ENVIRONMENTAL APPLICATIONS OF MARINE BIOTECHNOLOGY

Summary of a Program Development Workshop

Sponsored by the California Sea Grant College System

Bradley M. Tebo

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August 2, 1994
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, California

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EXECUTIVE SUMMARY

At the present time, one of the most important objectives of environmental science and policy is "sustainable development"—that is, "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (National Science and Technology Council, 1994). One particular area of concern to coastal states in general, and California in particular, is the environmental health of its coastal and estuarine waters. Sustained, continuing development of our coastal regions and watersheds requires a long-term commitment to science, education, and outreach activities focused on (1) an ongoing effort to assess the state of the system and to monitor changes that may occur, (2) prevention of damage to the system, and (3), development of remediation and restoration technologies where problems exist. Environmental marine biotechnologies offer promising approaches for achieving these goals and, at the same time, are emerging areas that can provide opportunities for commercialization and economic growth.

The California Sea Grant College System held a workshop on environmental applications of marine biotechnology on August 2, 1994 at Scripps Institution of Oceanography, La Jolla, California. The goal of this workshop was to bring agency representatives, researchers, and potential users together in an open dialogue on the needs, opportunities, and potential applications of biological and biochemical technologies in monitoring, remediation, and restoration of contaminated marine environments. Specifically the workshop assessed the current state of knowledge of "environmental marine biotechnology" in California, cited some examples of environmental problems, and attempted to identify some of the key scientific questions and areas for future Sea Grant research. An additional goal of the workshop was to promote technology transfer from terrestrial systems, where technology and science may be more mature, to the marine environment. California Sea Grant actively promotes collaborative efforts between academic and industry scientists in order to capitalize on academic research efforts and to help focus academic research efforts in relevant areas.

The workshop consisted of short presentations from participants (Appendix II) in which they summarized their areas of work and made recommendations for research needs and technology development. Abstracts of these presentations are given in Appendix I.

California Sea Grant recognizes that environmental application of marine biotechnology is an emerging area of potentially great economic importance to the State of California. This document is intended to clarify goals and opportunities for California Sea Grant-sponsored research.

INTRODUCTION

Sixty-five thousand tons of toxic wastes are released each year into California's air, water, and soil. These wastes result in significant anthropogenic input into our coastal waters. Contamination can result from either point or nonpoint sources. Point source pollution is of particular concern in areas adjacent to industrial activities, including oil refineries, power plants, and manufacturing plants. The coastal shelf off Palos Verdes, California, for example, contains over 200 metric tons of DDT and polychlorinated biphenyls (PCBs) which can be traced to releases from industrial manufacturers. In areas close to oil refineries, selenium and other trace elements are a major problem. The levels of other metal pollutants, like copper (used as an antifouling agent in boat paints), are increasing in our coastal waters (Stephenson and Leonard, 1994). New technologies for removing and recovering toxic wastes are necessary to restore already impacted environments and would also be effective for treating industrial effluents before they find their way into our coastal and marine waters.

Nonpoint source pollution arising from agricultural uses and urban runoff is also a crucial issue because of the arid nature of most of California and the extreme seasonality to weather conditions. A variety of chemical fertilizers and pesticides are applied to large areas of land during agricultural activities, while others accumulate from atmospheric deposition. Because the rainy season is limited to several winter months, pollutants accumulate on land, and the first heavy rains result in the mobilization of high concentrations of toxic substances in the runoff from the rains. This event, termed the "first flush," results in large inputs of toxic substances to our bays, estuaries, and coastal environments. There is a critical need to assess and monitor the impacts of these events, which, with population growth in California, will be magnified in the future.

Like many other coastal areas in the United States, California's harbors and bays contain sediments contaminated with polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and toxic elements including metals. Sediments are a constant source of pollutants into the overlying water, a problem that is magnified when they are disturbed as a result of either natural or anthropogenic causes, such as storm events or dredging activities. Millions of cubic yards of sediments are dredged every year in the United States to maintain waterways and berthing areas, a significant fraction of which has to be treated as toxic waste. Sediment contamination is under increasing legislative and regulatory scrutiny. Sediment management standards already in place or in the discussion and planning stages at the state and federal level, as well as the lack of affordable or available upland or offshore dumping sites, will require some form of treatment for contaminated sediments from dredging operations before disposal. Bioremediation or biological pretreatments employing marine organisms or their products that are active under normal marine environmental conditions promise to be economical solutions for a variety of organic and inorganic pollutants.

The area of marine biotechnology is only now becoming recognized as an important field for research, development, and commercialization. Research could lead to new applications and products, ranging from biochemicals and biological processes for water purification to biomarkers of environmental health and biocontrol of exotic species in the marine environment.

The very high standards that exist in California for environmental protection present opportunities for commercialization and economic growth. Already California has a $20 billion environmental technology industry with over 180,000 people employed in management and clean-up efforts. The opportunities for business development and manufacturing are enormous and not limited only to California. Coastal resource users and communities will benefit from new marine biotechnological products such as biosensors and monitoring equipment, pollution prevention, waste treatment and bioremediation technologies, and new environmentally friendly processes.

The intent of this document is not to exhaustively review all the existing problems within the State of California, nor all the current and future applications of marine environmental technologies, but rather to touch upon some of the examples where marine biotechnology may make important contributions and to make some recommendations with regard to future program initiatives.

CURRENT STATE OF KNOWLEDGE—
SUMMARY OF PRESENTATIONS

EXAMPLES OF ENVIRONMENTAL PROBLEMS WITHIN THE STATE OF CALIFORNIA

A variety of environmental problems in coastal and estuarine marine environments exist within the State of California. For example, San Diego Bay sediments have been severely impacted by a variety of operations by industries that have leases from the San Diego Unified Port District or by Naval operations (Maher, Appendix I). Among the elements and compounds of concern are copper, mercury, zinc, lead, polycyclic aromatic hydrocarbons, PCBs, and petroleum hydrocarbons. The Navy is currently in the process of dredging 12 million cubic yards of sediments in the bay of which about 200,000 cubic yards are contaminated with petroleum hydrocarbons, mercury, PCBs and other heavy metals (Maher, Appendix I). With the recent decision to relocate and establish the home ports of two or three aircraft carriers in San Diego, the bay will require further dredging operations.

In the Palos Verdes coastal shelf near the site of the White's Point sewage outfall, sediments contain over 200 metric tons of DDT and PCBs. These compounds and their breakdown products can be very toxic and can act as environmental estrogens or dioxins, which interfere in reproduction or cause cancers.

Contamination of coastal waters by selenium (Se) and other trace elements is a major problem in areas close to oil refineries (especially in San Francisco Bay), which release substantial amounts of toxic metals and metalloids in their wastewater discharge. To date, the few available technologies are either costly or marginally effective.

Copper pollution in coastal California waters has been increasing in recent years. Copper pollution occurs through urban runoff, waste from the loading and offloading of metal ores from ships, and from copper-containing paint scrapings from boat hulls. Typically, clean-up and monitoring programs strive for a reduction in total metal concentrations without regard to the biological effect (bioavailability) of the metal. A site with high levels of copper in a less available form may be less toxic than one with lower levels in a more available form. There is clearly a need for better methods of rapid environmental monitoring for toxic metal pollution that incorporate the degree of bioavailability of the metals involved and can be used by environmental regulators and managers to prioritize and monitor the clean up of polluted sites.

AGENCY ISSUES AND CONCERNS

A number of issues and concerns were raised by agency representatives. One of the most important issues was the apparent large gap between research and development in the laboratory as compared to that being applied in environmental monitoring and clean-up. Agency representatives also perceived a lack of realism in many laboratory studies relative to what happens in the real world. They called for improvements in the transfer of information from the lab to the real world and for research that allows better environmental prediction. Studies conducted in the laboratory should mimic the natural environment to the fullest extent possible, and *in situ* studies should be more commonly employed.

Our bays and estuaries have been largely overlooked by the EPA with regard to clean-up. Bioremediation is a particularly important approach for these environments because conventional techniques are so disruptive of the environment. In particularly sensitive habitats, such as mud flats and wetlands, which would be destroyed by conventional clean-up techniques, novel approaches that minimize disturbance are required (Sowby, Appendix I).

Research is needed to develop methods to assess the bioavailability of pollutants. Current measurements usually measure total concentration of a pollutant; however, because these pollutants may be complexed or tied up (for example, in sediment matrices), these measurements do not take into account the amount of pollutant that the biota actually sees. In many cases the results of EPA-approved tests are difficult to interpret. And in site remediation, the question often comes up, "how clean is clean?" Clearly new approaches for assessing and monitoring pollution and its effect on the biota are needed.

Finally, an issue that is extremely important to address in environmental monitoring and assessment is the heterogeneity of natural systems. What sort of spatial scales are necessary to evaluate environmental impact, and how can one best map the distribution of environmental pollution? Methods that are better, cheaper, and faster are required.

SCIENTIFIC ISSUES

The potential applications of environmental marine biotechnology generally fall into two areas: (1) assessment and monitoring, and (2) pollution prevention, remediation, and restoration. We can see from the experience in terrestrial environments that there are significant challenges, but also that substantial progress toward practical and effective methods and applications have been made. The marine environment has received very little attention, even though it is clear that there is a great need for knowledge of and technologies for dealing with marine pollution. Terrestrial research is more advanced and some of the same principles developed in terrestrial systems need to be elaborated and applied to marine systems. However, questions arise as to whether there are characteristics of marine environments that pose unique challenges or opportunities and thus require new insights, not just application of terrestrial knowledge.

ASSESSMENT AND MONITORING

In order to develop sound policy and management decisions regarding the discharge of wastes into our coastal regions, it is important to be able to accurately evaluate the degree of pollution and its biological effects, to distinguish between natural and anthropogenic causes, and to monitor changes that occur with time, in particular with regard to changes that might occur as a result of mitigation and remediation or policy and management decisions.

To adequately predict the effect and fate of environmental toxicants requires a thorough understanding of the toxicity and bioavailability of different pollutants and their cause and effect relationship. Human and marine pathogens, such as bacteria and viruses, or toxins produced by marine organisms may pose a threat to human health either directly through exposure or via consumption of seafood. Marine biosensors and biomarkers offer promising tools for both environmental assessment and monitoring. Biosensors typically employ a biochemical or intact organism or tissue in combination with a transducer that converts a biochemical signal into an electrical or photometric signal. Sensors for testing toxins in the environment or in seafood can lead to a better understanding of the processes that cause toxin production or bioaccumulation as well as providing a faster technique for evaluating contamination on site, for example, as field test kits during fish harvesting.

What better way to evaluate environmental impact than to determine the effect on the organisms present in the environment? Organisms respond to environmental cues and stresses by varying their biochemical composition (e.g., enzyme activities). With sufficient understanding of these biochemical responses, it is possible to correlate the presence or absence of specific biomolecules to the environmental parameter and thus use that molecule as a biomarker for the impact of the corresponding parameter. For example, metal-binding proteins (metallothioneins) in animals are produced in response to metal stress and thus are good indicators of metal pollution.

POLLUTION PREVENTION, BIOREMEDIATION, AND BIORESTORATION

The ideal fate of organic pollutants in the environment is their complete degradation to carbon dioxide and water. Certain organic compounds persist in the environment because they are resistant to biodegradation. The most difficult organic compounds to degrade, such as DDT and PCBs, are only able to be degraded by microbial consortia–no single organism is capable of the complete degradation. These organic compounds are often not used as carbon, nutrient, or energy sources by the microbes, rather their degradation is due to fortuitous metabolism (cometabolism) of the substrate by an enzyme that serves another purpose. In soils, for example, the breakdown of PCBs involves three groups of bacteria: the biphenyl degraders, the chlorobenzoate degraders, and the chloroacetate degraders (Focht, Appendix I). Organisms that degrade biphenyl cometabolically are ubiquitous in soils; however, the chlorobenzoate degraders are not. Thus, this step represents the rate limiting step in PCB degradation in soils. When chlorobenzoate is eventually degraded, the product is chloroacetate, a compound that inhibits the tricarboxylic acid cycle and is therefore a suicidal product. Thus, the third group of organisms, the chloroacetate degraders, is necessary to dehalogenate the substrate and thereby bypass the toxic effects on cellular metabolism. There are two general approaches to engineer this consortium to enhance the degradation of PCBs. One could try to maximize the consortium either by adding nutrients to enhance the organisms whose activities are rate-limiting or by adding the rate-limiting organisms themselves. Alternatively, the catabolic genes might be combined into a single organism, either by genetic engineering in the laboratory or in natural genetic exchange in the environment. The major question with the genetic recombination approach is whether the different pathways are compatible within a single organism. In addition, if the organism can be engineered in the laboratory, then regulatory problems associated with introducing genetically engineered microorganisms in the environment become of concern.

One of the issues that has been addressed time and again in terrestrial systems is the effectiveness of naturally occurring or indigenous microorganisms versus that of foreign microbes introduced into a polluted environment. Frequently what happens is that the foreign microbes are unable to compete and are lost from the environment. Although the basis of this observation isn't completely understood, it is generally believed that the native microbes are better adapted for their environment. This is an important area of research for the future, because the application of molecular tools for strain improvement in the laboratory will ultimately require the ability of these tailored microbes to express the desired activities in the environment. Just as we are learning to use microbes as biocontrol agents in the prevention of plant disease, microbes will be used in the prevention and clean-up of environmental pollution.

The use of microbial and plant consortia offers great promise in organic pollutant removal because the plants can provide the carbon and energy source that drives the bacterial cometabolism of organic pollutants and thereby minimize the need for fertilization (Fan, Appendix I). Certain plants also enhance the competitiveness of genetically engineered microorganisms and therefore may be used to increase the efficacy of exogenously added microorganisms.

Inorganic pollutants such as metals and metalloids, on the other hand, require a completely different strategy, since they are neither degraded nor destroyed. Applications for metal removal are required by a variety of different industries and are particularly necessary for the prevention or treatment of metal pollution. Metal pollutants can be found in liquid and solid industrial effluents and as by-products of numerous manufacturing processes such as mining, smelting, and refining operations, leather tanning operations, film processing plants, and in the manufacturing of circuit boards. The long-term solution to metal pollution is the prevention of metal release into the environment. The conventional technologies that are employed for metal removal or detoxification include chemical fixation or precipitation, solvent extraction, ion exchange resins, or adsorption onto activated charcoal. However, these technologies are expensive, are frequently unable to reduce pollutant concentrations to the desired levels, and often produce a large amount of hazardous waste by-product that must be buried in a toxic landfill. As a consequence, metal and metalloid pollutants are released into our waters where they impact a variety of terrestrial, aquatic, and marine environments, interfere with our recreational activities, and jeopardize our water supplies and food resources. Thus, new technologies are needed in which metals can be efficiently removed and, if possible, recovered for recycling.

Biotechnologies for metal removal have been shown to be feasible alternatives to physical and chemical treatment technologies (Gadd, 1992; Tebo, Appendix I). Microbes interact both passively and actively with metals and may bring about metal removal in a variety of different ways, including metal binding (biosorption), volatilization, intracellular accumulation, precipitation, and redox reactions (Tebo, 1995). There is a potential for microorganisms and/or their activities to be used in the bioremediation or biotreatment of metal pollution through the detoxification of toxic metal species, for example, by altering the chemical species or form, thereby decreasing the bioavailability of the toxic species, or causing the removal of metal from solution. Terrestrial plants are also being studied for their ability to hyperaccumulate heavy metals and radionuclides from soils.

One attractive, cost-effective approach for improvement of water quality is the use of constructed wetlands, which are living systems designed for the treatment and purification of waters. They are effective at removing organic as well as inorganic compounds, such as nitrogen compounds, phosphorus, and trace elements (metals and metalloids). Constructed wetlands have been most frequently employed in the treatment of acid mine drainage, where their efficacy for metal removal has been proven (Brierley et al., 1989). In constructed wetlands, both plants and microorganisms contribute to metal removal.

Although strategies exploiting biological metal removal or detoxification may have applications for a variety of metals in a number of environmental situations, a more ideal long-term solution requires not only change in form of the metal but also physical separation and recovery of the metal pollutant from the environment. A number of different issues come up when pollutants, such as metals, are separated from the environment. Separation usually results in waste minimization; however, the resulting waste is more concentrated and toxic, which then has to be dealt with. Such waste is usually buried in a specially contained landfill site. Another issue concerns how to handle mixed wastes. How do you treat and process such wastes? What interactions exist between different contaminants, and how do those interactions affect the ability to use bioremediation? Are treatments targeting different pollutants in sequence more efficient and, if so, in which order do you treat: metals first, then organics, or vice versa? This probably depends on the pollutants and sample type; but are there generalizations that can be made? Are there separation technologies that are effective at removing a broad spectrum of pollutants?

Finally, biological processes that may be more environmentally friendly may be used instead of traditional chemical or physical processes and thereby serve as a mechanism for pollution prevention. For example, instead of the industrial processes currently employed, metal precipitating or solubilizing activities of marine bacteria may be used for separating metals from low-grade ores or for immobilizing or detoxifying metal pollution. New processes in marine organisms might circumvent the need for environmentally unfriendly chemical syntheses. Technologies developed for bioremediation may have dual application in pollution prevention.

FUTURE NEEDS AND OPPORTUNITIES

The area of marine biotechnology is only now becoming recognized as an important field for research, development and commercialization. The area of environmental applications of marine biotechnology has to date been largely unexplored, particularly when one considers the myriad possibilities that exist. The workshop identified several areas where future research could lead to new applications and products, from biochemicals and microbial processes to biomarkers of environmental health and biocontrol of exotic species in the marine environment (Table 1).

The future directions of environmental marine biotechnology can be many. These range from applications of live organisms or their biochemical products (such as proteins, enzymes, and carbohydrates) for use in processes such as bioremediation, biomonitoring, or biosensing to solutions for more recently recognized problems such as the control of nonindigenous species. There is an important need to continue searching for organisms that have unique biochemicals or processes that might be exploited in environmental technology. For example, finding organisms with enhanced abilities to degrade oil or chlorinated solvents will enable characterization of the metabolic pathways of degradation and perhaps permit engineered organisms to be better at degrading multiple pollutants or removing toxic substances that are present at extremely low but toxic levels. Biochemicals that have useful properties may, for example, be added as chemical fertilizers to speed the breakdown of refractory organic compounds, as surfactants for cleaning purposes, or as agents to immobilize or detoxify metals *in situ*. Applications such as these offer much promise and circumvent the problem associated with introducing foreign microbes or unnatural compounds into the environment. They also promise to provide safe and rapid treatment for catastrophic events such as oil spills. In environmental assessment and monitoring, biological responses of marine organisms may be employed in technologies for sensing a variety of pollutants, pathogens, or toxins. In particular, when it comes to sensing marine toxins or pathogens, the most exquisitely specific and sensitive response pathways are likely to be of marine origin. The challenge will be to develop these tools for rapid and sensitive detection in the field.

Table 1. Examples of Research and Development Needs in Marine Environmental Biotechnology

BIOTECHNOLOGY	APPLICATION
Probe and sensor development for analysis of specific anthropogenic toxicants (assessment of bioavailability, detection of pollutants, toxins, pathogens, and viruses).	Environmental monitoring
Development of new technology for assessing environmental insults.	Environmental monitoring
Quick and inexpensive technologies for detecting organic and inorganic pollutants, pathogens, and toxins.	Environmental monitoring
Nonpoint-source-pollution-technologies to monitor environmental sources and effects.	Environmental monitoring
Biotechnologies to respond to catastrophic events (e.g., organisms, biological products with useful applications).	Environmental monitoring and pollution remediation
Nonpoint-source-pollution-technologies to remove or detoxify toxic substances.	Pollution prevention and remediation
New marine biochemicals/products or microorganisms for water and wastewater treatment, metal recovery, etc.	Pollution prevention, pollution remediation, and biomining
Improved biological systems as scrubbers to avoid release of toxic substances to the environment (e.g., engineered systems or constructed wetlands).	Pollution prevention
Contaminated sediment remediation. *In situ* vs. *ex situ* treatment; biological treatment.	Pollution remediation
Recycling and reuse of valuable resources.	Pollution prevention
Biocontrol of exotic species in the marine environment	Biorestoration

SPECIFIC RECOMMENDATIONS

The workshop participants were in agreement that the California Sea Grant College System should work to develop a new program to stimulate research and development of new commercially-viable biotechnologies for the assessment, monitoring, clean-up, and prevention of pollution in bays, estuaries, wetlands, and coastal waters. The following broad goals were suggested:

- To identify, research, and develop marine biotechnologies for the assessment, monitoring, remediation, and restoration of polluted marine environments.
- To develop novel biology-based strategies for the prevention, control, and monitoring of marine pollution.
- To develop modern molecular biology tools for the application of marine biotechnology to environmental problems and stimulate the commercialization of these biotechnologies.

The most profitable directions for environmental marine biotechnology from a programmatic point of view will be those that promise new approaches to solving problems and answering questions in the near term. Successful demonstration of environmental applications of marine biotechnology will fuel the development of this broad discipline and provide new opportunities for commercialization and economic growth.

CONCLUSIONS

California Sea Grant has an opportunity to accelerate progress in the field of environmental marine biotechnology, which should lead to better technologies for monitoring environmental pollution, ameliorating environmental problems, and at the same time providing a basis for marketable technologies. Through outreach efforts, California Sea Grant can also help coordinate and stimulate technology transfer between universities, federal and state agencies, and the private sector.

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APPENDIX I
ABSTRACTS OF PRESENTATIONS

Abstract

Sabine Apitz
Remediation Research Laboratory
Naval Command, Control, and Ocean Surveillance Center
San Diego, California

I am currently principal investigator of the Remediation Research Laboratory at the Naval Command, Control and Ocean Surveillance Center, RDT&E Division (NRaD) in San Diego. This is a Navy R&D laboratory. Our division has focused on marine environmental issues, from modeling to remediation, for about 30 years. The primary focus of the work in my group is currently on the biodegradation of organic components, particularly PAHs, in marine sediments. Projects range from basic research (looking at issues of contaminant-mineral interactions and bioavailability) to applied technology (working on methods to enhance *ex situ* bioreactor technology for marine sediments).

Since I began at NRaD, I have focused on issues of contaminant-mineral interaction in soils and sediments, and how those affect site assessment, bioavailability, and biodegradability. Recent experiments have looked at how sediment composition will affect biodegradability, how to optimize microbial populations for optimal biodegradation, and how the fluorescent signature of contaminated sediments changes during biodegradation (for the eventual real-time tracking of biodegradation *in situ*).

Abstract

Pao C. Chau
AMES
University of California, San Diego

Our laboratory is involved in two projects that are related to bioremediation technology. On the numerical front, we are trying to improve the algorithms of simulating multiphase transport of contaminants in groundwater. In many general schemes that simulate groundwater transport, the entire set of model equations are solved even in regions where no nonaqueous phase liquid is present. Since single-liquid phase regions may cover a large portion of the entire simulation domain, much computational time is used inefficiently. In this project we are investigating numerical schemes, including the use of imaging processing techniques, that may allow us to solve a smaller subset which only includes grid points or elements that may involve multi-phases over one discrete time step.

Our second project is experimental. We attempt to make use of chemotaxis, as opposed to more standard selective enrichment, to isolate or enrich microbial subpopulations that have adapted to the utilization of an organic contaminant. Currently, we are using *Pseudomonas putida* and more common organics such as benzene, toluene, xylene, and naphthalene. Degradation rates and induced enzyme levels are correlated to subpopulations that exhibit different chemotaxtic behavior and to control populations that have or have not adapted to each organic contaminant.

Abstract

John Cubit
Damage Assessment—Southwest Region
National Oceanic and Atmospheric Administration

At NOAA's Damage Assessment Center we are developing projects to determine injuries to marine resources caused by factors such as oil spills and long-term contaminant releases and then assuring that the injured resources are restored and replaced. As we build this program, we are committed to using the best expertise and technology available, as well as improving the science and technologies needed by our program.

The Damage Assessment Center relies primarily on outside experts and laboratories to conduct and evaluate these environmental studies. Our interest is to contribute perspectives on particular areas of research and technology that are needed for marine environmental research problems on the Western Coast. In addition, we are interested in becoming acquainted with the researchers and institutions that can contribute to our program.

Abstract

Teresa W. Fan
Department of Land, Air, and Water Resources
University of California, Davis

Remediation and restoration of coastal environments (e.g., major harbors) have become pressing issues. Besides the physical and chemical methods that have been practiced, bioremediation via microbial and plant consortia is receiving increasing attention because of its potential efficacy and low cost. However, there are a number of gaps in our basic understanding in implementing bioremediation. These gaps were addressed nicely in a recent DOE Phytoremediation Workshop that I attended.

First, it is becoming clear that the single-genetically-engineered-organism approach will not work effectively with removing the common marine pollutants (e.g., various metals, PCBs, and DDT). It is also clear that the choice of bioremediation means (e.g., *in situ*, dredge land-fill, and bioreactor types) will depend on the specific characteristics of polluted sites. *In situ* (e.g., salt marsh) and land-fill bioremediation, in particular, can benefit greatly from research into microbial and indigenous plan consortia that cooperate in pollutant removal. This is because plants can provide the energy source for microbial cometabolism of pollutants, thereby minimizing the need for expensive "fertilizer." Plants can also help sequester pollutants and directly degrade them. Moreover, it is possible to enhance the competitiveness of genetically engineered microbes by selecting the proper plant partners. However, plant/microbe interactions and their application to bioremediation have not been examined in much detail. This area of research should receive a high priority.

Second, it is recognized that the effectiveness of bioremediation will not only depend on the development of proper organisms but also on the bioavailability of contaminants in soils and sediments. Yet our understanding of the latter issue is very limited. This is particularly so regarding the role of humic substances, even though these substances have been implicated as major adsorption sites for both metals and organic pollutants. Since the humic characteristics from each polluted site are presumed to be variable, a mechanistic understanding of the structure/function relations for contaminant binding to humate should be the key, instead of superficial characterization that only applies to a few specific cases. Recent advances in several areas of analytical chemistry hold great promises for in-depth structure/function in investigations that are not previously possible. These include pyrolysis/GC-MS (Saiz-Jimenez, C., In: *Humus, Its Structure and Role in Agriculture and Environment,* J. Kubát, ed., Elsevier Science Publisher B.V., pp. 27-38, 1992) and multinuclear NMR (Fründ, et al., Z. Pflanzenernähr, Bodenk, 157, 175–186, 1994). Thus, studies on humic characterization and effects must be performed in conjuction with biotechnological development of appropriate organisms to enhance the chance of bioremediation success in the real world.

Abstract

Dennis D. Focht
Department of Soil and Environmental Sciences
University of California, Riverside

Our work is predicated on the concept that the catabolic gene pool exists in nature for the complete destruction of any synthetic (xenobiotic) chemical. The problem is that the genes which code for the total destruction of persistent compounds such as polychlorinated biphenyls (PCBs) do not reside in a single species, nor are the requisite organisms present in all environments. Therefore, the challenge is to identify the members of the microbial consortia that participate in total catabolism of the target compound, isolate them, identify the catabolic pathways, and combine the requisite gene pool from specific parental strains. Three physiological types of bacteria can be considered in the total aerobic destruction of PCBs, for example. The first group consists of biphenyl-utilizing bacteria which are indigenous in soil and fortuitously metabolize biphenyl by enzymes designed for some other natural substrate. The second group consists of chlorobenzoate-degraders, which are not readily culturable from soil, and appear to have evolved from natural selection. The third group are chloroacetate-degraders, which dehalogenate the substrate to glycolate instead of fortuitously passing it into the TCA cycle as a suicidal product. Complementary catabolic pathways can also be attained by enhancement of natural genetic exchange in soil through inoculation with the "missing" complementary strain or by the improvement of stable microbial consortia by addition of exogenous substrates which fortuitously catalyze xenobiotic substrates.

Abstract

Russell P. Herwig
Department of Microbiology
University of Washington, Seattle

The primary organic contaminants found in marine sediments that are of major concern are polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). These compounds are found to be problems in many nearshore urbanized or industrialized environments. The degradation of these compounds may occur with bacteria having enzymes that oxidize these compounds.

My long-term research goal is to develop and use rapid techniques to enumerate and identify aerobic PAH and PCB-degrading bacteria found in marine sediments or in biological treatment systems designed for the remediation of contaminated marine sediments. Presently, we are isolating and characterizing bacteria primarily from Puget Sound, Washington sediments that are capable of degrading PAHs and PCBs. In addition, we are attempting to isolate bacteria from San Diego Harbor and other marine environments that are capable of similar activities. We discovered a new bacterial genus called *Cycloclasticus pugetii* from Puget Sound sediments, which is capable of degrading a variety of PAHs and lightly chlorinated PCBs. We believe that this organism may be widely distributed in marine sediments. Phylogenetic analysis suggests that *C. pugetii* is a unique organism, not closely related to the more thoroughly studied genera of aromatic-degrading and mostly terrestrial bacteria. Work is also in progress to clone and characterize the catabolic genes from *C. pugetii* that are responsible for the initial attack on aromatic compounds.

Several areas exist for potential research in California and the rest of the United States. In some of these areas collaborative and multidisciplinary research should be encouraged because of the complexity of the problems or environment. Research areas include: (1) development of rapid diagnostic methods for detection and monitoring of bacteria and marine toxins of public health concern in seafoods, seafood growing and harvesting areas, and public beaches; (2) development of bioremediation technologies for the cleanup of contaminated marine sediments by *in situ* and *ex situ* treatment methods; (3) development of "inexpensive" probe and sensor technologies for monitoring and detecting pollutants from anthropogenic sources and remediation treatment systems.

Abstract

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Selenium occurs as a toxic metal pollutant in groundwater, agricultural and municipal wastewater, power plant cooling reservoirs, oil refining waste streams and in leachate from waste disposal sites. At high exposure levels selenium is lethal to microorganisms, animals, and plants. At lower levels of exposure, selenium causes reproductive defects in fish, shellfish, wild fowl, and animals. Subsurface geological deposits throughout the central region of California contain high concentrations of selenium. Deep agricultural irrigation and oil- well recovery streams are consequently contaminated with selenium. Wastewater from San Francisco Bay area oil refineries introduces over ten pounds per day of selenium into the Bay. Agricultural drainage originating in the Central Valley also introduces a large selenium burden into the Bay and other Northern California coastal environments.

No current technology exists for the cost-effective removal of selenium contaminants from high-volume liquid waste streams. Attempts to deploy ion exchange, reverse osmosis, and precipitation chemical treatment processes have been unsuccessful. The goal of our research is to develop and deploy a new microbial treatment process for the bioremediation of selenium contaminated wastewater. We have discovered that well-characterized laboratory strains of *Bacillus subtilis* possess the selenium detoxification characteristics previously found in bacilli isolated from selenium-contaminated aquatic environments (Kesterson Reservoir). Growth of *B. subtilis* in liquid-medium-containing selenite, the selenium species found in oil refinery waste streams, results in the quantitative conversion of selenite to a bound elemental form of selenium localized in the cell wall matrix.

We have been able to select mutants with enhanced selenium biovalence transformation capability. These mutant strains have elevated expression of the selenium detoxification genes (sel genes). We are genetically mapping and cloning the sel genes. The DNA sequence of genes of interest will be analyzed and the gene products overexpressed to increase the efficiency of selenium biovalence transformation. Concurrently, a biochemical approach is being used to identify the gene products and reductants required for selenium detoxification. A bioengineering approach will quantify the impact of environmental, nutritional and metabolic factors on selenium detoxification and resistance. Such information is essential for developing rationally engineered bioprocess systems for the remediation of selenium. Other toxic metal species—including chromium and arsensic—may be detoxified by similar or identical mechanisms to those functional for selenium. Hence, this research is relevant not only to selenium treatment processes, but will also serve as a model for bioremediation studies of other heavy metals which adversely impact coastal ecosystems in many areas of the United States.

B. subtilis, a common spore-forming soil bacterium, is one of the best characterized prokaryotic systems for genetic, biochemical, and physiological research. The organism has the advantage of being able to form dormant endospores—cell forms ideal for bioremediation

processes. Endospores can be inexpensively mass produced, are resistant to heat and desiccation, can be easily stored and transported, and are readily germinated into vegetative cells. *B. subtilis* has been widely used industrially, is nonpathogenic, and has an extensive array of recombinant DNA tools.

We have collaborative toxic metal site restoration projects with (1) the EXXON oil refinery in Benecia, California, to remove selenium and other heavy metals from liquid waste streams entering the San Francisco Bay; (2) the Panoche Water District to design, construct, and demonstrate a pilot plant for the bioremediation of nitrate and selenium contaminants in San Joaquin Valley agricultural wastewater (which eventually enters Northern California coastal waters); and (3) the Alameda Naval Air Station to design, construct, and demonstrate a system for the *in situ* biovalence transformation of hexavalent chromium. The runoff from the Alameda site ultimately enters the Pacific Ocean by way of the San Francisco Bay.

Abstract

Mary E. Lidstrom
Environmental and Engineering Science
California Institute of Technology
Pasadena, California
(not present)

My work involves the use of marine methanotrophic bacteria in bioremediation of small halogenated solvents, such as trichloroethylene (TCE). The possibility of using these bacteria to clean up sites contaminated with halogenated solvents is quite promising. However, remediation of contaminated marine sediments, seawater, and estuarine systems will require methanotrophs adapted to a range of salinities. We are studying methane and TCE utilization by marine methanotrophs both in pure culture and in estuarine samples, with the long-term goal of developing *in situ* bioremediation processes for marine systems.

Abstract

Eileen Maher
Port of San Diego
San Diego Unified Port District

The San Diego Unified Port District (Port) was invited to participate in the Sea Grant workshop on Environmental Applications of Marine Biotechnologies to discuss sediment contamination in San Diego Bay.

Port leaseholds through years of industrial operations contain different types and amounts of contamination in the soil, groundwater, and sediments. The following leaseholds have sediment contamination:

America's Cup Harbor, formerly Commercial Basin, has five boatyards which are under Cleanup and Abatement Orders to remove contaminated sediment from their leaseholds. The cleanup levels have been established at 530 parts per million (mg/kg) copper and 4.8 mg/kg for mercury; 25,000 cubic yards of sediment may need to be removed to achieve cleanup standards.

Convair Lagoon is also under a Cleanup and Abatement Order to remove PCB contaminated sediments. An EIR was performed and the regulators decided that the contaminated sediments could be capped, then remediated at a later date when technology is available.

Campbell Shipyard is awaiting the issuance of a Cleanup and Abatement Order for contaminated sediments. Contamination includes copper, zinc, lead, mercury, tributyltin, polycyclic aromatic hydrocarbons, PCBs, and petroleum hydrocarbons.

Sediments containing copper ore were dredged from the National City Marine Terminal. The ore below hazardous levels was treated by chemical fixation and placed in a landfill at the Marine Terminal. The remainder of the copper ore was treated by physical separation and a chemical fixation process, then recycled to a mining facility in Arizona.

A & E Industries sediment contains heavy metals from boat repair operations. Currently there are no plans to remediate this site.

Coronado Cays is the site of the former City of Coronado burn dump. The burn dump ash contains lead, copper and hydrocarbons.

Coronado Boat Yard (the former ferry boat landing) sediments contain lead, copper, chromium, and tributyltin contamination.

The Navy is currently in the process of dredging 12 million cubic yards of sediments in the bay. They have discovered about 200,000 cubic yards that are contaminated with petroleum hydrocarbons, mercury, PCBs, and other heavy metals.

Abstract

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Marine microorganisms have substantial potential for industrial exploitation. One possible application is bioremediation of polluted marine resources by mesophilic or psycrophilic anaerobes. Another application is catalysis of processes in the chemical industry by thermostable enzymes derived from organisms which live in marine thermal vents. Growth levels of such organisms as a result of unknown culture requirements, and to obtain sufficient biomass to develop such applications requires large culture volumes. Large-scale cultivation of such organisms presents additional difficulties because of the corrosive effect on stainless steel equipment of salt and other medium components, such as, sulfide. Lack of a means to produce sufficient biomass for experimentation has hampered investigation of the industrial potential of these organisms.

UCLA faculty with expertise in anaerobic, halophilic, thermophilic, and/or methanogenic microbiology and biochemical engineering have formed the UCLA Extremophile & Biotechnology Center to overcome these difficulties and facilitate the industrial exploitation of microorganisms from extreme environments. The Center's pilot-scale, glass-lined steel fermentor provides a growth facility which is unique within the United States in its resistance to corrosive growth conditions. Equipment for cell harvesting and protein purification is also available.

The Center seeks academic and industrial collaborators who can use its unique resources to facilitate research with organisms that grow in harsh environments.

Abstract

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The Office of Oil Spill Prevention and Response (OSPR) is funding studies to examine the feasibility of employing bioremediation techniques to clean spilled crude oil from the mud flats and wetland regions of California's bays and estuaries. Bioremediation potentially presents a nondestructive approach to removing spilled oil from sensitive habitats, such as mud flats and wetlands.

The OSPR has contracted with Dr. Bess Ward of the University of California, Santa Cruz, to identify factors controlling biodegradation of petroleum in the selected habitats and to determine the feasibility of employing bioremediation as a cleanup tool. San Francisco Bay has been selected as the primary study site.

Abstract

Biotechnologies for Metal Removal and Recovery Applications

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Biotechnologies for metal removal have been shown to be feasible alternatives to physical and chemical treatments technologies (Gadd, 1992). To date, a variety of technologies based on biosorption have achieved the most interest for applications in metal removal because they do not require live or growing microbes. Metals are removed from solution simply via passive adsorption to biomass. In fact, biosorption is one of the primary means by which materials are removed in classic sewage treatment facilities. Biosorption is very rapid and often inexpensive, employing waste biomass from other biotechnologies (e.g., fermentation). Frequently the loaded biomass can be treated so that valuable elements can be recovered for recycling or waste minimization. Thus, biosorption has been shown to be effective for removing metal and radionuclide pollutants from liquid waste (Gadd, 1992). However, biosorption often does not remove metals to the requisite levels.

Active metal removal can occur via extracellular precipitation, redox reactions, intracellular accumulation, or volatilization, and is brought about by a variety of microorganisms and plants (Brierley, 1990; Terry and Zayed, 1994; Tebo, 1995). Volatilization reactions such as the methylation of mercury, selenium, or arsenic, or the reduction of mercuric ion, result in metal removal via mobilization of the metal in the gaseous phase. The other active metal removal processes often result in the formation of solid phase materials (i.e., metal precipitates and minerals) either extracellularly or intracellularly. In any case, the result is a particulate form of metal that is less toxic than the dissolved form and can be more readily recovered.

Active metal removal processes have a number of different advantages depending on the specific process and the application. Frequently, active metal removal processes can reduce metal concentrations to levels lower than those achieved through biosorption or chemical or physical treatments, and may have a higher degree of metal specificity or metal affinity. Metals or metalloids transformed into volatile species are much less toxic and rapidly escape into the atmosphere where they can be diluted to undetectable levels. Metals that are transformed into particulate phases become "fixed" and are less susceptible to desorption that could be induced by changes in pH, chemical complexation, or competition with other metals. Because metal removal is brought about by organisms that are metabolically or catalytically active, there is more versatility for the development of tailored processes. Perhaps one of the greatest advantages is that in some cases these active metal removal processes may lend themselves to genetic manipulation.

Technologies exploiting active biological metal removal have applications for pollution prevention and remediation. One attractive cost-effective approach for improvement of water quality is the use of constructed wetlands which are living systems designed for the treatment and purification of waters. They are effective at removing organic as well as inorganic compounds such

as nitrogen compounds, phosphorus, and trace elements (metals and metalloids). Constructed wetlands have been most frequently employed in the treatment of acid mine drainage where their efficacy for metal removal has been proven (Brierley et al., 1989). In constructed wetlands, both plants and microorganisms contribute to metal removal.

One of the more promising biotechnological approaches to metal removal and recovery is biomagnetic separation (Ellwood et al., 1992). Biomagnetic separation involves the use of microorganisms to precipitate metals on the cell surface, producing a coating that can adsorb a variety of metal ions and organic compounds. In the process of metal precipitation, the microbes become either paramagnetic or ferromagnetic and can then be concentrated and removed from suspension by high gradient magnetic separation (HGMS). The activities of the microbes are involved in three ways: (1) the bacteria enhance metal precipitation through their metabolic (biochemical) activities; (2) the metal precipitates that form via microbial action have more favorable surface properties (e.g., higher surface area) than those produced by alternate chemical means and act as good adsorbents; and (3) the microbes can be manipulated (e.g., through the use of nutrients or genetically) to enhance the magnetic properties of the precipitates, the microbes themselves, or both. Biomagnetic separation has applications for pollution prevention and environmental remediation, and can lead to the recovery and reuse of precious non renewable resources.

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