquid scintillation counting. Water samples were also processed as described above with 10 ml of water per bottle. Water sample incubations were terminated with the injection of 0.5 ml of 1 M HCl, and macromolecules were precipitated with the addition of 9.5 ml of 2 M HCl.

Nucleic acid synthesis rates. DNA and RNA synthesis rates were determined with 15 cm^3 of wet sand amended with 0.5 ml (50 μCi) of [2-^{3}H]adenine solution (specific activity, 21.6 Ci/mmol). The isotope was mixed thoroughly into the sand with a spatula. At various times, 1 cm^3 of the incubating sediment was injected into 5 ml of boiling 60 mM phosphate buffer to extract the ATP and [^{3}H]ATP for determination of the ATP pool specific activity as described by Winn and Karl (22). After 60 and 120 min, 1 cm^3 of the incubating sediment was added to 60 ml of cold 1 M HCl, and the RNA and DNA were extracted by the method of Craven and Karl (3) for type 3 sediment. Rates of DNA and RNA synthesis were calculated by the ATP integrated specific activity method of Winn and Karl (22).

Specific growth rate determinations. Mean microbial community specific growth rate was estimated by two independent methods. The first estimate was derived from the measured rates of DNA synthesis and the ATP biomass data, i.e., (doublings per day) × 0.693, assuming a DNA/ATP ratio of 5. The rationale for this extrapolation is presented elsewhere (11). The second estimate was derived from the direct measurement of the turnover time of the adenine nucleotide pool, assuming that the pool turns over 40 times per generation. The rationale for this extrapolation is presented elsewhere (9). It should be emphasized that these methods are based upon totally independent assumptions and experimental data; however, both methods assess only that portion of the total population which is capable of assimilating labeled adenine.

DNA and RNA determinations. Total RNA and DNA in the sediments were determined by using standard colorimetric analyses after nucleic acid isolation and purification procedures. For RNA, 1 cm^3 from a homogenized 15- to 30-cm^3 sample of each sediment core was extracted in 60 ml of 1 M HCl to dissolve the $CaCO_3$ and to precipitate the nucleic acids. Three 15-ml portions were placed into clean tubes containing 1.5 g of diatomaceous earth (Celite) to serve as a centrifugation aid. The samples were mixed, centrifuged, and washed with 12 ml of 95% ethanol. Two milliliters of 5% trichloroacetic acid was added to each tube, and the samples

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TABLE 1. Chemical and microbiological analysis of slime material removed from rocks collected at the SI outfall site[a]

ATP	72.5 μg (SD = 28.2; n = 6)
C/ATP	2,480
C/N	10.2
DNA	21.9 mg (SD = 2.11)
RNA	10.6 mg (SD = 1.91)
DNA synthesis rate	20.5 nmol/h (SD = 0.05)
RNA synthesis rate	198.0 nmol/h (SD = 0.14)
DNA/RNA	0.10
Heterotrophic uptake	11.4 μg of glutamate/h (SD = 0.62)
DNA turnover rate	35.9 days
RNA turnover rate	41.1 h
Specific growth rate[b]	1.16/day
Carbon production[c]	30.4 mg/day
Biomass-specific values	
DNA synthesis	0.28 pmol of adenine/ng of ATP per h
RNA synthesis	2.73 pmol of adenine/ng of ATP per h
Heterotrophic uptake	0.16 g of glutamate/g of ATP per h

[a] All values are means of three determinations (except as noted) and are reported per gram (dry weight) as appropriate.

[b] Specific growth rate = doublings per day × 0.693. Doublings per day were calculated as carbon production/biomass of carbon determined from ATP × 250.

[c] Carbon production was extrapolated from rates of DNA synthesis by assuming that 1 pmol of DNA is equivalent to 1,236 pg of DNA produced and that DNA accounts for 2% of total cell carbon.

were boiled for 30 min, cooled to room temperature, and centrifuged. A 1-ml portion of the supernatant was assayed for RNA by using the orcinol reaction (14) with AMP as the primary standard. For DNA, 1-cm^3 subsamples of each sediment homogenate were extracted in 1 M HCl and processed with Celite as described above for RNA determinations. After DNA isolation, the samples were incubated with 1 M NaOH for 1 h at 37°C to selectively hydrolyze the RNA, which, if present, will interfere with quantitative determinations of DNA. The NaOH hydrolysate was subsequently acidified and centrifuged; the precipitate was washed with 12 ml of ethanol. After the addition of 2 ml of 5% trichloroacetic acid to each tube, the samples were boiled for 30 min, cooled to room temperature, and centrifuged. A 1-ml portion of the supernatant was assayed for DNA by using the diphenylamine reaction (1) with deoxyadenosine as the primary standard.

RESULTS

The sediment at all three sampling sites was predominantly carbonate sand. At the SI outfall, the sand bottom was devoid of debris and organic matter accumulation. The bottom also appeared devoid of all but a few epibenthic fauna; however, the rocks supporting the outfall pipe were covered with a variety of corals and sponges. The entire outfall area supported a diverse community of local reef fishes. Many of the rocks near the diffuser ports were also covered with a slimy mat approximately 2 to 3 mm thick. Microscopic examination of this mat revealed a consortium of bacterial cells and filaments. No other organic matter was observed, and it was assumed that the mat was composed exclusively of microbial cells and extracellular products. Dislodged fragments of these mats were observed floating in the water column and also appeared in the sediment traps that were deployed during the time of our sampling. A chemical and microbiological description of this mat material (means ± standard deviations of three determinations, except as noted) is presented in Table 1. An analysis of the rate of metabolic activity as a function of salinity (10 to 100% seawater) revealed that maximum activity for heterotrophic uptake and nucelic acid synthesis occurred in 75% seawater (data not shown), suggesting that these bacteria did not originate from the effluent, but rather were bacteria of marine origin growing at the expense of carbon and energy derived from the outfall effluent.

At the BP outfall, neither slime nor mat was observed on the rocks, and the faunal communities were sparse compared with the SI outfall site. In the vicinity (within 10 m) of the pipe, the sand was covered with a layer of debris 1 to 5 mm in thickness. This debris layer appeared to be composed of sewage solids, including fibers. This layer supported an active community of meiofauna.

The DNA and RNA synthesis rates and the heterotrophic uptake activity of all samples are summarized in Table 2. These rates are also shown for surface (0 to 1.5 cm) sediment samples in Fig. 1 through 3. The only samples showing any activity significantly greater than the control samples were those collected within 10 m of the BP outfall. Data for DNA synthesis throughout the entire sediment profile (to a depth of 4.5 cm) are shown in Fig. 4. These data are from the SI outfall site, but their pattern is representative of all of the data collected for both sites; elevated activity was only detected in the surface horizon, and only for samples taken within 10 m of the diffuser pipe. The DNA/RNA synthesis rate ratios varied little within or between both outfall sites (SI average, 0.18; SI range, 0.13 to 0.23; BP average, 0.13; BP range, 0.10 to 0.17). The DNA/RNA synthesis rate ratios from the control sands were higher than the outfall sites (average, 0.28), but were also relatively constant among cores (range, 0.22 to 0.33).

The biomass-specific rates for nucleic acid synthesis and heterotrophic uptake are tabulated for all samples in Table 3. Representative data are shown for the biomass-specific RNA synthesis rates for all the horizons from the BP outfall site in Fig. 5. Although there is some variation in the data, there are no significant deviations from the data collected at the control site.

The ATP and GTP-UTP content of the sediment samples is shown in Table 4. Both ATP and GTP-UTP displayed substantial variation in the sediments examined. The values presented in Table 4 represent the means of triplicate determinations. To investigate this variability, over 200 samples were analyzed from cores and surface scoop samples. In all cases, the 95% confidence intervals were approximately ±50% of the mean. Even so, it appears that there are larger amounts of both ATP and GTP-UTP in the sediment near the BP outfall than in either the SI outfall or control site sediment. The concentration of DNA and RNA contained in the sediments (Table 4) exceeds the amount which can be attributed to living cells (as determined by ATP values), suggesting that a large (>75%) portion of the total nucleic acids are detrital. Figure 6 presents a series of representative time course experiments used to measure the turnover time of the total adenine nucleotide pool and to estimate specific growth rates, which were compared with independent estimates derived from DNA synthesis and ATP data (Table 5). Table 5 also presents the estimated rates of total carbon production derived from DNA synthesis rates. Again, the only significant deviation from the rate of carbon production at the control site is in the surface sediment at the sampling

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TABLE 2. Nucleic acid synthesis and heterotrophic uptake activity of microbial populations from outfall and control sediments[a]

Site	Core[b]	Horizon[c]	Synthesis (pmol g [dry wt]$^{-1}$ h^{-1})		Heterotrophic uptake	
			DNA	RNA	ng of glutamate g (dry wt)$^{-1}$ h^{-1}	% Respired
SI	1	A	726.8 (103.1)	3,662.2 (227.9)	40.4 (1.6)	17.6
		B	325.4 (16.3)	1,410.3 (214.4)	31.9 (1.8)	18.7
		C	269.7 (40.9)	1,204.5 (19.6)	25.6 (4.6)	18.8
SI	2	A	215.3 (6.2)	1,473.7 (123.5)	36.3 (5.6)	26.9
		B	173.6 (6.6)	1,199.2 (64.8)	34.0 (0.4)	28.4
		C	84.4 (13.7)	644.0 (134.7)	27.4 (1.5)	26.1
SI	3	A	404.4 (43.7)	1,925.3 (255.1)	20.1 (1.0)	21.5
		B	404.1 (9.0)	2,091.3 (114.4)	23.8 (1.6)	22.9
		C	314.5 (43.7)	1,740.9 (267.0)	25.2 (0.5)	23.1
BP	1	A	1,759.8 (153.0)	11,192.3 (785.3)	97.5 (12.8)	44.6
		B	685.5 (33.9)	3,965.7 (189.1)	76.8 (3.1)	44.9
BP	2	A	318.3 (9.2)	2,471.0 (140.3)	66.3 (3.3)	41.3
		B	155.2 (9.9)	1,396.3 (67.4)	48.6 (8.0)	31.3
		C	102.3 (6.8)	951.1 (47.4)	35.6 (7.0)	32.6
BP	3	A	171.7 (6.2)	1,493.5 (160.0)	15.9 (0.3)	45.1
		B	184.7 (20.4)	1,714.2 (347.1)	14.5 (0.6)	45.1
		C	217.1 (8.4)	1,689.5 (23.5)	15.0 (1.2)	45.3
Control	1a	A	284.3 (13.6)	1,300.6 (23.8)	28.7 (0.3)	30.1
		B	298.1 (22.8)	1,148.2 (48.1)	34.3 (2.0)	27.1
		C	215.3 (19.1)	857.3 (66.9)	28.1 (12.5)	30.1
Control	1b	A	220.5 (30.2)	810.6 (63.8)	36.1 (1.9)	29.5
		B	296.1 (25.4)	1,195.4 (53.9)	41.1 (2.9)	37.0
		C	260.0 (27.5)	878.9 (74.9)	26.1 (4.4)	37.8
Control	1c	A	373.3 (56.4)	1,126.6 (76.5)	51.8 (3.2)	27.6
		B	361.0 (49.2)	1,163.3 (90.7)	22.4 (2.1)	28.2
		C	242.5 (36.7)	762.0 (101.8)	20.0 (2.6)	29.2

[a] Values reported are means of three determinations. The standard deviation is shown in parentheses.
[b] Core 1 was taken within 10 m of the outfall; core 2 was taken between 30 and 60 m from the outfall; core 3 was taken between 90 and 120 m from the outfall; control cores 1a, b, and c were taken randomly at the control site.
[c] Horizons: A, surface to 1.5-cm depth; B, 1.5 to 3.0 cm; C, 3.0 to 4.5 cm.

point closest to the outfall. The specific growth rates of the microbial communities in the sediment generally decrease with depth at both the SI and the BP site. At the control site, the specific growth rates of the microbial communities are generally depth independent. Even in the surface sediment samples closest to the outfalls, the specific growth rates at the oufall sites are generally lower than those measured in the control sediment.

DISCUSSION

The design of the effluent diffuser systems posed difficult sampling problems. Rather than a single point source of discharge, effluent is discharged from numerous points along both sides of a long pipe. Each diffuser port, therefore, is a separate discharge site. Away from the diffuser pipe the effect of numerous discharge points is slight, but for samples close to the pipe variability due to sample collection must be considered. Using the submersible *Makali'i* it was possible to take sediment samples that were precisely positioned relative to the diffuser discharge sites. It was also possible to mark accurately the location for sediment core collection, although the accuracy of the ship-deployed gravity corer was estimated to be only ±5 m. The greatest variability in the data collected was with the ATP determinations. This variability, however, appeared to be both intra- and intersample variability. Even in well-mixed single samples the standard deviation was a large percentage of the mean (Table 4). It was concluded that a rigorous, statistical analysis of these data was impossible under the scope of the present study. The variability was caused in part by the coarse nature and varied grain sizes of the sand and the small subsample size (1 cm^3). Due to the carbonate content of the sand (nearly 100%), a large volume of acid is needed for extraction. Increasing the subsample size would be unwieldy with this technique; perhaps the development of a different extraction technique would be useful for future studies.

The only sampling difficulty encountered was in taking core samples. Coring sand proved to be more difficult than expected and was impossible with several conventional coring devices. The only device successfully used was a Shipek corer, which is a spring-loaded bucket design. Even with this device, only 4.5 cm of sediment could be recovered, with the exception of 1 BP outfall station where only 3.0 cm of sediment were recovered. It was assumed that there was rock or hard packed sand below 3 cm, and therefore no C horizon is reported for this site. All of the biochemical and microbiological techniques worked well,

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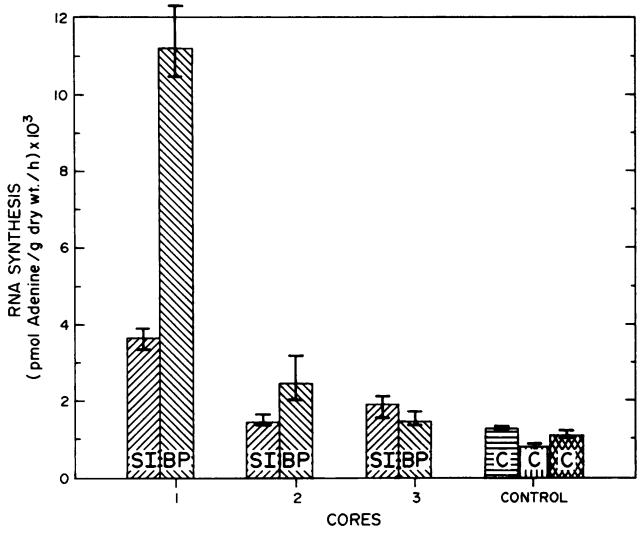


FIG. 1. RNA synthesis in surface (0- to 1.5-cm) sediments as measured by the incorporation of [^{3}H]adenine. The main bars represent the means of three determinations; the smaller bars represent the ranges. Core 1 was taken within 10 m of the outfall, core 2 was taken between 30 and 60 m from the outfall, core 3 was taken between 90 and 120 m from the outfall, and the control cores were taken randomly at the control site (C).

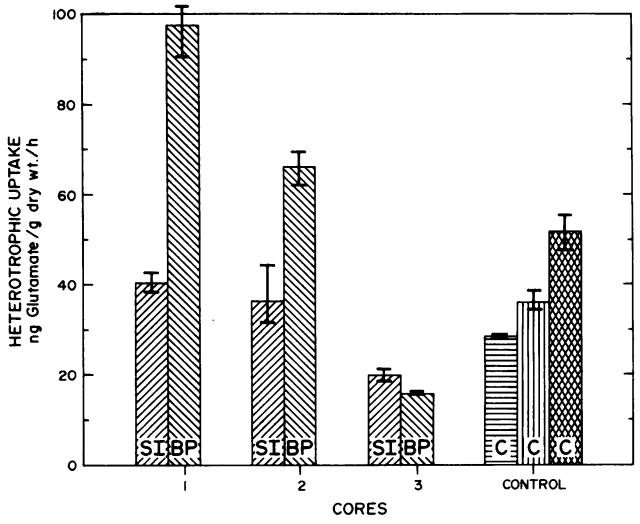


FIG. 3. Heterotrophic uptake of glutamic acid in surface (0- to 1.5-cm) sediments. The main bars represent the means of three determinations; the smaller bars represent the ranges. Sites and cores are as in Fig. 1.

except for the ATP extraction procedure. A previous study (2) and preliminary experiments indicated that the use of Triton X-100 increased the extraction efficiency of ATP from these sediments. During the present study it was determined that the use of Triton X-100 resulted in an apparent or real loss of ATP during the extraction procedure, since we concentrated our extracts with activated charcoal. Although this loss can be and was corrected for, the routine use of Triton X-100 for sediment ATP extraction can only be recommended with caution. The uptake of glutamic acid was found to be linear for the first 45 min of uptake (data not shown); thereafter, only a single determination at 45 min was used to extrapolate an hourly rate. Sand that was repeatedly washed with filtered bottom water and assayed in filtered seawater showed the same heterotrophic activity as unwashed sand assayed in unfiltered water. Although it appeared from these experiments that the microbial population was firmly attached to the sand grains, biochemical interaction with the pore water cannot be ruled out. Amino acid concentrations in the pore water were below our detection limits for the high-pressure liquid chromatography system used (0.5 nM). The added amount of [^{14}C]glutamate, therefore, exceeded the amount of [^{12}C]glutamate present,

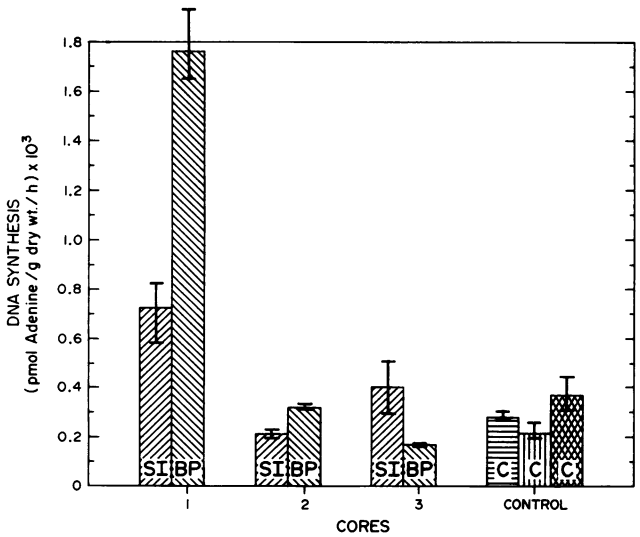


FIG. 2. DNA synthesis in surface (0- to 1.5-cm) sediments as measured by the incorporation of [^{3}H]adenine. The main bars represent the means of three determinations; the smaller bars represent the ranges. Sites and cores are as in Fig. 1.

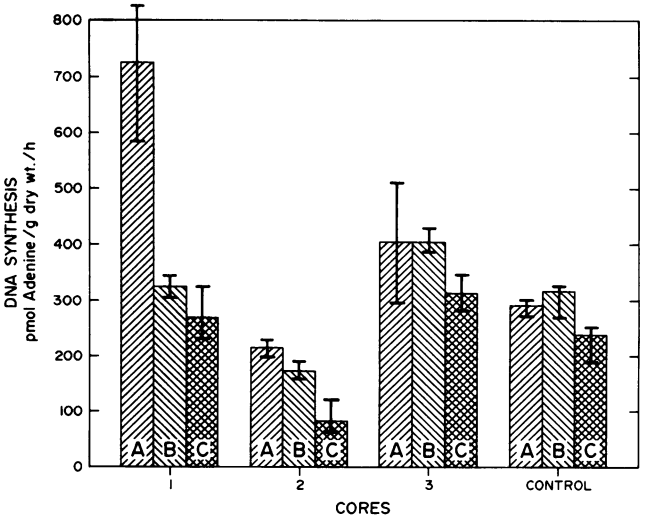


FIG. 4. DNA synthesis in SI outfall sediments as measured by the incorporation of [^{3}H]adenine. The main bars represent the means of three determinations; the smaller bars represent the ranges. Cores are as in Fig. 1. Horizons: A, surface to 1.5 cm; B, 1.5 to 3.0 cm; and C, 3.0 to 4.5 cm.

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TABLE 3. Biomass-specific rates of DNA and RNA synthesis and heterotrophic uptake for microbial populations from outfall and control sediments[a]

Site	Core	Horizon	Biomass-specific rates		
			Synthesis (pmol ng of ATP^{-1} h^{-1}		Heterotrophic uptake (g of glutamate g of ATP^{-1} h^{-1}
			DNA	RNA	
SI	1	A	1.0	5.2	0.06
		B	0.5	2.1	0.05
		C	0.8	3.5	0.07
SI	2	A	0.3	1.8	0.05
		B	0.2	1.6	0.05
		C	0.1	0.9	0.04
SI	3	A	0.5	2.2	0.02
		B	0.4	1.9	0.02
		C	0.2	1.3	0.02
BP	1	A	0.5	3.0	0.03
		B	0.2	1.2	0.02
BP	2	A	0.2	1.9	0.05
		B	0.2	1.7	0.06
		C	0.2	1.4	0.05
BP	3	A	0.4	3.2	0.03
		B	0.3	3.0	0.03
		C	0.4	2.9	0.03
Control	1a	A	0.6	2.9	0.06
		B	0.4	1.7	0.05
		C	0.3	1.2	0.04
Control	1b	A	0.3	1.2	0.05
		B	0.5	1.9	0.06
		C	0.5	1.8	0.05
Control	1c	A	0.9	2.7	0.12
		B	1.6	5.3	0.10
		C	1.0	3.3	0.09

[a] Sites, cores, and horizons as in Table 2.

and the heterotrophic uptake rates must be considered as relative rates only. Increasing the amount of [^{14}C]glutamate added in these experiments increased the measured rates, indicating that the rates measured were less than the maximum rate (V_{max}). Experiments for the determination of the rates of DNA and RNA synthesis were conducted for 2 h, but it was determined that in all cases the [^{3}H]adenine was exhausted between 1 and 2 h. Rate calculations were therefore made within the initial 60 min of incubation.

Although the main purpose of this study was to examine the microbiology of the sediment surrounding the outfalls, we thought that it would also be interesting and useful to make some preliminary measurements of the effluent and ambient seawater. Effluent collected from the treatment plants just before release showed a heterotrophic uptake activity of 7 to 10 ng of glutamate ml^{-1} h^{-1}. Effluent collected in situ from a diffuser port at the SI outfall showed essentially the same activity. The residence time of the effluent in the diffuser pipe varies with the flow rate, but on an average day the residence time is less than 50 min. Apparently this short residence time has little effect on the metabolic activity of the microorganisms in the effluent water. Ambient seawater collected at the control site exhibited activity approximately 2 orders of magnitude less. Likewise, the effluent water contained 9.9 ng of ATP ml^{-1} compared with a concentration of 25.1 pg of ATP ml^{-1} in the control seawater. Due to the fact that the sewage is chlorinated as it enters the treatment plants, we measured the chlorine content of the water at the diffuser port and found it to be essentially chlorine free (less than 5 ppb [5 nl/liter]). Apparently the chlorine addition has no effect on the microbial activity of the effluent. Water collected in situ within 1 m of a diffuser port at the BP outfall contained 0.52 ng of ATP ml^{-1} and exhibited a heterotrophic uptake rate of 0.30 ng of glutamate ml^{-1} h^{-1}; both values more indicative of ambient seawater than of sewage effluent. Apparently, the effluent is effectively and rapidly mixed with a large volume of ambient seawater. In addition to the physical mixing, the rapid change in salinity may also contribute to the decrease in microbial activity. Obviously designed for this purpose, from a physical standpoint, it appears that the diffuser system is working well. On a larger scale, the diluted effluent is further mixed and transported by local currents. Although specific data are lacking, on several occasions the submersible encountered strong currents which we estimated to be in excess of 1 knot.

An unexpected discovery was the presence of a well-developed microbial slime covering the rocks near the diffuser ports at the SI outfall. The slime was determined to be composed of microbial cells and filaments. Examination of the slime reveals a rather firm mat adhering to the rocks. The surface of the mat is smooth and white; the underside is black. Large quantities of the mat material have the odor of reduced sulfur compounds, and we assumed that the underside portions of the mat are partially or totally anaerobic. Our studies of the mat community in a graded series of salinities indicate that the microbial mat is marine in nature, with optimal growth and metabolic activity at a salinity that is slightly reduced from that of the ambient seawater. Located near a source of fresh water input, the community has physiological characteristics that match its environment perfectly. Unfortunately, time and logistics did not permit a more detailed study of this intriguing microbial community.

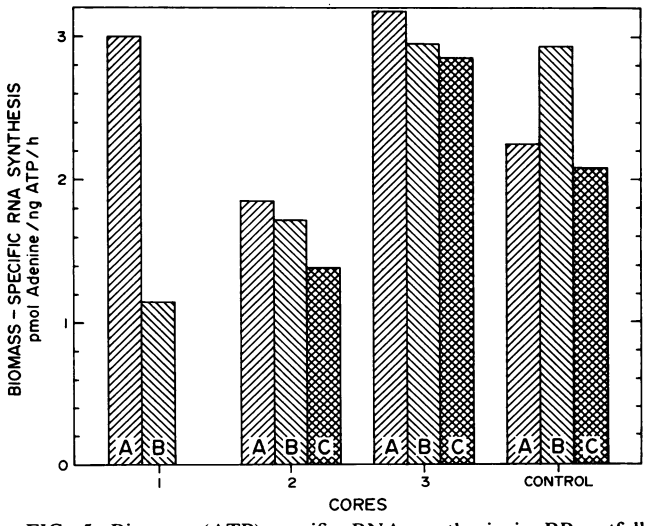


FIG. 5. Biomass (ATP)-specific RNA synthesis in BP outfall sediments as measured by the incorporation of [^{3}H]adenine. Cores and Horizons are as in Fig. 1 and 4. The datum presented for each control horizon is the mean of three determinations.

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TABLE 4. ATP, GTP-UTP, DNA, and RNA concentrations in sediments from outfall and control sites[a]

Site	Core	Horizon	Concn (per g [dry wt] of sediment)				
			ATP (ng)	GTP-UTP (ng)	GTP-UTP/ ATP[b]	DNA (μg)	RNA (μg)
SI	1	A	699.3 (228.6)	539.1 (131.5)	0.75	97.2 (13.0)	117.2 (4.4)
		B	671.4 (224.3)	439.8 (104.6)	0.64	125.4 (12.9)	93.4 (8.2)
		C	348.1 (67.0)	311.4 (96.4)	0.87	50.1 (2.9)	82.2 (8.3)
SI	2	A	808.9 (133.7)	674.8 (153.4)	0.81	54.7 (6.6)	131.3 (32.7)
		B	754.7 (275.7)	270.5 (56.0)	0.35	36.3 (5.7)	105.9 (4.7)
		C	743.3 (246.7)	319.7 (82.7)	0.42	274.2 (16.6)	88.3 (7.8)
SI	3	A	860.4 (91.5)	519.1 (29.2)	0.58	50.3 (4.0)	91.5 (8.6)
		B	1,113.1 (86.3)	614.1 (248.4)	0.54	59.2 (10.6)	113.3 (4.9)
		C	1,359.5 (111.8)	1,153.7 (161.0)	0.83	36.8 (6.0)	128.7 (14.0)
BP	1	A	3,647.6 (321.2)	5,362.2 (220.4)	1.43	78.4 (2.3)	466.8 (226.1)
		B	3,441.7 (240.4)	4,511.1 (2333.0)	1.27	57.1 (1.6)	251.9 (19.0)
BP	2	A	1,325.9 (145.7)	1,435.8 (255.7)	1.05	226.5 (14.6)	303.5 (62.8)
		B	812.0 (30.5)	502.8 (71.5)	0.60	230.2 (15.7)	218.1 (44.2)
		C	684.9 (98.0)	388.5 (100.0)	0.55	225.4 (19.3)	161.5 (21.7)
BP	3	A	470.0 (147.3)	68.3 (69.6)	0.15	241.4 (20.9)	217.5 (44.7)
		B	580.4 (33.1)	68.1 (23.3)	0.12	222.3 (16.9)	220.5 (37.3)
		C	593.4 (69.2)	216.2 (34.6)	0.35	234.7 (21.6)	278.5 (27.3)
Control	1a	A	448.7 (100.0)	243.7 (61.6)	0.53	214.2 (8.8)	122.6 (14.9)
		B	675.5 (27.5)	413.4 (10.7)	0.59	234.3 (11.0)	116.5 (8.9)
		C	691.2 (90.5)	439.1 (90.1)	0.62	196.6 (19.2)	102.8 (14.8)
Control	1b	A	703.9 (13.3)	364.8 (33.2)	0.51	226.0 (16.6)	110.7 (8.3)
		B	637.2 (191.7)	303.5 (117.8)	0.47	246.2 (8.7)	141.4 (11.8)
		C	503.0 (21.2)	247.1 (38.7)	0.48	209.8 (21.6)	139.1 (9.8)
Control	1c	A	419.2 (26.0)	189.5 (35.8)	0.44	215.6 (8.3)	159.9 (16.4)
		B	221.4 (77.1)	44.8 (61.5)	0.19	190.8 (13.2)	114.2 (11.8)
		C	233.3 (50.1)	47.5 (22.4)	0.19	199.8 (20.9)	140.4 (9.8)

[a] All values reported are means of three determinations; standard deviations are given in parentheses. Sites, cores, and horizons as in Table 2.
[b] Molar ratio.

It is difficult to compare the mat to the sediment on a dry weight basis due to the large mineral component of the sand. On a dry weight basis, therefore, it is no surprise that the mat material contains significantly more ATP than does the sediment. On an ATP biomass-specific basis however, both the mat and the sediment are about equal in their per capita rates of nucleic acid synthesis, but the mat has a greater heterotrophic activity on a per cell basis. The specific growth rates for the sediment and mat are also approximately equal. On a per capita basis, the microbial communities, therefore, appear to be equivalent, but the high density of microbial biomass in the mat makes it a focal point of heterotrophic activity and biomass production for the outfall ecosystem. Likewise, it may also be a locus of decomposition, nutrient regeneration, and perhaps pollutant concentration and transformation. By carefully removing measured portions of the mat we determined its abundance to be approximately 5.2 mg (dry weight) cm^{-2}. We estimated visually that each diffuser port supported an area approximately 5 m in diameter where mat growth was evident. A conservative estimate for the entire diffuser system is a standing crop of 290 kg (dry weight) of mat material producing 8.8 kg of microbial carbon per day (1.58 g of C m^{-2} d^{-1}). The estimate is conservative because we made no attempt to quantify the large amount of mat material floating in the water column. By comparison, the rate of primary production measured in these waters (13) is only 350 mg of C m^{-2} d^{-1}. In addition to the cellular carbon, the mat community may also be producing a large amount of extracellular polysaccharide, as evidenced by the high carbon/ATP (2,480) and high C/N (10.2) ratios of the mat. Overall, the slime-covered rocks at the SI site appear to be analogous to a conventional secondary sewage treatment trickling filter system. To our knowledge, this is the first submarine system described, but in principle the operation should be the same: the microbial populations develop to mineralize the dissolved organic wastes; filamentous organisms and cells that are able to attach to the rocks are favored; and the mat continues to develop until the bacterial population is drastically reduced, first by protozoa and second by the thickness of the film which reduces the oxygen and the available nutrients (16). Under anaerobic conditions, gas (H_2S and possibly methane) and organic acids would be expected to be produced and could cause pieces of the mat to slough off by pushing the slime away from the rock surface or by decreasing the local pH and killing the lower layer of cells (21). For these reasons, in addition to the hydraulic shear, the mat is detached and floats away. The floating bits of mat which were observed even on the surface can represent the transport of considerable amounts of microbial carbon away from the discharge site. Although large amounts of the mat material were noted at the SI outfall, no mat or slime was observed at the BP outfall during

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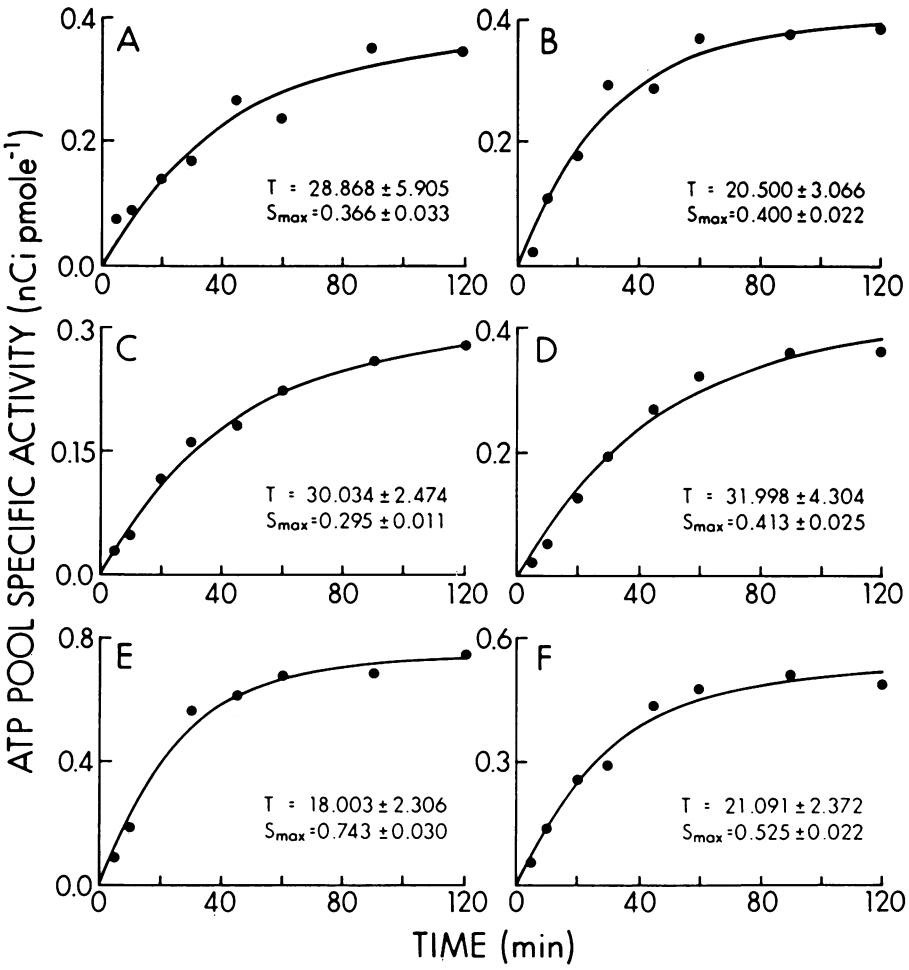


FIG. 6. Representative data on the time-dependent changes in the specific activity of the ATP pool after the addition of [^{3}H]adenine. The results were analyzed with a computer-assisted, nonlinear least-square fit to the expected experimental relationship described by Karl and Bossard (9). From this analysis, T (the turnover time of the total adenine nucleotide pool) and S_{max} (the specific radioactivity at isotopic equilibrium) are derived. Samples: A and B, SI; C and D, BP; E and F, Control. Specific growth rate was extrapolated from adenine nucleotide turnover time by assuming that the adenine nucleotide pool turns over 40 times per generation (9).

this study. The reasons for this are unknown. Essentially the only differences between the two are the ages of the sites (the SI outfall has been in operation for 9 years; the BP outfall has operated for 5 years) and the amounts and types of sewage discharged (SI large volume, primary treatment; BP lower volume, no treatment). In addition, we do not know whether these mats are a transient or permanent feature of ocean outfalls.

The differences between the two outfall sites were also seen in the microbial activity of the sediment. The BP outfall discharges less sewage, but, because it receives no prior treatment, is believed to discharge higher concentrations (and possibly higher amounts) of both dissolved and particulate organic matter. Even when the type and amount of organic matter in the effluent are known, it is difficult to measure how much actually reaches the sediment. The SI sewage is treated before release, removing approximately 70% (2.5×10^4 kg per day) of the suspended solids. Indeed, particulates were never observed in the sediment surface or in the water column at the SI site, whereas large amounts of debris were noted at the BP site (see Results). Although the microbial activity in the sediment at the BP outfall was greater than that measured at the SI outfall, the difference was not great, and it was confined to an area immediately surrounding the diffuser pipe.

The vertical profiles of microbial activity throughout the sediment showed slightly greater activity in the surface horizons, but again the effect is limited to the areas closest to the discharge. The reasons for such a limited impact on the microbiology of the area probably include the rapid mixing of the effluent with the ambient seawater, local currents in the area, and, in the case of the SI outfall, the presence of a microbial mat community removing organic material from the effluent before it can reach the sediment. The main effect of the organic discharge on the microbial community seems to be an increase in the microbial carbon production rate. Although the carbon production rates at the outfalls are much higher than at the control site, the specific growth rates as estimated by two independent methods are about equal (Table 5). In addition, at the outfall sites the biomass-specific rates of RNA and DNA synthesis and heterotrophic uptake are essentailly the same as, if not slightly lower than, those measured at the control site. This indicates that the growth rates of the populations are not nutrient limited and that the additional nutrient input is translated into a larger standing crop, and production, of biomass. In light of these data, the

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specific growth rate of the control populations does not seem exceedingly low. The relationship between nutrient levels and growth rate, therefore, must be viewed and interpreted cautiously. This study emphasizes the fact that there is no a priori reason to conclude that a slow growth rate indicates a starving microbial population. Likewise, from the heterotrophic uptake and respiration data, it does not seem that the outfall populations are mineralizing large amounts of carbon without a concomitant production of biomass. The percentage of the substrate respired does not exceed 45%, a value in agreement with other sediment populations that do not receive anthropogenic organic matter input (7, 17, 18). The pattern of heterotrophic uptake in these sediments generally follows the pattern of DNA and RNA synthesis, but the correlation between them is not good (r^2 of 0.53 and 0.56, respectively). This is in agreement with results from other studies (Karl and Novitsky, unpublished data).

Although the activity of the microbial populations does not seem to have been affected drastically by the input of sewage effluent, we have no indication of its effect on the microbial community structure. The fate of the large microbial community of the effluent itself is unknown. Grimes et al. (6) were able to isolate coliforms up to 3.7 km from an ocean outfall in Puerto Rico. Whether some of the effluent community can establish itself in the surrounding sediment is also unknown. The fact that the sediment organisms appear to be firmly attached to the sand grains indicates a permanent, nontransient population. This is in agreement with results from other microbial sand populations (MacSween and Novitsky, unpublished data) and is not believed to be unique to the outfall systems.

TABLE 5. Carbon production by and specific growth rates of microbial communities in sediments from outfall and control sites[a]

Site	Core	Horizon	Carbon production[b] (μg g dry wt^{-1}) day^{-1}	Specific growth rate (per day) DNA[c]	Specific growth rate (per day) AN turnover[d]
SI	1	A	1,078.0	4.27	0.92
		B	482.7	1.99	1.22
		C	400.0	3.19	1.39
SI	2	A	319.3	1.09	2.15
		B	257.4	0.95	0.92
		C	125.2	0.47	1.14
SI	3	A	599.8	1.93	0.86
		B	599.4	1.49	1.69
		C	466.5	0.95	0.91
BP	1	A	2,610.1	1.98	1.97
		B	1,016.7	0.82	0.85
BP	2	A	472.1	0.99	2.17
		B	230.2	0.79	1.79
		C	151.7	0.61	1.54
BP	3	A	254.7	1.50	0.66
		B	273.9	1.31	0.83
		C	322.1	1.50	1.17
Control	1a	A	421.7	2.61	0.61
		B	442.2	1.81	1.18
		C	319.3	1.28	1.33
Control	1b	A	327.1	1.29	1.26
		B	439.1	1.91	1.66
		C	385.6	2.12	0.85
Control	1c	A	553.7	3.66	0.89
		B	535.4	6.70	0.70
		C	359.6	4.27	1.39

[a] Sites, cores, and horizons as in Table 2.
[b] Carbon production was extrapolated from rates of DNA synthesis by assuming that 1 pmol of DNA is equivalent to 1,236 pg of DNA produced and that DNA is 2% of total cell carbon.
[c] Specific growth rate is doublings per day × 0.693. Doublings per day were calculated as carbon production/biomass carbon determined from ATP × 250.
[d] Specific growth rate is doublings per day × 0.693. Doublings per day calculated from adenine nucleotide (AN) turnover time assuming that the adenine nucleotide pool turns over 40 times per generation.

ACKNOWLEDGMENTS

We thank the captain and crew of the M/V *Holokai* and the submersible *Makali'i* (Hawaii Undersea Research Laboratory) and acknowledge the field assistance of K. Orrett. We are grateful to U. Magaard for excellent technical assistance and diligence throughout this project. J.A.N. thanks D. Karl and the Department of Oceanography, University of Hawaii, for their facilities and cooperation that made this research possible during his sabbatical leave there.

This research was organized by S. Dollar under grant CR811180-01-1 from the U.S. Environmental Protection Agency, S. V. Smith, Principal Investigator.

This research was funded, in part, by grant PYI83-51751 from the National Science Foundation and grants A-6548 and T-1925 from the National Research Council of Canada.

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