2003

# The effects of sediment grain size and oil exploration on microbial ATP biomass

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# THE EFFECTS OF SEDIMENT GRAIN SIZE AND OIL EXPLORATION ON MICROBIAL ATP BIOMASS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Oceanography and Coastal Sciences

by

Eric Tyson Guilbeau
B.S. Louisiana State University and Agricultural and Mechanical College, 2001
December 2003

The ultimate thanks go to my wife, Leah Guilbeau and my daughter Isabella Guilbeau. You are my best friends and the strength from which I stood on when confronted with the many obstacles associated with this thesis and in life. You have been my inspiration everyday. I love you.

## ACKNOWLEDGEMENTS

I would like to express my sincere and appreciative thanks to my committee: Dr. Paul A. LaRock, Dr. Charles A. Wilson, and Dr. Vincent L. Wilson. Without their patience, aid and guidance this thesis would never have been made to completion. They were always available and eager to advance my understanding of this subject and of the intricacies of life itself. I owe you more than I can return; I am forever in your debt.

For being subjected to long hours of sometimes-pointless conversations, I owe a special thanks to my lab partner and research cohort Neal Satterly. He was always there for idea bouncing and musical enjoyment. Neal, a driven man, I expect great things from you.

I would also like to express my appreciation to:

- The Minerals Management Service for their funding through the Continental Shelf associates.
- Dr. John Trefry at Florida Atlantic University for conducting the trace metals and oxygen analysis.
- Dr. Wayne Isphording at the University of South Alabama for the use of his sediment grain-size data.

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## ABSTRACT

Adenosine triphosphate (ATP) is a unique biochemical indicator of active microbial biomass and its relationship to environmental conditions. Its assay in sediments is complicated, however, by adsorptive loss to the sediment matrix and subsequent interferences in the luciferin-luciferase assay by compounds released during the extraction process. Corrections must be applied to correct for these losses and we describe a novel approach using radioactive ATP to correct for ATP adsorption. The sediment matrix also plays a significant role in determining both the magnitude of the ATP pool and the extent of the ATP adsorptive loss. Coarser sediments were found to have greater ATP levels and little adsorption, whereas silts and clays had significantly lower ATP levels and up to 95% adsorptive loss. Application of the ATP assay to very fine-grained marine sediments in off shore oil producing areas revealed a sedimentary ATP biomass of approximately 40ngg$^{-1}$ prior to oil development but after drilling the ATP level dropped 10 to 15 fold to 2 to 3 ngg$^{-1}$. Post-drill sediments contained high levels of barium which is associated with drilling fluids, and had no detectable oxygen at a depth of 3mm, and the mean grain size decreased indicating the bottom was being coated over by the drill spoils.

# INTRODUCTION

The nature and magnitude of the sedimentary microbial community has been the subject of a number of studies. The initial approach to quantify sedimentary bacteria was to separate them from the sediment matrix and count the resultant cells using epifluorescent microscopy. A consensus had developed that the size of the bacterial community was inversely proportional to sediment grain size (Dale, 1974: DeFlaun and Mayer, 1983: Yamamoto and Lopez, 1985), and that it also varied directly with organic carbon. The reasoning for the increase in bacterial numbers with decreasing grain size was the greater availability of sediment surface area for colonization. Early experiments at sediment oxygen uptake indicated that more oxygen was consumed by small grain-size sediments than by coarser materials. However, the experiment was carried out by suspending less than a gram of material into 28ml BOD bottles and following oxygen loss (Hargrave, 1972). This approach ignores *in situ* conditions and the fact that very fine materials may have more pore space, but the pores are so small that flow into the sediments is greatly inhibited. This condition limits the introduction of nutrients and oxygen into the sediment matrix (Maier et al., 2000). The work of Yamamoto and Lopez (1985) revealed that increased surface was only one aspect of microbial colonization, and recognized that the size and shape of sediment particles affect sediment packing. Larger more sorted particles, as found in sands, would actually make nutrients more available to the sedimentary bacterial community. Both DeFlaun and Mayer (1983) and Yamamoto and Lopez (1985) noted that clays were a poor substrate for bacterial attachment.

Biochemical approaches were subsequently employed to probe the microbial activities in sediments. One such example was the fine work of Koster and Meyer-Reil (2001) who experimented with shallow coastal sediments whose compositions varied from sand to sand-mud mixtures. Measurements of phospholipid, chlorophyll a and ATP were made to assess structural components, photosynthetic producers and physiologically active biomass respectively. Parallel determinations of total carbon and nitrogen were carried out to assess the relative availability of nutrient resources based on the notion that sediments with low C:N ratios indcated the presence of easly available DOC, or new material, whereas high C:N ratios indicated the presence of resilient compounds. Their results indicated that in sandy sediments both the phospholipid and ATP were high, but as the sediments became progressively more muddy (finer grain size) the phospholipid became the dominant biomarker (accumulated) but ATP decreased significantly. Similarly, the carbon analysis revealed that available DOC was a large fraction (about 25%) of the total organic pool in the sandy sedimentes, but as the sediment mud content increased the available DOC fraction became an absolute minor component of the carbon pool. In sum, Koster and Meyer-Reil found that as sediment grain size decreased available carbon resources were diminished, and that compounds that are microbial structural components accumulated but the community itself was less physiologically active.

The ATP assay offers considerable advantage over conventional microbiological methods when assessing the effect of the environment on microbial communities because

it is non-cultural, avoids the issue of nutrient limitations and reflects the *in situ* properties of the community. ATP is present in physiologically active cells and thus offers a means of assessing the relationship between the microbial community and the suitability of the prevailing environment. Environmental ATP determinations can, therefore, provide quantification of the effects of toxicants, nutrient enrichment or other alteration of the environment. A few of the more recent applications of ATP technology have been to examine issues in marine ecology (Karl, 1986; Karl, 1993; Bjorkman and Karl, 2001), to study the effects of nutrient loading in coastal waters (Malin et al., 2001), to quantify hydrothermal communities (Atkinson et al., 2000), to assess river water and sediment quality (Dutka et al., 1991), to follow the toxic effects of zinc released from trout farms (Martinez-Tabche et al., 2000), to determine the toxicity of pollutants on waste water treatment (Dalzell and Christofi, 2002; Dalzell et al. 2002), and to assess the effects of wastewater-borne heavy metals in mangroves (Yim and Tam, 1999).

Conceptually the ATP assay can be divided into two components, the initial extraction and the subsequent laboratory assay of the ATP using the luciferin-luciferase reaction. The earliest efforts of a quantitative ATP assay applied to marine materials was done by Holm-Hansen and Booth (1966) using boiling TRIS buffer to extract the ATP from water samples and stabilize it for optimum measurement. Boiling extractants, however, do not work with sediments because thermal gradients are set up and the necessary temperatures needed to stabilize the ATP are not reached. Subsequently Karl and LaRock (1976) used cold sulfuric acid as an extractant while working with soil and marine sediments to avoid the problems caused by heating. Subsequent modifications to the extraction process were made by Karl (1993) who substituted phosphoric acid as the extracting medium.

As the ATP assay became more widespread it became evident that there were substances that resulted in a loss of ATP during extraction and had to be compensated for. It was shown that suspended material in water samples, for example, was capable of reducing ATP quantification by either affecting the extraction efficiency or by inhibiting the luciferin-luciferase reaction (Sutcliffe and Orr, 1976; Lee et al., 1971). Sutcliffe and Orr (1976) found that high levels of suspended particles reduced the yield of ATP by as much as 50 to 66 percent of that known to be in the sample. Efforts were made to compensate for this adsorptive loss by diluting the sample thus reducing the particulate load (Sutcliffe and Orr, 1976; Lee et al., 1971), but more importantly the findings raised an awareness for the need to correct for adsorptive ATP loss.

The nature of the ATP extractant also plays a role in the overall efficacy of the determination. Bancroft et al. (1976) suggested that extractant interactions with the individual sample must be considered especially when using an acid as this promotes the mobilization of otherwise insoluble ions. This brings up the second type of difficulty encountered with the ATP assay, that ionic interference. To address this issue EDTA was initially used to complex cations (Karl and and LaRock, 1976), but Bancroft et al. (1976) indicated that EDTA addition was a time-consuming step that lost an additional 25 percent of the sample ATP and consequently it is no longer used. Along these lines, Cunningham and Wetzel (1978) found that samples that were acid extracted had a

problem with fulvic acids that accounted for a loss of 70 to 80 percent of the sample ATP. These acid soluble organic compounds sequester and bound charged species including ATP, and in the case of fulvic acids the inhibitory effect was not reversible. The preceding discussion serves to illustrate the importance of establishing a protocol that incorporates ATP standards that allow correction for the all the potential losses encountered along the way. An obvious mechanism is to prepare replicate samples that contain a known ATP standard and note the efficiency of recovery. This necessitates multiple samples and perhaps a range of ATP standards in order to cover all possibilities (Koster and Meyer-Reil, 2001). The use of a radioactive ATP standard circumvents the need for duplicates as will be demonstrated later in this publication.

Once the ATP is extracted and quantified how does one use the findings to interpret ecological events? ATP values have been translated to organic carbon content of the sample using a value of 250 to 1 for the ratio of carbon to ATP (Karl, 1980). This ratio might well serve as an average value but in actuality it is a function of growth phase and the effects of external stresses. In chemostat experiments (LaRock et al., 1988) active cells were found to have an ATP content of 1.3 fg each. When the cells were allowed to enter into stationery or resting phase, however, the ATP concentration dropped by ten to twenty-fold. Tuovila et al.(1987) also reported that intracellular ATP values varied considerably when bacteria were exposed to stresses caused by UV radiation and hypersaline conditions. The increased ATP levels presumably were used for repair of DNA damage or to adjust internal osmolarity. When instances such as this occur the ATP to carbon will vary considerably and for this reason we have chosen to report our findings in terms of the actual ATP content rather than to translate them into some other format that is not directly interpretable.

The work we now report deals with the effects of oil development on the sedimentary microbial community using ATP determinations as the primary analytical tool. Given the work schedule, the number of samples to be processed, the chemical nature of the material and the very fine muds encountered, it became necessary to refine the ATP assay protocol and assess the effects of sediment grain size on ATP recovery. As a result of these considerations we developed a rapid and sensitive means of assessing adsorptive loss in each sample processed. We also investigated the effects of sediment grain size on the sedimentary community and the changes mediated by oil exploration.

## MATERIALS AND METHODS

*ATP Analysis*: The ATP was extracted using 0.6 or 1.25N sulfuric acid as previously described (Karl and LaRock, 1976) since phosphoric acid resulted in copious precipate. The major alteration in the technique was separate corrections for adsorptive loss encountered during sediment extraction and subsequent interference during the assay procedure. A one-quarter square meter box core was used to obtain the sediment from the sea floor. Next the top two cm of sediment were collected for a total volume of 60 cc which was then quickly stirred, and sub-sampled using syringe corers. Two cc of sediment was added to each of four centrifuge tubes (15 x 100 mm) and 5 ml of 0.6N sulfuric acid and 0.1 ml of $^{14}$C-ATP (total activity 10,000 dpm, NEC-417 or equivalent, New England Nuclear/Perkin-Elmer, Boston, MA). The mixtures were vortexed for 5 min, centrifuged in a clinical centrifuge until settled (generally 15 min) and 4 ml withdrawn to be adjusted to pH 7.8 with a graded series of sodium hydroxide solutions. The volume was then brought up to 7.0ml with TRIZMA 7.8 (Sigma-Aldrich Chemical Co., St. Louis, MO) and the extract frozen for subsequent laboratory analysis. The addition of a radioactive ATP internal standard allowed every sample to be corrected for adsorptive losses, which was a critical concern when working with very fine-grained sediments.

The ATP was measured by adding 1 ml of the extract to a 22 ml scintillation vial along with 0.5 ml TRIZMA 7.8 buffer to which 200 μl of luciferin-luciferase mixture (FL-AAM ATP kit, Sigma-Aldrich Chemical Co., St. Louis, MO) was injected. After a 30 second delay the emitted light was detected using a Turner 20e Luminometer (Turner Industries Inc., Sunnyvale, CA) and the ATP determined relative to standards run with each enzyme preparation. Substances interfering with the assay were corrected for by recounting each sample and substituting a 10 to 50 ng ATP standard in 0.5 ml of TRIZMA for the plain TRIZMA used in the initial count. Adsorptive loss was corrected by determining the $^{14}$C-ATP recovered in the extract relative to that which was added. In all calculations careful attention was paid to all the dilution factors used in the various steps of the process.

*Grain Size Analysis*: Particle size distribution was carried out by combined sieve and hydrometer methodologies as specified in ASTM D-422. The process entails drying the sediment sample in air or oven at 110$^{o}$ C and weighing approximately 40 g in a Mason Jar containing 300 ml distilled water. Next, 20 ml of a 10% sodium hexametaphosphate solution was added, the suspension stirred and allowed to stand over night. The sample was transferred to a soils stirrer on medium setting for 5 min then poured into a 1 liter hydrometer cylinder which was then brought up to a volume of approximately 800-900 ml. After standing for 6 hours the volume was brought to 1 liter if there was no flocculation. The sample was vigorously stirred and hydrometer readings were taken with a 152H ASTM hydrometer at intervals of 2, 5, 15, 60, 240, 720 and 1200 min. Following the 1200 min. reading the sample was then washed through a 230 mesh (62.5 micron) or 270 mesh (53 micron) sieve and the retained material transferred to a drying pan, dried and then subjected to a standard sieve analysis.

## RESULTS

The use of ATP is a powerful means of determining factors affecting microbial biomass. There are, however, corrections that must be applied to overcome inaccuracies that are associated with both the extraction of the ATP from the sediments and its subsequent assay by the luciferin-luciferase method. One approach to correct for these interferences is to add an internal standard to a second set of replicates and determine the recovery after subtracting the actual sample ATP from the sample plus standard mixture. This approach essentially doubles the workload, and the ultimate accuracy of the correction depends upon the number of replicates used. For this reason we decided it would be better to differentiate between the adsorptive loss in the extraction process and the interference encountered during the actual ATP assay itself.

The approach we used to determine adsorptive loss was to add radioactive ATP, either $^{14}C$ or $^{3}H$ labeled, to every sample and calculate an extraction efficiency as the difference between the added and recovered radiolabeled standard. The use of a radiolabeled material allows every sample to be treated, is the more sensitive approach, and does not add measurable ATP to the sample. To demonstrate the efficacy of this approach we conducted a series of experiments in which a stable internal ATP standard was added to one set of samples (done in triplicate) and $^{14}C$-ATP to a second set of samples (also done in triplicate) and the recovery of each standard determined. The results of this experiment are shown in Fig. 1, and reveal close agreement between the two approaches ($r^2 = 0.82$), but the stable ATP standard has a recovery that is only about 80% of that determined by the use of the radioisotope. We attribute the difference to the increased sensitivity of the radioassay and the fact that isotope detection is not affected by potential ionic interference as might be encountered when assaying the stable internal ATP standard.

As may be noticed in Fig. 1, the recovery of the extracted ATP varied from 10 to 100%. The principal factor affecting ATP loss in the extraction process is sediment grain size, with the finer materials being more adsorptive relative to the coarser sands. To illustrate the point we compared the ATP extraction efficiency (determined with a radioactive standard) to sediment grain size ratio. The results are seen in Fig. 2 and demonstrate that as the proportion of fines increases (i.e.; the grain size decreases) the ATP extraction efficiency decreases.

In order to interpret our current findings determined on samples collected in oil lease areas, it is necessary to examine results of earlier work done off the west coast of Florida with particular emphasis on the effects of sediment grain size on microbial ATP biomass. In 1975 baseline data was determined on three cruises from sediment samples collected along transects radiating out from the Florida coastline as shown in Fig. 3. These were all relatively shallow stations ranging from 10 to 180m in depth, with six to seven boxcore samples taken along each transect to be analyzed for ATP and/or grain size. Water depth did not have a pronounced effect on ATP variability, but distance from shore did exert some influence. To illustrate this point the ATP results of the three cruises along transect 24 were averaged and plotted against the distance from the nearest

coastline. This particular transect was chosen because it had a uniform sediment grain size (see below) and thus eliminated any effects that could be attributed to sediment size. As one might expect the sediment ATP biomass decreased as one moved progressively

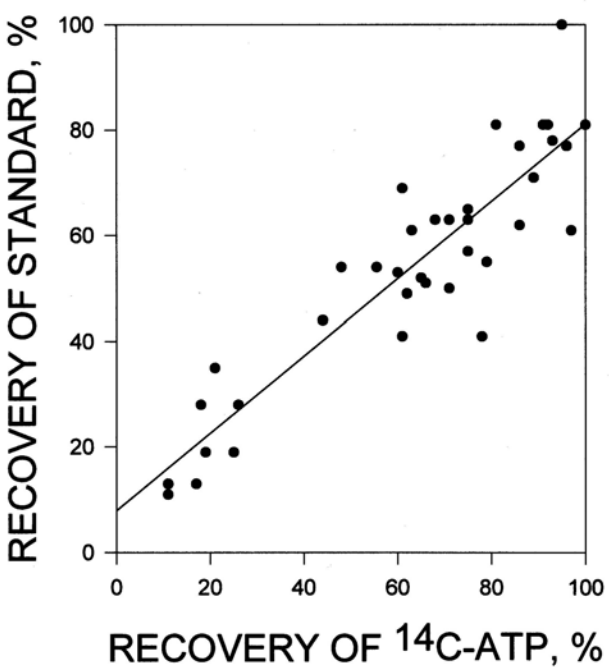


Fig. 1. A comparison between the recovery of a stable internal ATP standard to the recovery of the $^{14}C$ ATP adsorption standard.

seaward as illustrated in Fig. 4, although there are "hot-spots" that cloud a clear-cut relationship caused by land effects ($r^2 = 0.51$).

Of greater interest, however, was the overall effect sediment grain size had on controlling microbial biomass. The relationship between ATP and grain size for transects 21 through 24 off Florida's west coast is shown in Fig 5, with graphs representing the results of cruises in June (Fig. 5A), Sept.-Oct. (Fig. 5B) and Dec. (Fig. 5C) which illustrate seasonal effects. Transects 21 and 24 had a uniform sediment granularity of about 0.3mm and in these cases the decline in ATP reflects the seaward progression of the sampling sites. Interestingly, the ATP concentrations along these two transects did not show an appreciable seasonal variation and remained fairly constant when Figs. 5A through 5C are compared.

Transects 22 and 23, on the other hand, had a wide range of sediment grain size. For all three cruises we found that as the sediment grain size decreased, so did the ATP concentration. There were some differences between the cruises at these two locations, with the Sept.-Oct. samples having more than twice the ATP concentrations found in the previous and following efforts. Passing regression lines thorough the data points and

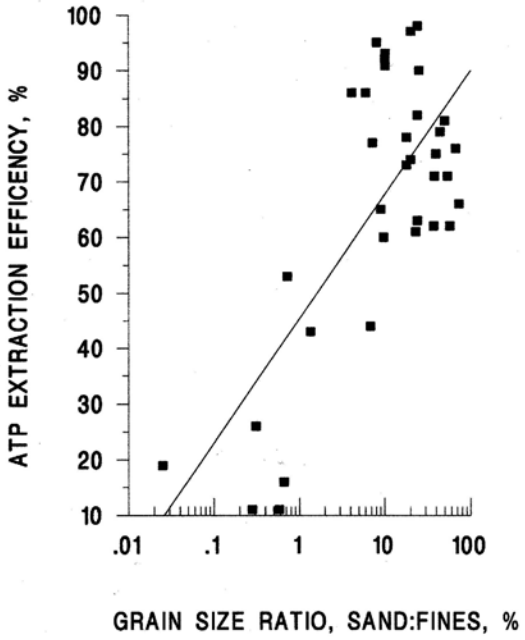


Fig. 2. The effect of sediment grain size on the recovery of the $^{14}C$ ATP extraction standard. Note that as the sediment grain size decreases adsorption of the extracted ATP becomes significant reducing the efficiency of extraction to as little as 10%.

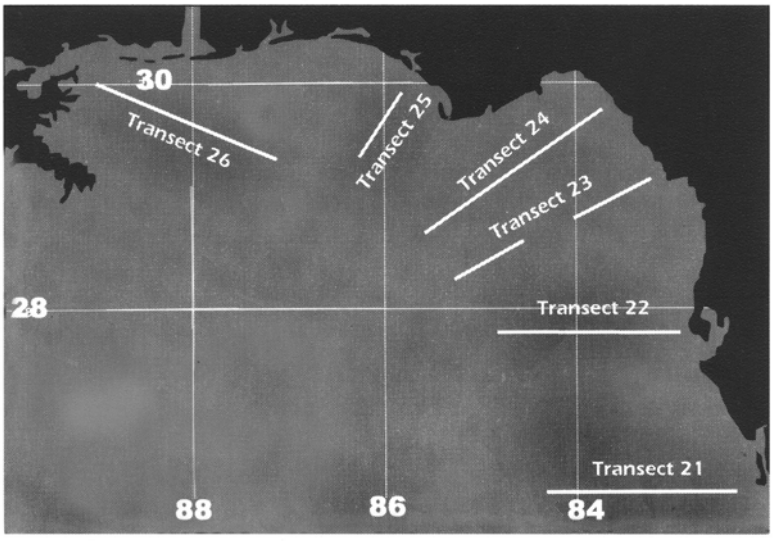


Fig. 3. The locations of the six sampling transects on the Florida shelf that were used to establish baseline ATP and sediment information. Water depths ranged from approximately 10m near the coastline to a maximum of 180m at the most distant sample locations. There were six sampling sites along transects 21, 22, and 23 and nine sampling sites along transects 24, 25 and 26.

extrapolating to zero ATP we find a potential minimum sediment size of about 0.002 to 0.003mm that will allow bacterial development.

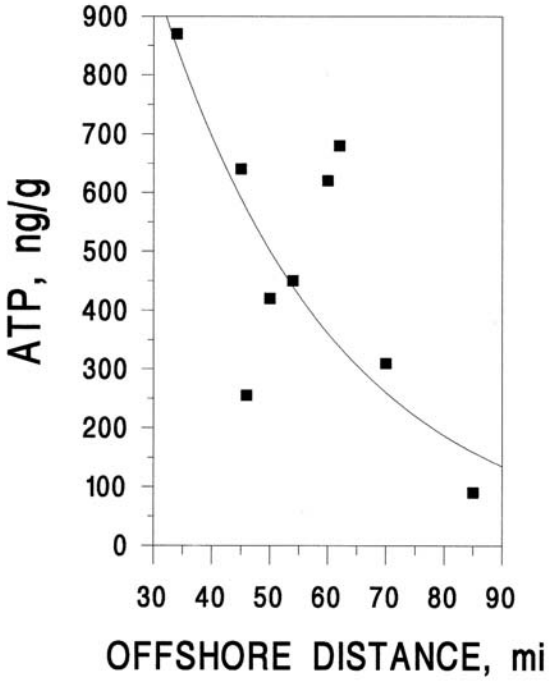


Fig. 4. The effect of distance from shore on sedimentary ATP content is shown for transect 24. This particular transect was chosen to illustrate terrestrial influence as it has a relatively constant sediment grain size.

Transects 25 and 26 bordered the coasts of Florida and Alabama. Transect 25 (Figs. 6A – C) had a relatively consistent sediment grain size except for the two most distant sampling locations, and maintained a consistent ATP pattern throughout the sampling period. Transect 26 (Figs. 6A – C), however, differed in that, 1) there was considerable grain size variation, and 2) there was one instance where the ATP concentration actually increased with decreasing grain size, which is contradictory to all our other observations. We speculate that this unique situation reflected the summer transport of Mississippi River water, and its associated sediment burden, to the east as opposed to its usual westward movement. This flow pattern has been demonstrated in satellite images as well as with drifter buoys from which it was shown that the river discharge moved from the delta, jogged northeastward to just below Mobile Bay and then migrated along the coast line between the 200- and 1000-m isobaths (Walker et al., 1994; Walker et al., 1996). Extrapolating the regression lines for the ATP-grain size data

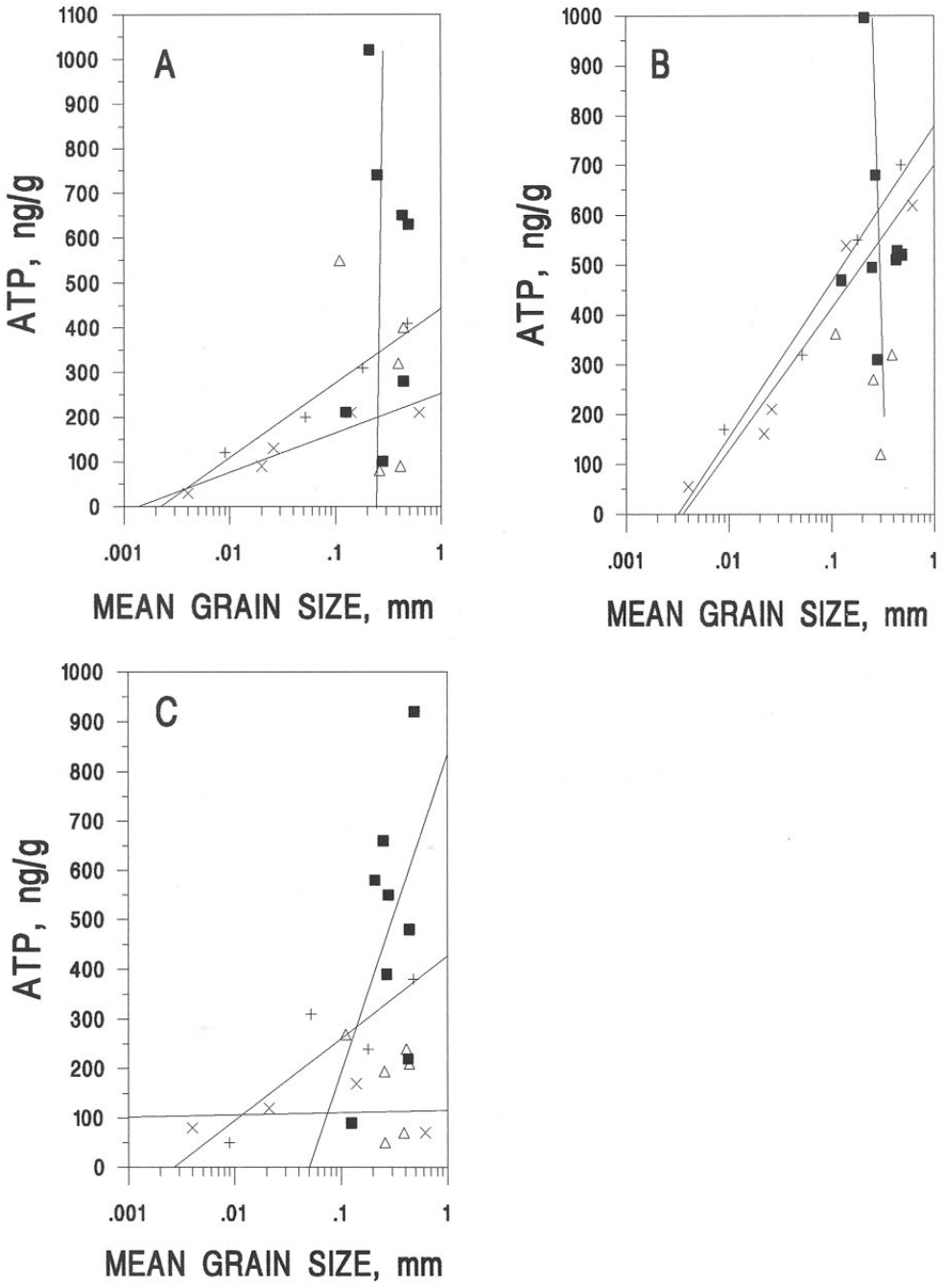


Fig. 5. The relationship between sedimentary ATP, corrected for adsorptive and counting losses, as a function of sediment mean grain size for transects 21 to 24. The figures represent collections made in June (A), collections made in September (B), and collections made in December(C). Note that no regression line was drawn for transect 21 as all of the data points tended to cluster around a sediment size of 0.3mm and ATP values between 200 – 400 ng/g. Symbols: Xs are transect 22, plus signs are transect 23 and solid squares are transect 24.

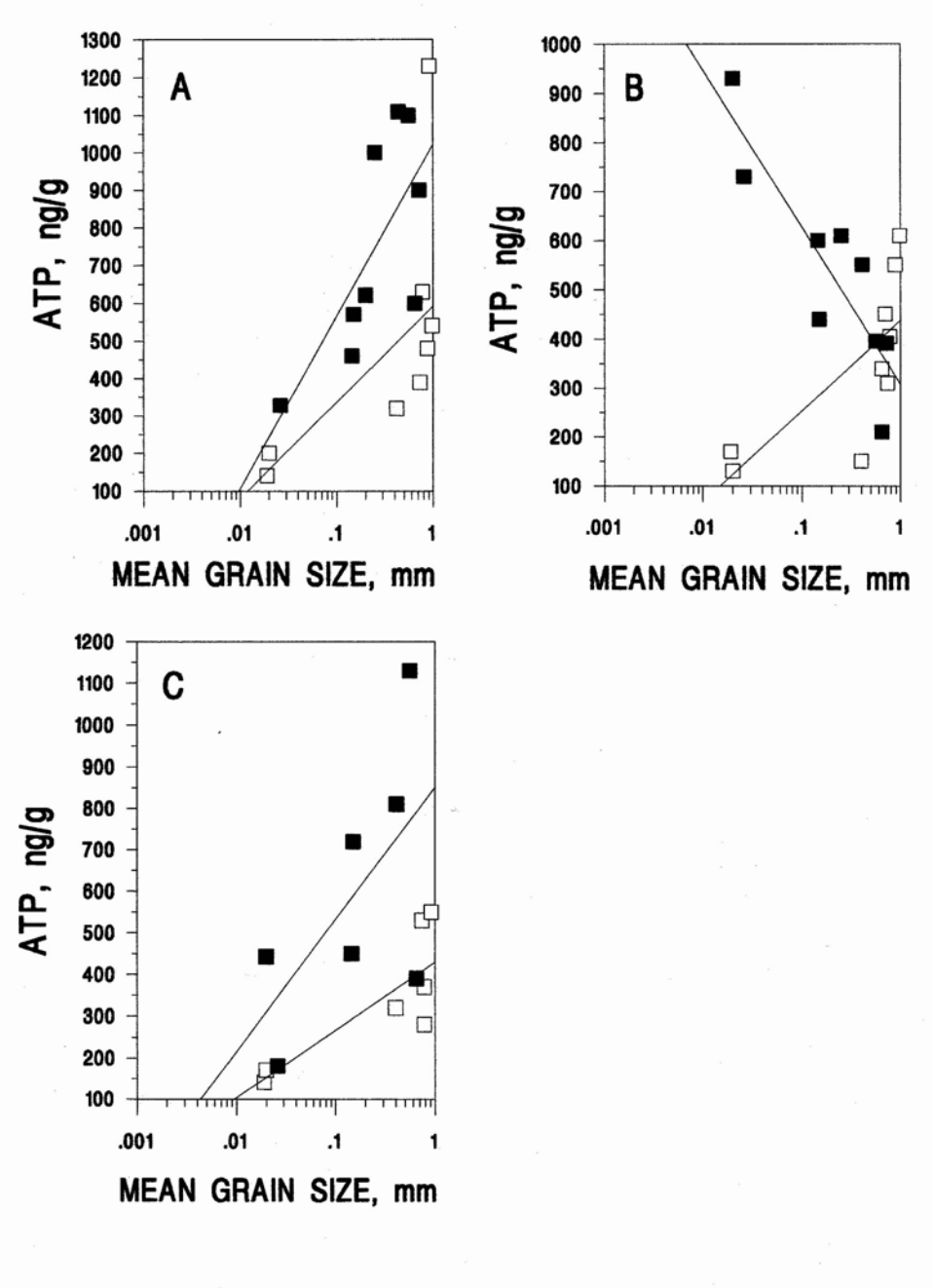


Fig. 6. The relationship between sedimentary ATP, corrected for adsorptive and counting losses, as a function of sediment mean grain size for transects 25 and 26. The figures represent collections made in June (A), collections made in September (B), and collections made in December(C). As noted in the previous figures sedimentary ATP decreases as the grain size decreases with the exception of the Sept-Oct. collection at transect 26. See text for explanation. Symbols: open squares are transect 25, and solid squares are transect 26.

(except for the Sept.-Oct. data of transect 26) we find a minimum grain size of approximately 2μm that will allow bacterial metabolism as determined by ATP production. We can use this relationship between grain size and ATP biomass as a starting point in evaluating what one might reasonably expect in other sedimentary regimes.

The thrust of the current effort was to determine the effects of oil exploration on sedimentary microbes. A series of sites was established at a depth of approximately 1,000m in the oil lease areas of Garden Banks 516 (GB 516) and 602 (GB 602), Mississippi Canyon 292 (MC 292), and Vioska Knoll 916 (VK 916) off the Louisiana Coast (see Fig. 7 for site map). The sampling strategy was to take 12 box cores in the immediate vacinity of where drilling was or will be taking place, termed the near field sites and identified by the designation NF, and 12 box cores approximately 10 miles distant from drilling center and termed the far field sites and identified by the designation FF. A total of 12 near field stations were established and are identified by the location (VK, GB etc.), near field (NF) and by station number (01 through 12). An example for Vioska Knoll would be VK916 NF 01 through 12. Six far field stations were established at each location, but in this instance each station was sampled twice and the resultant cores were denoted with the location (VK, GB etc.) as a far field sample (FF), with the site number (1 through 6), and box core number (1 or 2). An example for Vioska Knoll would be VK916 FF 01-01 or 02). A map of the sample locations for VK 916 is shown in Fig 8, but the same general pattern holds for all of the other stations. Of the four sites two, GB 602 and MC 292, had been developed, whereas GB 516 and VK 916, were sampled twice, once before drilling and again after development and, therefore, are of particular interest.

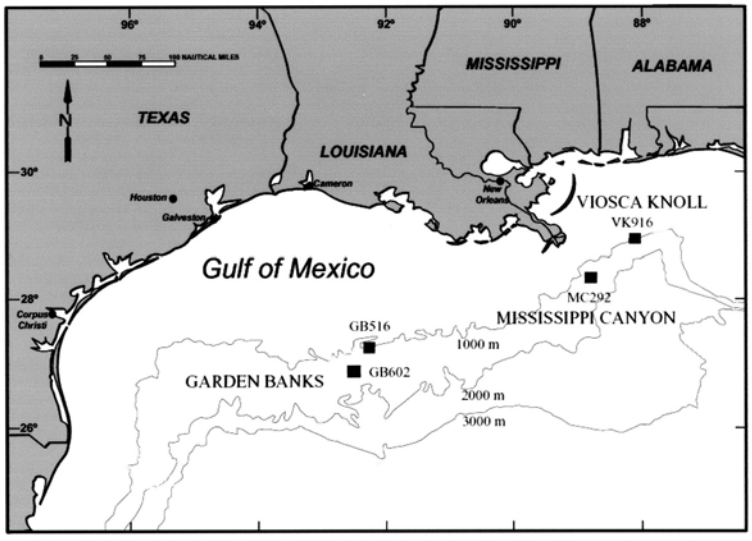


Fig. 7. Collection sites in the oil lease areas off the Mississippi and Louisiana coasts. The four locations essentially follow the 1000m isobath.

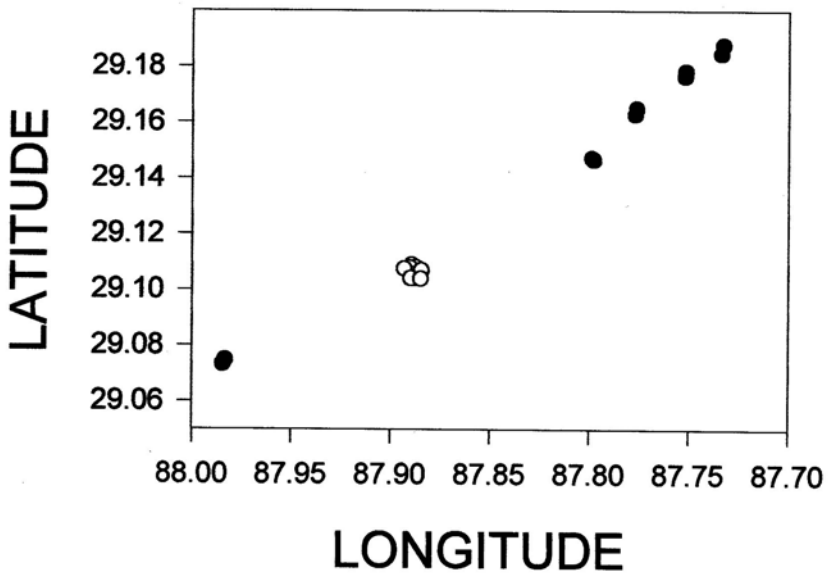


Fig. 8. Conceptual sampling strategy that was used to obtain sediments at the near field locations surrounding the drill site (open circles), and the more distant far field locations (solid circles). The locations in this figure are for VK-916 but the same rationale was used for all of the sampling sites.

Trace metal analysis was performed on all of the stations but only the data for VK 916 will be shown to illustrate the salient points bearing in mind that drilling fluids are a part of the sedimentary makeup. The pre drill conditions are shown in Table 1, post drill conditions are shown in Table 2 and both are compared to values that might be expected in average marine sediments. Relative to average marine sediments prior to drilling, barium is from two to three times the average sediment concentration in the FF and NF locations respectively, manganese is nearly an order of mangitude greater throughout and mercury is about one-half the average concentration. All other metals are approximately the same as would be expected relative to the average concentrations. Samples from the post drill collection have values similar to the pre drill conditions except for barium, which increased dramatically at all of the NF stations by factors up to 100 fold. Increased barium levels are also noted at the FF sites, but the concentrations only go up by about 50%. Barium is a major component in the fluids that are used in the drilling process and as such would be expected to be most concentrated in the immediate vacinity of the oil platform. The increased barium at the FF sites indicates that some component of the drill muds had been carried beyond the point of application, which as will be detailed below are the very finest of particles.

Changes in the oxygen profile in the sediment column illustrates one of the more dramatic changes mediated by the drilling. Measurements at 1mm intervals using a microelectrode indicated the presence of oxygen down to the 2cm depth in the pre drill

Table 1. Trace Metal and Total Organic Carbon Concentrations in Sediment Samples (dry weight) for Vioska Knoll 916 Pre Drill[1].

| Sample Identification | Al (%) | As (µg/g) | Ba (µg/g) | Cd (µg/g) | Cr (µg/g) | Cu (µg/g) | Fe (%) | Hg (µg/g) | Mn (µg/g) | Ni (µg/g) | Pb (µg/g) | V (µg/g) | Zn (µg/g) | TOC (%) | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VK916 NF-01 | 7.17 | 12.9 | 1200 | 0.31 | 62.1 | 28.0 | 3.31 | 0.070 | 10600 | 41.3 | 31.5 | 137 | 107 | 1.87 | |
| VK916 NF-02 | 7.53 | 13.0 | 1190 | 0.27 | 68.0 | 27.5 | 3.51 | 0.068 | 8110 | 40.5 | 34.2 | 142 | 110 | 1.79 | |
| VK916 NF-03 | 7.48 | 12.1 | 1190 | 0.28 | 43.7 | 28.5 | 3.44 | 0.074 | 9620 | 42.8 | 32.3 | 140 | 110 | 1.30 | |
| VK916 NF-04 | 7.20 | 13.7 | 1160 | 0.33 | 51.6 | 27.1 | 3.36 | 0.075 | 10000 | 40.7 | 32.6 | 133 | 106 | 1.36 | |
| VK916 NF-05 | 7.31 | 12.6 | 1150 | 0.33 | 34.1 | 25.1 | 3.33 | 0.072 | 8780 | 37.6 | 31.6 | 130 | 106 | 1.90 | |
| VK916 NF-06 | 7.37 | 13.4 | 1290 | 0.28 | 38.8 | 27.3 | 3.44 | 0.068 | 9350 | 44.0 | 34.8 | 141 | 109 | 1.50 | |
| VK916 NF-07 | 7.16 | 12.6 | 1210 | 0.28 | 41.1 | 25.4 | 3.45 | 0.070 | 7300 | 37.3 | 31.0 | 131 | 107 | 1.51 | |
| VK916 NF-08 | 6.92 | 11.9 | 965 | 0.29 | 65.4 | 26.7 | 3.67 | 0.065 | 9200 | 40.7 | 30.0 | 135 | 109 | 1.57 | |
| VK916 NF-09 | 7.13 | 11.1 | 837 | 0.24 | 64.4 | 26.4 | 3.45 | 0.073 | 9430 | 40.9 | 25.6 | 138 | 107 | 1.55 | |
| VK916 NF-10 | 7.57 | 11.3 | 802 | 0.18 | 31.2 | 25.5 | 3.75 | 0.069 | 5780 | 38.3 | 25.6 | 137 | 110 | 1.33 | |
| VK916 NF-11 | 7.16 | 13.0 | 1070 | 0.28 | 66.1 | 26.2 | 3.44 | 0.073 | 6620 | 41.4 | 25.0 | 136 | 108 | 1.33 | |
| VK916 NF-12 #1 | 7.52 | 13.6 | 972 | 0.27 | 65.6 | 28.1 | 3.53 | 0.069 | 10900 | 42.2 | 30.0 | 144 | 110 | 1.44 | Lab Duplicate |
| VK916 NF-12 #2 | 7.35 | 13.2 | 963 | 0.25 | 67.1 | 27.3 | 3.53 | 0.070 | 10500 | 42.4 | 29.4 | 143 | 110 | 1.51 | Lab Duplicate |
| VK916 FF2-01 | 7.29 | 13.2 | 1180 | 0.22 | 70.4 | 24.9 | 3.53 | 0.071 | 6950 | 44.9 | 29.9 | 131 | 105 | 1.34 | |
| VK916 FF2-02 | 7.77 | 14.4 | 1080 | 0.22 | 65.8 | 27.0 | 3.34 | 0.072 | 8420 | 24.5 | 29.7 | 136 | 106 | 1.34 | |
| VK916 FF3-01 | 7.79 | 12.9 | 823 | 0.24 | 71.8 | 23.1 | 3.42 | 0.078 | 6850 | 46.9 | 28.9 | 133 | 98.7 | 1.35 | |
| VK916 FF3-02 | 5.78 | 13.5 | 1020 | 0.23 | 78.3 | 25.9 | 3.38 | 0.074 | 10000 | 52.4 | 30.9 | 144 | 106 | 1.55 | |
| VK916 FF4-01 | 7.26 | 10.3 | 656 | 0.23 | 75.8 | 25.0 | 3.24 | 0.070 | 4710 | 34.0 | 26.7 | 143 | 106 | 1.58 | |
| VK916 FF4-02 | 7.12 | 13.3 | 855 | 0.22 | 71.7 | 26.4 | 3.27 | 0.067 | 6480 | 55.6 | 29.6 | 128 | 106 | 1.36 | |
| VK916 FF5-01 | 6.68 | 13.5 | 852 | 0.17 | 78.4 | 24.5 | 3.18 | 0.070 | 3810 | 41.1 | 27.5 | 123 | 100 | 1.46 | |
| VK916 FF5-02 | 6.91 | 9.6 | 698 | 0.23 | 76.2 | 25.1 | 3.35 | 0.062 | 2810 | 33.3 | 25.3 | 129 | 102 | 1.48 | |
| VK916 FF6-01 | 6.88 | 11.8 | 789 | 0.20 | 67.5 | 24.7 | 3.37 | 0.071 | 6490 | 26.7 | 27.2 | 132 | 102 | 1.58 | |
| VK916 FF6-02 | 6.87 | 10.3 | 747 | 0.23 | 76.3 | 25.5 | 3.35 | 0.075 | 5990 | 36.6 | 27.2 | 128 | 104 | 1.32 | |
| Average Marine Sediment[2] | 7.2 | 7.7 | 460 | 0.17 | 72 | 33 | 4.1 | 0.19 | 770 | 52 | 19 | 105 | 95 | - | |
| Continental Crust[3] | 7.96 | 1.7 | 584 | 0.1 | 126 | 25 | 4.32 | 0.04 | 716 | 56 | 14.8 | 98 | 65 | - | |

1. Chemical analyses performed and generously provided by John Trefry, Florida International University.
2. From Solomons and Forstner, 1984.
3. From Wedpohl, 1995.

Table 2. Trace Metal and Total Organic Carbon Concentrations in Sediment Samples (dry weight) for Vioska Knoll 916 Post Drill[1].

| Sample Identification | Al (%) | As (µg/g) | Ba (µg/g) | Cd (µg/g) | Cr (µg/g) | Cu (µg/g) | Fe (%) | Hg (µg/g) | Mn (µg/g) | Ni (µg/g) | Pb (µ9/9) | V (µg/g) | Zn (µ9/g) | TOC (%) | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VK916 NF-01 | 4.75 | 19.9 | 171000 | 0.32 | 62.7 | 48.0 | 2.42 | 0.071 | 6880 | 29.2 | 27.8 | 87.6 | 142 | 2.76 | |
| VK916 NF-02 #1 | 7.05 | 14.2 | 1990 | 0.23 | 75.3 | 25.7 | 3.37 | 0.078 | 9670 | 42.7 | 27.8 | 125 | 106 | 1.68 | Lab Duplicate |
| VK916 NF-02 #2 | 7.03 | 14.0 | 2030 | 0.23 | 74.4 | 26.0 | 3.33 | 0.076 | 9620 | 42.1 | 28.0 | 127 | 107 | 1.65 | Lab Duplicate |
| VK916 NF-03 | 7.16 | 14.2 | 3350 | 0.20 | 71.6 | 25.8 | 3.28 | 0.073 | 7850 | 41.4 | 28.6 | 130 | 105 | 1.59 | |
| VK916 NF-04 | 6.97 | 14.4 | 4300 | 0.22 | 77.9 | 26.3 | 3.18 | 0.084 | 9030 | 41.0 | 27.8 | 125 | 99.6 | 1.45 | |
| VK916 NF-05 | 7.22 | 11.6 | 36600 | 0.32 | 75.4 | 29.7 | 3.13 | 0.075 | 1400 | 31.7 | 27.4 | 112 | 104 | 2.22 | |
| VK916 NF-06 | 6.73 | 11.3 | 86800 | 0.27 | 67.2 | 32.6 | 2.78 | 0.089 | 1180 | 25.7 | 34.4 | 96.2 | 99.0 | 3.43 | |
| VK916 NF-07 | 5.65 | 12.5 | 81500 | 0.27 | 62.9 | 39.1 | 2.63 | 0.153 | 1040 | 23.2 | 36.9 | 89.8 | 87.6 | 3.45 | |
| VK916 NF-08 | 7.27 | 1.2 | 9420 | 0.21 | 78.4 | 28.0 | 3.35 | 0.085 | 9770 | 41.6 | 31.9 | 129 | 104 | 1.39 | |
| VK916 NF-09 | 7.44 | 12.6 | 2150 | 0.14 | 80.2 | 27.3 | 3.49 | 0.072 | 4610 | 40.1 | 26.5 | 130 | 108 | 1.50 | |
| VK916 NF-10 | 7.69 | 15.4 | 1050 | 0.14 | 78.8 | 27.3 | 3.68 | 0.085 | 7480 | 43.6 | 28.9 | 129 | 109 | 1.56 | |
| VK916 NF-11 | 7.00 | 14.4 | 1070 | 0.17 | 79.4 | 27.1 | 3.52 | 0.073 | 10400 | 42.5 | 26.3 | 129 | 108 | 1.53 | |
| VK916 NF-12 | 6.92 | 10.1 | 30500 | 0.24 | 75.2 | 34.8 | 3.09 | 0.145 | 7910 | 37.8 | 33.6 | 90.6 | 102 | 1.68 | |
| VK916 FF1-01 | 7.62 | 14.8 | 1040 | 0.15 | 81.5 | 28.0 | 3.57 | 0.080 | 9210 | 42.5 | 27.0 | 133 | 116 | 1.67 | |
| VK916 FF1-02 | 7.72 | 13.0 | 811 | 0.21 | 83.2 | 28.7 | 0.61 | 0.087 | 4830 | 41.5 | 27.4 | 137 | 111 | 1.44 | |
| VK916 FF2-01 #1 | 7.23 | 12.8 | 1390 | 0.19 | 76.2 | 26.1 | 3.49 | 0.076 | 6780 | 37.7 | 25.9 | 127 | 108 | 1.63 | Lab Duplicate |
| VK916 FF2-01 #2 | 7.26 | 13.0 | 1390 | 0.19 | 76.0 | 26.2 | 3.44 | 0.077 | 6760 | 37.9 | 25.5 | 127 | 108 | 1.61 | Lab Duplicate |
| VK916 FF2-02 | 5.93 | 14.0 | 1240 | 0.15 | 70.3 | 24.4 | 3.34 | 0.069 | 6570 | 36.1 | 22.4 | 118 | 102 | 1.52 | |
| VK916 FF3-01 | 7.41 | 13.4 | 954 | 0.19 | 75.8 | 26.9 | 3.43 | 0.080 | 9050 | 42.1 | 26.3 | 127 | 110 | 1.37 | |
| VK916 FF3-02 | 6.80 | 14.5 | 1090 | 0.17 | 76.2 | 26.4 | 3.45 | 0.086 | 12000 | 42.7 | 26.8 | 117 | 106 | 1.73 | |
| VK916 FF4-01 | 7.62 | 12.5 | 880 | 0.19 | 76.9 | 26.5 | 3.48 | 0.071 | 6530 | 41.2 | 26.0 | 125 | 109 | 1.42 | |
| VK916 FF4-02 | 7.01 | 12.1 | 881 | 0.18 | 75.0 | 26.7 | 3.36 | 0.087 | 7120 | 39.3 | 25.5 | 123 | 104 | 1.48 | |
| VK916 FF5-01 | 7.20 | 11.9 | 828 | 0.14 | 74.1 | 26.2 | 3.28 | 0.077 | 5830 | 37.7 | 24.2 | 115 | 103 | 1.48 | |
| VK916 FF5-02 | 6.29 | 11.3 | 804 | 0.17 | 75.0 | 25.6 | 3.27 | 0.064 | 5250 | 37.5 | 24.6 | 115 | 101 | 1.65 | |
| VK916 F1[7]6-01 | 7.66 | 8.9 | 693 | 0.17 | 76.4 | 26.8 | 3.37 | 0.069 | 2070 | 38.9 | 25.0 | 127 | 106 | 1.67 | |
| VK916 F1[7]6-02 | 6.76 | 10.0 | 674 | 0.17 | 75.1 | 26.4 | 3.35 | 0.074 | 5890 | 39.1 | 24.1 | 128 | 109 | 1.35 | |
| Average Marine Sediment[2] | 7.2 | 7.7 | 460 | 0.17 | 72 | 33 | 4.1 | 0.19 | 770 | 52 | 19 | 105 | 95 | - | |
| Continental Crust[3] | 7.96 | 1.7 | 584 | 0.1 | 126 | 25 | 4.32 | 0.04 | 716 | 56 | 14.8 | 98 | 65 | - | |

1, Chemical analyses performed and generously provided by John Trefry, Florida International University.
2. From Solomons and Forstner, 1984.
3. From Wedpohl, 1995.

samples even thought it is only 1.8% of saturation value (Table 3). In marked contrast the post drill samples had zero oxygen detectable at a sediment depth of 3mm.

Characterization of the sediment granularity for a sample from VK 916 is presented in Fig. 9 and briefly summarized in Table 4 indicating that the material is overwhelming composed of clay with a mean sedimentary particle diameter of 2.32μm prior to oil development. Over 90% of the sediment consisted of components smaller than 10μm (Fig. 9). The analyses for the other locations were not appreciably different, and thus we were dealing with materials that are very fine-grained, highly porous but very impermeable (Maier et al., 2000). Post development grain-size analyses showed no appreciable difference in the NF stations but the FF stations contained a higher percentage of finer materials (Table 4). A more detailed comparison of the changes in grain size distribution at FF BO2 (Fig. 9) reveals little difference in the percentage of accumulated material down to 100μm grain diameter between the pre and post drill samples. However, below that value the NF station accumulated more coarse material, whereas the FF station had approximately 15% of its total mass below 1μm (mean grain size of 1.37μm) indicating that very fine grained debris had drifted from the drill site to more distant locations. In other words, the barium analysis, the sediment grain size analysis and the depleted oxygen indicate contributions of fine materials from the drill muds to the sediments that essentially seals them.

The ATP results for all stations, pre and post drill, are summarized in Table 5 and although there are some hot spots, they show that for all the post drill conditions the ATP ranges between 2 to 5ng/g. In contrast, a comparison of the pre and post drill ATP values at GB 516 and VK 916, on the average, show a marked decrease of from 10 to 15 fold after development. These findings are shown more clearly in Figs. 10 and 11, noting that the graphs are plotted logarithmically. Referring back to Figs. 5 and 6 we might expect a reasonable sedimentary ATP value at GB 516 and VK 916 pre drill of 30 to 40ng/g at a mean grain size of 2 to 3μm provided that sediment grain size was the controlling factor. Our measured ATP results are in good agreement with the projected values based on grain size criteria for GB 516 and VK 916 pre drill conditions, but the post drill results are significant lower.

Table 3. Dissolved Oxygen in the Sediment Column at VK

| | PRE DRILL | | POST DRILL | |
|---|---|---|---|---|
| DEPTH cm | OXYGEN % | OXYGEN um | OXYGEN % | OXYGEN um |
| Bottom Water | 13.8 | 184 | 9.5 | 132 |
| 0.1 | 11.9 | 158 | 4.8 | 67 |
| 0.2 | 9.8 | 130 | 1.2 | 17 |
| 0.3 | 8.1 | 108 | 0 | 0 |
| 0.4 | 6.6 | 88 | | |
| 0.5 | 5.4 | 72 | | |
| 0.6 | 4.8 | 64 | | |
| 0.7 | 4.1 | 55 | | |
| 0.8 | 3.6 | 48 | | |
| 0.9 | 3.2 | 43 | | |
| 1 | 2.9 | 39 | | |
| 1.1 | 2.5 | 33 | | |
| 1.2 | 2.3 | 31 | | |
| 1.3 | 2.3 | 31 | | |
| 1.4 | 2.2 | 29 | | |
| 1.5 | 2.2 | 29 | | |
| 1.6 | 2.1 | 28 | | |
| 1.7 | 2 | 27 | | |
| 1.8 | 1.9 | 25 | | |
| 1.9 | 1.8 | 24 | | |
| 2 | 1.8 | 24 | | |

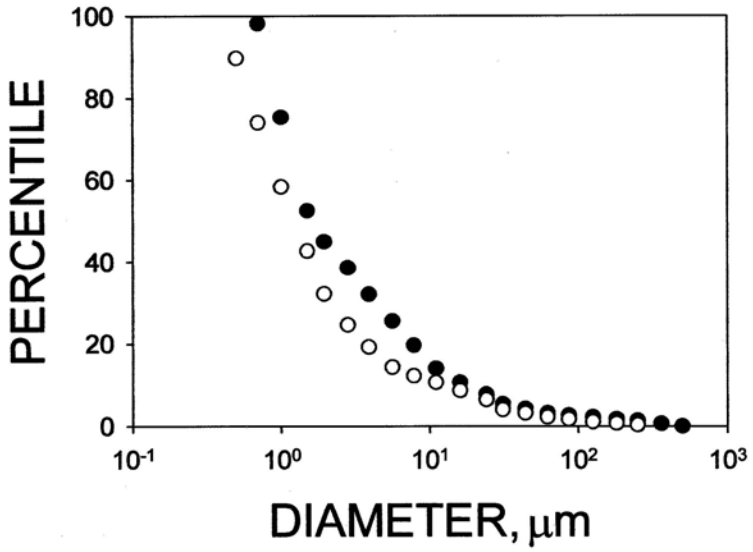


Fig. 9. The cumulative distribution of sediment grain size for samples collected at VK 916. Note that the sediment distribution becomes finer after drilling had begun (open circles) relative to the pre drill collections (solid circles). In both instances however approximately 90% of the sediment material is less than 10 μm.

Table 4. Sediment Characterization at VK 916 Pre and Post Drill.

| Site | % Sand | % Silt | % Clay | Mean Size | Comment |
|---|---|---|---|---|---|
| NF | 3.81 | 29.96 | 66.23 | 2.46 | Pre Drill |
| FF | 3.16 | 29.95 | 66.89 | 2.27 | Pre Drill |
| NF | 3.18 | 28.53 | 68.28 | 2.32 | Post Drill |
| FF | 2.27 | 16.66 | 81.07 | 1.37 | Post Drill |

Table 5. Sediment ATP Concentrations (dry weight) for all Near and Far Field Stations Pre and Post Exploration[1].

| Sample Identification | GB-516 Predrill ATP ng/g | GB-516 Postdrill ATP ng/g | VK-916 Predrill ATP ng/g | VK-916 Postdrill ATP ng/g | GB-602 Postdrill ATP ng/g | MC-292 Postdrill ATP ng/g |
|---|---|---|---|---|---|---|
| NF-BO1 | 288.3 | 2.1 | 47.5 | <1.7 | | 4.5 |
| NF-BO2 | 418.2 | 0.6 | 42.1 | <1.3 | 3.4 | 1.2 |
| NF-BO3 | 13.1 | 7.9 | 30.2 | <1.8 | 3.3 | 1.4 |
| NF-BO4 | 27.9 | 0.5 | 34.5 | <1.8 | 2.3 | 0.9 |
| NF-BO5 | 26.4 | 1.1 | 29.3 | 15.3 | 14.1 | 4.4 |
| NF-BO6 | | 11.9 | 48.3 | 25.6 | 4.7 | 4.4 |
| NF-BO7 | 33.5 | 4.4 | 30.1 | 41.5 | 2.3 | 1.1 |
| NF-BO8 | 104.1 | 1.5 | 21.9 | <0.3 | 17.6 | 5.4 |
| NF-BO9 | 39.2 | 9.6 | 25.1 | 1.1 | 2.7 | 1 |
| NF-B10 | 29.2 | 0.7 | 23.3 | 1.3 | 15.2 | 1.6 |
| NF-B11 | 56.3 | 5.1 | 23.4 | <1.5 | 16.2 | 5.5 |
| NF-B12 | 68.7 | 6.8 | 29.1 | 1.1 | N.D. | 1.7 |
| FF1-BO1 | 28.7 | 5.5 | | 61.1 | 2.1 | 2.9 |
| FF1-BO2 | 43.4 | 5.8 | | 1.1 | 1.1 | N.D. |
| FF2-B01 | 102.4 | 2.3 | 57.9 | 1.3 | 1.7 | 2.5 |
| FF2-B02 | 25.26 | 2.8 | 61.4 | 1.1 | 1.9 | 66.6 |
| FF3-BO1 | 92.3 | 0.5 | 34.2 | 2.7 | 3.6 | 1.7 |
| FF3-BO2 | 35.3 | 3.1 | 29.6 | <1.5 | 2.4 | 2.8 |
| FF4-BO1 | 29.1 | 5.4 | 19.7 | 2.7 | 1.1 | 2.1 |
| FF4-BO2 | 38.1 | 1.1 | 28.4 | 2.3 | 2.2 | 0.7 |
| FF5-BO1 | 64.4 | 0.5 | 24.5 | 2.3 | 3.2 | 1.1 |
| FF5-BO2 | 14.2 | 0.6 | | 2.7 | 216.3 | 2.2 |
| FF6-BO1 | 29.3 | 1.4 | 36.4 | 0.9 | 2.6 | 1.5 |
| FF6-BO2 | 16.7 | 0.8 | 32.1 | 5.6 | 2.7 | 1 |

1. A < symbol indicates that interference reduced the sensitivity of the assay to the value indicated thus ATP may be present at a lower comcentration but be undectable.

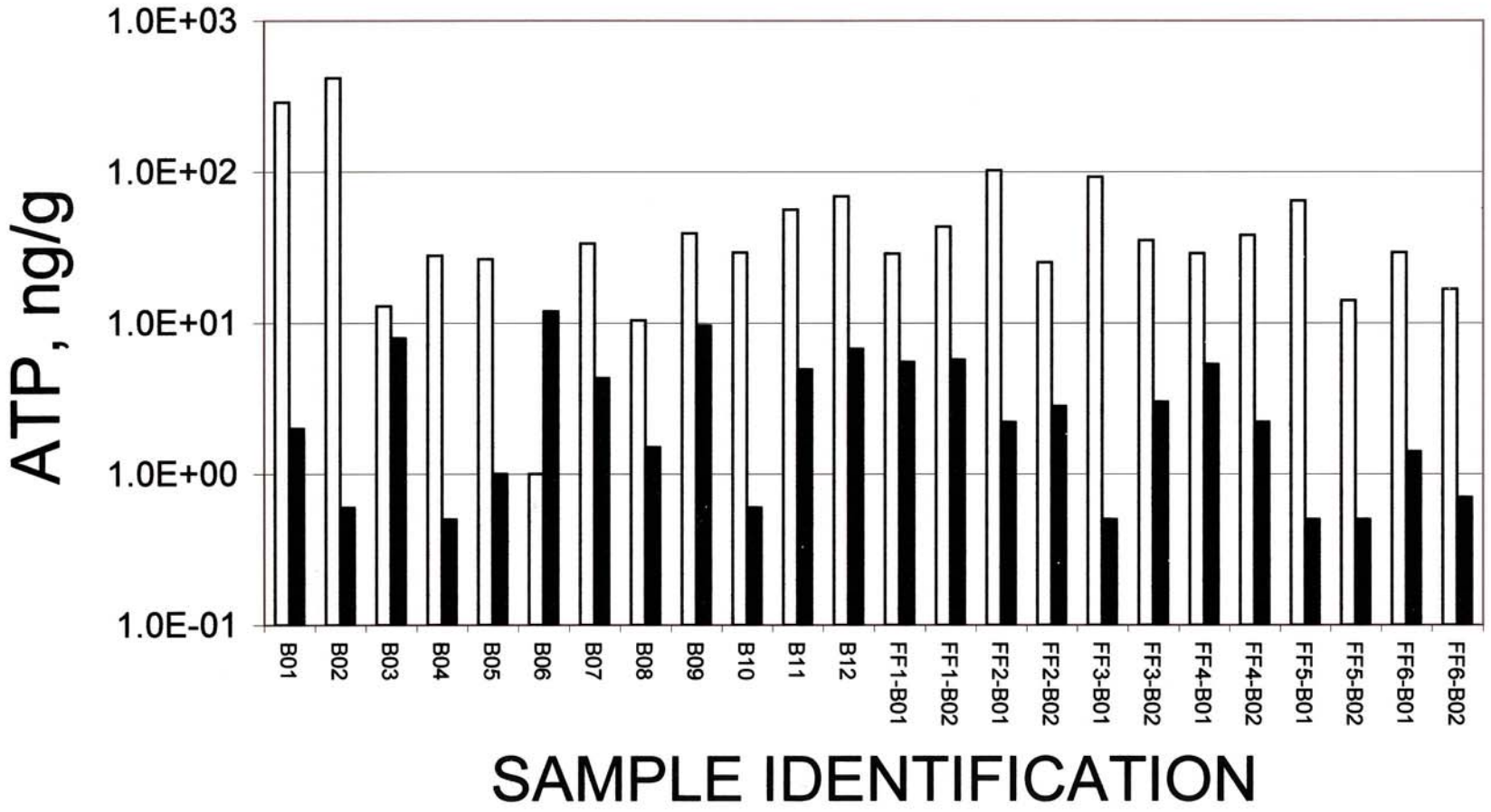


Fig. 10. A comparison between the pre drill and post drill ATP sedimentary concentrations for all sampling locations at GB 516. Pre drill results are open bars, post drill results are solid bars.

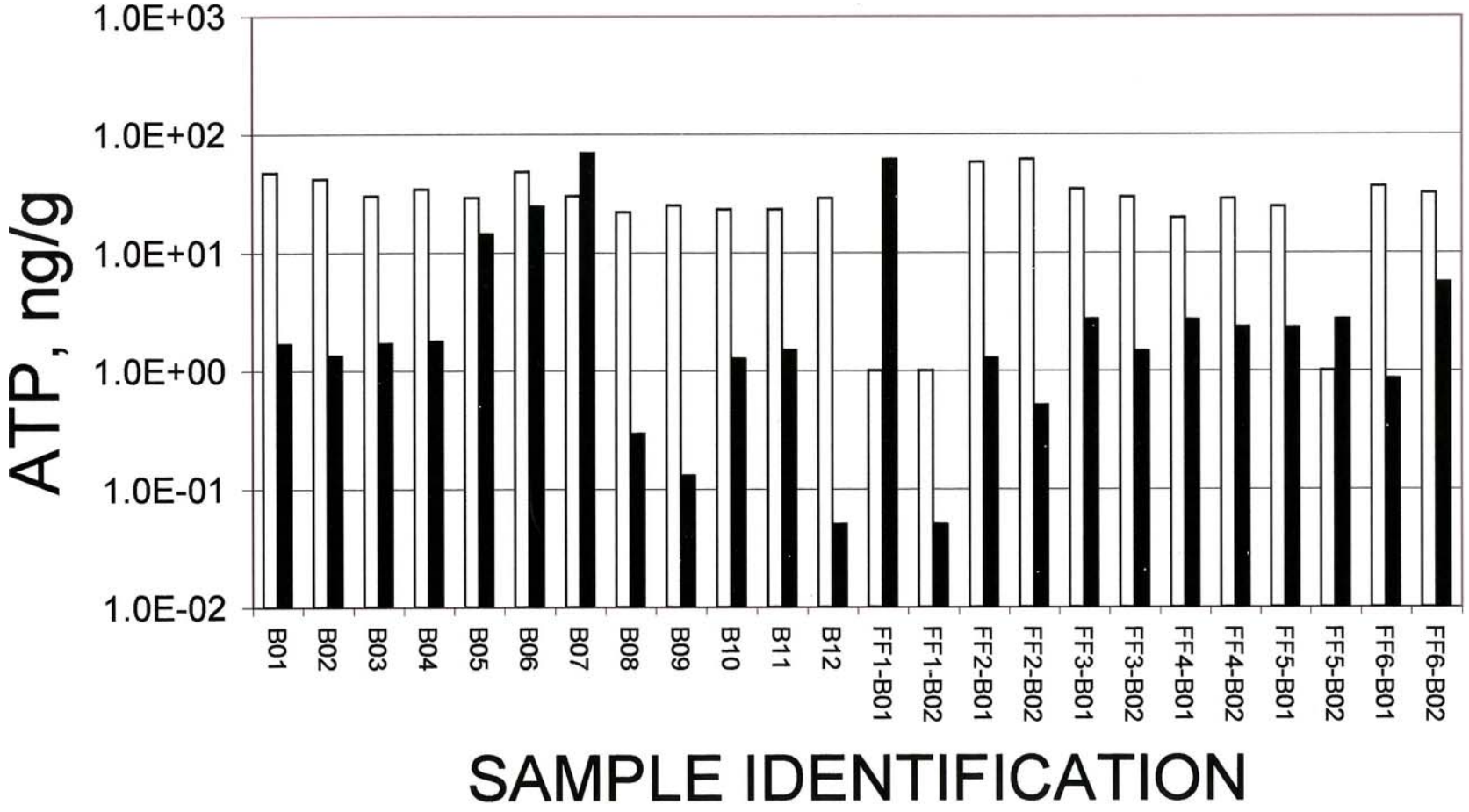


Fig. 11. A comparison between the pre drill and post drill ATP sedimentary concentrations for all sampling locations at VK 916. Pre drill results are open bars, post drill results are solid bars.

## DISCUSSION

The major issues covered in this effort were, 1) an improved means of determining interference in the sediment ATP analysis, 2) the relationship between sediment grain size and microbial ATP levels, and 3) the effects of oil development on microbial ATP biomass. While there are a variety of means of extracting sedimentary ATP, a major problem for all of them is a means of correcting for interference in various aspects of the process. Essentially there is loss of ATP during sediment extraction by adsorption to the sediment matrix followed by a secondary interference encountered during the luciferin-luciferase assay. The simplest means to tackle the problem would be to add an internal standard to a duplicate set of samples and determine the overall loss in the final counting. This approach essentially doubles the work effort and limits the number of replicates that can reasonably be processed, and for very fine-grained materials, the initial loss from adsorption to the sediments may be so great that too much of the standard is lost to be of value.

Our solution was to use radioactive ATP to follow the adsorptive loss followed by an internal ATP standard added at the time of assay to determine counting efficiency. By separating the interference into two components every sample can be used to determine adsorptive loss (the radioactive ATP is essentially at a concentration that is below detection limits) thus expediting the field aspect. When counting the extracted ATP the internal standard can be adjusted in the laboratory if, as was the case with our work, there is severe interference in the counting process. As seen in Fig. 1, there is agreement between both approaches, although the radioisotope approach gave a better estimate of recovery. Our findings also show that as the sediments became finer in composition, the extraction efficiency fell off appreciably (Fig. 2). Samples taken from the oil lease area typically had an extraction efficiency of 8%.

There are a considerable numbers of reports describing the bacterial biomass found in a variety of sediments. One general conclusion is that bacterial numbers increase as sediment grain size decreases as determined by direct microscopic evaluation. The question that arises is whether these bacteria are active or whether an alternative

metabolic assay, such as ATP, would be a better measure of sediment suitability and microbial colonization? Where grain size was found to vary (Figs. 5 and 6) we observed that the ATP levels declined with reduced sediment particle size which is contrary to the microscopic observation, but in good agreement with the findings of Koster and Meyer-Reil (2001). Furthermore, by extrapolating the ATP-grain size regression lines to a zero ATP concentration we arrived at a theoretical minimum grain size of about 2μm that would support bacterial development (Figs. 5 and 6).

These conclusions seem to be offset by the summer curve for transect 26 in Fig. 6B which shows increasing ATP with smaller particles. A plausible explanation for this one anomaly lies in its location relative to the Mississippi River and Mobile Bay. Major freshwater inflow to this part of the Gulf comes from the eastward flow of the Mississippi during the summer (Walker et al., 1994: Walker, 1996) as well as flow from the Mobile

River through Mobile Bay. The Mississippi in particular carries a large sediment burden, depositing sands in the delta but transporting fine material for some distance to sea. The increased ATP may represent newly deposited particulate material from either the Mississippi River or Mobile Bay especially since the phenomenon is not seen in either the early summer or late fall (Figs. 6A and 6C).

Information on deep-sea sedimentary microbial biomass is limited and information that is integrated to sediment grain size is even more scarce. One of the first measurements of deep-sea ATP biomass was reported by Karl et al., (1976) who found that the upper 2cm of sediment collected from the abyssal plain in the Atlantic Ocean had a value of 2.4ng $g^{-1}$. More recent work by Egeberg (2000), done in conjunction with the Ocean Drilling Program, suggested that particle size may be a determing factor in regulating sediment microbes. Egeberg made down-core measurements from 1.38 to as deep as 57.8m below the sea floor and reported ATP values as high as 6 to as low as 0.23 ng $g^{-1}$, with an average value of 0.75ng $g^{-1}$. No determinations were made on surface sediments, however.

Koster and Meyer-Reil made a suite of measurements in shallow coastal waters of the Baltic Sea that included ATP, chlorophyll a, phospholipid, and carbon species. The sediments they worked with were characterized as sandy, slightly muddy sand and muddy sand. The total organic carbon increased as the sediments became finer although the available dissolved organic carbon (the more labile component) decreased leading to the conclusion that the higher organic content is composed of more refractory, or aged material that is less capable of supporting microbial metabolism. With increasing mud content the microbial biomass, based on phospholipid determinations, increased whereas the active biomass, based on ATP determinations, decreased. This result neatly illustrates the disparity that we observe between measurements that seek to quantify the total quantity or number of microbes without regard to whether they are functional or not. In this case, as well as with direct AODC counts, the measured parameter (phospholipid or cell number) reflects only whether bacteria are present, and not whether they are growing or in a metabolically active state. In other words, the finer sediments may harbor more microbes but based on ATP measurements their geochemical potential is diminished.

The ATP values obtained by Koster and Meyer-Reil are not directly comparable to our measurements because they reported their results as a function of sediment volume, i.e.: ng $cm^{-3}$ as opposed to ng $g^{-1}$. Since our sediments were about 60 – 70 % water, one g of sediment is a close approximation to one $cm^3$ of sediment slurry. With this difference in mind, the ATP values reported ranged from 673 to 2843ng, which agrees well with the values we reported for the Florida shelf off the Gulf coast. The question now is what ATP levels might one reasonably expect at 1000m in the sediments of the Gulf of Mexico if there were no anthropogenic impacts? A reasonable expectation might range from 1000 ng $g^{-1}$ in the shallow coastal zone to 2 ng $g^{-1}$ in the most distant and deepest regions of the open ocean. The data of Fig. 4 do not extend far enough to predict what might occur under the present circumstances, but an approximate value might be between 50 and 100 ng $g^{-1}$ if distance from land is a relevant factor.

The predrill ATP values for GB-516 (Table 5) show considerable spatial variability which may, in part, be the result of gas and oil seeping from the sea floor (see the dedicated issue of Geo Marine Letters 14:2/3, 1994 for an extensive overview of the Louisiana seep zone). In calculating an average ATP concentration for the area we did not include the exceptionally high values of NF-BO1 and 2 as these samples may be affected by gas or oil seepage. An average ATP concentration for the NF was 44.2 ng $g^{-1}$ and for the farfield predrill sites the average ATP concentration was 43 ng $g^{-1}$. The average predrill ATP concentration for VK-916 NF was 32 ng $g^{-1}$ and for the FF it was 36 ng $g^{-1}$. These values are within reason based on the information of Fig. 4. The postdrill ATP averages for GB-516 NF (4.3 ng $g^{-1}$) and FF (2.5 ng $g^{-1}$) are reduced by a factor of 10 to 15 as a result of oil exploration. The post drill averages for VK-916 NF (7.7 ng g-1) and FF (7.1 ng g-1) indicate a five fold reduction in the microbial ATP biomass although it should be noted that again there is spatial heterogeneity in the data.

The major effect of drilling was the addition of drill spoils to the sea floor. This is seen in the chemical analyses in Tables 1 and 2 where it is apparent that the primary change is with the addition of barium to the sedimentary matrix. Barite is a major constituent to the synthetic based fluids (SBF) that are used to aid in the drilling process and to prevent blowout. Based on the change in barium concentrations alone, more of the drill spoils were deposited in the NF sites than was found in the FF locations. In otherwords, heavier materials are quickly deposited whereas the finer materials are more dispersed, a fact noted in the sediment grain size distributions of Fig. 9 and Table 4. Interestingly, the mean grain size for these sediments borders on the hypothetical 2 μm threshold we postulate to be the lower limit that will support bacterial growth. Clay sediments, because of the extremely small particle size, are highly porous but the pores are very small and small pores do not readily allow the passage of water relative to larger pore sizes; to put it another way, clay materials are relatively impervious. This has the effect of inhibiting bacterial movement either into or out of the clay sediments as well limit the passage of oxygen or nutrients (Maier et al., 2000).

How might drill spoils affect the sedimentary microbial community? There are three possibilities: 1) the solid phase of the SBF simply burys the existing community, 2) there is an inhibitory component in the SBF that affects bacterial metabolism or the environment, or 3) the outcome is a combination of 1 and 2. From the oxygen data (Table 3) it is obvious that the sediments quickly became anoxic as a result of drilling. The base chemical of SBFs (representing up to 75% of the total volume) is deliberately made biodegradable which is intended to expedite recovery of the sediments. However, this organic enrichment has the effect of creating anoxia and reducing species diversity. The synthetic chemicals, usually internal and poly alpha olefins, have low solubility and bioavailability, but emulsifiers that include detergents and other additives may contribute some level of toxicity (Jerry Neff, personal communication; Neff et al., 2000). To test this possibility a bioassay was done by the Lumitox Co., Inc. and indicated that water extracts made from the post drill sediments did exhibit toxicity, whereas the pre drill sediment extracts did not.

How long do these deleterious effects last? That is difficult to say, but they do not appear to be permanent. GB-516 had been developed and then abandoned a number of years ago and the redeveloped in 2000-2001. Based on ATP and toxicity measurements prior to the secondary development it is apparent that the sediments had fully recovered from the earlier drilling.

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## VITA

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