Integration of Biotechnology in Remediation and Pollution Prevention Activities

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> ***"Convinced of the importance of the conservation, protection and enhancement of the environment in their territories and the essential role of cooperation in these areas in achieving sustainable development for the well-being of present and future generations ..."***
>
> *preamble to the North American Agreement on Environmental Cooperation*

The *North American Free Trade Agreement/North American Agreement on Environmental Cooperation* provides a mechanism for an international collaboration between the United States, Canada, and Mexico to jointly develop, modify, or refine technologies that remediate or protect our environment. Our countries have a vested interest in this type of collaboration because contaminants do not respect the boundaries of a manufacturing site, region, city, state, or country.

The Environmental Sciences Division (ESD) at Oak Ridge National Laboratory (ORNL) consists of a diverse group of individuals who address a variety of environmental issues. ESD is involved in basic and applied research on the fate, transport, and remediation of contaminants; environmental assessment; environmental engineering; and demonstrations of advanced remediation technologies. The remediation and protection of the environment includes water, air, and soils for organic, inorganic, and radioactive contaminants. In addition to remediating contaminated sites, research also focuses on life-cycle analyses of industrial processes and the production of green technologies. I will focus this discussion on subsurface remediation and pollution prevention; however, our research activities encompass water, soil and air and many of the technologies are applicable to all environments. The following discussion focuses on the integration of biotechnology with remediation activities and subsequently linking these biological processes to other remediation technologies.

Environmental Remediation/Risk Assessment: The large number of contaminated sites throughout the North American continent necessitates the evaluation and ranking of these sites according to contaminant concentration and type, extent of both vertical and horizontal contaminant dispersion, and the potential immediate and long-term impact to human health and the environment. The evaluation must take into account the current state of technology development and the finite funding available for remediation activities. This need for ranking sites does not suggest that all sites must be evaluated prior to initiating cleanup activities. It will be relatively simple to select a site or sites that have the highest immediate human and environmental impact/threat and can be remediated through the use of current technologies. Risk analyses groups within ESD are involved in these types of activities.

The sites chosen for the initial collaborative remediation will require site characterization and risk assessment to provide the information needed to design a remediation plan and time frame. Community support is very important; therefore, choosing sites that can be cleaned up with current technologies not only increases public acceptance and confidence but also provides full-scale evaluation of many current pilot-scale technologies. Furthermore, these relatively simple demonstrations will provide invaluable field experience to further development of new technologies that are required for the more difficult and unique sites.

Most contaminated sites can benefit from a combination of physical, chemical, and biological, remediation methods. Research within the ESD Microbial Interactions Group has focused on bioremediation activities combined with physical treatments, such as deep soil mixing and vapor extraction and chemical treatment through the use surfactants. The specific topics we are examining include:

1) the selection of bacteria capable of degrading contaminants;
2) enhancing the bioavailability of contaminants for microbial degradation through the use of surfactants and biosurfactants;
3) monitoring the distribution of bacteria, nutrients, surfactants, etc. by using classical microbiological methods and innovative, real-time tracers; and
4) using engineered microorganisms as bioreporters to measure the physiological status of the microbial community during remediation activities.

The simultaneous investigation into these parameters as well as a close collaborations with engineers, geologists, chemists, and hydrogeologists minimizes the risk of failure.

The remediation strategy will be designed on the basis of site assessment, environmental impact analyses, and previous experience. Recommending natural contaminant attenuation (intrinsic bioremediation) may be possible and economically feasible. This treatment involves monitoring the contaminant concentration levels in combination with the natural bacterial populations for their ability to degrade the contaminants with limited to no input, such as nutrients and oxygen. If natural attenuation is not possible or does not proceed at an acceptable rate, the addition of bacteria (i.e. bioaugmentation) could significantly increase the rate of contaminant degradation.

Bacteria capable of degrading a variety of contaminant compounds have been isolated from both pristine and historically contaminated sites (water, soil, and air), and their capacity to degrade a variety of contaminants (organic and inorganic) has been measured. Research has shown that under defined conditions nonindigenous bacteria can outperform indigenous microorganisms. The bacteria used in amendment technologies are currently not genetically engineered. However, the eventual approved use of genetically engineered bacteria in in-situ bioremediation has the potential to significantly increase the rate of contaminant degradation and thus, decrease the time required to bring a site to closure.

In addition to bacterial and nutrient/oxygen amendment, the rate of contaminant degradation can be further enhanced by the addition of surfactants. These surfactant compounds can be used to (1) increase the rates of in situ bioremediation and (2) increase contaminant solubilization for soil washing followed by aboveground treatment (e.g., bioreactors). Our recent laboratory work has shown that adding low concentrations of surfactant can enhance the overall rates of in situ bioremediation while keeping the costs at a minimum. High surfactant concentrations, although useful as a soil washing technology, can reduce the contaminants ability to be degraded by microorganisms as well as being more costly.

In addition to examining contaminant degradation by a variety of microorganisms, we have also used microbial bioreporters to directly measure the physiological processes of contaminant degradation and have used molecular methods to monitor the bacterial population active in contaminant degradation. These bioreporters provide a real-time analysis of the overall contaminant degradation rates. Thus, if oxygen becomes a limiting factor or the contaminants are not available for degradation, the system can be immediately adjusted to maintain optimal operating (i.e., degradation) conditions.

These bioreporters have also been used as an efficient and inexpensive screening tool to detect contaminants within groundwater samples. The bioreporters can provide a real-time analysis of a waste stream to determine sporadic leaching or accumulation of volatile organic compounds. Through these research activities, we are integrating basic microbial ecology with molecular biology, which enables us to design and optimize remediation regimes that are both cost effective and efficient.

Pollution Prevention/Life Cycle Analyses: In addition to in situ contaminant remediation, research has also focused on the use of microorganisms and bioreactors in pollution prevention and waste minimization technologies. Technologies for recycling chemical compounds at the industrial sites can not only reduce operating costs but can be used to enhance public relations. If recycling is not a viable or economically feasible option, degradation of the waste stream on-site can reduce disposal costs and prevent the generation of potential, future pollutants.

Information gained from in situ bioremediation experiments/demonstrations (discussed above) has enabled the design of unique bioreactors that operate as end-of-pipe technologies. Bacteria isolated from previous bioremediation and site characterization studies have been used as inoculum in bioreactors and in land-farming activities. These activities occur on-site and have the potential to significantly reduce environmental impacts because they do not generate hazardous waste. Furthermore, they increase positive public relations between the communities and industry.

Collaborations/Site Closure Levels: In addition to other work within the United States, our group has recently expanded research activities to include unique contamination problems in the south of Mexico and in the northern regions of the United States and Canada. The work in Mexico is focusing on the remediation of weathered crude in regions with high environmental temperatures. These temperatures are not typical in the United States; thus, this project is a collaboration between the United States and Mexico to jointly develop and modify existing technologies for use at these sites. In addition to high temperatures, low temperature are also an area of interest. We currently have several projects in the United States and Canada that are examining the degradation of glycols in the subsurface as pollution prevention activities.

Regardless of the contaminated matrix (air, water, subsurface) or location (United States, Canada, Mexico) the ultimate goal is to reach a cleanup level that permits site closure. In many cases, this level is not standardized between states let alone between countries. It will be a particular challenge to establish acceptable remediation levels and measure them accurately; keeping in mind the costs and balancing that with the level of immediate human and environmental impact.

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