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13. ABSTRACT (Maximum 200 words) To examine the effect of disturbance on bacterial PAH transformation in sediments, <sup>14</sup> C-labeled sediment was resuspended into anoxic overlying water column in a flowthrough microcosm system (I-SORES) at resuspension frequencies of 8-12, 4, 1, 0.25 and 0 d <sup>-1</sup> . Rates of mineralization of <sup>14</sup> C-naphthalene, phenanthrene and benzo(a)pyrene, modeled as first order processes, were found to increase, along with cumulative mineralization as a function of resuspension frequency. Flux of dissolved compound from the bed was also found to increase with increasing resuspension for all compounds. For the most quiescent treatments (0 and 0.25 d <sup>-1</sup> ), multi-order kinetics suggested acclimation of bacterial populations. First order rate constants normalized to the fraction of bioavailable solute in porewaters were within an order of magnitude for all three compounds which span three orders of magnitude in hydrophobicity, suggesting that bioavailability is an important control on mineralization. Similarities in the rate and extent of mineralization between naphthalene and phenanthrene was explained by a greater resistnace to desorption for naphthalene relative to phenanthrene. Model predictions of phenanthrene mineralization rates, based upon the degree of oxygenation in the different treatments, <del>bracketed measured rates, demonstrating oxygen's control over mineralization</del>				
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FINAL REPORT

Grant #: N00014-93-1-0849

R&T Code: 4410005meq01

PRINCIPAL INVESTIGATOR: Profs. G. Taylor, B. Brownawell, G. Lopez

INSTITUTION: SUNY Stony Brook, Marine Sciences Research Center

GRANT TITLE: Effects of Episodic Disturbances on Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs) in Coastal Sediments

AWARD PERIOD: 1 July 1993 - 31 December 1996

OBJECTIVE: Examine effects of intermittent resuspension of suboxic marine sediments into oxic waters on remobilization and bacterial mineralization of PAHs. Characterize how disturbance effects vary between PAH compounds. Tease apart the effects of redox oscillation, desorption and resuspension on mineralization rates of PAHs.

APPROACH: Cause mixing of sediment porewater and solid phase into overlying water via physical resuspension using the Intermittent Sediment Oxygenation & Resuspension Experimental System (I-SORES). Compare release of  $^{14}\text{C}$ -labeled PAHs, polar metabolites and  $^{14}\text{CO}_2$  through time in sediments disturbed 0, 0.25, 1.0, 4, and 8-12 times  $\text{d}^{-1}$ . Follow bacterial dynamics through time. Introduce worms to  $^{14}\text{C}$ -labeled sediments and follow PAH dynamics. Subject thin plugs of sediment labeled with PAH to overlying water that is continually oxic, continually anoxic or oscillating between oxic and anoxic. Monitor mineralization, release of parent and partitioning of parent PAH under the different resuspension treatments.

ACCOMPLISHMENTS: Sediment from Port Jefferson Harbor, a muddy sand sediment with high native PAH concentrations ( $10 \pm 3$  ppm total PAH) was employed for all studies. Batch slurry experiments to determine mineralization under oxic conditions were performed using  $^{14}\text{C}$ -spiked radiolabel and revealed no lag period for naphthalene and phenanthrene assays, suggesting the presence of an acclimated PAH-degrading bacterial population. First order mineralization rates for naphthalene, phenanthrene and benzo[a]pyrene were 0.2, 0.08 and  $0.00003 \text{ d}^{-1}$  respectively.

I-SORES was constructed to allow computer-controlled resuspension of sediments into an overlying oxic water column. Mineralization at different resuspension frequencies was compared in separate experiments for phenanthrene, B(a)P and naphthalene, and in an experiment where phenanthrene and naphthalene mineralization were examined in side-by-side trials

First order rates of mineralization and remobilization were observed for  $^{14}\text{C}$ -phenanthrene at resuspension frequencies greater than  $0.25 \text{ d}^{-1}$  and varied by a factor of two across all frequencies ( $0.013 - 0.021 \text{ d}^{-1}$ ). Multi-order kinetics observed for the 0 and  $0.25 \text{ d}^{-1}$  treatments suggest an acclimation of the PAH-degrading community in these treatments. Model predictions of mineralization, based upon the application of rates

determined from oxic and anoxic slurries and applied to the corresponding oxic and anoxic fractions of the sediment bed, were close to measured initial rates. Incubation of unlabeled sediment resuspended in microcosms at  $12 \text{ d}^{-1}$  and  $0 \text{ d}^{-1}$  frequencies for 30 days, resulted in enhanced rates of phenanthrene mineralization in spiked batch slurry assays when compared to fresh sediment ( $k = 0.27$  and  $0.14 \text{ d}^{-1}$  vs.  $0.08 \text{ d}^{-1}$ ). Addition of fresh phytodetritus to aged, labeled I-SORES sediments stimulated mineralization of  $^{14}\text{C}$ -phenanthrene by 45% in muds disturbed  $12 \text{ d}^{-1}$  but had negligible effect on muds disturbed  $0$  and  $0.25 \text{ d}^{-1}$ . These results suggest that sediment resuspension enhances oxygenation of sediments, which is a major controller of mineralization in these sediments. Labile carbon also plays a role, and the interaction between bulk carbon degradation and PAH concentration may stimulate PAH-degrading bacteria.

Time courses comparable to those performed with phenanthrene were also completed for benzo[a]pyrene (89 d) and naphthalene (19 d) in I-SORES. The ranges of initial mineralization rates ( $k$ ) were  $0.005$ - $0.033 \text{ d}^{-1}$ , and  $0.00014$ - $0.00018 \text{ d}^{-1}$  for naphthalene, and benzo[a]pyrene, respectively. Remobilization and mineralization rates were positively correlated with resuspension frequency for all three PAHs and were consistent with differences in hydrophobicity ( $K_{ow}$ ) of these PAHs. However, cumulative mineralization of naphthalene was slightly less than for phenanthrene. Subsequent side-by-side experiments demonstrated greater resistance to desorption for naphthalene than phenanthrene, as measured by slurry incubations with XAD resin. Results are consistent with recent findings in the literature and may be due to greater penetration into sediment micropores by naphthalene compared to larger PAH molecules, such as phenanthrene. Predictions of PAH partitioning into porewaters using a kinetic radial diffusion model, shows the time to reach equilibrium ( $\sim 10$  minutes for naphthalene and phenanthrene,  $0.5 \text{ min.}$  for BaP) is rapid relative to porewater exchange rates induced by sediment resuspension, suggesting that resuspension is not a mechanism for porewater depletion.

First order rate constants were calculated for all compounds in I-SORES and batch slurry experiments as a function of porewater (bioavailable) fraction. Results showed that rate constants calculated in this manner varied by only an order of magnitude across all resuspension frequencies and between the three compounds which span three orders of magnitude in hydrophobicity, suggesting that partitioning into porewater is an important control on mineralization. The mineralization rates of these PAHs are comparable to those at which soluble labile organic substrates are mineralized. Ultimately, low rates of B(a)P degradation may be attributable to its extreme hydrophobic nature as measured by the octanol-water partition coefficient ( $k_{ow}$ ). Resulting aqueous concentrations may be beneath a threshold level required by bacteria for successful exploitation.

Total bacterial abundances, measured by ATP and direct counts, increased with resuspension frequencies in two I-SORES runs. Bacterial abundances were also affected by age of sediment and presumably availability

of labile substrates; systematically increasing early in the experiment and decreasing later especially in the most disturbed sediments. Observations suggest that availability of co-substrates affect bacterial communities' abilities to grow and degrade PAHs. Characterization of sediment extracts by thin layer chromatography indicate that 20-40% of phenanthrene and naphthalene exist as non-parent, i.e., modified by metabolism, over 30 days, while B[a]P extracts from sediments were >95% parent over a 3 month period.

First order rate constants for mineralization in thin plug (1.5 mm deep) experiments performed on phenanthrene and naphthalene ranged from 0.001(anoxic)-0.051 d<sup>-1</sup>(oxic) for phenanthrene and 0.0184(anoxic)-0.127 d<sup>-1</sup>(oxic) for naphthalene. Redox-oscillated (0.4-1 d<sup>-1</sup>) treatments gave intermediate rates in both cases and frequency of oscillation did not significantly affect rate. Anaerobic mineralization rates of naphthalene were only a factor of 7 slower than aerobic, indicating the potential importance of alternate electron acceptors. In contrast, anaerobic mineralization rates were 50 time slower than aerobic rates for phenanthrene. Partitioning of parent compound between aqueous and sediment phases seemed to be controlled to some extent by degree of oxidation of mineral phases. In both anoxic and mostly anoxic treatments, partitioning of both phenanthrene and naphthalene between aqueous and sediment phases in thin plugs was about 5-fold less in the aqueous phase compared to continuously oxic plugs and was significantly lower than predicted by equilibrium partitioning.

In order to determine whether enhanced diffusive loss of phenanthrene by organism-induced bioturbation can deplete porewater concentrations sufficiently to limit mineralization, desorption rates determined in our experiments were input into a transport model developed by Chen (1993). Results for all three compounds showed that for a wide range of bioturbation rates (10<sup>-6</sup> to 10<sup>-4</sup> cm<sup>2</sup> s<sup>-1</sup>) depletion of porewater only occurs at the sediment-water interface (0.01 mm depth). Results from this exercise have been used in planning experiments with macrofauna, which will be completed this spring.

CONCLUSIONS: Oxygen availability and bioavailability are major controls on bacterial degradation rates of PAHs in contaminated sediments. Sediment resuspension will influence both of these parameters and thus systematically affect the rates at which PAHs are degraded. The magnitude of the treatment effect varies for different PAHs partially due to differences in relative amounts of aerobic and anaerobic degradation. Naphthalene has the highest potential for anaerobic degradation and mineralization rates are least tightly coupled to disturbance and input of oxygen. It also appears that to some extent, oxidation state, controlled by oxygen availability, of sediments exerts some control on bioavailability. Aqueous concentration of large PAHs are sufficiently low under oxic conditions, and possibly lower under anoxic conditions, that bacteria are unable to exploit them due to kinetic limitations.

SIGNIFICANCE: While oxygen availability is believed to be the major rate limitation for PAH degradation in predominantly anoxic coastal sediments, our studies have shown that bioavailability (controlled by desorption) is equally important. Recent studies demonstrate that in field-aged sediments, desorption rates are much slower than in our spiked sediment systems, and so bioavailability may assume even more importance in the field. Natural processes which supply oxygen to sediments, such as tidal resuspension and macrofaunal bioturbation, may affect bioavailability both positively and negatively. The interplay between oxygen availability and aqueous activity (bioavailability) in regulating PAH degradation is under further examination.

PUBLICATIONS AND ABSTRACTS:

- 1995 Taylor, G. T., J. Gulnick, L. LeBlanc, B. Brownawell and G. Lopez. Episodic disturbances and microbial degradation of polycyclic aromatic hydrocarbons (PAHs) in coastal sediments. Benthic Ecology Mtgs., Rutgers Univ, Mar 17-19, 1995.
- 1995 LeBlanc, L. A., J. D. Gulnick, B. J.. Brownawell, G. T.. Taylor. Effects of sediment resuspension on the degradation of phenanthrene. SETAC N. Atlantic Chapter Mtg., Plymouth, MA, July 15-16.
- 1995 LeBlanc, L. A., J. D. Gulnick, B. J.. Brownawell, G. T.. Taylor. Effects of sediment resuspension on the degradation of phenanthrene. SETAC World Congress, Vancouver, BC, Nov. 5-9.
- 1996 LeBlanc, L. A., J. D. Gulnick, B. J.. Brownawell, G. T.. Taylor. Effects of sediment resuspension on the degradation of polycyclic aromatic hydrocarbons: I. System description and characterization of initial degradation of phenanthrene. Ocean Sciences Mtg., San Diego, CA Feb. 12-16.
- 1996 Gulnick, J. D., L. A. LeBlanc, G. T. Taylor, B. J.. Brownawell. Effects of sediment resuspension on the degradation of polycyclic aromatic hydrocarbons: II. Comparison of 2, 3 and 5 ringed compounds Ocean Sciences Mtg., San Diego, CA Feb. 12-16.
- 1996 LeBlanc, L. A., J. D. Gulnick, B. J.. Brownawell, G. T.. Taylor. Factors regulating PAH Transformations in disturbed coastal sediments. SETAC N. Atlantic Chapter Mtg., Newport, RI, Apr 26-27.
- 1996 Taylor, G. T., J. D. Gulnick, L. A. LeBlanc, B. J.. Brownawell. Microbial degradation of polycyclic aromatic hydrocarbons in cyclically disturbed coastal sediments. ASM 96<sup>th</sup> General Mtg., May 19-23.
- 1996 Taylor, G. T. and J. D. Gulnick. Enhancement of marine bacterial growth by mineral surfaces. Can. J. Microbiol. 42:911-918.

Attachment: Copies of papers published to date, and abstracts presented at various meetings

**Effects of Sediment Resuspension on the Degradation of Phenanthrene.** L. A. LeBlanc, J. Gulnick, B. J. Brownawell, G. T. Taylor, Marine Sciences Research Center, SUNY Stony Brook, NY, USA. Degradation of bulk organic matter in sediments is enhanced by oxic/anoxic cycling, a feature common in coastal sediments which are resuspended into overlying waters. We are examining the effect of periodic cycling of sediment between an oxic water column and a reducing sediment bed on polycyclic aromatic hydrocarbon (PAH) degradation by altering resuspension frequency in controlled laboratory exposures. Rates of initial degradation in coastal sediments have been studied for <sup>14</sup>C labeled phenanthrene in sediments that were suspended at the following frequencies: 12/day, 6/day, 1/day, 0.25 day and 0/day in liter-sized flow through chambers. Results to date show that degradation rates are initially log linear, with the greatest rates (2.4-2.7%/day) occurring in the first three treatments. In treatments resuspended less frequently, this rate decreases with time and is followed at 10-12 days by another rate increase, which may indicate the stimulation of a bacterial subpopulation. Rates of PAH mineralization are tied to the lability and mineralization of other pools of sediment organic matter, that in turn are also affected by oxic/anoxic cycling. Addition of fresh diatom detritus stimulated the rates of phenanthrene degradation in resuspension experiments where labile organic matter had already been consumed and microbial activity was low; in contrast diatom addition depressed phenanthrene degradation in sediment exposures with more labile organic matter remaining. We are further addressing the behavior of phenanthrene in exposures where we vary the concentration of initial phenanthrene and the concentration and nature of co-substrates. Presented as a poster at the 2nd SETAC World Congress (16th Annual Meeting), 5-9 November, 1995, Vancouver, BC, Canada.

**Factors Regulating PAH Transformations in Disturbed Coastal Sediments.**

L. A. LeBlanc, J. D. Gulnick, B. J. Brownawell, G. T. Taylor, Marine Sciences Research Center, SUNY Stony Brook, NY, 11794-5000. Important limitations to bacterial transformations of polycyclic aromatic hydrocarbon (PAH) compounds in sediments include compound structure, dissolved concentration and oxygen availability. Here we present evidence suggesting that dissolved concentration is as important as structure in controlling degradation in radiolabeled sediments in flow through microcosms. Results from experiments where the mineralization rates of naphthalene (2 ring PAH), phenanthrene (3 ring PAH) and benzo[a]pyrene (5 ring PAH) were related to the degree of oxygenation of suboxic sediments via sediment resuspension, showed that differences between mineralization rates and percent maximum degradation never varied by more than a factor of two between naphthalene and phenanthrene, despite phenanthrene having sixteen-fold greater hydrophobicity. Our results also showed that dissolved naphthalene concentrations in the overlying water from frequently resuspended sediments were much farther below equilibrium predictions than dissolved concentrations of phenanthrene and B[a]P. We postulate that kinetic limitations to desorption into the aqueous phase limits the biodegradation of naphthalene to a greater extent than for phenanthrene and B[a]P. Results from experiments relating desorption from sediment to mineralization in side-by-side comparisons showed that labeled sediments undergoing resuspension in microcosms desorbed much less naphthalene than phenanthrene, and that the amount desorbed was largely underpredicted by a kinetic radial diffusion model, especially for naphthalene. Desorption rate limitation in our system may help explain the similar degree of mineralization of naphthalene and phenanthrene seen in our experiments. Presented as a Platform at the SETAC 17th Annual Meeting, 17-21 November 1996, Washington, DC.

**Factors Regulating PAH Transformations in Disturbed Coastal Sediments.**

L.A. LeBlanc, J. D. Gulnick, B. J. Brownawell, G. T. Taylor, Marine Sciences Research Center, SUNY Stony Brook, NY. The effect of intermittent oxygen exposure on microbiological mineralization rates of sediment-sorbed polycyclic aromatic hydrocarbons (PAHs) has been examined in flow through sediment-water microcosm systems. Sediments radiolabeled with either naphthalene, phenanthrene or benzo[a]pyrene were suspended into overlying oxic water at the following frequencies: 12, 4, 1, 0.25, 0 per day. Results show that an increasing frequency of resuspension enhances mineralization, but the relationship between individual resuspension frequencies and mineralization kinetics is complex and varies between the three compounds. The greatest separation between treatments was seen for B[a]P (5-ring PAH), followed by phenanthrene (3-ring PAH) and naphthalene (2-ring PAH), which only had significant differences between the non-disturbed and the mean of the disturbed treatments. In addition, differences between naphthalene and phenanthrene mineralization rates were much less than in our own aerobic slurry experiments and similar reports in the literature. We postulate that desorption into the aqueous phase limits the biodegradation of naphthalene to a greater extent than for phenanthrene and B[a]P. Our results show that dissolved naphthalene concentrations in resuspended sediments are much farther below equilibrium predictions than dissolved concentrations of phenanthrene and B[a]P, and may over time have a bioavailable concentrations that is similar to phenanthrene, despite having more than an order of magnitude greater octanol-water partition coefficient. Preliminary results show that sediments labeled with naphthalene and undergoing resuspension in microcosms desorb much less naphthalene than phenanthrene and are largely underpredicted by a kinetic radial diffusion sorption model for hydrophobic organic compounds. Desorption rate limitation in our system may help to explain the similar degree of mineralization of naphthalene and phenanthrene seen in our experiments. Platform presented at the SETAC North Atlantic Chapter Meeting, 26-27 April, 1996, Newport, RI.

Taylor, G., J. Gulnick, L. LeBlanc, B. Brownawell & G. Lopez. Marine Sciences Research Center, SUNY Stony Brook, NY 11794-5000

### **EPISODIC DISTURBANCES AND MICROBIAL DEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN COASTAL SEDIMENTS**

Effects of intermittent resuspension and bioturbation on remobilization and bacterial degradation of PAHs in marine sediments were examined using a flow-through, computer-controlled system (I-SORES) producing 5 disturbance regimes in triplicate microcosms. Mineralization of  $^{14}\text{C}$ -phenanthrene exhibited multiphasic time dependence in all treatments and sediments disturbed 12, 4, 1, and  $0.25\text{ d}^{-1}$  were mineralized 3.5, 3.8, 2.0, 1.2 times faster than undisturbed sediments. Mineralization rates of  $^{14}\text{C}$ -phenanthrene bound to sediments in I-SORES microcosms were intermediate to sediment slurries held under constant oxic or anoxic conditions. At 29 d, < 9% of the  $^{14}\text{C}$ -phenanthrene was remobilized and < 20% was mineralized even in the most active muds. Patterns suggest that remobilization as well as oxygen strongly control PAH degradation rates.

Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAH) in  
Cyclically Disturbed Coastal Sediments. G.T. TAYLOR\*, J.D. GULNICK,  
L.A. LeBLANC, B.J. BROWNAWELL, SUNY, Stony Brook, NY 11794-  
5000

Shallow coastal sediments are periodically resuspended by physical and biological processes thereby altering chemical gradients which control the fate of adsorbed organic matter. The effects of cycling anoxic sediments into an oxic water column on microbial degradation of PAH were examined in a computer-controlled device containing 15 flow-through microcosms resuspended 12, 4, 1, 0.25, and 0 d<sup>-1</sup>. Kinetics and responses to disturbance varied among the three PAH examined (phenanthrene, benzo(a)pyrene, naphthalene). Initial rates of mineralization of <sup>14</sup>C-phenanthrene (<sup>14</sup>CO<sub>2</sub> production) were linear and in treatments disturbed 12, 4 and 1 d<sup>-1</sup> were as much as 2.8 times more rapid (2.4 - 2.7% d<sup>-1</sup>) than undisturbed sediments. In contrast, mineralization of benzo(a)pyrene lagged for two weeks and in the most frequently disturbed sediments was 3.6 times greater than in undisturbed sediments. Resuspension frequency influenced mineralization of benzo(a)pyrene more strongly than that of phenanthrene, i.e., resuspension frequencies of 1 d<sup>-1</sup> or more produced nearly the same effect for phenanthrene whereas mineralization of benzo(a)pyrene increased as a function of resuspension frequency. Bacterial mineralization rates of all tested PAH in cyclically disturbed sediments are compared to those measured in anoxic and oxic slurry incubations. The importance of sediment redistribution and oxygen availability on microbial activity and PAH degradation will be discussed.

**Effects of Sediment Resuspension on the Degradation of Polycyclic Aromatic Hydrocarbons: I. System Description and Characterization of Initial Degradation of Phenanthrene.** L.A. LeBlanc, J. Gulnick, B. J. Brownawell and G. T. Taylor (Marine Sciences Research Center, SUNY at Stony Brook, Stony Brook, NY 11794-5000; 516-632-8688; email: gtaylor@cmail.sunysb.edu. Degradation of bulk organic matter in sediments is enhanced by oxic/anoxic cycling, a feature common in coastal sediments which are resuspended into overlying waters. We examine the effects of periodic cycling of sediment between an oxic water column and a reducing sediment bed on polycyclic aromatic hydrocarbon degradation by alternating resuspension frequency in controlled laboratory exposures. Rates of initial degradation in coastal sediments have been determined for  $^{14}\text{C}$ -labeled phenanthrene in harbor sediments that were suspended at the following frequencies: 12, 4, 1 0.25, and 0  $\text{day}^{-1}$  in flow through microcosms. ISORES (Intermittent Sediment Oxygenation and Resuspension Experimental System) is a system of fifteen liter-sized chambers with computer controlled sediment resuspension. Results show that remineralization rates are initially linear, with the greatest initial rates (2.4-2.7 %  $\text{day}^{-1}$ ) occurring in the first three treatments. In treatments resuspended less frequently, the rates decreased with time and is followed at 10-12 days by another rate increase, which may indicate the stimulation of a bacterial subpopulation. Preliminary evidence that depletion with time of more metabolizable substrates contribute to this rate increase will be presented. Initial rates are slower than rates determined for the same (and other hydrocarbon-impacted) sediments in oxic slurry experiments but faster than initial rates determined for anoxic slurry experiments. The effect of sediment resuspension into anoxic overlying water is currently being investigated and will be discussed. Platform presented at the 1996 Ocean Sciences Meeting, February 12-16, San Diego, California.

**Effects of Sediment Resuspension on Degradation of Polycyclic Aromatic Hydrocarbons:**

**II. Comparison of 2, 3 and 5 Ringed Compounds**

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The effects of resuspension of organic rich, fine grained sediment into oxic overlying water on rates of microbial degradation of polycyclic aromatic hydrocarbons were examined. Experiments employed one of three model PAHs; phenanthrene, benzo(a)pyrene or naphthalene, which encompass a wide range of physicochemical properties. Sediments equilibrated with  $^{14}\text{C}$ -PAH were resuspended using I-SORES (Intermittent Sediment Oxygenation and Resuspension Experimental System) at frequencies of 12, 4, 1, and  $0.25 \text{ day}^{-1}$ . In all cases, resuspension resulted in increased mineralization rate as measured by  $^{14}\text{C}$ - $\text{CO}_2$  evolved from the system. Kinetics varied among PAHs. Benzo(a)pyrene, after a two week lag period where no microbial degradation was detected, showed the most pronounced response to resuspension frequency, the most frequently disturbed treatment resulting in 3.6 times greater remineralization than undisturbed control. Progressively less benzo(a)pyrene remineralization occurred with decreasing resuspension frequencies. Phenanthrene degradation was enhanced with sediment resuspension of at least  $1 \text{ day}^{-1}$  compared to undisturbed control, but increased frequency showed no greater mineralization rate. Naphthalene was remineralized more rapidly than the other two PAHs. Reasons for differences will be discussed in terms of physical properties of individual PAH relative to geochemistry of experimental system.

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# Enhancement Of Marine Bacterial Growth By Mineral Surfaces<sup>1</sup>

Gordon T. Taylor and Jeanne D. Gulnick

**Abstract:** The effects of sorptive inert surfaces and partitioning of organic substrates on growth of marine bacteria and metabolism were examined in microcosms inoculated with bacterioplankton from a local salt marsh. Introduction of organic-free glass beads to a dilute seawater medium (tryptic soy broth) increased yields of ATP, a surrogate for bacterial biomass, by 187% within the entire microcosm (attached + free-living). Growth efficiencies (bacterial C / media C) were 30% for bacteria grown in microcosms with beads compared with 16% without beads. Surface enrichment increased rates of proteolytic enzyme activity and cell-specific [<sup>3</sup>H]leucine incorporation into protein by factors of 6.8 and 2.2, respectively. Scanning electron microscopy revealed obvious organic coatings on all beads after 2 h of exposure, but few strongly attached bacteria were evident, even after 40 h of exposure. Results support the hypothesis that mineral surfaces facilitate bacterial utilization of complex organic matter through physical-chemical processes that increase conversion efficiencies of labile substrate despite possible kinetic limitations. Furthermore, firm attachment by bacteria to these surfaces is apparently not a requirement to produce surface-enhanced activity.

**Key words:** epibacteria, sorption, interfaces, hydrolytic enzymes, growth efficiency.

**Résumé:**

**Mots clés :**

## Introduction

Numerous biological, geochemical, and biotechnological processes occur at interfaces between aqueous and solid phases, a realm distinct from either bulk phase that provides unique habitats for microorganisms. On a global basis, diagenesis of a significant portion of organic matter (OM), both native and xenobiotic, is controlled by its association with surfaces of sediments, soils, and seston and their indigenous microbial communities. It is known that the presence of surfaces and their sorptive capacity for nutrients affect bacterial metabolism. However, to what extent the effects are positive or

negative depends on a variety of factors, including substrate composition and concentration, substratum composition and surface properties, aqueous ionic strength and pH, temperature, hydrodynamics, and microbial community composition (ZoBell 1943; Fletcher and Marshall 1982; Bright and Fletcher 1983; Samuelsson and Kirichman 1990; van Loosdrecht et al. 1990; Zheng et al. 1994; Taylor 1995). One set of conditions, such as small molecules sorbed to porous clay phases, may reduce the bioavailability of nutrients and produce negative surface effects for bacteria, leading to sorptive protection of OM (e.g., Gordon and Millero 1985; Dashman and Stotsky 1986; Mayer 1994; Keil et al. 1994). Whereas another set of conditions, such as macromolecules sorbed to less porous clay or siliceous phases, may improve bioavailability and produce positive surface effects for bacterial metabolism and degradation of OM (e.g., Estermann and McLaren 1959; Griffith and Fletcher 1991; Taylor 1995).

In a dilute medium, such as most natural aquatic systems, organic matter can concentrate at the interface through adsorption, which in turn can stimulate bacterial activity (ZoBell 1943; Kjelleberg et al. 1982). However, variable effects of surfaces have been noted in the literature. For example, bacterial isolates (*Pseudomonas* sp., *Flavobacterium* sp., *Vibrio*

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Table 1. Effect of surface enrichment on maximal bacterial growth rate ( $\mu_{max}$ ), ATP yield, and growth yield efficiency.

Sample	$\mu_{max}$ <sup>*</sup> (h <sup>-1</sup> )	Yield ( $\mu\text{g ATP} \cdot \text{flask}^{-1}$ )	Growth yield efficiency <sup>†</sup> (%)
- beads, suspended	0.38(0.12)	7.1(2.3)	16(5.2)
+ beads, suspended	0.26(0.08)	7.6(0.1)	17(5.2)
+ beads, associated	0.25(0.06)	5.7(1.0)	13(3.1)
+ beads, total <sup>‡</sup>	0.26(0.06)	13.3(2.4)	30(6.9)

Note: Summation of experimental results are presented in Fig. 1; values presented are the mean from triplicate microcosms with 1 SD in parentheses.

\* Calculated from paired time points representing steepest slopes in Fig. 1 using

the equation  $\mu_{max} = (\ln Y_t - \ln Y_{t-1}) / t$  where  $Y = \text{ATP yield}$  and  $t = \text{time}$ .

$\Sigma \text{ATP}$   $\mu_{max}$   $(\ln Y_t - \ln Y_{t-1}) / t$   $\leftarrow$   $(\ln Y_t - \ln Y_{t-1}) / t$   
 $\uparrow$   $\leftarrow$   $\text{substr.}$   
 $\uparrow$   $\leftarrow$   $(\text{Karl 1980})$

† Total biomass yield/added substrate, i.e.,  $\text{ATP} \times 222 \times 100 / \text{TSB} \times 0.33$ ; this assumes an average C/ATP ratio of 222 for marine aerobic microheterotrophs and a C/TSB ratio of 0.33, which was measured directly on a Carlo Erba CHNS analyzer (courtesy of D. Arwood).

‡ Calculated by summing measured values for sus-ATP and bead-ATP.

preserved in 2.5% glutaraldehyde prior to exposure, and after 2- and 40-h exposures. At the University Microscopy Imaging Center (State University of New York, Stony Brook), samples were gently washed three times with sterile seawater, fixed in 2% OsO<sub>4</sub>, washed in distilled water, dehydrated in an ethanol series, critical point dried, and sputter coated with Au-Pd. Samples were observed on a Jeol model 5300 scanning electron microscope and photographed with a Polaroid camera. Images were digitized via a flat-bed scanner and processed on a MacIntosh computer with Adobe PrintShop™ software and reproduced on a Kodak ColorEase™ PS printer.

### Bacterial uptake and protein synthesis

Bacterial amino acid uptake and subsequent protein synthesis were examined to determine whether interfacial communities provide labile substrates for suspended populations or sequester them and to determine if mineral phases inhibit growth of suspended cells through some undefined mechanism. Bacterioplankton populations (0.8  $\mu\text{m}$  seawater filtrate) were grown in saline TSB to midexponential growth phase. <sup>3</sup>H-leucine was added in tracer concentrations (0.2 nM final concentration; specific activity = 141 Ci mmol<sup>-1</sup>, 1 Ci = 37 GBq) to 400 mL of this suspension, which was then immediately split between two flasks, one empty (control) and one with 100 g of precombusted glass beads (surface rich). Both flasks were aerated while incubating at 22°C for 6 h. Radioactivity measured in solution at 0 and 15 min confirmed that essentially all <sup>3</sup>H-leucine remained in solution and was not sorbed to surfaces. At 6 h, triplicate aliquots of the overlying suspensions were removed and fixed with 5% trichloroacetic acid (final concentration) to precipitate protein from cells. Samples were processed for <sup>3</sup>H incorporation into protein according to Kirchner (1993). Aliquots were also removed and fixed with 2% borate buffered formaldehyde (final concentration) and bacteria were enumerated by epifluorescence microscopy using the fluorochrome acridine orange (Hobbie et al. 1977). No attempts to mix or dislodge attached cells beyond the turbulence created by aeration were made, so cell counts and <sup>3</sup>H incorporation rates are representative of cells that were suspended and loosely associated with beads. Using this method, efflux of hydrolytic products from interfacial populations and their utilization by suspended populations were assessed, but protein synthesis rates of attached cells was not.

### Bacterial hydrolytic activity

An incubation similar in all respects to the cell yield experiment described above was performed to evaluate surface effects on proteolytic enzyme activity. Aminopeptidase activity was estimated by

adding a saturating concentration (250  $\mu\text{M}$  final concentration) of a fluorogenic dipeptide analog, l-leucine-2-naphthylamide (Fluka Biochemika), to microcosms with and without glass beads at the initiation of the experiment. After 18 h of incubation, hydrolysis of the dipeptide was measured by production of the naphthylamide fluorochrome in a Shimadzu RF-551 fluorimeter (Hoppe 1993). Separate control experiments verified that sorption of the dipeptide and the hydrolyzed fluorochrome to surfaces in the microcosms was negligible. Therefore, we assume that most of the reactant and product remained in solution throughout the experiment.

## Results and discussion

### Surface-enhanced cell yield

This experiment is similar in design to those of Heukelekian and Heller (1940) and van Loosdrecht et al. (1990), except that only a single TSB concentration was examined instead of a 200-fold range of glucose-peptone concentrations. In those experiments, differences in suspended *E. coli* concentrations between treatments with and without beads were inversely proportional to nutrient concentration, becoming indistinguishable at glucose-peptone concentrations exceeding 50 mg · L<sup>-1</sup>. Our experiment approximates their highest concentration, but we used a medium that was composed of peptones from casein and soybeans instead of animal tissues and had a lower glucose content (11 versus 33% by weight). We also provided 70 times more surface area with smaller beads (total = 20 125 versus 287 cm<sup>2</sup>). These combined factors favored more partitioning of substrate from aqueous to solid phases than the cited experiments.

Unlike the studies discussed above, production of bead-associated as well as suspended bacterial biomass was measured through time (extractable ATP) instead of as end-point determinations. One can see that by merely examining suspended biomass (Fig. 1, open symbols), surface enrichment appears to delay initiation of exponential growth phase and has no apparent effect on growth yields, i.e., equivalent production (Student's *t* test;  $P < 0.90$ ) of sus-ATP (7.1 and 7.6  $\mu\text{g ATP} \cdot \text{flask}^{-1}$ ) (Table 1). In reality, inclusion of bead-associated biomass (Fig. 1, solid triangles =  $\Sigma \text{ATP}$ ) illustrates that lag phases did not differ between treatments, although maximal growth rates ( $\mu_{max}$ ) may have been greater for surface-poor

Table 3. Effect of surface enrichment on [ $^3\text{H}$ ]leucine turnover, protein synthesis rates of bacterioplankton, and hydrolytic enzyme activity.

Treatment	[ $^3\text{H}$ ]leucine turnover time (h)	[Bacteria] ( $\times 10^7 \text{ mL}^{-1}$ ) <sup>super</sup>	[ $^3\text{H}$ ]leucine incorporation rate ( $\times 10^{-6} \text{ dpm cell}^{-1} \text{ h}^{-1}$ )	leucine-naphthylamide hydrolysis
- beads	217(17)	6.26(0.27)	3.93(0.36)	0.58
+ beads	159(14)	3.86(0.24)	8.70(0.94)	3.95

Note: Values in parentheses are 1 SD of triplicate analyses

numbers may have remained relatively constant and the intracellular ATP content may have decreased in proportion to cell size after the onset of nutrient limitation. The actual reason for such rapid declines in ATP pools is unknown and was not further evaluated in this study.

#### Sorption and colonization

For surfaces to positively affect bioavailability, organic substrates must be structurally complex and surface active (ZoBell 1943; Kjelleberg et al. 1982; Taylor 1995). To assess structural complexity, nominal molecular mass distribution of saline TSB was examined using ultrafiltration. At least 44% of the solutes in the medium exceeded 1 kDa and were likely to be oligo- or poly-meric (Table 2). Proteinaceous solutes >1 kDa are expected a priori to be highly surface active in seawater (Taylor et al. 1994a and references therein).

Sorption of TSB solutes to glass surfaces was confirmed in two ways: by measuring disappearance from solution and visually by SEM observations (described below). As much as 38% of the TSB appeared to be bound to beads and sorption was related linearly to mineral surface area (Fig. 2). The slope of this relationship represented the average surface concentration, yielding  $200 \text{ ng} \cdot \text{cm}^{-2}$ . This concentration is equivalent to a protein monolayer for which rapid degradation is predicted (Taylor et al. 1994a, 1994b). Mean carbon surface concentrations were the equivalent of  $65 \text{ ng C} \cdot \text{cm}^{-2}$ , as calculated from direct measurement of TSB carbon content (0.33) by CHN analysis and the sorption experiment (significance discussed below).

Scanning electron microscopy revealed that beads equilibrated for 2 h in saline TSB were coated with conspicuous organic layers (Fig. 3). Beads averaged  $172 \mu\text{m}$  in diameter although much smaller beads fused to larger beads were commonly observed (Fig. 3b). Consequently, effective surface area of the beads actually exceeded our calculated value of  $20 \text{ 125 cm}^2 \cdot \text{flask}^{-1}$ . Many irregularities in surface coverage were apparent, especially along margins of adjoining beads. Fissures in organic coatings were common, probably resulting from desiccation during sample processing (Fig. 3c). At high magnification ( $\times 10 \text{ 000}$ ), a fuzzy texture was readily apparent for coatings on beads incubated for 40 h (Fig. 3d), but not for those incubated for only 2 h. Visible alteration of the coating from early to late time points may signify enzymatic degradation of sorbed OM.

Surprisingly few bacteria were apparent on bead surfaces, even in the 40-h exposures. Almost all bacteria had bacillus morphology ( $0.5 \times 2.0 \mu\text{m}$ ) and the majority were clustered in microcolonies (Fig. 3d). Most cells in these microcolonies did

not appear to be intimately attached to bead surfaces but were attached primarily to other cells and projecting away from the substratum. The basal cells had few points of contact with the substratum and no conspicuous pili, fimbriae, or exopolymers were evident. Although these samples were processed according to standard SEM protocols for biological materials, possible ablation of attached cells was a concern. Two lines of evidence suggest that this did not occur. Firstly, the organic coatings appeared to be intact at both time points with no indication of erosion or peeling. Secondly, parallel samples were examined nonquantitatively by epifluorescence microscopy, which has milder processing requirements, and sparse colonization was also apparent by this analysis. A plausible explanation is that much of the observed bacterial ATP and activity is attributable to populations loosely associated with the substratum, its coating, and boundary layer, as well as pore waters, and not attributable to firmly attached cells. It has been shown that glass coated with proteinaceous materials inhibit sorption of plaque bacteria (Østravik 1977). In the present study, beads became coated minutes after initiation of the experiment while exposed to a very dilute bacterial suspension ( $\sim 10^3 \text{ mL}^{-1}$ ), so perhaps access to bare substratum and the ability to firmly adhere were limited by the organic film during the course of the experiment.

#### Bacterial processing of substrate

Rates of microbial processing of OM should be accelerated throughout surface-rich systems (ZoBell 1943), if physical sorption facilitates enzymatic hydrolysis and utilization of TSB (as demonstrated for protein (Griffith and Fletcher 1991; Taylor 1995)), if suspended polymers continue to sorb replacing degraded OM, and if hydrolysates diffuse from vicinal waters to fuel suspended cell metabolism (Cho and Azam 1988; Azam and Smith 1991). The first hypothesis is supported by evidence presented below, as well as from other studies. The second hypothesis was not investigated in these experiments. The last hypothesis was examined by comparing turnover of [ $^3\text{H}$ ]leucine, a tracer of free hydrolysates, by exponentially growing bacterial suspensions shortly after adding beads. During a 6-h exposure, unconditioned beads partitioned 38% of the bacterioplankton out of the overlying suspension (Table 3). Nonetheless, suspended bacteria turned over dissolved [ $^3\text{H}$ ]leucine 136% faster in the surface-rich microcosm than in the control (159 versus 217 h). Moreover, cell-specific rates of [ $^3\text{H}$ ]leucine incorporation into protein were 220% faster ( $P < 0.01$ ) in surface-rich microcosms;  $8.70$  versus  $3.93 \times 10^{-6} \text{ dpm} \cdot \text{cell}^{-1} \cdot \text{h}^{-1}$  (Table 3)

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were apparent and by the high rates of [ $^3\text{H}$ ]leucine incorporation measured in cells suspended above the beads. Therefore, measured protein synthesis rates probably reflect activity of both suspended and interfacial bacteria.

### Ecological context

Surface concentrations of TSB (mean = 65 ng C·cm<sup>-2</sup>) fall within the range of organic monolayers (60–90 ng C·cm<sup>-2</sup>; Fig. 2, broken lines) reported recently for subsurface coastal sediments (Mayer 1994; Keil et al. 1994). In contrast with undefined monolayers of complex OM associated with heterogeneous aged sediments, our monolayers did not appear to be sorptively protected (Mayer 1994; Keil et al. 1994). In fact, sorbate was quite labile and efficiently utilized by bacteria. We offer several reasons why these results diverge. Preservation of sorbed materials is favored if mesopores that sequester OM and exclude bacteria are abundant on mineral phases, such as some clays (Estermann and McLaren 1959; Marshman and Marshall 1981; Mayer 1994). Likewise, mono- or oligo-mers sorbed from solution or remaining behind as refractory residues from degraded polymers will be preserved if irreversibly bound to surface sites (Dashman and Stotsky 1986). In contrast, degradation and bioconversion of sorbed OM will be enhanced if mineral surfaces are relatively nonporous, like biogenic opal, quartz, and basalts, and if sorbates are largely polymeric (ZoBell 1943; Griffith and Fletcher 1991; Taylor 1995).

The surface effect as elaborated by ZoBell (1943) can be explained through a combination of kinetic and thermodynamic controls. In essence, mineral surfaces and their boundary layer serve a catalytic function. They concentrate reactants (colloids, polymers, bacterial hydrolases and uptake systems, and hydrolysates), induce conformational changes (i.e., denaturation) that relax steric limitations, and control transport of labile substrates. Concentrating reactants favors faster hydrolytic and uptake kinetics locally (ZoBell 1943). Denaturation of sorbed polymers is thermodynamically favored because interfacial free energies decrease when hydrophobic moieties associate with the substratum and entropy of the bulk fluid increases when hydrophilic moieties are exposed at the water–film interface (Norde and Lyklema 1991). Consequently, the process of denaturation exposes more bonds susceptible to enzymatic hydrolysis, permitting more efficient functioning of hydrolases (Taylor 1995).

Hydrolases produce free peptides and amino acids that may be taken up immediately. Alternatively, some portion of the hydrolysate escapes the uptake systems of proximal hydrolytic bacteria and diffuses away to be further hydrolyzed or taken up by other bacteria (Azam and Smith 1991). Unlike the isotropic diffusion of hydrolysates in solution, diffusion of hydrolysates from sorbed parent molecules is anisotropic, theoretically confined to approximately a 180° hemisphere by the interface (Taylor 1995). Furthermore, movement of hydrolysates within the vicinal layer is controlled almost exclusively by molecular diffusion, whereas molecules in bulk phase are subject to both advective and diffusive processes. Therefore, probabilities of further encounter with bacterial hydrolytic and transport systems are enhanced by at least a factor of two within the boundary layer of solid phases compared with similar encounters in the bulk phase. Consequently, interfacial bacteria can capture more hydrolysate per unit enzyme activity and couple hydrolytic and

uptake processes more effectively as suggested by Taylor (1995). Hence, we observe doublings in bacterial growth yield efficiency and protein synthesis rates, as well as stimulated proteolytic activity merely by introducing inert beads.

The present study should be considered representative of the near-term fate of labile polymers associated with sandy surficial deposits, coarser materials (e.g., gravel, rock) and synthetic surfaces. Findings may also have biotechnological applications, particularly in fermentor and other bioreactor technologies. In nature, surface effects on bioconversion of OM are undoubtedly more heterogeneous and complex than portrayed here, varying spatially, temporally, and among mineral phases, as well as being influenced by interactions with other sorbates. However, our results conclusively demonstrate that glassy sediment surrogates can exert a positive surface effect on bacterial productivity and degradation of proteinaceous substrates in marine microcosms. The questions of whether all labile polymers and those associated with nonsiliceous mineral phases and biogenic debris behave similarly merit closer examination.

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