REMEDIATION WITH ENCAPSULATED ENZYMES

PROVIDING AN ALTERNATIVE IN-SITU SOIL REMEDIATION TREATMENT STRATEGY TO MINIMIZE IMPACTS TO SENSITIVE ECOSYSTEMS

Battelle has developed a patented process (International Patent WO2016/040266) for the use of encapsulated enzymes to remediate recalcitrant pollutants and is actively seeking commercialization partners.

When compared to invasive technologies, such as dredging and excavation, the application of encapsulated enzymes to surface soil and sediment for contaminant reduction has multiple benefits, including:

- Broad spectrum of treatment
- Continuous release of enzyme
- No generation of harmful byproducts
- Ease of application
- Minimum disruption to ecosystems
- Time and cost savings

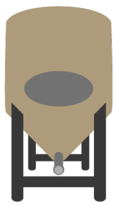
This enzymatic-based treatment technology has been demonstrated to rapidly decrease total petroleum hydrocarbons (TPHs), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) concentrations after application to soil samples in the laboratory environment. It reduces contaminant bioavailability through adsorption and effectively reduces contaminant mass in the sediment over time via direct and enzymatic-mediated degradation.

Our work using encapsulated enzymes such as lignin peroxidase, manganese-dependent peroxidase and laccases show enhanced conversion of hydrophobic aromatic compounds to less harmful/nonharmful forms. Through laboratory research and bench-scale testing, we have purified, encapsulated and stabilized a unique amendment that eliminates the need to grow fungal species at the contaminated site, reducing the restoration timeframe.

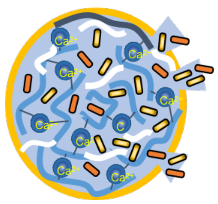
Application and slow dissolution of the enzyme formulation through the beads provide continuous and time-released delivery of a catalyst that facilitates release of compounds bound to sediment particles and promotes biodegradation in groundwater. For example, we have demonstrated that extensive degradation can be achieved for PAHs containing up to six-ring compounds, including highly carcinogenic benzo[a]pyrene, benzo[a]anthracene, pyrene and chrysene in liquid medium, and sediment matrix, which cannot be adequately achieved using conventional in situ technologies. We also have demonstrated that byproducts formed during the process are readily biodegraded by the native microbial community and natural attenuation of any residual compounds is enhanced.

From Concept to Execution

- **Fungal culture growth parameters**
- **Enzyme production and encapsulation**



- **Fungal culture scale up**
- **Fungal supernatant with enzymes transported for encapsulation**



- **Highly concentrated enzymes encapsulated**
- **Encapsulated enzymes transported to site**

- **Application to contaminated sediments via spray, rototill or injection**

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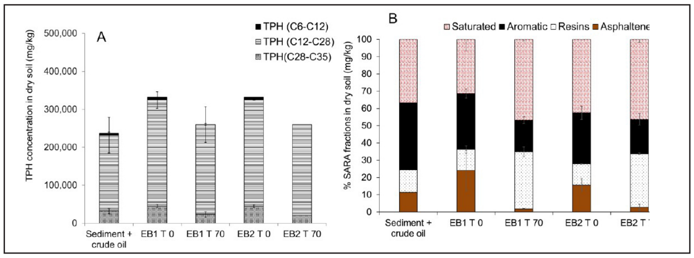
CASE STUDY

Fungal ligninolytic enzymes are attractive candidates for PAH degradation due to their broad substrate specificity and high reactive potential of the radical generating reactions. Recent studies have shown that intracellular fungal enzymes are important in degradation of TPHs and PAHs. Since free radicals produced by fungal enzymes occur mostly in the extracellular environments, a broad range of substrates may be transformed.

Battelle has encapsulated laccase and manganese peroxidase in alginate microcapsules to successfully stabilize these enzymes and achieve their slow uniform release. Results show that the relative catalytic activity of the encapsulated enzyme blends is higher than the crude enzymes. The encapsulation process increases enzyme tolerance to temperature and pH variation, providing an environment for longer enzyme viability.

In general, the enzyme loading per bead is independent of the degree of crosslinking, but the increase in the concentration of crosslinkers results in delayed release rates. Two enzyme blends of varied degree of crosslinking have been tested. Results showed that degradation of TPH in crude and weathered crude oil was rapid and reached 80% for the residual range organics in 14 days. The addition of encapsulated enzymes to the sediments aided slower but consistent degradation of heavy asphaltene fractions.



Concentrations of: (A) total petroleum hydrocarbons and (B) SARA fractions in sediment samples spiked with crude oil and treated with Enzyme Blend 1 and Enzyme Blend 2.

Our bench-scale pilot study demonstrated extensive degradation of up to six-ring compounds. The degradation of PAHs and oil compounds characteristic of a high ring number resulted in production of byproducts that were readily biodegraded by microorganisms ubiquitous in the environment.

Examples of Battelle's project outcomes, where encapsulated enzymes were utilized to degrade recalcitrant compounds in laboratory studies.

Key projects	Outcomes
Application of Encapsulated Enzymes to Soils and Sediments Contaminated with Heavily Weathered TPHs	Encapsulated enzymes were applied to real-world weathered crude impacted soil samples to investigate the ability of the enzymes to reduce TPH to below the regulatory value of 1% or 10,000 mg/kg. Degradation efficiency and generation of petroleum byproducts were tested over time in sediment microcosms and revealed a decrease in heavy hydrocarbon fraction (C28-C40) of 40%.
Enhanced Biodegradation of Sediment-Bound Heavily Weathered Crude Oil with Ligninolytic Enzymes Encapsulated in Calcium- Alginate Bead	Battelle applied encapsulated enzymes to crude, weathered crude and crude oil contaminated sediments in a laboratory study. The concentration of heavy >C40 hydrocarbon fractions in sediment samples treated with encapsulated enzymes decreased by 80% during the 72-day incubation time. Results show almost complete (96%) degradation of asphaltene fractions and 80% degradation of saturates and aromatics; 80% degradation of benzopyrene was also observed. Additional 16S sequencing analysis before and after application of encapsulated enzymes to the contaminated sediments revealed an enrichment in oil-degrading microorganisms due to an increase in bioavailability of shorter chain hydrocarbon fractions.
Assessment of PCB Degradation after Application of Encapsulated Enzymes	Encapsulated enzymes were applied to the liquid stocks of Aroclors and sediments contaminated with PCBs. The data showed gradual decrease in concentration of PCB compounds and decrease in their level of chlorination over time. PCB degradation ranged from 40 to 66% during the 72-day incubation. Two-dimensional gas chromatography coupled with mass spectrometry showed that no phenol-containing byproducts were produced, signifying that this technology could be applied to sensitive ecosystems where risk of endpoint exposure is of great concern. 16S sequencing analysis of sediment samples prior to and after application of encapsulated enzymes showed an enrichment in diversity of microorganisms involved in degradation of polychlorinated compounds.

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It can be done

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