Encapsulated Bacteria for In Situ PAH Bioremediation

LEAD AGENCY: U.S. Navy

LAB: Naval Research Laboratory

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PROBLEM STATEMENT: This project addresses Requirement 1.I.4.m, and is for the continuation of an existing SERDP- funded project entitled "Encapsulated or Immobilized Enzymes, Bacteria and Nutrients for Remediation of Fuel Spills" and is applied research (6.2/6.3).

The soils and sediments at Naval refueling facilities are often contaminated with petroleum products that are classified as hazardous wastes. The petroleum products that remain as long term contaminants, include polycyclic aromatic hydrocarbons (PAHs; hazardous waste CFR#K001). Removal and cleanup of these materials is expensive and disruptive to Naval operations. Current methods for treatment of contaminated harbor sediments involve dredging with subsequent off-site detoxification. Development of remediation technologies that treat contaminated soils and sediments in situ, would save the Navy considerable expense and eliminate procedures disruptive to Naval operations.

This project addresses the SERDP goals of lowering environmental cleanup costs, compliance with environmental regulations, and unencumbrance of military operations. It focuses on the Thrust Area of cleanup of soil, sediment, groundwater and subsurface water. The overall goal of this project is to develop an in situ treatment strategy using microencapsulated bacteria for low-cost bioremediation of petroleum products that are poorly degraded by naturally-occurring bacteria. In situ treatment strategies lower the cost over ex situ treatments by eliminating the need for expensive excavation, transportation and storage of the hazardous waste prior to detoxification.

If successful, this treatment strategy may be useful for remediation of some toxic hot spots (PAH contamination) in San Diego Bay sediments. The magnitude of the problem, in terms of PAH contamination, is considerable as the sediment of nearly every harbor is contaminated from ship oil and creosote treatment of docks. In San Diego Harbor alone, nearly 4.5 million cubic yards of sediment is being dredged for Naval harbor deepening projects (e.g. carrier turnaround basin, Pier Bravo), with an expected 0.5 million cubic yards being too contaminated for oceanic dumping or beach replenishment projects. According to the San Diego Regional Quality Control Board, once sites in the Bay are declared "toxic hot spots", dredging and off-site disposal will not be legal means of detoxification as a precursor to harbor deepening. In lieu of effective in situ remediation technologies, such as this microencapsulated bacteria strategy, the Navy will either have to receive an exemption, for the law (which may lead to public relations difficulties) or will have to limit access of Naval vessels to the base. These harbor and pier deepening projects should be considered crucial for continued Naval vessel access to the base. Even if this proposed strategy is not successful at complete removal of the toxicant, treatment may lower PAH levels to the threshold that would allow for the dredging and further off-site cleanup of the sediment.

PROJECT DESCRIPTION: With previous SERDP funding, strains of petroleum-degrading bacteria were microencapsulated in an alginate matrix cross-linked with divalent cation bridges (calcium). The published system has been adapted using an internal set with solid calcium particles. As the calcium slowly solubilizes in the alginate matrix, the cross-linked form which encapsulate the bacteria. This adaptation is expected to reduce ionic stress to the bacteria, resulting in increased encapsulation efficiencies and higher cell viability with long term storage. Higher encapsulation efficiencies and cell viability lowers the cost of the bioremediation treatment strategy.

For the bacterial microencapsulation strategy to be successful and low-cost, the conditions for encapsulation have been optimized to enhance cell viability and petroleum-degrading activity. By developing such a system, fewer microcapsules are needed per volume of treated material, thus lowering the overall treatment cost. Bacterial growth conditions prior to encapsulation have been examined for their effect on cell viability. In addition, the effect of various storage conditions (lyophilization, refrigeration, freezing) on cell viability of encapsulated bacteria was determined to enhance treatment effectiveness. These tasks address the primary environmental concern of ensuring the use of effective and affordable remediation technology.

Microencapsulation of petroleum-degrading bacteria allows for the storage and delivery of strains that are normally found in nature, but may be present in reduced abundance at the spill site. These strains were originally isolated from Superfund sites that were contaminated over decades with creosote. Over time, similar strains will increase in abundance at the spill sites resulting in eventual degradation of the petroleum, but this process may take fifty years or more. The goal is to use microencapsulated bacteria to reduce the contamination level over time scales of months instead of decades. This would reduce exposure of Naval personnel to toxic and carcinogenic waste, and enable the site to be used by either the Navy or the public sector within a reasonable time frame and at an acceptable cost.

Poor performance of in situ treatments involving the addition of bacteria have been due to the unknown effects of site conditions on the ability of bacteria to degrade contaminants. Naval Research Laboratory (NRL) is currently developing specialized indicator strains of bacteria that produce light in response to the presence of bioavailable polycyclic aromatic hydrocarbons (PAH). The indicator strains would enable us to predetermine, whether or not the appropriate nutrient and environmental conditions exist at a site making it amenable to the in situ treatment using microencapsulated bacteria. This system would allow us to alter the site conditions, with nutrients or buffers, prior to adding the bacteria. Though this project involves the construction of genetically engineered strains for assays, it does not involve the release of genetically engineered bacteria into the environment.

Previously, it has been demonstrated that disturbing contaminated soils has resulted in abiotic removal of the toxin from the site. These treatments have often erroneously been characterized as "bioremediation." To determine whether or not the microencapsulation treatment is resulting in actual bacterial degradation of PAH at the site, newly developed molecular probing techniques (16S rRNA hybridization) will be used to identify the added PAH-degrading bacteria in the soils and sediments. These tasks address the primary environmental concern to implement affordable methods for site characterization, namely the field location and abundance of PAH-degrading bacteria.

The microencapsulated bacteria will be tested as a bioremediation strategy in model microcosm systems (5 liter) and subsequently in pilot-plant scale systems (100 liter). These series of tests will be designed to demonstrate the potential of employing microencapsulated PAH-degrading bacteria, along with essential inorganic nutrients and electron acceptors, as part of an in situ bioremediation strategy. Finally, the system will be field tested in coordination with an EPA project examining the use of stable isotopic methods for determining efficacy of bioremediation treatments. This important collaboration will help determine the effectiveness of microencapsulated bacteria at degrading PAH under field conditions.

EXPECTED PAYOFF: Using microencapsulated bacteria to detoxify a site of hazardous waste compounds would be a low- cost alternative to excavation and off-site treatment of contaminated soils. Aside from the lower cost of removal of PAH from Naval soils and sediments, an important aspect of the strategy is that Naval operations in the treated areas are less disrupted when compared with treatment requiring excavation and off-site cleanup. It is exceedingly difficult to estimate the dollar value of this savings to the Navy. Also, because of the potential of causing collateral damage to areas adjacent to the treated area, there is sometimes no alternate ex situ treatment that can be used for cost comparison. In situations where soils and sediments are co-contaminated with heavy metals and PAH, removal of PAH allows for the immobilization of the metals with subsequent land filling of the treated materials.

TRANSITION PLAN: The technology will be transitioned to the industrial collaborator, SBP Technologies, Inc. SBP and its parent company, Eicon, have the technology base and expertise and equipment necessary to use this treatment strategy for eventual cleanup of Naval sites. Their research facilities are on-site at the EPA's Gulf Breeze Environmental Research Lab in Pensacola, FL where they work on encapsulated and immobilized cell technologies for creosote degradation in bioreactors.