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Effect of Temperature and Yeast Extract on Microbial Respiration of Sediments from a Shallow Coastal Subsurface and Vadose Zone

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ABSTRACT

As a part of our study on microbial heterogeneity in subsurface environments, we have examined the microbial respiration of sediment samples obtained from a coastal site near Oyster, VA. The sediments at the site are unconsolidated, fine to coarse beach sand and gravel. A Columbus Instruments Micro-Oxymax Respirometer was used to measure the rate of carbon dioxide (CO_2) production during the respiration of the sediment samples. The rate of respiration of the sediment samples ranged from 0.035–0.6 μL CO_2/h/g of the sediment. The sediment samples showing maximum (0.6 μL CO_2/h/g) and minimum (0.035 μL CO_2/h/g) production of CO_2 were selected to study the effect of micronutrient–yeast extract (0.5 and 1.0 μg/g of the sediment) and water (0.5 and 1.0 mL) on the rate of CO_2 production. The rate of CO_2 production increased with the addition of water, but increased approx 2 orders of magnitude (from 0.26 to an average of 23.5 μL CO_2/h/g) when 1.0 g/g yeast extract was added to the sediment samples. In these coastal sediments, temperature, depth, and addition of water influenced microbial activity, but the addition of 1.0 μg/g yeast extract as a micronutrient rapidly increased the rate of CO_2 production 2 orders of magnitude.

Index Entries: Subsurface sediments; respiration; carbon dioxide (CO_2); yeast extract; micro-oxymax respirometer; water; production.

INTRODUCTION

Bioremediation, the use of microorganisms to degrade organic wastes or immobilize inorganic contaminants in the subsurface, is among the many technologies proposed for the restoration of contaminated sediments and ground water *(1)*. However, the promotion of *in situ* bioremediation presents complex problems. The

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interactions of physical, chemical, and microbial processes need to be understood to ensure rapid, effective bioremediation. We are examining some physical, chemical, and biological interactions that could influence bioremediation in a simple, shallow, uncontaminated aquifer and vadose zone. One aspect of our study is the examination of microbial respiration.

In recent years, much attention has focused on the activity of microorganisms in soils and sediments *(2)*. The measurement of respiration rates is used for estimating microbial activity. Because biodegradation depends on microbial activity and biomass, determining the rate of respiration as accurately as possible is appropriate.

Total microbial biomass is often estimated from direct microscopic counts *(3)*. However, microscopic counts of bacteria in soil and sediment samples are time-consuming, subjective, and not very accurate *(4)*. Microbial diversities and numbers of viable cells have been examined in many surface and subsurface environments, including deep coastal sediments, using measurements of colony forming units (CFU) and colony morphology *(5)*. However, bacteria in sediments can be difficult to grow. Thus, methods that do not require growth are advantageous. Respiration rate, measured as CO_2 production, estimates the activity of microbial populations, without requiring growth on a defined medium. The observed metabolic activity may be expressed as a specific respiration activity (called a metabolic quotient of CO_2 by Anderson and Domsch) *(6)*, which represents the CO_2 production/U biomass and unit time $(MM^{-1}\ t)^{-1}$ *(7)*.

We examined the rate of respiration and the relationships among respiration and other selected physical and biological properties of a subsurface site using a Columbus Instruments Micro-Oxymax Respirometer. Further, We also examined the effect of temperature and yeast extract solutions on the respiration rate of the sediment samples collected from the field site. This article is part of a large field effort funded by the US Department of Energy (DOE). Many other complex relationships will be addressed in other articles.

MATERIALS AND METHODS

Description of Study Site

The field site selected for this study is near Oyster, VA, a site used for the DOE, Office and Health and Environmental Research (OHER), Subsurface Science Program (SSP), heterogeneity and bacterial transport field studies (Fig. 1). The Nature Conservancy owns the site, and the site is operated and maintained by University of Virginia investigators. Sediments of the Coastal Plain physiographic province underlie the Typic Hapludult (coarse loam) soil at the site. The surface and shallow subsurface sediments belong to the Butler's Bluff Member of the Nassawadox Formation. This formation was deposited during late Pleistocene and consists of unconsolidated, fine to coarse beach sands and gravel that are clean and well-sorted *(8)*. Preliminary examination showed that ground water at this site contained <10 mg/L of dissolved organic carbon.

Sampling

Core samples were obtained using steam-cleaned Lexan core liners with split-spoon coring tools and a hollow-stem auger system, as used in the past by the OHER SSP *(9)*. A microsphere tracer was used as quality control for most of the cores collected. A total of 19 wells were drilled in June and August 1994. Nine of the wells

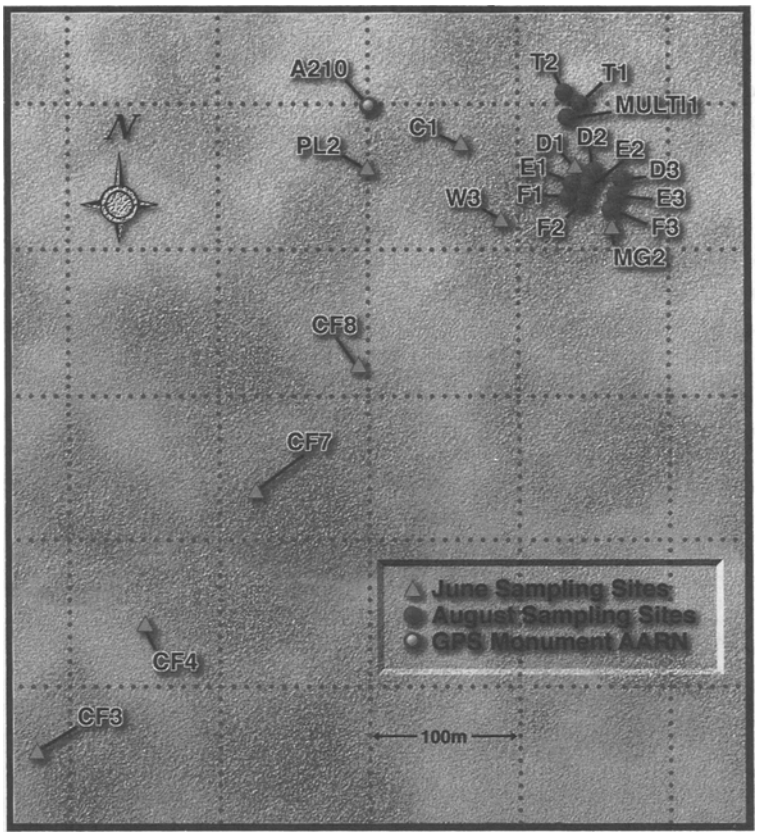

Fig. 1. Map of the study site showing the locations of the wells sampled during August 1994 at the Oyster site (D2, D3, E1, E2, E3, F1, F2, F3, T1, and T2). Also shown are wells placed in June 1994 and earlier. Grid lines are spaced at 100-m intervals, and north is to the top of the figure.

were in a grid with dimensions of 20 × 30 m (Fig. 1) to yield regularly spaced samples. The rest of the wells were distributed surrounding the grid system to obtain samples over a wide area around the central gradient and cover the entire depth sampled. Water levels within these wells ranged from 1.4–22 m with an average of 1.8 m below the land surface. Water content (based on weight loss during air-drying of samples) above the water table ranged from 3.3–17% of sediment dry wt with an average of 10.4%. The water content of the samples below the water table ranged from 17.6–24% with an average of 19.8%. Further heating at 103°C for 24 h did not significantly change weight loss.

Sample Handling in the Field

Undisturbed sediment cores were removed from the spilt-spoon sampler and described for general physical features before being sectioned for sampling. A hand saw was used to section the core material. The fresh face of the sectioned cons was pared away with a sterile spatula, and only the center sediments were collected into sterile Whirl-pak bags for biological analyses. After the outer layer of the sample was placed into 40-mL EPA vials, the vials were gassed with N_2 and saved for

chemical and physical analyses. Subsamples from the inner core were immediately distributed for initiation of on-site aerobic bacterial enumeration by plating or prepared for express shipment to Selma University (SU) for respiration studies. Samples for respiration studies were immediately stored in a refrigerator at SU.

Measurement of Respiration

Microbial respiration within the sediments and the effect of yeast extract additions were measured using a Micro-Oxymax Respirometer (Columbus Instruments, Columbus, OH) in which head space levels of CO_2 and O_2 were continuously monitored. The instrument used gas sensors to measure the changes in the oxygen and CO_2 concentrations in the head space of vessels, and the CO_2 production rates were calculated. When two or more samples were being monitored, the gas sensors sequentially switched from one measuring vessel to the next. Since the system was closed, the head space gas in the vessel was returned after it had been analyzed by the gas sensors. An IBM-PC-compatible computer connected to the respirometer was used for monitoring the system and calculating the data.

Ten grams of each sediment sample collected from Oyster, VA, were placed into a measuring vessel. A test tube containing 5 mL of water was placed in the center well of the vessel to prevent the drying of the samples. The rate of respiration of the sediment samples was measured by monitoring CO_2 produced at 4-h intervals for 24 h using the Micro-Oxymax Respirometer. The effect of different temperatures (20, 25, 30, and 35°C) on the rate of respiration of the sediment samples was measured by monitoring the amount of CO_2 produced at different temperatures for 24 h.

Effect of Yeast Extract Additions on the Rate of Respiration

The effects of a micronutrient—yeast extract—and water addition on the sediment samples showing the maximum and minimum rates of CO_2 production were studied using the respirometer. Ten grams of the selected sediment samples were combined with yeast extract (0.5 and 1.0 g/g of the sediment sample)/water (0.5 and 1.0 mL). The rates of CO_2 production by the sediment samples were measured at 4-h intervals for 24 h and compared with the respiration in an unattended control.

CFU Determination

As a measure of biomass, we determined total numbers of CFU in the samples using published plate count procedures *(5,10)*. Briefly, 10-g subsamples were suspended into 100 mL of a sterile pyrophosphate buffer and mixed in a Waring blender. Serial dilutions of the blended samples were made into phosphate buffer and, along with aliquots from the original pyrophosphate, were plated on dilute peptone, tryptone, yeast extract, glucose (PTYG) medium prepared by adding glucose 0.1 g, yeast extract 0.1 g, peptone 0.05 g, tryptone 0.05 g, magnesium sulfate 7-hydrate 0.60 g, calcium chloride dihydrate 0.07 g, and 17 g Bacto agar to 1 L of filtered distilled water and autoclaving for 20 min.

RESULTS

Effect of Temperature on CO_2 Production

The changes in the CO_2 production rate at different temperatures of 20–35°C are presented in Fig. 2. Low rates of CO_2 production (0.008 μL CO_2/h/g) were

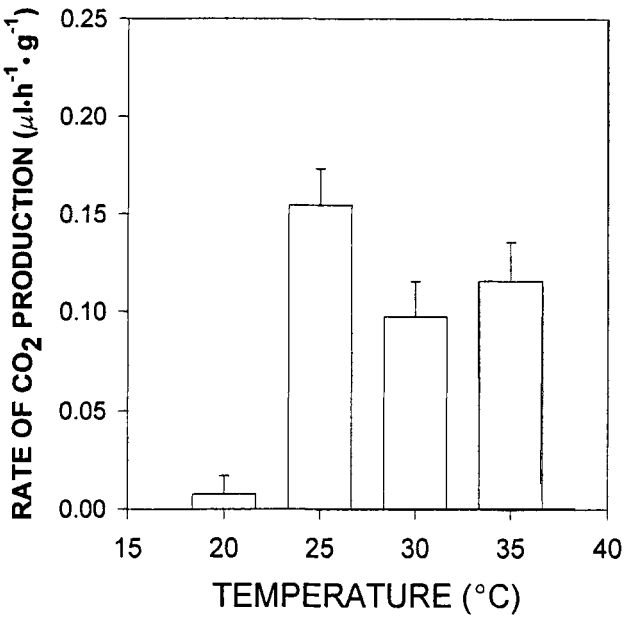

Fig. 2. Effect of temperature on the respiration of the sediment samples. Error bars indicates 1 SE.

observed at 20°C. Maximum CO_2 production (0.15 µL CO_2/h/g) was observed at 25°C (Fig. 2) as compared with 30°C (0.096, µL CO_2/h/g) and 35°C (0.116 µL CO_2/h/g). The temperature for optimal respiration of these sediments was 25°C. Based on these results, 25°C was selected to examine the relative differences in activity among the samples assayed.

CO_2 Evolved and Depth of the Sediment Samples

The sediment samples exhibited different rates of CO_2, production and the rate of CO_2 production ranged from 0.035–0.6 µL CO_2/h/g (Fig. 3A). The rates of respiration within 1 m of the surface (mean and variance = 0.234 ± 0.024 µL CO_2/h/g) were higher than those in the vadose zone between 1 and 2.5 m depths (mean and variance = 0.168 ± 0.003 µL/h/g) (Fig. 3A). Samples from the capillary fringe and water table >2.5 m depths exhibited respiration rates similar to surface soils and much higher than vadose zone samples (mean and variance = 0.247 ± 0.035 µL/h/g) (Fig. 3A).

CO_2 Evolved and CFU

There was no clear relationship between CFU and respiration rate (Fig. 3B) or number of bacteria counted using acridine orange direct counts (AODC) (data not shown). At both low CFU levels ($<10^3$) and at high CFU levels ($>10^6$), respiration varied over almost the entire range seen in the study. The pattern for AODC was similar (data not show) except that direct counts were much higher (10^6–10^8) than the CFU numbers (10^2–10^7).

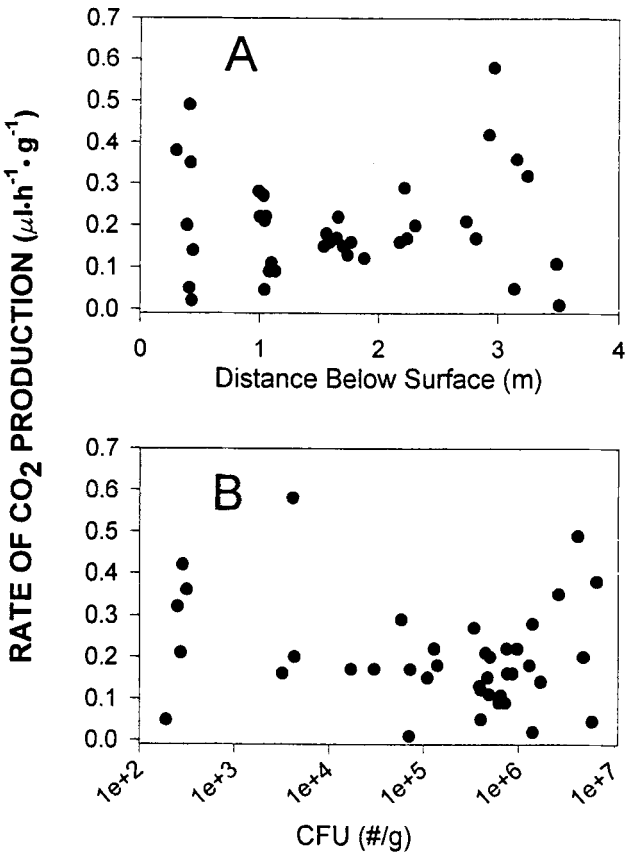

Fig. 3. Relationship between respiration and (A) depth of the sediment samples and (B) viable cells as indicated by CFU.

Effect of Yeast Extract and Water on Soil Respiration

Changes in the respiration rates of the sediment samples with addition of yeast extract solutions and water are presented in Figs. 4 and 5. With the addition of 0.5 μg/g yeast extract, the rate of respiration increased more than tenfold from 0.267–4.29 μL CO_2/h/g. Addition of 1.0 μg/g yeast extract resulted in approx 100-fold (to 23.53 μL/h/g) increases in CO_2 production rates (Figs. 4 and 5). The addition of 0.5 mL of water to the sediment samples increased the respiration rate from 0.267–0.281 μL CO_2/h/g. However, the CO_2 production rate doubled to 0.583 μL/h/g with the addition of 1.0 mL water (Figs. 4 and 5).

DISCUSSION

Measurement of the CO_2 production rate of the sediment samples provided an analytical tool for examining the activity of resident microorganisms. In the present study, the optimal temperature for the respiration of the sediment samples was 25°C, and higher rates of CO_2 production were observed from surface or water saturated sediments. Stimulation of respiration rates above those in unamended samples was most pronounced with the addition of 0.05 or 1.0 μg/g yeast extract as a micronutrient solution, but water alone also increased respiration.

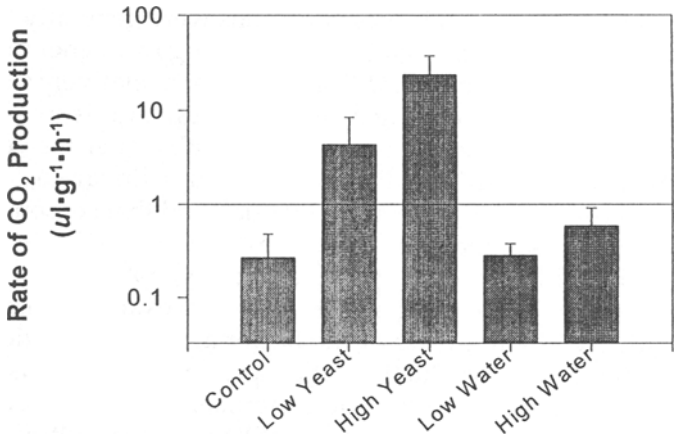

Fig. 4. Effect of addition of yeast extract and water to all sediment samples.

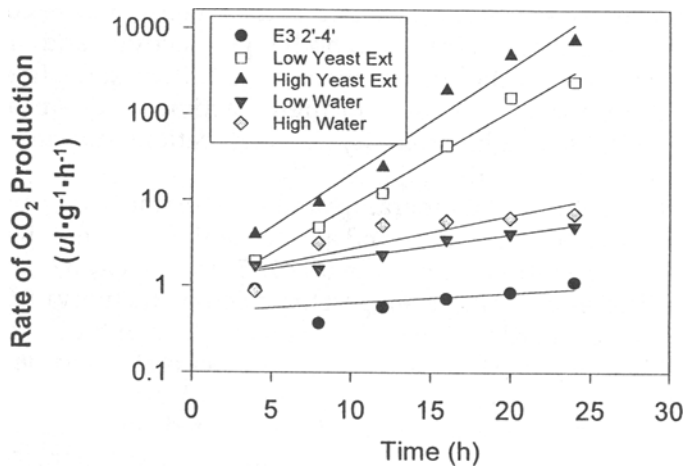

Fig. 5. Effect of addition of yeast extract and water to the sediment sample from well E3 from the 2–4 ft core.

The potential for higher rates of respiration in the sediment samples was evident from the strong response to the yeast extract. The rate of CO_2 production significantly increased when the yeast extract was added to the sediment samples and the rate of CO_2 production increased with an increased concentration of the yeast extract.

The rate of respiration doubled with the addition of 1.0 mL of water. This observation suggests that the rate of CO_2 production depended on the availability of organic carbon, water, and nutrients in the sediment samples. However, the addition of 0.5 mL water had no significant effect on the rate of respiration of the sediment samples. Phelps et al. *(11)* showed a greater stimulation of microbial activity with water on deep confined samples, and Palumbo et al. *(12)* shown the same for deep arid sediments. However, the effect of water on these well-flushed shallow sediments at Oyster was unexpected. The magnitude of the response may have been greater if the samples from an earlier sampling in June were analyzed

for the water response. In June, CFU concentrations were generally lower than in these August samples (data not shown), perhaps owing to a generally dry period prior to the June sampling. Bloem et al. *(13)* showed that a relatively small decrease in water potential after 1 mo of drying caused a significant decrease in respiration and a small decrease in bacterial numbers. Also, subsequent rewetting caused increased respiration. Orchard et al. *(14)* reported a linear-log relationship between surface soil respiration and water potential, and that seasonal respiration changes are a response to soil moisture variation.

It was evident that differences in microbial activity, not differences in population numbers (Fig. 3A and 3B), were critical in the differences among samples. In some samples with relatively low numbers of the bacteria, there was evidence for the potential for high rates of CO_2 production. Thus, per-cell activity must have varied over a wide range. Even comparisons with total numbers of bacteria (AODC), rather than the viable counts from the CFU gave essentially the same result. Similarly, in the Bloem et al. *(13)* study, per-cell activity must have changed significantly in response to drying, since respiration changed significantly, but numbers changed only slightly.

The higher activities at the surface and at the water table are consistent with published studies. Beloin et al. *(15)* found both higher activity and higher biomass in the saturated zone and at the surface than in the vadose zone. In our study, we saw a similar pattern for the potential for higher activity near surface and in the water table, but there was high variability at both locations, and many samples in these regions exhibited low activity.

In summary, the effects of temperature and yeast extracts on the rates of microbial respiration in these coastal vadose and aquifer sediments were significant. At 25 and 35°C, average respiration rates were 0.1–0.15 μL of CO_2/h/g vs <0.01 μL of CO_2/h/g at 20°C. These observations suggest that microbial activity under mesophilic temperatures could be an order of magnitude greater than at typical sediment temperatures of 15–20°C. Depth in the vadose zone sediments also may be a determinate of subsurface microbial activity; the highest rates (0.23 μL of CO_2/h/g) only occurred in sediments within 1 m of the surface or in the saturated zone. The 2 order of magnitude increase in CO_2 production with the addition of yeast extract indicates that subsurface microorganisms are poised for rapid activities and amenable to biostimulation. Preliminary evidence from samples taken in July and August also supports the importance of water in these sediments. Samples taken in July after a relatively low rainfall period exhibited lower CFU than samples taken in August after several heavy rains. The respiration rate provides insight into metabolic potentials fundamental to site characterization. Measurement of respiration activity may be an effective technique for evaluating the microbial responses to nutrients. Despite the limitation of temperature, significant information or the relative activity of different samples and the response to nutrient additions can be gained using this technique.

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