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TECHNICAL PROGRESS REPORT

THE FLUX AND RECYCLING OF BIOACTIVE SUBSTANCES IN THE
SURFACE SEDIMENTS OF DEEP BASINS OFF SOUTHERN CALIFORNIA

June 11, 1990

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The Flux and Recycling of Bioactive Substances in the Surface
Sediments of the
Deep Basins Off Southern California

Progress Report - 11 June 1990

Richard A. Jahnke

The overall goals of this project are to develop an understanding of the processes responsible for the recycling of biogenic debris in the surface sediments of Santa Monica Basin and to quantitatively determine the rate at which dissolved chemicals exchange across the sediment-bottom water interface. This information will provide fundamental information about organic matter decomposition and oxidation at the seafloor and provide a quantitative maximum estimate of the lateral input of organic matter from the adjacent continental shelf.

Because this program is being terminated, the focus of our efforts during the past year has been primarily on data assimilation and interpretation. Toward this goal, one manuscript (Jahnke, R. A. 1990, Early diagenesis and recycling of biogenic debris at the seafloor, Santa Monica Basin, California, *J. Mar. Res.* 48, 1-24.) has been published and another manuscript (attached) has been submitted and reviewed. In addition, we have continued our field investigation of the processes that recycle organic debris at the seafloor by participating in the January 1990 expedition to the Santa Monica Basin. On this expedition, the following was accomplished:

1. We recovered three, good-quality box cores suitable for fine-scale analyses.
2. Four subcores were sectioned at 1 mm intervals for future solid phase analysis to examine the near-surface distribution of oxidation products of chemotrophic metabolism. These results will be used to estimate the relative roles of manganese, iron, and sulfur oxidizing organisms in near-surface metabolism.
3. We directly measured the pore water oxygen distribution at 1 mm sampling intervals using shipboard microelectrodes. These results will strengthen and refine our understanding of the relative roles of oxic and anoxic processes influencing metabolic utilization of organic materials and authigenic mineral formation in the sediments.
4. A total of 12 subcores were incubated for near-surface microbial growth-rate measurements. This essentially doubles the total number of measurements of this kind for the basin sediments. These results, when combined with those presented in the attached manuscript, will provide new constraints on the relationships between benthic microbial processes and sea floor fluxes of metabolic reactants and by-products.

In the coming year, we propose to continue to process the microbial incubation samples and advance the interpretation of them. Because of the large number of samples involved, the extractions and determinations will not be fully completed until the end of this year. Interpretation and write-up will then follow. We also intend to perform a variety of elemental analyses on the fine-scale sediment samples recovered during the January expedition. These analyses will be begun within the next month and will also be completed at approximately the end of the year.

**MICROBIAL UTILIZATION AND TURNOVER OF ORGANIC CARBON
IN SANTA MONICA BASIN SEDIMENTS**

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Abstract

Sediment microbial community biomass and activity in Santa Monica Basin, a nearshore basin in the California Continental Borderland, were examined in October 1985, 1986 and 1987, May 1986, April 1987 and January 1990. Millimeter-scale ATP profiles and incubation of intact cores with ^3H -adenine indicated a high-biomass interface microbial population in the low-oxygen central basin, which was absent in samples from the basin slope sediments. A majority of microbial activity and organic matter mineralization occurred in the top cm of sediment. Comparison of measured ATP and total organic carbon profiles suggest that the C:ATP ratio (wt:wt) ranges between 47:1 and 77:1 in central basin interfacial populations, substantially lower than reported for other aquatic environments. Carbon production estimated from DNA synthesis measurements via ^3H -adenine incorporation was compared with TCO_2 fluxes measured by *in situ* benthic chamber experiments. Within the uncertainty of the C:ATP ratio, an overall microbial carbon assimilation efficiency of 75-90% was indicated. The low C:ATP ratios and high carbon assimilation efficiencies significantly affect estimates of microbial growth and respiration and are substantially different than those often assumed in the literature. These results suggest that without independent knowledge of these ratios, the uncertainty in tracer-derived microbial growth and respiration rates may be larger than previously reported.

1. Introduction

The mineralization of detrital organic carbon and regeneration of inorganic nutrients in sediments are dominated by microbially-mediated reactions. Accurate assessment of growth and respiration in sediment microbial populations must be achieved before a clear understanding of the role of benthic microbial metabolism can emerge (Blackburn, 1987). Much recent research effort has been directed toward studying microbial processes in sediments, but methodological limitations and the complex nature of the sediment system have restricted progress. It is difficult to examine sediment populations without altering natural gradients and conditions and thereby altering natural rates.

The employment of radioactive tracers to examine the activity of a single subset of microorganisms, i.e., sulfate reducers (Jorgensen, 1978; Christensen, 1984) or methane oxidizers (Reeburgh, 1980; Alperin and Reeburgh, 1985), or the use of a single labile organic compound can provide useful information but of limited application when the questions pertain to the activity of the entire microbial community. While the metabolism of specific compounds can be assessed, it is not clear that utilization of any single compound or group of compounds mimics the utilization of natural substrates. Consequently, tracers intended to measure a community-wide process, such as nucleic acid synthesis, have been developed, primarily in the water column, to assess bacterial (Fuhrman and Azam, 1982) or microbial (Karl, 1981) growth. These techniques have also been applied to sediments (cf. Moriarty and Pollard, 1981, 1982; Fallon *et al.*, 1983; Findlay *et al.*, 1985 for thymidine; Craven and Karl, 1984; Craven *et al.*, 1986; Smith *et al.*, 1987; Novitsky and Karl, 1985, 1986 for adenine).

In this study, we have used [2,³H]-adenine to estimate the rate of DNA synthesis. We chose this tracer for several reasons. The specific radioactivity of ATP (which is in isotopic equilibrium with the immediate intracellular DNA precursor dATP; Karl, 1981) can be extracted and measured directly. The specific activity estimate is not biased by sediment adsorption. The radioactivity incorporated into DNA can be determined directly using a modified Schmidt-Thannhauser technique (Munro and Fleck, 1966; Craven and Karl, 1984) to separate RNA from DNA and avoid substantial contamination by labeled protein (via histidine synthesis from labeled ATP; Brittain and Karl, 1990). Also, the adenine is specifically labeled at the 2 position of the purine base so that the tritium is lost as ³H₂O if the adenine is catabolized or converted to guanine. Thus guanine production from labeled adenine and subsequent incorporation into DNA does not interfere with establishment of accurate precursor-product relationships. Finally, adenine is utilized via extant salvage pathways by bacteria, microalgae, fungi, and yeasts (Hochstadt, 1974). The hypothesis that assimilation of ³H-adenine by microorganisms <20 μm in size occurs in about the same relative proportion as their biomass in most mixed populations appears well-validated (Karl and Winn, 1986; Winn and Karl, 1986; Bell and Riemann, 1989). In addition, the utilization of adenine by anaerobic microorganisms has been directly addressed in the literature with pure cultures of two anaerobic heterotrophs (Karl *et al.*, 1987), where estimates of ATP pool turnover time via kinetics of labeling with ³H-adenine were highly correlated with measured generation time, and fit the same Mangelsdorf transformation plot as did results with pure cultures of aerobic heterotrophs and phototrophs. A variety of studies have also employed ³H-adenine in environments where anaerobic populations are significant or dominant. Novitsky and Karl (1985, 1986) measured nucleic acid

synthesis rates with ^3H -adenine in organic-rich, coastal marine sediments, where anoxic microniches with active populations of sulfate reducers are likely to occur within oxidized sediment layers (Jorgensen, 1977), while Karl and Knauer (1988) report adenine-based synthesis rates across the chemocline and into the anoxic portions of the Black Sea Water column. Thus, it appears that adenine is incorporated by a large portion of the benthic microbial community and that through direct measurement, product-precursor activity relationships can be accurately assessed.

To utilize tracer-derived DNA synthesis rates to estimate microbial growth and respiration rates, the DNA:cell carbon ratio and organic carbon assimilation efficiency must be known. Because of the complex and heterogeneous nature of sediment systems, the large and often dominating detrital organic carbon pool relative to living biomass carbon, and abundant, diverse mineral surfaces that may interfere with standard biochemical extraction procedures, most sediment studies do not independently determine these required ratios. Most often, values that were determined from pure laboratory cultures or from water-column studies are used.

In the following, we report independent estimates of benthic biomass derived from total organic carbon measurements, sedimentary ATP standing stock, microbial carbon production from DNA synthesis measurements, and benthic community respiration rates, based on *in situ* benthic CO_2 flux-chamber measurements in Santa Monica Basin. Slurry incubation at four isotope concentrations of the top 0-5 mm was performed to estimate the length of incubation and the concentration of adenine necessary to achieve optimum labeling. DNA synthesis measurements were performed at 2 mm depth intervals on intact sediment cores using ^3H -adenine to trace DNA production

rates and compared to results derived concurrently with divided cores. Comparison of the organic carbon production rates in intact cores with the measured benthic flux rates provides a direct measure of microbial community organic carbon incorporation efficiency.

2. Materials and Methods

2.1 Sample Collection

Santa Monica Basin is a flat-bottomed, steep-walled basin approximately 910 m deep in the California Continental Borderland (Fig. 1). Deep water circulation is restricted by the depth of the deepest sill at 737 m. The bottom waters contain approx. 5 μM oxygen, so macrobenthic organisms are generally absent and the central basin sediments are varved.

Sediments were collected with a Soutar box corer from Santa Monica Basin on the dates and at the locations given in Table 1. A summary of analyses performed is also included in Table 1. Subcores were collected in 50 ml plastic syringe barrels modified as described elsewhere (Jahnke *et al.*, 1986; Craven *et al.*, 1986) or in glass barrels of the same inner diameter as the plastic barrels, also beveled on one end to allow smooth insertion into the sediment. The subcores were collected so that 3-4 cm of overlying water remained above the sediment. Both core barrel types were capped at each end with syringe plungers and stored upright at 4°C until use, less than one hour.

2.2 Sediment Analyses

Sediments in plastic core barrels were used to measure total ATP biomass at 1- or 2-mm intervals, as described previously (Craven *et al.*, 1986), with cold 0.5 M H_3PO_4 + 1% Triton X100, v/v as extractant. Additional subcores were sectioned at the same intervals and placed in prebaked glass dram vials, dried with an IR lamp, capped and stored (October 1985 samples only). Sediment porosity was later determined by AgNO_3 titration of the deionized water-extractable Cl^- (Jahnke *et al.*, 1986). Total organic carbon was measured on a Hewlett-Packard Carbon-Hydrogen-Nitrogen Analyzer after treating the sediment with sufficient HCl to dissolve all calcium carbonate (Hedges and Sterns, 1984; Jahnke *et al.*, 1982).

The top 5 mm of sediment plus associated porewaters were collected from several cores and frozen for subsequent laboratory estimation of ATP adsorption (Station U only). Samples were thawed, mixed and dispensed in 0.2 ml aliquots, and chemical ATP was added over a 100-fold concentration range to replicates. Samples were mixed and incubated at 4°C. At one h and 16 h, one set of replicates was centrifuged and the pellets and supernatants were separately acidified with 1.5 ml 0.5 M H_3PO_4 . Extracts were then processed as ATP samples (Craven *et al.*, 1986).

2.3 Microbial Incubations

The top 0-5 mm were collected from several syringe cores (Station T) and divided into four aliquots, which were incubated with increasing concentrations of isotope ($[2,^3\text{H}]$ -adenine, New England Nuclear; diluted with cold adenine to provide a final specific activity of 1 Ci mmol⁻¹; 0.5 mCi ml⁻¹) and sampled in time series and extracted in cold 0.5 M H_3PO_4 (no Triton X100 for incubated samples). Glass syringe

cores were mounted on a labjack and ringstand, and the top plunger was removed, leaving 3-4 cm of water above the core top. A Hamilton repeater syringe assembly containing isotope was mounted above the core top, and the needle was inserted down the center of the core using the labjack to move the core upward until the deepest intended injection site was reached with the syringe needle tip. At 4-mm (October 1985) or 2-mm intervals (all other times), 10 μ l of isotope solution were injected and the labjack was then adjusted to move the core away from the needle tip to the next shallower interval. After injection, each core was recapped with the plunger, using care to exclude any air bubble between the water surface and the plunger. Injected cores were incubated at *in situ* temperature and one atmosphere pressure for 14-72 h. Incubations were terminated by sectioning the cores at 2-mm intervals in the same manner as the ATP cores and extracting the sections as for the slurry samples. Extracts were frozen until processing, at which time total and radiolabeled ATP were measured in the supernatant and radiolabeled DNA was separated from the particulates and measured as described elsewhere (Craven *et al.*, 1986). DNA synthesis rates were calculated from incorporation of label into DNA divided by integrated ATP specific radioactivity (Winn and Karl, 1984).

For comparison to previously-used methods and to examine the effect of physical disruption on sediment microbial populations, an additional subcore from three sites (B, C and E only) was sectioned at mm intervals and slices (approx. 0.53 cm³ each) were placed into Vacutainer tubes. Following an N₂ flush, 10 μ l of the ³H-adenine solution was added to each tube in addition to 2 or 3 drops of filtered seawater collected from above the core prior to sectioning, and incubations commenced at *in situ*

temperature after mixing. Extractant (cold 0.5 M H_3PO_4) was added to each tube to terminate the incubation. Storage and processing followed that described above for intact incubations. Station E samples were lost in processing.

2.4 Pore Water Sampling

Pore waters were extracted by a pressurized core barrel technique (Jahnke, 1988) from 8.9 cm diameter subcores removed from the Soutar box corer. Briefly, the sediment subcore was retrieved in a coring barrel that had sampling ports located at specific intervals along the length of the barrel wall. During subcoring the ports were sealed with nylon screws. After subcoring the box core, pistons were placed in both ends of the subcore-barrel, sealing the sediments in the coring tube. The nylon screws were removed from the depths at which pore water samples were to be retrieved, and a plastic syringe with prefilter was attached. The pistons were gently driven toward each other, pressurizing the sediment subcore and expelling the pore waters out of the sampling ports and into the plastic syringes. Once collected, the pore waters were filtered through 0.45 μm Gelman membrane filters and stored cold until analysis. All manipulations were performed at *in situ* temperatures.

2.5 Benthic Flux Chamber Experiments

In situ benthic chamber experiments were performed with the MANOP Bottom Lander in 1986 and the Benthic Experimental Chamber Instrument (BECI) in 1987 and 1988 (Jahnke and Christensen, 1989; Jahnke, 1990). A detailed description of the free-vehicle instruments is provided in the above references.

Briefly, the experiments were performed in 30 cm X 30 cm thin-walled titanium chambers. Once the chamber was emplaced in the surface sediments, the top of the chamber was sealed by a lid constructed of polyvinylidene fluoride, PVC and aluminum, trapping approximately 10 cm of bottom water above the sediments in the chamber. A stirring mechanism, attached to the chamber lid, mixed the chamber waters constantly throughout the experiment.

At preset time intervals, samples of the chamber waters were drawn into spring-loaded syringes or glass ampules (O_2 analyses only). Once the sample had been collected, the syringe plunger was locked into position, minimizing the possibility of exchange between samples and chamber waters during vehicle recovery. The volume of water removed during sampling was replaced by bottom water. This results in a small but significant dilution of chamber waters. The results presented here have been corrected for this dilution. At the termination of the experiment, a spade was closed to capture the sediments in the chamber and weights were jettisoned, permitting the vehicle to float to the sea surface for recovery by a surface ship.

2.6 Pore Water and Chamber Water Analyses

Pore water and chamber samples were analyzed for pH ($\pm 0.03\%$) by glass electrode (NBS Buffer scale) and for titration alkalinity (T.A.) ($\pm 0.5\%$) by Gran titration (Edmond, 1970). TCO_2 was calculated from pH and T.A. using the dissociation constants of Lyman (1956) and Mehrbach (1973). Pore water samples were also analyzed for SO_4^{2-} ($\pm 1.5\%$) by the turbidometric method of Tabatabai (1974), Fe^{2+} ($\pm 5\%$) by the ferrozine method (Stookey, 1970) and Mn^{2+} ($\pm 5\%$) by the dioxime method (Brewer and Spencer, 1971). Porewater aliquots (1 ml each) were acidified with 34 μ l

concentrated H_3PO_4 and processed as ATP samples (see Section 2.2), to estimate dissolved pore water ATP (Jan. 90 only).

3. Results

ATP was measured at 1- and 2-mm intervals at two slope stations (Stations B and E; Fig. 2a and b) and in the central basin (Station C; Fig. 2c). The two slope stations contained only slight gradients in ATP concentration with depth in the cores, and only modest near-surface enrichments. At Station B, the sediments were contorted and virtually no macrofauna were present, although the slope bottom waters are well-oxygenated, suggesting that the sediments slumped recently. Overlying water in the box core was clear, and there were no sediment features associated with the corer walls, suggesting that the sediments were not disturbed during the coring operation itself. The shallower slope station (E) contained an active macrofaunal population, and displayed more of a gradient in ATP than did the sediments of Station B.

ATP profiles in the central basin (Fig. 2c, d) were dominated by a surface layer of extremely high concentrations. The values indicate very high surface microbial biomass provided that abiotic pools of ATP such as dissolved or adsorbed fractions do not contribute significantly to the total concentration. Pore water ATP was below the limit of detection ($<2 \text{ ng ml}^{-1}$ detection limit for the sensitivity level required to measure surface sediment ATP; pore water measured at Station U only), indicating that dissolved ATP contributes $<0.1\%$ to the total ATP in surface sediments.

Several strategies were used to assess the potential contribution of ATP adsorbed onto sediment surfaces to the total measured. Mean total ATP for the 0-5 mm surface layer for all cores from Stations C, F, T, H and O was $3.65 \mu\text{g cm}^{-2}$ (Table 2) while

porewater ATP was less than 2 ng cm^{-3} . If adsorbed ATP contributed significantly to the total measured, the distribution ratio for adsorbed ATP to dissolved ATP must be greater than 1000:1 (or 3650:2). ATP adsorption experiments using freeze-thaw sterilized Santa Monica surface sediment (see Methods) showed no greater than 60:40 apportioning between sediment and porewater after 16 hours of contact. Consequently, it appears that adsorbed ATP which is extractable in H_3PO_4 accounted for little more of the total ATP signal than did porewater ATP, or $<0.1\%$. It is important to note that the proportions of dissolved vs. adsorbed extractable ATP were not dramatically different between the 1 hour and 16 hour sampling, suggesting that the sorption process had reached near steady-state conditions.

Although the relative distribution did not change significantly during the adsorption experiment, total extractable ATP did decrease significantly. Thus, enzymes that survive freezing were able to hydrolyze a major portion of the added ATP, further suggesting that adsorbed ATP cannot be an important contributor to the total values displayed in Figure 2.

The use of internal standards and the extraction procedures described in Craven *et al.* (1986) permit the assessment of ATP adsorption onto freshly precipitated iron oxyhydroxides. The naturally-occurring ferric oxyhydroxides are dissolved by the acid extraction procedures but re-precipitate when the extract is neutralized prior to analysis. Because we typically recover $>70\text{-}90\%$ of the added internal standards in the presence of this fresh precipitate, rapid adsorption cannot represent a significant loss of dissolved ATP.

Preserved surface samples (Station C, 0-2mm) examined with acridine orange

epifluorescence microscopy, were dominated by dense populations of a large filamentous microorganism. Lesser populations of a smaller filamentous microorganism were interspersed with the large species in the microscopy samples. Since the cores were sectioned at 2 mm intervals and each slice mixed with preservative, any *in situ* stratification of the two groups at less than 2 mm intervals would not be observed by microscopy. A variety of rods and cocci and some ciliates were also apparent as minor populations in the microscopic samples. Subsequent preserved samples of surface (0-2mm) sediments from Station U were stained with proflavin and 4'6-diamidino-2-phenylindole (DAPI) and sizes of the two dominant filamentous populations were estimated using an Olympus BH-2 epifluorescence microscope with a Hitachi DK-7000 microsurgical 3-chip color CCD image analyzer. The larger filamentous form was 3.1 μm in diameter (coefficient of variation (C.V) = 18.7%; n = 31) and individual filaments were found as long as 1350 μm . Entire cell contents appeared to be stained very heavily with the DNA-specific DAPI stain. Filaments occurred individually in the preserved samples, and were not observed in clumps, although they often appeared to be attached to or associated with detrital particles. The smaller filamentous form was 0.5 μm (C.V = 22.0%; n = 28) in diameter, with individual filaments as long as 1060 μm . The smaller organism was often observed in tangled, threadlike masses in the preserved samples, so that filament lengths could not be determined. Neither filament appeared to be sheathed.

The slope stations (A, B, E) contained much less TOC and displayed lower porosities than central basin stations (C and D) throughout the depth interval examined (Fig. 3). An important feature of the organic carbon profiles from the central basin was the change in slope observed in the upper 5 mm which corresponded to the

extremely high ATP surface layer concentrations.

Time series incubations of undiluted slurried surface sediment from the central basin (Station T) showed that additions of 1 nmol ^3H -adenine ml^{-1} slurry were too low to remain available for the time required to achieve isotopic equilibrium in the ATP pool (Fig. 4a; Winn and Karl, 1984). Added label was apparently utilized prior to the first time point (5 h), and production of $^3\text{H}_2\text{O}$ was not linear over 20h at the 1 nmol ml^{-1} addition, but was linear at 5-20 nmol ml^{-1} additions (Fig. 4b). Linearity of $^3\text{H}_2\text{O}$ production is a reasonable indicator of adequate tracer supply throughout an incubation.

DNA synthesis experiments were performed on intact syringe cores and syringe cores divided into mm intervals and incubated in test tubes (Stations A and C only; Fig. 5a-b). Measured synthesis rates decreased in a generally exponential fashion downcore, but the magnitude of production is considerably greater in the central basin (panels c and d; note scale change on Figure). Although four replicate cores were incubated intact and extracted as a time series at each site in October 1985 (B, C, E), it was found that virtually all added label had been utilized in the upper sediments by the second time point as indicated by a decrease in $^3\text{H}_2\text{O}$ production rate with time (data not presented). Estimates of synthesis made from incubations where exogenous label is depleted are inaccurate based on theoretical principles (see Winn and Karl, 1984). In the central basin cores (C), mass balance inventories of isotope pools also indicated that in the surface sediments, added tracer was utilized before intracellular isotopic equilibrium was achieved. Tracer additions for subsequent studies at the central basin site were adjusted such that complete utilization of tracer did not occur dur-

ing the incubation period and such that isotopic equilibrium was achieved in the precursor pools. Comparison of the October 1985 and later results (Fig. 5c and d) do not indicate major differences but in light of the above considerations, the October 1985 data were not included in carbon production calculations that follow.

Although no absolute rates can be derived from the October 1985 study, we present the results because they provide our only direct comparison of intact vs. divided incubations. The results indicate an underestimate of relative DNA synthesis in the slope station (B) and an overestimate in the central basin (C), particularly in the upper few mm. Since the slope population was predominantly aerobic (overlying water ~ 45 μ M oxygen), flushing with N₂ prior to incubation may have inhibited obligate aerobes and decreased the activities of facultative anaerobes. Mixing of stratified populations with new sources of nutrient via sectioning and homogenizing could be responsible for apparent stimulation of the top few mm in the central basin. Below the interfacial populations, DNA synthesis rates were very similar between methods.

Highest rates of DNA synthesis from intact cores from the central basin were measured in the upper few mm at all times studied (Fig. 5d), with measured rates decreasing downcore until they were not reliably above adsorptive background below 20 mm (measured October 1985 only). Rates could have been determined deeper in the cores if incubations had been continued for longer periods to increase labeling above background adsorption for the DNA fraction. The ATP fractions were labeled at all depths examined (down to 40 mm for October 85 only); their determination by methods employed here is not interfered with by residual adsorption of ³H-adenine onto the sediments, as is true with the DNA fraction.

The benthic flux chamber T.A. and TCO_2 results are presented in Figure 6. Geochemical interpretations are discussed in detail elsewhere (Jahnke, 1990). A measurable flux out of the sediments was observed in all cases. Because TCO_2 was calculated from pH and T.A. and different pH electrodes have been used through the course of this study, small variations in electrode calibration may account for the variability in TCO_2 values observed between expeditions. This uncertainty does not influence flux calculations, however, because fluxes are based on the relative concentration changes in chamber waters within a deployment. Pore water solute distributions are presented in Figure 7. The major profile features may be attributed to the heterotrophic breakdown of organic matter. Because bottom waters contain little O_2 ($\sim 5\mu\text{M}$), anoxic reactions dominate throughout the depths sampled, as may be inferred from the decrease in SO_4^{2-} and increase in T.A. and NH_4^+ with increasing sediment depth and the elevated concentrations of Fe^{2+} and Mn^{2+} throughout the profiles.

4. Discussion

4.1 Biomass Production Estimates

Tritium in the 2 position of the purine ring in adenine as employed here labels only the adenine base in DNA (Brittain and Karl, 1990), such that precursor-product relationships can be accurately established. Although confidence can therefore be placed in the relative accuracy of the rate of incorporation of labeled adenine into DNA, at least three additional kinds of information are required to provide an accurate estimate of carbon production and growth; 1) ATP:biomass carbon ratios; 2) biomass carbon:DNA ratios; and 3) the mole percent of adenine in DNA of microbial popula-

tions studied with ^3H -adenine as a tracer.

An accurate biomass carbon:DNA ratio of the microbial community must be known to estimate microbial carbon production from measured DNA synthesis rates. C:DNA values have frequently been inferred from measured C:ATP or DNA:ATP ratios. A C:ATP weight ratio of 250:1 has been reported or inferred for a wide range of planktonic (Karl, 1980, 1986 and references therein; Laws *et al.*, 1984; Costa-Pierce and Craven, 1987), and sedimentary environments (Karl *et al.*, 1987; Novitsky, 1987). However, living biomass carbon estimated using this ratio and the measured ATP content of the top 5 mm of central basin sediments accounts for approximately 40% [$(3.65 \times 10^{-6} \text{ g cm}^{-2} \times 250) = 9.13 \times 10^{-4} \text{ g C}$; $9.13 \times 10^{-4} / 2.29 \times 10^{-3} = 39.8\%$; see Table 2] of the measured total organic carbon. We could speculate that the nonliving mass in the surface layer was primarily carbon-free, composed of native sulfur and iron oxides, by-products of metabolism of sulfide (and possibly iron) oxidizers, but bulk chemical analysis indicates that the top 4 mm is composed of 10-20% each of calcium carbonate, silica and iron, 3-6% each of magnesium and aluminum, and only minor amounts (<1%) of sulfur (Finney and Huh, 1989). Additionally, if 40% of the total organic carbon at the sediment surface was in living biomass, the detrital organic carbon content of the surface sediments would be approximately 3% by weight, less than that measured at 5-10 mm depth. While the input of sewage to the basin has changed in the last 10 years, the majority of the organic matter reaching the seafloor is derived from surface water productivity, for which no consistent change has been observed (Jackson *et al.*, 1989; Eppley and Holm-Hansen, 1986). It is therefore unlikely that the sediment surface could be depleted in detrital organic carbon to such an extent. Although the discrepancy could arise from dissolved or adsorbed ATP, (Azam and

Hodson, 1977; Nawrocki and Karl, 1989) we consider that such an artifact is unlikely in this case, based on the porewater and adsorbed ATP results presented previously.

Therefore, it appears that a biomass carbon:ATP ratio less than 250:1 is required to explain the observed results. To approximate the C:ATP ratio of the microbial community in 0-5 mm depth, we estimated the detrital organic carbon content of the surface 5 mm in two ways. For a maximum estimate, we assume that the organic carbon content at 5 mm represents primarily detrital carbon (i.e., biomass C is low relative to TOC) and that it is constant throughout the upper 5 mm (represented by the dotted lines in Fig. 3c and d). Thus, the difference (after correction for differences in porosity) between this value and those at shallower sediment depths is a measure of living biomass carbon. For a minimum estimate, the trends in the organic carbon profile from 1.5 to 0.5 cm were linearly extrapolated to the surface (dashed lines in Fig. 3c and d) and the difference between the extrapolated and measured value was assumed to represent living carbon. The range of estimates of the C:ATP ratio of the surface microbial community are listed in Table 2.

Biomass production estimates also require knowledge of the DNA:ATP ratio of the community. Jeffrey and Paul (1986a and b) report a DNA:ATP ratio range from approximately 3:1 to 10:1 in natural marine planktonic microbial populations. The influence of the C:ATP and DNA:ATP ratios on calculated carbon production is displayed in Figure 8. Using the mean DNA synthesis rate for the four experiments in Santa Monica Basin surface sediments (calculated from data in Figure 4d), the biomass carbon production rate is estimated for the indicated values of C:ATP and DNA:ATP. The values included within the DNA:ATP range quoted above and our estimated

C:ATP range are indicated by the shaded area and result in biomass carbon production rates that range from 9-50 mmol C m⁻²d⁻¹. Note that a carbon production rate of 50-164 mmol C m⁻²d⁻¹ would be obtained if the frequently cited C:ATP ratio of 250:1 had been used in the calculation. Without independent knowledge of these conversion factors a large range of results are possible.

Results from earlier studies measuring carbon production are often defended on the premise that since reasonable growth rates were measured, the carbon production estimates must also be reasonable. However, growth rate is carbon production per unit carbon biomass, such that any error in extrapolation from the measured quantity to carbon essentially cancels out in the growth rate calculation. The error remains in the estimate of production alone. For an example of the extreme cases, growth rates calculated from our DNA synthesis and ATP results would be identical whether C:ATP is 250:1 or 47:1 (assuming a constant DNA:ATP ratio), since the ratio of ATP to carbon occurs in both numerator and denominator. However, calculated production would be 5.3 times greater at a C:ATP of 250:1 than at 47:1.

A third criterion required for estimating accurate production rates is information about the relative mole percent of adenine in the DNA of the majority of the microbial populations incubated. An adenine mole percent of 25% has been employed in the absence of additional information (see Karl, 1981 and references therein), and Figure 8 was generated using that value. The Santa Monica surface sediment microbial population is dominated by the two filamentous bacterial types described earlier, so that the adenine mole percents of the two groups would represent a major portion of the biomass. Based on filament width and length, visual (microscopic) appearance and

habitat, the larger organism is tentatively identified as *Desulfonema limicola* (Pfennig *et al.*, 1981), while the smaller filamentous form is possibly a *Beggiatoa* species (Wiessner, 1981). Mole % of G + C of *Beggiatoa* species examined to date is 37-51% (Mandel and Lewin, 1969; Strohl, 1989), making adenine mole % approximately 25-32%. G + C mole percent for *Desulfonema limicola* is 34.5% (Pfennig *et al.*, 1981; Widdel *et al.*, 1983), so adenine mole % is approx. 33 mole %. A change in the mole % value used from 25 to 33 would decrease our calculated DNA synthesis rates by 25%.

We presently have no concrete justification for using a mole percent value different from that recommended in previous studies employing adenine as a nucleic acid precursor (cf. Karl, 1981; Karl and Winn, 1984). We have not yet been successful in culturing the filamentous organisms in order to directly measure their DNA:carbon, ATP:carbon, and adenine mole percent compositions.

4.2 Respiration and Utilization Efficiency

TCO₂ benthic fluxes measured at this same site (Figure 5) can be compared to production rates to assess community incorporation efficiency. CaCO₃ dissolution does not contribute significantly to the TCO₂ benthic flux in Santa Monica Basin (Jahnke, 1990) and measured fluxes are assumed to be the result of respiration.

Benthic fluxes represent the total sum of reactions occurring within the sediment column whereas our DNA synthesis incubations were restricted to the upper 2 cm. To compare the resulting data, therefore, it is first necessary to subtract the contribution of reactions deeper than 2 cm to the TCO₂ flux. The diffusive pore water flux of TCO₂

at 2 cm depth was calculated from the observed gradient (Fig. 7) and the effective diffusion coefficient ($D_{\text{eff}} = D_{\text{mol}} \Phi^2$) using the molecular diffusion coefficient for HCO_3^- reported by Li and Gregory (1974). Calculated carbon production was also corrected for potential chemolithotrophic carbon uptake (Table 3), since autotrophic production consumes CO_2 . This correction was estimated by calculating the maximum amount of organic carbon (as CH_2O) that could be produced from the oxidation of the four major reduced species potentially present in the surface sediments and pore waters, Fe^{2+} , Mn^{2+} , NH_4^+ and S^{2-} . Maximum oxidation rates of Fe^{2+} , Mn^{2+} and NH_4^+ in the surface layer were estimated from the pore water flux calculated from the measured near-surface gradients (Fig. 7), assuming molecular diffusivities that had been corrected for use in sediments via the Archie relationship and the measured surface porosities (Table 3).

The potential contribution of S^{2-} oxidation to autotrophic production is more difficult to estimate. Free HS^- was not detectable ($< 1 \mu\text{M}$) in the pore waters, most probably due to the high concentration of Fe^{2+} and formation of FeS in surface sediments. As an initial estimate, we adopt the integrated SO_4^{2-} reduction rate reported by Jahnke (1990) based on the observed mean pore water gradient. In bioturbated sediments, SO_4^{2-} reduction rates can be larger than pore water gradients would indicate due to the oxidation of reduced S to SO_4^{2-} . Santa Monica Basin sediments are not bioturbated, however. Also, the total flux of oxidants (O_2 , NH_4^+ and SO_4^{2-}) into the sediments is consistent with measured CO_2 fluxes, indicating no unaccounted oxidant flux that would be required if SO_4^{2-} was being reduced and re-oxidized in the sediments at a significantly greater rate than revealed by the pore water profile.

Assuming that the products were completely oxidized and that the chemoautotrophically fixed carbon was reduced to the level of carbohydrate, we estimate the potential autotrophic production in the surface layer (Table 3). This is clearly a maximum estimate because no metabolic losses are included. This calculation suggests an estimated maximum heterotrophic CO_2 production rate of $2.98 \text{ mmol m}^{-2}\text{d}^{-1}$ in the surface layer. Incorporation efficiency contours that are consistent with this estimated respiration rate are included in Figure 8. Obviously, as production rate increases, the organisms must become more efficient to keep the respiration rate at the measured value.

It is clear from Figure 8 that if our DNA synthesis rates are valid, an estimated C:ATP range is more realistic for this environment than is C:ATP of 250:1, which would require theoretically impossible assimilation efficiencies much greater than 90% (Calow, 1977), or extremely high DNA:ATP ratios. The C:ATP and DNA:ATP ratios used here produce a range in DNA of approximately 4-21% of cell carbon. The DNA:C ratio of marine microorganisms has been the subject of ongoing debate with DNA reported to range from 1-43% of cell carbon (cf. 1-3%: Holm Hansen, 1969; 6-43%: Paul and Carlson, 1984; ~1-17%: Paul *et al.*, 1985). Our estimates fall between the extremes. The filamentous microorganisms dominating the surface sediment biomass stained extremely brightly with DAPI, which is DNA-specific, to the extent that the individual cells appeared to be filled with stained material. We have no evidence other than such qualitative visual observations to suggest that the filamentous forms may be DNA-enriched relative to other marine microbial populations.

The shaded area in Figure 8 is the range of carbon production bracketed by our

C:ATP range and the DNA:ATP range of Jeffrey and Paul (1986a and b) for natural samples. Within that overlap, carbon utilization efficiency ranges from approximately 75-94%. If the DNA:ATP ratio is restricted to values ≥ 5 , maximum required efficiencies are lowered to $\leq 90\%$, which fit relatively well with the results of several studies measuring efficiencies exceeding 80% for utilization of simple labile organics by sediment microbial populations (Griffiths *et al.*, 1983; Benner *et al.*, 1986; Wirsen and Jannasch, 1986; Novitsky and Karl, 1985, 1986). If our speculation about the high adenine mole percent in the DNA of the filamentous populations is borne out by further study, then calculated efficiencies would decrease by several percent. Since microbial populations appear to be inefficient primarily under conditions of nutrient limitation, particularly N limitation (Calow, 1977; Goldman *et al.*, 1987; Linley and Newell, 1984; Griffiths *et al.*, 1983), it is reasonable to expect that the community studied in the ammonium- and dissolved organic nitrogen-rich Santa Monica Basin sediments would be relatively efficient.

From the results we conclude that most of the microbial activity and organic matter mineralization occurs in the upper one cm of the sediment column. Comparison of sediment ATP and TOC suggests that the commonly-employed C:ATP ratio of 250:1 is too large for this environment and we suggest a ratio between 47:1 and 77:1. With the uncertainty of this ratio in mind, the measured CO_2 and biomass production rates suggest an overall microbial assimilation efficiency of ca. 75-90%. Note that this apparently 'reasonable' range of assimilation efficiencies covers a threefold range in carbon production (9.4-28.1 mmol C m⁻²d⁻¹). The ability to bracket even that broad a range arises only because we have independent estimates of CO_2 produc-

tion and C:ATP ratio in these sediments. Within these constraints, the microbial and geochemical results provide an internally consistent description of the fate and turnover of detrital organic carbon reaching the Santa Monica Basin sea floor. However, if further work reveals C:ATP ratios and/or assimilation efficiencies significantly different from the above estimates, then the benthic flux and DNA synthesis measurements cannot be reconciled.

Clearly, this work demonstrates the extreme sensitivity of tracer-derived production and respiration rates to variations in C:ATP ratio, C:DNA ratio and assimilation efficiency and provides evidence suggesting that in Santa Monica Basin these parameters are different from those commonly assumed by other investigators. In light of this, we conclude that estimates of carbon turnover reported in studies where independent determinations of the necessary conversion factors were not made may be subject to extremely large errors. While DNA synthesis rate measurements by themselves are useful for comparison of sediment communities, the uncertainties in carbon turnover rates associated with potential unknown variations in conversion factors preclude the current use of these studies for understanding sea floor organic carbon dynamics. It is evident that in the absence of information about cellular C:ATP and C:DNA ratios and carbon utilization efficiency, the measurement of DNA synthesis has current limited application. Comparison of relative variations within or among sediment communities may be made using DNA synthesis rate as the metric; however, more rigorous interpretations should be approached cautiously. This conclusion applies to the measurement of any cellular process or constituent and not only to the ^3H -adenine-based DNA synthesis measurement employed here. Unless the measured parameter can be reliably extrapolated to carbon produced or mineralized, only very broad and

generalized information of geochemical significance is produced.

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Figure Legends

Figure 1. Map of Santa Monica Basin with boxcore locations labeled as given in Table 1. Depth is in fathoms.

Figure 2. ATP concentration determined at one (■) and two (O) mm intervals at three locations in Santa Monica Basin, October 1985 (a = Station B; b = Station E; c = Station C)) and at two mm only in the central basin (d) in October 1985 (■ = Station C), May (● = Station F) and October (O = Station T) 1986, April (Δ = Station H) and October (□ = O) 1987. Letters correspond to samplings listed in Table 1.

Figure 3. Total organic carbon in weight percent and sediment porosity at mm intervals at five locations in Santa Monica Basin, October 1985.

Figure 4. ATP specific radioactivity (a) and $^3\text{H}_2\text{O}$ production (b) in sediment slurry from Station T at additions of 1 (□), 5 (O), 10 (●) and 20 (Δ) nmol ml⁻¹ slurry.

Figure 5. DNA synthesis measured at two-mm intervals in intact (□) or divided (O) cores from three locations in Santa Monica Basin, October 1985 (a = Station B; b = Station E; c = Station C) and in intact cores only the central basin (d). Symbols for 5d are as given in Fig. 2d.

Figure 6. Benthic fluxes of TCO_2 (□) and titration alkalinity (●) in central Santa Monica Basin as determined with a benthic chamber (Jahnke and Christensen, 1989).

Figure 7. Porewater distributions of TCO_2 , pH, ammonium, sulfate, manganese and iron in central Santa Monica Basin. All porewaters were collected via pressurized core unless indicated. □ = Station F; O = Station H, centrifuged; ■ = Station H;

Δ = Station I; $\bullet$ = Station L; ∇ = Station O.

Figure 8. Computer-generated plot of carbon production and assimilation efficiency for measured DNA synthesis rate ($1.99 \text{ mmol DNA} \cdot \text{C m}^{-2} \text{d}^{-1}$) over a DNA:ATP ratio range of 1-20 and a C:ATP ratio range of 30-105. Shaded area is the range enclosed by our measured C:ATP and DNA:ATP range of Jeffrey and Paul (1986a and b). Three-dimensional graphics from Surfer (Golden Software, Inc.)

Fig. 1

Fig 2

Fig 3

ATP specific radioactivity

μ Ci tritiated water

Fig. 4

DNA synthesis ($\text{pmol h}^{-1} \text{g}^{-1}$)

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Table 1

Station location, depth, and summary of analyses performed

Designation	Date	Box Core I.D.	Station Location	Depth, m	Analyses ^a
A	Oct. 85	BC 12	33°50'N 118°41'W	750	TOC, Φ
B		BC 40	33°51'N 118°38'W	398	ATP, II, DI, TOC, Φ
C		BC 89	33°41'N 118°56'W	908	ATP, II, DI, TOC, Φ
D		BC 102	33°45'N 118°50'W	908	TOC, Φ
E		BC 113	33°51'N 118°39'W	572	ATP, II, DI, TOC, Φ
F	May 86	BC 1A	33°41'N 118°51'W	908	ATP, II, PW, TSS
T	Oct. 86	BC 3	33°45'N 118°54'W	908	ATP, II
H	Apr. 87	BC 1B	33°44'N 118°49'W	910	ATP, II, PW
I	Apr. 87	BC 4H	33°46'N 118°48'W	907	PW
L	Sept. 87	BC 2	33°44'N 118°49'W	907	PW
O	Oct. 87	BC 1C	33°44'N 118°49'W	908	ATP, II, PW
U	Jan. 90	BC 1	33°45'N 118°51'W	908	dATP, adATP

^aATP = ATP at 1 or 2 mm interval; II = intact incubation; DI = divided incubation; TOC = total organic carbon; Φ = porosity; dATP = dissolved ATP in porewaters; adATP = adsorbed ATP; PW = pore water solutes; TSS = time series slurry.

Table 2

Living biomass carbon in surface sediments of central Santa Monica Basin

Station	ATP, g X 10 ⁻⁶ cm ⁻² in 0-5 mm
C	5.01
F	2.14
T	2.97
H	4.98
O	3.17
$\bar{X}$	3.65

Station	Organic Carbon, g X 10 ⁻³ cm ⁻² in 0-5 mm		
	Total	Excursion Min	Max
C	2.10	0.148	0.230
D	2.47	0.191	0.334
$\bar{X}$	2.29	0.170	0.282

Excursion C: ATP

$$\text{min} \quad \frac{1.70 \times 10^{-4} \text{ g C}}{3.65 \times 10^{-6} \text{ g ATP}} = 47:1$$

$$\text{max} \quad \frac{2.82 \times 10^{-4} \text{ g C}}{3.65 \times 10^{-6} \text{ g ATP}} = 77:1$$

Table 3

**Calculation of chemolithotrophic consumption and
heterotrophic respiration of CO₂**

Reactants	Products	$\Delta\text{Reactant}:\Delta\text{C}_{\text{org}}$	Flux, mmol m ⁻² ·d ⁻¹ ^a
HCO ₃ ⁻ , Fe ²⁺	CH ₂ O, Fe ³⁺	4:1	0.133
HCO ₃ ⁻ , Mn ²⁺	CH ₂ O, Mn ⁺⁴	2:1	0.056
CO ₂ , S ²⁻	CH ₂ O, SO ₄ ²⁻	1:2	0.660
NH ₄ ⁺ , CO ₂	CH ₂ O, N ₂	4:3	0.004
Maximum chemolithotrophic uptake			0.85
Net CO ₂ produced (0-20 mm)			2.13
Total heterotrophic CO ₂ release			2.98

^aCalculations include measured porosity Φ at 0-20 mm depth = 0.93; effective diffusion coefficients $\times 10^{-6}\text{cm}^2\text{s}^{-1}$ at 5°C: $D_{\text{Fe}} = 4.08$; $D_{\text{Mn}} = 3.80$; $D_{\text{NH}_4} = 11.7$ and NH₄⁺ adsorption coefficient $K = 1.3$ ($D/(1+K) = 0.44 \text{ cm}^2\text{d}^{-1}$).

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