Presented by Washington Sea Grant Program and Marine Bioremediation Program

Research advances & practical applications

PROCEEDINGS: Marine Extremozymes and Bioremediation

A workshop for researchers and industry

Washington Sea Grant Program · 3716 Brooklyn Avenue N.E. Seattle, Washington 98105

Marine Extremozymes and Bioremediation

Research and Practical Applications

Conference Proceedings

September 24-25, 1996

University of Washington, Seattle

Washington Sea Grant Program
University of Washington

Support for this workshop and publication of the proceedings was provided in part by grant number NA36RG0071 from the National Oceanic and Atmospheric Administration to the Washington Sea Grant Program. The views expressed herein are those of the authors and do not necessarily reflect the view of NOAA or any of its sub-agencies.

Washington Sea Grant Program
3716 Brooklyn Ave. N.E.
Seattle, WA 98105
March, 1997

Keywords
1. Bioremediation. 2. Marine biotechnology. 3. Extremozymes. 4. Extreme temperatures.
5. Marine Sediments. 6. Polyaromatic hydrocarbons (PAHs). 7. Microorganisms.

WSG-WO 97-1

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Introduction

Andrea E. Copping, Assistant Director, Washington Sea Grant Program

The fields of marine bioremediation (the cleanup of hazardous and toxic wastes by living organisms) and marine extremozymes (the study and use of enzymes extracted from organisms living in extreme environments) are in their infancy. Bioremediation of terrestrial and freshwater environments is developing into a major industrial sector world-wide; however, bioremediation in the marine environment is still in the exploratory and startup phases. The discovery of deep ocean vents and their associated microbial communities has given rise to new possibilities for commercial applications using enzymes formed within high-temperature environments. Enzymes created by microorganisms living in other extreme environments—including cold ocean water and polar ice, very high salinity waters, and waters of great depths and pressures—have added to the scientific interest in and commercial potential of marine extremozymes.

Building on the core of researchers working in marine bioremediation and marine extremozymes at the University of Washington, Washington Sea Grant Program brought together scientists and engineers from around the country to discuss these two emerging and fascinating topics. While these topics seem unrelated, researchers in universities, industry, and the government are seeing connections between bioremediation in the marine environment and the capabilities of certain extremozymes.

During a one-and-a-half day workshop held in September 1996 at the University of Washington in Seattle, over 130 researchers and students shared the results and potential ramifications of their work through a series of oral and poster presentations. This proceedings provides summaries of the talks, titles of the posters, and a listing of workshop participants. Washington Sea Grant Program is pleased to have had the opportunity to present this outreach effort in biotechnology. We hope to have similar opportunities in the future.

About Washington Sea Grant Program

Established in 1968, Washington Sea Grant Program (WSGP) began as a federal experiment in local investment. Designed to identify marine resource issues at the grass-roots level and bring the scientific expertise of university researchers to bear in addressing them, WSGP draws on a wealth of talent from the University of Washington (UW) and other participating institutions. The program grew as part of the UW's major interest in marine science, engineering, and policy. In 1971 WSGP became one of the first four programs designated nationally as a Sea Grant College. Today WSGP is part of a national network of 29 Sea Grant programs administered by the National Oceanic and Atmospheric Administration (NOAA) in the U.S. Department of Commerce.

Washington Sea Grant Program uses a partnership approach in all its activities. Some of WSGP's frequent partners include other universities and educational institutions; tribes and local, state, and federal agencies; business and industry; marine-oriented interest groups; and citizens of the state of Washington. The participation involves financial contributions to program and project activities, advice and counsel, and involvement in projects.

Washington Sea Grant Program is located in the Office of Marine Environmental and Resource Programs (OMERP), one of five principal operating units of the UW's College of Ocean and Fishery Sciences.

In addition to funding research projects in marine products, microorganisms in the marine environment, and ocean engineering, WSGP continues a long-term effort to inform marine scientists, decision makers, and the public about emerging issues in marine biotechnology.

Marine Molecular Biotechnology Laboratory, University of Washington

The extremophile and bioremediation research efforts at the University of Washington benefit from the resources of the Marine Molecular Biotechnology Laboratory (MMBL), a new facility in the College of Ocean and Fishery Sciences. In this unique suite of laboratories, excellent facilities are available for analysis of nucleic acids and proteins and for recombinant DNA work. In addition to all the facilities routinely found in a molecular biology laboratory, the MMBL boasts a fast protein liquid chromatography apparatus for purifying novel enzymes, an automated DNA sequencer/gene scanner, and a fluorescence imager. The latter two instruments permit a wide range of analyses—including sequencing, electrophoretic analysis, and membrane-based assays—using fluorescent labeling techniques instead of traditional and more hazardous radioactive labeling methods.

In addition to bioremediation-related work, current research in the MMBL deals extensively with the conservation, management, and aquacultural genetics of marine organisms, and fundamental aspects of gene expression in marine microalgae. Although dedicated primarily to research and training of new scientists within the College of Ocean and Fishery Sciences, the MMBL is available for contract support from industrial and other interests. For more information contact Paul Bentzen (206/685-9994, pbentzen@fish.washington.edu) or Ginger Armbrust (206/616-1783, armbrust@ocean.washington.edu).

Abstracts

Overview of Extremozymes and their Biotechnological Potential

John A. Baross, School of Oceanography,
University of Washington, Seattle

Microorganisms have been isolated from or detected in environments once thought to be too extreme for biological systems. The range of physical and chemical conditions in these environments includes temperatures from below freezing to greater than 110°C, pH ranges from 0 to 12, saturated salt concentrations, hydrostatic pressure equivalent to the depth of the deepest ocean trenches, and levels of heavy metals and xenobiotics once thought to be toxic. The basis for microbial growth and enzyme stability under such extreme conditions is just starting to be addressed. So are implications of the existence of life in these environments to theories of the origin and evolution of life on Earth and elsewhere. In addition, biotechnological applications for microorganisms from these extreme environments are just beginning to be explored. Particular attention has focused on microorganisms isolated from deep-sea hydrothermal vent environments and growing at temperatures greater than those necessary to boil water (hyperthermophiles) and microorganisms isolated from cold marine environments (psychrophiles).

A high diversity of microbial species is expected to exist in these environments, based on preliminary examination of the sequence differences of specific genes and on the incidence and properties of specific enzymes. This high diversity among species from extreme environments has greatly expanded our search for enzymes, called extremozymes, that have biotechnological applications, such as the thermostable polymerases used to synthesize DNA. Extremozymes can also be used to hydrolyze proteins and complex carbohydrates, and to modify other enzymes, such as by converting glucose to fructose. Metabolic products, such as novel bioactive compounds including antibiotics, and new applications, including the ability to remediate environments polluted with heavy metals or toxic organic chemicals, are examples of future prospects that may result from the study of microorganisms that live in extreme environments.

Overview of the UW Marine Bioremediation Program

Jody W. Deming, School of Oceanography,
University of Washington, Seattle

Terrestrial bioremediation, the use of native or introduced microorganisms to enhance the degradation of contaminants in groundwater and soil, is a maturing field of international inquiry. In contrast, the parallel roles and possible applications of microorganisms in bioremediation in the marine environment remain poorly understood, even though high concentrations of contaminants, including organics and heavy metals, have entered and persist in vast and heavily populated coastal areas of the world. The University of Washington received a five-year University Research Initiative Award from the Office of Naval Research to establish an interdisciplinary research program to advance the new field of marine bioremediation. With additional support from the University, we have built a research and student training program focused on the degradation of organic contaminants, especially polyaromatic hydrocarbons (PAHs) but also polychlorinated biphenyls (PCBs), in marine sediments, the repository of these hydrophobic and toxic compounds.

As a model site for exploring native microbial community structure and PAH-degradative capabilities, faculty and students conduct empirical research and modeling studies at, or in relation to, an EPA Superfund site in Puget Sound called Eagle Harbor; it was heavily contaminated over a period of decades with PAHs from a now-defunct creosote wood-treatment plant. In 1994 Eagle Harbor was capped with a thick (~1 m) layer of relatively clean sediment dredged from a nearby waterway, thus introducing the opportunity for researchers in the Marine Bioremediation Program (MBP) to combine microbial studies with the implications of sediment capping, the apparent treatment of choice today for contaminated marine sediments (second only to "no action").

In the course of these studies, MBP microbiologists and molecular geneticists have determined that the total numbers of bacteria residing in the contaminated sediments are higher than expected, given the toxic nature of the environment, and higher than in surrounding cleaner sediments; that community diversity based on DNA extraction, amplification, and sequencing methodology is also surprisingly high; and that significant portions of the community are viable (culturable) oxygen-requiring specialists feeding on PAHs. MBP engineers and oceanographers have further determined that sub-communities capable of degrading PAHs (and PCBs) in the absence of oxygen, as occurs beneath a sediment cap, also exist in the contaminated sediments. First estimates of their degradation rates in situ suggest a multi-year to decadal time scale for removing PAHs dissolved in the sediment pore waters. An analysis of results to date reveals the continuing need for sorption studies under marine conditions that would identify means to enhance the bioavailability of sediment-sorbed and aged contaminants (e.g., use of surfactants, nutrient augmentation, even "extremozymes"). It also leads to the hypothesis that acceptable degradation rates and protection of the overlying ecosystem may be achieved with a thin capping approach to contaminated marine sediments.

Protein Stability at Extreme Temperatures

Michael W.W. Adams, Department of Biochemistry and Molecular Biology,
University of Georgia, Athens, Georgia

The so-called hyperthermophiles are a recently discovered group of microorganisms that grow at temperatures of 90°C and above. All have been found in geothermally-heated environments and most are of marine origin. Several enzymes and proteins have been purified from these organisms. Many, although not all, are extremely thermostable with half-lives at 100°C of many hours and, in some cases, days. The mechanisms by which protein "hyperthermostability" is achieved, however, are far from clear. Aligning the amino acid sequences of hyperthermophilic proteins with the equivalent protein from more conventional organisms gives no insight into potential stabilizing mechanisms, and it has become clear that comparisons must be made at the level of three dimensional structures. As model systems we have chosen two redox proteins, rubredoxin (Rd) and ferredoxin (Fd), purified from the hyperthermophilic marine archaeon *Pyrococcus furiosus* (T_{max}, 105°C). The proteins Rd and Fd are small (~60 amino acids) and extremely stable ($T_m \geq 117$°C). Both have been structurally characterized by X-ray crystallography and/or NMR spectroscopy. Their genes have been cloned and expressed in a mesophilic host, thus enabling specific mutant forms to be produced. These mutant forms are being used to investigate potential stabilizing mechanisms.

A related issue is whether hyperthermophiles contain intracellular solutes that serve as general "thermoprotectants." For example, many of these organisms contain high concentrations of the novel sugar di-inositol, 1, 1'-phosphate (DIP). A large scale purification scheme has now been devised to obtain DIP from the hyperthermophilic marine bacterium *Thermotoga maritima* (T_{max}, 90°C).

Proteases and Glycosyl Hydrolases from Hyperthermophilic Microorganisms: Biological and Biotechnical Considerations

Robert M. Kelly, Department of Chemical Engineering, North Carolina State University, Raleigh,
North Carolina

Hyperthermophilic microorganisms are known to produce an impressive array of intrinsically thermostable enzymes which have been studied for their scientific and technological potential. Since many such organisms isolated to date are heterotrophs growing on an array of peptide- and oligosaccharide-based media, hydrolytic enzymes responsible for recruiting these as nutritional sources have received particular attention. In this regard, we have isolated a number of proteases and glycosyl hydrolases from the hyperthermophilic archaeon *Pyrococcus furiosus* and from hyperthermophilic bacteria in the genus *Thermotoga*. These enzymes have been studied for their physiological importance, their biochemical characteristics, and, in some cases, their biotechnological potential.

Recombinant-Based Approaches for Enzyme Discovery

Eric J. Mathur, Jeffrey L. Stein, Dan E. Robertson, Ronald V. Swanson, and Jay M. Short, Recombinant BioCatalysis, Inc., La Jolla, California

The myriad microorganisms inhabiting diverse biotopes represent a vast repository of potentially valuable biocatalysts for industrial applications. Molecular phylogenetic analyses suggest that greater than 99% of the organisms existing in the environment remain uncultured. At present, all commercially available extremozymes are derived from the small fraction of microbes amenable to growth in pure culture.

Our principal route to enzyme discovery involves expression cloning using genomic libraries constructed with nucleic acids extracted directly from the environment. The screening of these information-rich "environmental libraries" is accelerated by use of high-throughput, robotic systems in which thousands of clones are rapidly screened against multiplexed regimes of chromogenic substrates. Following confirmation and sequencing of positive clones, open reading frames are identified and the genes subcloned for overexpression. High throughput, automated expression screening of recombinant genes from eubacterial and archael origin has yielded over 300 industrially relevant enzymes. These recombinant enzymes, which have evolved naturally for more than three billion years, provide unique starting molecules for the application of non-natural, directed evolution techniques. The combination of natural enzyme diversity with directed, iterative evolution enables the optimization of specific biocatalytic activities for a wide range of industrial and pharmaceutical applications.

Sorption and Biological Interaction for PAH Removal from Marine Sediments

H. David Stensel, Tom Poeton, Department of Civil Engineering, University of Washington, Seattle; and Stuart Strand, College of Ocean Resources, University of Washington, Seattle

Removal of polyaromatic hydrocarbons (PAHs) during bioremediation of marine sediment was assumed to occur by a process of desorption of PAHs from the sediment and biodegradation of the resulting dissolved PAHs in the sediment pore spaces. This process was described by a mechanistic model and individual experiments were carried out to quantify the mechanisms of solids-liquid partitioning, desorption kinetics, and biodegradation kinetics in a PAH-degrading marine environment. These studies were followed by experiments in which PAHs were degraded by the environment with and without sediment present. Blakely Harbor sediment was used and phenanthrene was the model PAH compound. The fate of phenanthrene was followed using radio-labeled carbon.

The research results showed that when phenanthrene was present only in the dissolved phase, it was degraded two to three times faster in environments with sediment than in those without sediment. When sediment was present, solids partitioning resulted in less than 10% of the phenanthrene in the test vials being in the dissolved phase. One explanation for this result is that the bacteria attached to the sediment and were able to feed on phenanthrene located on or near the particle surfaces. Epifluorescent measurements showed bacteria attachment did occur and that the portion of added bacteria that attached increased from 20% to 50% when phenanthrene was present on the sediment. These results imply that the presence of PAH-degrading bacteria that attach to sediment can increase bioremediation rates.

Models for Rates Processes in Contaminated Marine Sediments

B. Krieger-Brockett, Chemical Engineering, University of Washington, Seattle

It is necessary to predict the rate of biodegradation of xenobiotics or contaminants in order to compare natural remediation or biological treatment processes to more traditional options such as dredging. However, some biological degradation rates are not optimal owing to reduced bioavailability of the substrate, limited supply of a needed nutrient, or slow transport of nutrients or metabolites in the sediment. Predictions have been made of physical, chemical, and biological rates occurring in marine sediments in Eagle Harbor, Wash.

Cold Regions Bioremediation

Hal Marlow, Hart Crowser, Inc., Anchorage, Alaska

Petroleum hydrocarbons are generally biodegradable if the indigenous microorganisms that acclimate to using these fuels as a carbon source are provided an adequate source of oxygen and inorganic nutrients. Bioventing is a remediation technique that provides the naturally-occurring microorganisms with favorable conditions. This technique has been used in several locations in Alaska. Natural attenuation does occur at some sites, and microorganisms may eventually degrade residual hydrocarbons present in the soil. Such natural bioremediation is limited by many factors, but the process is primarily dependent on natural diffusion rates of oxygen. Consequently, natural biodegradation rates are frequently too slow to prevent contaminant migration and sites may require remediation.

Bioventing uses enhanced bioremediation to mitigate the effects of petroleum hydrocarbons (or other contaminants) that occur in soils located in the vadose zone. Bioventing systems are engineered to provide the naturally occurring, acclimatized microorganisms with conditions favorable for the in situ degradation of petroleum hydrocarbons by providing appropriate inorganic nutrients and an electron acceptor (oxygen), thus reversing the degradation-limiting conditions commonly encountered in petroleum impacted soils. The use of an air-based oxygen supply for enhancing biodegradation relies on airflow rates and configurations that will ensure adequate oxygenation for aerobic biodegradation while minimizing the production of hydrocarbon-contaminated off-gas.

Hart Crowser has designed or is currently operating 15 in situ and 11 ex situ bioventing systems in various locations throughout the state of Alaska. The objective of these projects was to design, install, and operate a remediation system capable of reducing the existing petroleum hydrocarbon levels to below the Alaska Department of Environmental Conservation clean up action levels. Prior to the design of the bioventing systems, Hart Crowser initiated site investigations including soil borings and installation of monitoring wells to determine site geological characteristics, and the extent of the hydrocarbon impacted soils. Laboratory biofeasibility testing or in situ respirometry testing was accomplished to determine the biological activity at the sites and provide information to optimize the remedial design. Degradation rates for the various sites ranged from 0.92 $mgkg^{-1}d^{-1}$ to 17.6 $mgkg^{-1}d^{-1}$. Three in situ bioventing case studies were presented. The results of treatability testing, considerations for the design of the bioventing systems, systems installation, and the results from two years of operation were outlined.

Aerobic Degradation of PAHs in Marine Sediments

J.T. Staley, Allison Geiselbrecht, and Brian Hedlund, Department of Microbiology, University of Washington, Seattle

Our laboratory studies aerobic bacteria that degrade polycyclic aromatic hydrocarbon (PAH) compounds in Puget Sound and the Gulf of Mexico. Of the areas we have studied, the highest concentrations of PAH degraders are found at the creosote-contaminated Eagle Harbor EPA Superfund site in Puget Sound. These high concentrations indicate that PAH-degrading bacteria are enriched at this contaminated site. We have not recovered terrestrial or freshwater PAH-degrading taxa from the marine habitats we have studied. Instead, several new PAH-degrading marine bacteria, including two new genera, have been isolated. A PAH-degrading genus, *Cycloclasticus*, has been found both in the Gulf of Mexico and Puget Sound. Other genera including an *Oceanospirillum*-like organism, a new *Vibrio* species, and a new strain or species of *Pseudoalteromonas haloplanktis* have been isolated from Puget Sound sediments. Each of these bacterial groups exhibits a unique pattern of utilization of PAH compounds. The initial critical step in aerobic PAH degradation is carried out by a complex high molecular weight enzyme termed a dioxygenase. We are interested in determining whether each bacterial group has its own characteristic dioxygenase enzyme that is responsible for determining the substrate range and specificity. Also, studies are in progress to determine the distribution of *Cycloclasticus* spp. in contaminated and noncontaminated Puget Sound sites. Preliminary results indicate that species of *Cycloclasticus* are found both in the water column as well as in aerobic, surficial sediments.

Anaerobic PAH Degradation: Novel Pathways and Strategies for Isolation

Robert Sanford, Department of Microbiology, University of Washington, Seattle

Polyaromatic hydrocarbons (PAHs) degrade fairly easily under aerobic conditions. Since these contaminants are often sequestered in anaerobic environments, however, it is important to investigate the microbially mediated degradation of PAHs in these types of ecosystems. Recently PAH degradation has been demonstrated in anaerobic marine sediment microcosms and in anaerobic enrichment reactors. Both sulfate reducing and denitrifying conditions have been used in PAH degradation experiments. Although no isolates have been obtained from marine systems, we are a step closer to obtaining pure cultures. Degradation activity of phenanthrene has been obtained from mixed microbial population growth on agar plates.

To obtain isolates we have adopted strategies that have been successful for isolating anaerobic toluene degraders and chlorophenol degraders. Central to these strategies is the utilization of culture techniques that closely model the natural environment. As a result, it is often necessary to modify culture methods to better suit our needs. For example, although Artificial Seawater (ASW) is a defined medium, we have found evidence that actual seawater supported better microbial activity in PAH enrichments. Finally, the most important component of obtaining anaerobic isolates is having patience. It is not uncommon for six months to a year to pass before activity is observed in an enrichment and it may be twice that amount of time before a pure culture or defined culture is obtained.

There are several benefits associated with obtaining pure cultures that degrade PAHs anaerobically. 1) Novel catabolic pathways may be discovered and pure cultures will facilitate the study of those pathways. 2) Optimization of PAH degradation can be studied in detail. 3) Associations with other anaerobic microorganisms that may stimulate PAH degradation can be investigated. 4) New microbial diversity may be discovered. 5) Catalysis pathways may provide enzymes that have economic value. 6) It may be possible to manipulate a pure culture genetically to degrade PAHs more efficiently.

Alternatives to Oxygen-Driven Degradation of Organic Contaminants in Marine Sediments

Stuart E. Strand, College of Forest Resources, University of Washington, Seattle

Recent publications have provided convincing evidence for the degradation of benzene, naphthalene, and phenanthrene in anaerobic sediments. Recent results from work at the University of Washington were presented showing the effects of nitrate on phenanthrene degradation under denitrifying conditions. Along with earlier demonstrations of the degradation of creosotes, toluene, xylene, and other oxygenated aromatics, it is apparent that this class of hydrocarbons is more labile in sediments than previously thought. Anaerobic bacteria can oxidize these pollutants all the way to carbon dioxide and may play an important role in the ultimate fate of aromatic hydrocarbons located deep in sediments and under caps applied for remediation purposes.

Molecular Methods for Determining the Composition of Microbial Communities in Marine Sediments

Russell P. Herwig, School of Fisheries, University of Washington, Seattle

For many years microbiologists have attempted to determine the composition of the microbial communities that reside in the sediments of marine environments. Microorganisms that live in sediments are responsible for the degradation and recycling of the organic matter that falls onto sediments from the overlying waters. Study of sediment microorganisms is important to the study of marine bioremediation because many of the pollutants and toxicants that enter the marine environment are eventually deposited in the sediments. In the past, microbiologists have used culture methods to enumerate, isolate, and identify microorganisms that inhabit marine sediments. These methods depend upon the ability of the organisms present in a sediment sample to grow to large numbers under laboratory conditions. No bacteriological medium or laboratory condition supports the growth of the wide variety of organisms that are found in marine sediments. Some investigators have suggested that less than 1% of the organisms found in environmental samples can be cultivated. Staining methods are used to estimate the total number of microorganisms, but what can be done to describe the composition of the microbial community when we can cultivate less than 1% of it?

In recent years techniques developed for modern molecular biology have been applied in microbial ecology investigations. Our laboratory is examining the microbial composition of a variety of environments using a molecular protocol. We do not need to grow the environmental organisms on microbiological media to perform our analysis. Starting with less than one-half of a gram of sediment, we lyse or break open the cells that are present, isolate and purify the released DNA, amplify a particular segment of the DNA that can be used to identify bacteria, and then segregate the amplified DNA so that we know that a particular fragment of DNA came from a single bacterial cell present in the original environmental sample. We then characterize the composition of the microbial community based upon these DNA fragments, which we examine by sequencing. Our recent results suggest that the surficial sediments of creosote-contaminated Eagle Harbor contain a diverse population of bacteria with at least six different bacterial groups represented. We also examined the composition of the surficial sediments in Blakely Harbor, an uncontaminated site, and also found a diverse population of bacteria. Work is in progress to scale-up our methods so a larger number of samples can be analyzed.

Potential Roles for Protozoa in the Bioremediation of PAHs in Marine Sediments

Evelyn Lessard, School of Oceanography, University of Washington, Seattle

Understanding the natural processes of biodegradation of pollutants in marine sediments is an important first step in formulating rational bioremediation actions. Bacteria and protozoa exist together in close association in marine sediments, and therefore the interaction of protozoa and bacteria will affect biodegradation rates and pathways. Although protozoa often reduce the standing stock of bacteria through grazing, protozoa may also enhance the metabolic activity of bacteria and thus increase the overall degradation rate of a pollutant. To determine the potentially beneficial action of protozoa we have studied naturally occurring protozoa in PAH-contaminated sediments in Eagle Harbor, Puget Sound, and conducted laboratory work on protozoan enhancement of PAH degradation by bacteria.

Development of a General System for Biosensor Characterization: Application to Surface Plasmon Resonance (SPR) and Chemiluminescence

Clement E. Furlong and Richard G. Woodbury, Medical Genetics, University of Washington; Sinclair S. Yee, Department of Electrical Engineering, University of Washington; Lloyd Burgess and Heather Edberg, Department of Chemistry, University of Washington; Rick Carr, Jerry L. Elkind, Dwight Bartholomew and Jose L. Melendez, Texas Instruments, Dallas, Texas

The aim of the described research is to develop protein-based biosensor systems for a broad range of marine applications. In the process of pursuing this goal, we have developed a general system for sensor development and testing. The approach that we are using is applicable to signal transduction systems based on surface plasmon resonance, chemiluminescence, fluorescence, and mass, as well as other phenomena. One of our specific goals is to develop a general system that will allow for the systematic characterization of the effects of the affinity of the sensor specificity element for the target analyte, the effect of analyte mass on signal size, and the general performance of the sensor system with respect to sensitivity and selectivity. At the same time, this system should allow for the characterization of the distribution of biospecificity elements on the sensor surface. We chose the anti-fluorescein monoclonal antibody approach for this development system, because the antigen fluorescein can be attached to many different molecules and organisms through free amine groups via reaction with fluorescein isothiocyanate. Also, well-characterized monoclonal antibodies with a broad range of Kd values are available. The fluorescent epitope allows for both rapid and detailed examination of the distribution of the immobilized antibodies on the sensor surface.

Commercialization of sensor technology requires that both the electronic and biological components be manufacturable via cost-effective processes. The other component of our research is aimed at developing rapid, cost-effective procedures for producing proteins to use in biosensor applications.

Field Trials of Fertilization to Enhance Bioremediation of Contaminated Beach Sands

Albert D. Venosa, U.S. Environmental Protection Agency, Cincinnati, Ohio

In the summer of 1994, a field study was undertaken in Delaware by researchers from U.S. EPA-Cincinnati and the University of Cincinnati in cooperation with the state of Delaware. Light crude oil was intentionally released onto segregated plots to evaluate bioremediation. The study objectives were to obtain credible statistical evidence to determine if bioremediation with inorganic mineral nutrients and/or microbial inoculation enhanced the removal of crude oil contaminating a sandy beach, and to compute intrinsic and enhanced biodegradation rates. Biodegradation was tracked by GC/MS analysis of selected components, and the measured concentrations were corrected for abiotic removal by hopane normalization. Five replicates of three treatments were evaluated: an oiled no-nutrient control, addition of water soluble nutrients, and addition of water soluble nutrients supplemented with a natural microbial inoculum from the site.

Although substantial hydrocarbon biodegradation occurred in the untreated plots, statistically significant differences between untreated and treated plots were observed in the biodegradation rates of alkane and aromatic hydrocarbons. First order rate constants for disappearance of individual hopane-normalized saturates and PAHs were computed, and the patterns of loss were typical of biodegradation. Significant differences were not observed between plots treated with nutrients alone and plots treated with nutrients and the indigenous inoculum. The high rate of oil biodegradation that was observed in the untreated plots was attributed to the relatively high background nitrogen concentrations that were measured at the site.

Even though oil loss was enhanced by nutrient addition, active bioremediation in the form of exogenous nutrient addition might not be appropriate in cases where background nutrient levels are already sufficiently high to support high intrinsic rates of hydrocarbon biodegradation.

Panel Discussion: Federal Opportunities in Marine Bioremediation and Biotechnology

David Boron, U.S. Department of Energy; Jane Dionne, National Science Foundation; Albert D. Venosa, U.S. Environmental Protection Agency; Usha Varanasi, National Marine Fisheries Service; Al Mearns, Hazmat, National Oceanic and Atmospheric Administration

The panelists discussed their agencies' perspectives on the future of marine bioremediation and extremozymes.

David Boron, U.S. Department of Energy, discussed aspects of the Biological and Chemical Technologies Research Program at the U.S. Department of Energy Office of Industrial Technologies. The program's mission regarding bioprocessing in extreme environments is to stimulate and nurture the development, testing, and commercial deployment of advanced chemical and biological technologies. Research areas include non-aqueous phase bioprocessing, bioprocessing in other extreme environments, and product recovery and separation technologies.

Jane Dionne, National Science Foundation, reported on contamination at National Science Foundation research stations in Antarctica. Contamination includes hydrocarbons and heavy metals in sediments and on the sea floor, the result of poor waste disposal practices in the past. Dredging sediment and transporting it to the U.S. for treatment would be difficult and expensive. Therefore N.S.F. is investigating in situ remediation options.

Albert D. Venosa, U.S. Environmental Protection Agency, reported that the U.S. Environmental Protection Agency is emphasizing research on inland oil spills, which account for about half the nation's spills, and has established a competitive grant program for university researchers.

Usha Varanasi, National Marine Fisheries Service, reports that National Marine Fisheries Service researchers have determined that contaminant exposure impacts the health of fish, including runs of endangered salmon, possibly by causing immunological impairment. NMFS and its parent agency, the National Oceanic and Atmospheric Administration, are researching means to contain or reduce contaminants through bioremediation.

Al Mearns, Hazmat, National Oceanic and Atmospheric Administration, discussed oil spill cleanup. The toolbox of spill response technologies includes in situ burning and use of dispersants to keep oil from shore. Bioremediation and high-pressure hot water have been used in attempts to remove oil once it has reached shore. Currently the agency is in its sixth year of monitoring high-pressure wash sites vs. control sites in Prince William Sound, site of the Exxon Valdez oil spill.

About the Speakers

Michael W. Adams is a research professor of biochemistry, molecular biology, and microbiology, and co-director of the Center for Metalloenzyme Studies at the University of Georgia. His research interests involve the physiological, biochemical, spectroscopic, and structural characterization of a variety of metal-containing enzymes and redox proteins obtained from hyperthermophiles.

Dr. Adams' previous professional experience includes six years as a research and senior biochemist at the Corporate Research Laboratories of Exxon Research and Engineering Co. in Annandale, New Jersey, and two years as a post-doctorate research associate at Purdue University. He is currently editor of *Systematic and Applied Microbiology* and *FEMS Microbiology Reviews*. He received B.S. and Ph.D. degrees in Biochemistry from King's College, the University of London, England.

John Baross is a professor in the University of Washington's School of Oceanography. His research focuses on the isolation and taxonomy, physiology, and ecology of deep-sea hyperthermophiles and on the ecology of marine bacterial viruses.

Prior to coming to the University of Washington, Dr. Baross was a professor of oceanography at the University of Oregon. He received a B.S. and M.S. from California State University, San Francisco, Calif.; and a Ph.D. from the University of Washington, Seattle, Wash.

David Boron is a biologist with the Office of Industrial Crosscut Technology at the U.S. Department of Energy, Washington, D.C.

Jody W. Deming is a professor of oceanography and director of the Marine Bioremediation Program at the University of Washington. Her research interests include fundamental aspects of the behavior of natural microbial populations in marine sediments and in extreme environments such as deep-sea and polar sediments.

Prior to coming to the University of Washington, Dr. Deming was a research scientist and associate professor at Johns Hopkins University and a staff scientist at the Center of Marine Biotechnology, University of Maryland. She received a B.S. from Smith College and a Ph.D. from the University of Maryland.

Jane Dionne is program manager for Antarctic Environmental Research, Office of Polar Programs, at the National Science Foundation, Washington, D.C. Dr. Dionne has also worked at the U.S. Department of Energy in environmental and fossil fuel policy. She received a B.A. in geology and geography at the University of Connecticut, and an M.A. and Ph.D. in geology from George Washington University.

Clement Furlong is a research professor in the Division of Medical Genetics and the Department of Genetics at the University of Washington. In addition to his research in bioremediation and biosensors, Professor Furlong's laboratory is studying the genetic variability of organosphere sensitivity in humans.

Prior to coming to the U.W., Dr. Furlong was a research associate professor in the Biochemistry Department at the University of California, Riverside. He holds a B.A. in chemistry from San Jose State College, and a Ph.D. in biochemistry from the University of California-Davis. He held a postdoctoral appointment at Cornell University.

Pat Hardisty is a research scientist in the Environmental and Tactical Systems Department, Applied Physics Laboratory, University of Washington. Ms. Hardisty has extensive experience in graphic design and photography. She received B.A. degrees from the University of Washington.

Russell P. Herwig is a research assistant professor in the University of Washington's School of Fisheries. His research focuses on the composition of microbial communities in marine sediments, isolating characterizing organisms that degrade monocyclic and polycyclic aromatic hydrocarbons, and characterizing genes responsible for PAH degradation. He has been a visiting faculty member at the Remediation Laboratory at the U.S. Navy NRaD Laboratory in San Diego.

Robert M. Kelly is a professor of chemical engineering at North Carolina State University. His research interests include microbial physiology, biochemistry, and biochemical engineering, with a focus on extremophilic microorganisms.

Dr. Kelly's previous professional experience includes work at the DuPont Marshall Laboratory in Philadelphia and as a member of the faculties of chemical engineering at Johns Hopkins University and the Center of Marine Biotechnology, University of Maryland. Currently, he is chair of the Food, Pharmaceutical and Bioengineering Division of the American Institute of Chemical Engineers (AIChE) and a fellow of the American Institute for Medical and Biological Engineering (AIMBE). Dr. Kelly holds degrees in chemical engineering from the University of Virginia (B.S. and M.S.) and North Carolina State (Ph.D).

Barbara Krieger-Brockett is an associate professor in Chemical Engineering at the University of Washington. In addition to the study of polluted marine sediments, her research interests include fuel chemistry research, and wood pyrolysis and combustion.

Prior to coming to the University of Washington, Dr. Krieger-Brockett was a research engineer at the Institute Francais du Petrole (near Paris) and at General Motors Research Labs. He received a B.S. from the University of Washington and a Ph.D. from Wayne State University.

Evelyn Lessard is an associate professor in the University of Washington's School of Oceanography. Her research interests include marine microzooplankton ecology, the role of benthic protozoa in marine bioremediation, protozoan physiology, and the application of molecular techniques to ecological questions.

Dr. Lessard has been a participant in more than 25 oceanographic cruises and a chief scientist on two. She received a B.A. in biology from Middlebury College, an M.S. in microbiology from the University of Rhode Island, and a Ph.D. in oceanography from Rhode Island.

Hal Marlow is a senior project environmental scientist with Hart Crowser, Inc., in Anchorage, Alaska. He works on characterizing hazardous waste sites and determining the feasibility of bioremediation as a remedial alternative. Mr. Marlow received a B.S. in zoology from Western State College and an M.S. in environmental science and engineering from Rice University.

Eric J. Mathur is director of the Center for Molecular Diversity for Recombinant BioCatalysis, Inc. in La Jolla, California. His research interests include the identification and characterization of enzymes from hyperthermophiles. Prior to coming to Recombinant BioCatalysis, Mr. Mathur performed basic research at U.C. Riverside and the Scripps Research Foundation. He also worked at Stratagene where he set up the Thermophile Research Unit and cloned Pfu DNA polymerase. Mr. Mathur received a B.S. in biology from U.C. Riverside.

Alan Mearns is the leader of the Biological Assessment Team, Hazardous Materials Response and Assessment Division (HazMat), National Oceanic and Atmospheric Administration (NOAA), in Seattle, Wash.

Robert Sanford is a senior fellow in the University of Washington's Department of Microbiology, currently working in an integrated research effort to isolate anaerobic PAH-degrading microorganisms. After receiving a B.A. from Whitman College and an M.S. in microbiology from Colorado State University, he worked in the bioremediation industry for five years. He then received a Ph.D. in microbiology from Michigan State University.

James T. Staley is a professor of microbiology at the University of Washington. Professor Staley's research interests encompass microbial ecology, PAH degradation, and microbial evolution. He also studies polar sea ice bacteria. Since 1976 he has served as trustee and editorial board member of the *Bergey Manual of Systematic Bacteriology.*

David Stensel is a professor in the University of Washington's Environmental Engineering and Science

Program in the Department of Civil Engineering. His research interests over the past 10 years have included biological processes and design applications for hazardous site bioremediation.

Prior to coming to the University of Washington, Dr. Stensel was a professor at the University of Utah's Department of Civil Engineering. Before that he worked in private industry, serving as the director of environmental technology for Envirotech in Salt Lake City. Dr. Stensel is a registered professional engineer and a diplomate in the American Academy of Environmental Engineers. He received a B.S. in Civil Engineering at Union College, Schenectady, N.Y. and an M.S. and Ph.D. in Environmental Engineering from Cornell University, Ithaca, N.Y.

Stuart E. Strand is an associate research professor in the College of Forest Resources, University of Washington. Currently he is participating in an integrated research effort to isolate anaerobic PAH-degrading microorganisms. Dr. Strand received a B.S. in aeronautical engineering and a M.S. in environmental engineering from Ohio State University, and Ph.D. in environmental engineering from Pennsylvania State University.

Usha Varanasi is director of the Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle.

Albert D. Venosa is program manager of the Oil Spill Bioremediation Research Program, U.S. Environmental Protection Agency, National Risk Management Research Laboratory, Cincinnati. His research interests include developing protocols for testing the effectiveness of bioremediation products, developing microbiological and chemical analysis of oil spill bioremediation activities, and developing improved scientific understanding of biodegradation mechanisms.

Dr. Venosa received a B.S. in microbiology, an M.S. in environmental engineering, and a Ph.D. in environmental science from the University of Cincinnati.

Posters:

The following lists posters displayed during the reception September 24, 1996. For contact information, see the participants list starting on page 22.

Extremozymes

Shelly Carpenter and Jody Deming
An Approach to measuring in situ rates of extremophile activity in deep-sea hydrothermal vent fluids

James F. Holden, Andrea M. Hutchins, Michael W.W. Adams, and John A. Baross
Effect of temperature and pressure on the physiology and survival of the hyperthermophilic sulfur-reducing archaea *Pyrococcus* spp.

Adrienne Huston, Shelly Carpenter, and Jody Deming
Potential for cold-adapted enzymes from exztremely psychrophilic arctic bacteria

Karen Junge, J.T. Staley, and Jody Deming
Microbial community analysis of arctic sea ice—a source for extreme psychrophiles

Melanie Summit, Bradley R. Scott, Kirk B. Nielson, Eric A. Mathur, and John A. Baross
Pressure enhances the thermostability of DNA polymerase from three thermophilic organisms

Yves-Alain Vetter and Jody Deming
Freely-diffusing versus substrate-binding hydrolytic activity of enzymes released from eight marine bacteria

Sediment Bioremediation

Krista Anders and J. Ferguson
Anaerobic transformation of chlorophenolics in marine environments

Bernadita F. Anulacion, Tom Hom, William L. Reichert, and Tracy K. Collier
Temporal changes in CYPIA, DNA adducts, and contaminant residues in fish removed from contaminated areas

Xiao Hua Cai, Jaggat Adhiya, Samuel Traina, and Richard Sayre
Cadmium binding and tolerance in transgenic algae

Shelly Carpenter, W. Smith, Barbara Krieger-Brockett, and Jody Deming
An approach to estimating in situ PAH degradation rates in Eagle Harbor sediments

Shelly Carpenter and Jody Deming
Eagle Harbor superfund site: UW marine bioremediation study area

Andria M. Costello, Sergei Stoliai, and Mary E. Lidstrom
Multiple sets of genes for the copper methane monooxygenase in methanotrophs

Clement Furlong
Bacteria involved in hydrocarbon degradation

Allison Geiselbrecht, B.P. Hedlund, and Jim Staley
The distribution of a PAH-degrading genus in the marine environment

James Gray and Russell Herwig
Phylogenetic analysis of the bacterial consortia in Eagle Harbor and Blakely Harbor

Brian Hedlund, Allison Geiselbrecht, and Jim Staley
Dioxygenase and phylogenetic diversity among marine PAH-degrading bacteria

Russell Herwig, James Gray, J.E. Park
Fatty acid analysis of heterotrophic marine bacteria—development of an identification database

Jonathan Z. Kaye and John A. Baross
The bioremediation potential of cadmium-tolerant microbes isolated from hydrothermal vent environments

Barbara Krieger-Brockett
Rate comparisons for processes in bioremediation

Bing-Sun Lee, John L. Ballister
Carbon tetrachloride removal in anoxic and oxic marine environments

Evelyn Lessard, Kara Nakata, C. Laetz, D. Montagnes, and Mike Foy
Distribution and abundance of protozoa in PAH contaminated and uncontaminated sediments in Puget Sound

Mary Lidstrom
Enrichment of a marine methanotrophic population and its kinetics of methane and TCE oxidation

James Movius, M.A. Stoecker, S.M. Machlin, and Jim Staley
Cadmium resistance in Rhodoccus NO1-1

Tom Poeton and H.D. Stensel
Sorption and biological interaction for PAH removal from marine sediments

Karl Rockne, H.D. Stensel, and Stuart Strand
Transformations of polyaromatic hydrocarbons in marine nitrate reducing enrichments

Karl Rockne, H.D. Stensel, Russell Herwig, and Stuart Strand
Co-metabolic enhancement of PAH degradation by marine methanotrophic enrichment cultures

Robert Sanford, Joanne Chee-Sanford, K. Rockne, S. Strand, Jim Staley, and John Leigh
Isolation of PAH-degrading anaerobes

J.L. Schmidt, Jody Deming, P.A. Jumars, and R.G. Keil
A basic question for sediment bioremediation: What is the relevant reference frame of the bacteria?

Jill Schmidt and Jody Deming
A new technique to assess actively respiring bacterial in cold marine sediments

Matthew Stoecker, S.M. Machlin and Jim Staley
A two-component system controls expression of an emulsion-stabilizing capsule in Rhodococcus NO1-1

Cliff Wang
Cadmium removal by a *Pseudomonas* sp.

Marine Biotechnology

E.V. Armbrust and T. Rynearson
The development of genetic engineering in marine diatoms

Paul Bentzen and Adrian Spidle
Development and use of microsatellites for marine conservation genetics

Rose Ann Cattolico and E. Doran
How does light control phytoplankton growth?

Laurie Connell and Rose Ann Cattolico
Development of an oligonucleaotide based *Hetorosigma carterae* "kit " for use by aquaculturists

Lee Hadwiger
Anti-cancer gene regulatory properties of chitosan

Lyndal L. Johnson and John T. Landahl
Contaminant exposure and population growth of English sole (*Pleuronectes vetulus*) in Puget Sound

Marine Molecular Biotechnology Laboratory, University of Washington
Marine Molecular Biotechnology Laboratory web site

Gary Shigenaka and Rebecca Hoff
Prince William Sound—an ecosystem in transition

Scott L. Wallen, J. L. Fulton, D.M. Pfund, M. Newville and E.A. Stern
Ion hydration in supercritical water solutions

Washington Sea Grant Program (WSGP)

Megan Bailiff and Andrea Copping
WSGP fellowships

Nancy Blanton
WSGP on the world wide web

Nancy Blanton
WSGP's award-winning publications

Jim Bolger
Sound Boater program

Tom Dowd
Ports and marine transportation

Sarah Fisken, Steve Harbell, and Ed Melvin
Safety at sea

Kris Freeman
WSGP in the news

Robert Goodwin
Revitalizing small cities' waterfronts

Patricia Hardisty
Puget Sound CD-ROM

Teri King
Shellfish bed restoration

Edward Melvin
Reduction of seabird bycatch in salmon drift gillnet fisheries

Terry Nosho
Aquaculture

Eric Olsson
Education for preventing oil spills

Michael Spranger
Global environmental change education

Michael Spranger
MAS overview

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