FORMULATION AND TESTING OF FORAGING THEORY FOR MARINE BACTERIA

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LONG TERM GOAL

Our long-term goal is to understand the action of dissolved enzymes in organic material (OM) degradation and microbial growth. Microorganisms and their enzymes are essential for decomposition of much natural and pollutant OM. In marine environments, much of this material is unavailable for direct incorporation or degradation by microorganisms, either because it is itself too large to be ingested by the cell, or because it is sorbed to some large organic or inorganic substratum. Microbial enzymes, sufactants, and other dissolved cell products that act outside of the cell, hydrolyze, solubilize or otherwise reduce this OM to material that can be incorporated and/or degraded. This initial step often limits the overall rates of material cycling and microbial processes. Thus we wish to understand the action of dissolved enzymes in OM degradation and microbial growth in order to predict the fate of OM in marine environments. In so doing, we hope to contribute to understanding OM and microbe effects on sediment stability and the transmission of sound, as well as to the design of rational plans for pollutant remediation .

OBJECTIVES

Our scientific and technical objective is to design and test a predictive model of microbial foraging with cell-free enzymes. Specific objectives for the model are predictions of relative rates of microbial growth under different environmental and microbiological conditions including: different OM concentrations, compositions and distributions; different sediment porosities; different cell sizes; different enzyme properties, etc. Specific objectives for empirical testing are microbial growth rates, enzyme-substrate binding properties, and the relationship between rates and properties.

APPROACH

Our research takes an iterative approach to understanding hydrolysis of natural and pollutant OM, alternating modeling and laboratory research to predict and test hypotheses on important constraints on microbial foraging with cell-free enzymes. Model design and experimental data are refined as each guides or constrains the other. For example, preliminary modeling, completed prior to FY97, suggested specific enzyme-substrate binding as a key determinant of the potential efficacy (as reflected in microbial growth rates) of microbial foraging with cell-free enzymes. This inspired preliminary experiments that examined enzyme-substrate binding by cell-free enzymes of several bacterial isolates. These experiments, in turn, revealed important features of enzyme-substrate binding kinetics that were incorporated into the next iteration of the model. The refined model itself has now been used to design a series of experiments examining the relationship between enzyme binding kinetics and microbial growth rates that have been the principal subject of research during FY97.

WORK COMPLETED

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During FY97 a manuscript describing our numerical model of cell-free enzyme foraging by marine bacteria was completed and submitted for publication. This was the culmination of several years' theoretical and technical development that included experimental confirmation of some important model parameters. Concurrently, experiments on cell-free enzyme-supported microbial growth rates were performed. The results that were obtained from these experiments will be used in combination with data on enzyme kinetics that are currently being collected to test predictions of the completed model.

RESULTS

Modeling completed in FY97 has succeeded in predicting several features of enzyme activity observed in laboratory and environmental samples. These include: support of significant bacterial growth by cell-free enzymes; preponderance of particle-attached as opposed to dissolved cell-free enzymes; solubilization of particulate substrates in large excess of growth requirements of resident microbes; and constitutive, abundant enzyme release in some environments. The desire to understand the action of dissolved enzymes in OM degradation and microbial growth is well-served by the mechanistic understanding gained from this success. Other model predictions, such as restricted range for enzyme foraging and very high enzyme-substrate binding coefficients have implications for planning pollutant remediation.

Laboratory work completed in FY97 has determined growth rates of several bacterial isolates that were restricted to growing on the products of cell-free enzymatic hydrolysis of particulate chitin. Significantly, growth rates were variable between isolates within a restricted range. Cell numbers increased linearly during growth rather than exponentially, suggesting an absolute limit on the rate at which chitin hydrolysate was produced.

IMPACT

Significant impacts of our work for the Navy come in four areas:
Biofouling may be reduced by supplementing microbicides with enzyme-specific poisons.
Biodegradation may be enhanced by controlling bacterial growth with released enzymes.
Engineering of bioreactors, bacteria and enzymes may be improved by exploiting substrate specificity of released enzymes.
Acoustics of sediments may be influenced by enzymatic hydrolysis of organic films.

TRANSITIONS

Results have led to funding of a postdoctoral fellowship for the primary graduate student supported by this grant, Yves-Alain Vetter, to apply specific OM hydrolysis predictions to an innovative engineering approach to sediment biostimulation and consequent pollution bioremediation. Another graduate student, Jill Schmidt, supported temporarily on this grant, has moved her research findings on the constancy of bacterial abundance in sediments into the realm of sensing bacterial and OM influences on acoustic transmission in the upper sediment layer.

RELATED PROJECTS

This work has led directly to two new projects. One project on sediment biostimulation, which has implications for in situ bioremediation of pollutants, will be a collaboration of B Krieger-Brockett, YA Vetter and JW Deming, and is expected to be funded by Washington State Sea Grant. The second project on sediment acoustics is a collaboration of P Jumars, JL Schmidt, JW Deming and DJ Tang, and was awarded funding through an internal competition at the University of Washington Applied Physics Laboratory.

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