Myco-Treatment of Marine Oil Spills

By 2016, the top ten industrialized countries in the world consumed over 50 million barrels of oil per day. This demand for oil has led to an increase in oil spilled into the worlds' waterways from oil tankers, underwater pipelines, offshore drilling rigs and coastal facilities and refineries. Oil spills differ due to variations in oil type, location and weather conditions.

There are four basic responses to an oil spill:

• Leave the spill undisturbed to be broken down by weathering due to the dispersal actions of sunlight, wind, water currents and wave action.

• Contain the spill with booms; then either collect oil from the water with mechanical equipment (skimmers) or remove it by sorption. Sorbents are insoluble materials used to recover liquids through absorption or adsorption. To treat oil spills, sorbents must be both lipophilic and hydrophobic.

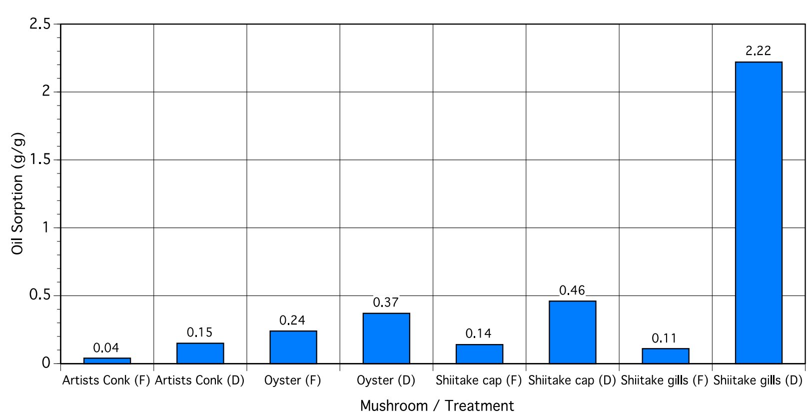
• Use dispersal agents to break up the oil spill and enhance natural degradation. Dispersal agents are mixtures of chemical surfactants used to improve interactions at the oil-water interface. Following application, surfactant molecules at the oil-water interface reduce surface tension which then allow the mixing actions of waves and wind then disperse the oil (as small droplets) into the underlying water column. At this point, microorganisms present in the environment can more readily break down the oil. The effectiveness of dispersants depends upon wave energy, water salinity, oil properties and temperature.

• Introduce biological agents into the water to stimulate the growth of naturally occurring micro-organisms. These microorganisms will then break oil down into harmless substances.
Mycoremediation is a form of bioremediation that uses fungi to break down contaminants in the environment. The mycelium, or vegetative part of a fungus, consists of a mass of branching, thread-like hyphae. These hyphae secrete extracellular enzymes that break down lignin and cellulose (the main components of plant fiber). These two organic compounds are composed of long chains of carbon and hydrogen with a structure similar to many organic pollutants, such as oil.
The research problem was a determination of mushroom potential to function in clean-up of marine oil spills. Research goals included an evaluation of:

- oil sorption potential of mushroom tissues
- mushroom exudates as potential oil dispersal agents
- oil spill remediation in marine environments by mushroom hyphae

Artist's conk, oyster and shiitake mushrooms were studied as oil spill treatments using Prudhoe Bay crude oil and gasoline.

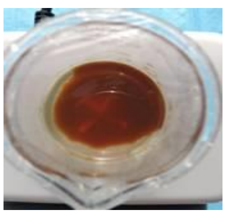
Oil sorption studies used both fresh and dried tissues of each of the 3 types that were equal size pieces, placed in beakers of seawater and oil. Pieces were then strained and weighed to determine oil sorbed. Oil was extracted from these pieces using cyclohexane as the solvent.



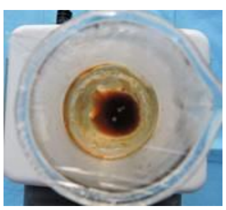
After initial sorption studies the best sorbent was the dried shiitake mushroom, with 0.46 g of oil sorbed per gram of sorbent. Later on, shiitake mushrooms with more defined gills present were re-tested, which yielded an almost 500% increase in sorption, at 2.22 g oil/g sorbent.

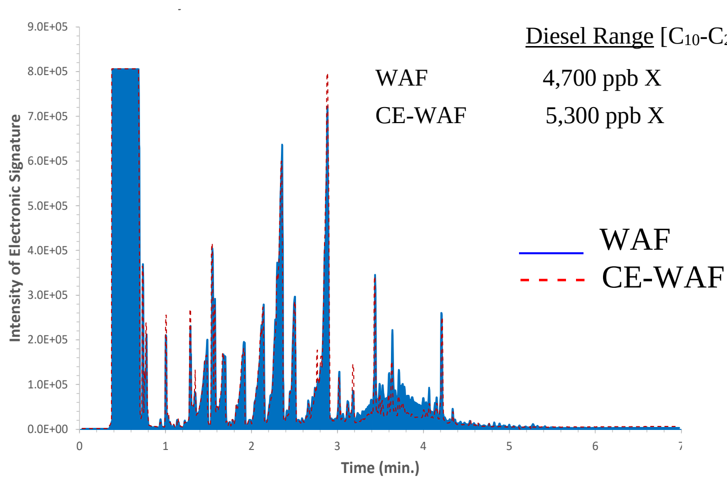
To preform dispersal tests, mushroom exudate was removed from spawn bags with a hypodermic syringe. Static testing was run in test tubes of seawater with oil to exudate ratios of 1/20, 1/15, and 1/10. From my visual results, experimentation advanced to active testing of the exudate. These tests were in beakers of seawater on a stir plate to mimic wave action. Oil/exudate ratios tested were 1/10 and 1/5. Vortex stability and oil droplet formation were noted. Normally, oil and water form a back and forth relationship, however, with the addition of a dispersant this becomes a one-way reaction where the droplets stay dispersed. From these tests oyster mushroom exudate proved to be the most promising to use for chemical analysis. This chemical analysis involved two large beakers with crude oil and seawater. In the beaker with just oil and water, was considered a WAF (water accommodated fraction) and the beaker with the oil, seawater and the exudate added to it, was considered a CE-WAF (or chemically enhanced water accommodated fraction). The beakers were placed on stir plate for four hours before 500 ml of water was extracted from each of the water column, using the arm. Chemical analysis of the total petroleum hydrocarbons (or TPH) was run by a commercial laboratory using a gas chromatography with a flame ionization detector (GC-FID).

Top view of control



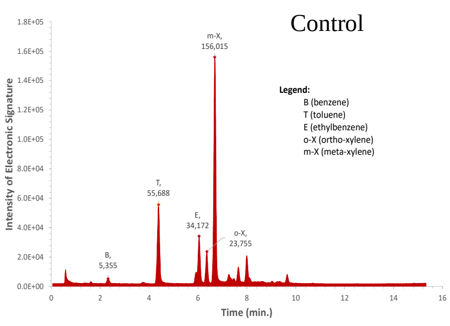
Top view of oyster exudate

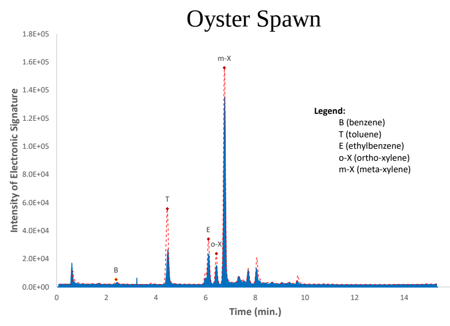


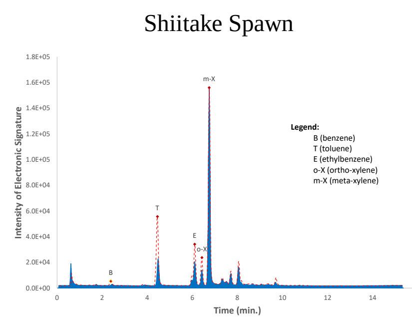


	Diesel Range [C_{10}-C_{25}]	Motor Oil Range [C_{25}-C_{36}]
WAF	4,700 ppb X	230 ppb X
CE-WAF	5,300 ppb X	400 ppb X

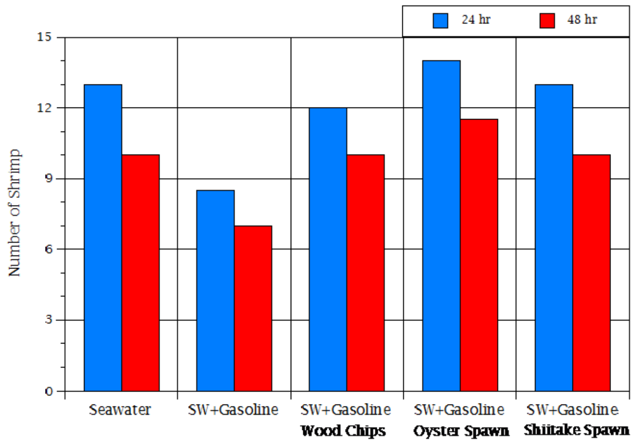
Four 20 gallon tanks were set up with 13 L of seawater and aerated. Small cloth bags were prepared with 70 g of oyster and shiitake spawn, a third bag with wood chips (to act as a control for the woodchip substrate from the spawn bags). The bags were added to the tanks along with gas, as the petroleum hydrocarbon, which was chosen for its high water solubility. A final tank with seawater and gasoline acted as a control. The tanks were left undisturbed for 2 weeks before samples were extracted below the surface and chemically analyzed through a commercial lab for gasoline BTEX TPH using gas chromatography with a photoionization detector (or a GC-PID).







There are several reductions in BTEX levels, as shown by the peaks, however mushroom spawn reduced these peaks the most, and woodchips by themselves had no effect.



The mysid shrimp has been shown to be exceptionally sensitive to toxic substances and a very good representative of marine life in a bioassay. Additional samples were taken from the tanks and used to complete two replicates of 2 day testing periods under these specific conditions and compared to a regular seawater control.

Bioassays showed enhanced shrimp survival (77%) in oyster mushroom remediated water.

Mushroom hyphae are cells surrounded by a tubular wall. Unique to fungi are a type of protein bound within this cell wall called hydrophobins. They form a water-repellent coating on the cell wall surface and have the ability to reverse the hydrophobic-hydrophilic nature of the walls and their surfactant capacity. Hydrophobins may allow oil sorption to the surface of individual hyphae in the mushrooms. The structure of mushroom gills may also allow oil to adsorb to/between individual gills. Initial testing for sorption was done with shiitake caps. Mushroom gills had not yet formed below the caps. Since shiitakes showed the highest oil sorption, studies were done on gill tissues. Fresh gill tissues showed minimal oil sorption while dried gill tissues sorbed 2.22 oil (g/g).

Oil dispersants do not reduce the total volume of oil - rather they change the chemical and physical properties of the oil. Dispersants contain a surfactant which lowers surface tension at the oil-water interface and small oil droplets break away with the help of wave action. Chemical analysis of the water accommodated fraction (WAF) for total petroleum hydrocarbons (TPH) was run. A similar analysis was run on the chemically enhanced-water accommodated fraction (CE-WAF) water where the enhancing chemical was oyster mushroom exudate. The CE-WAF water showed 5300 ppb (11% petroleum hydrocarbon increase) in the diesel range and 400 ppb in the motor oil range (42% petroleum hydrocarbon increase), indicating that dispersal of oil into the water column occurred.

From the remediation studies, gasoline BTEX testing was used to analyze total petroleum hydrocarbons; and revealed a >60 ppb drop in TPH levels when aquarium water was treated with oyster and/or shiitake mushroom spawn. Resulting graphs were digitized to clearly visualize treatment comparisons. Intensity of the benzene electronic signature was lowered by mushroom spawn by ≈15% more than just wood chips; toluene electronic signature intensity was lowered >50% more; Ethylbenzene signature levels were lowered >30% and o-xylene signature levels were lowered >37%. Not only were BTEX levels reduced, however the mushroom treated waters appeared to be much safer to the extremely sensitive mycid shrimp; reducing their mortality rates when compared to the control and even to just plain seawater.

Dried mushrooms are buoyant in seawater, so could be placed in booms to sorb oil. Shiitake mushrooms are not the most effective of all sorption materials, yet offer a unique feature. Tissues with sorbed oil can be composted into new cultures, where the oil will be utilized as an energy source for new fungal tissues. Oyster mushroom exudates dispersed petroleum hydrocarbons into the water column and are a natural alternative to man-made chemical dispersants (such as Corexit). Bioremediation studies showed up to a 40% reduction in total petroleum hydrocarbons, and mushroom-treated water appeared less toxic to marine invertebrates. Costs associated with cleaning up oil spills are high (fifteen billion dollars for the Deepwater Horizon (Gulf States) in 2010). My studies indicate use of mushroom treatments for oil spills would be most effective in more enclosed areas (rocky intertidal zones, bays and estuaries, boat marinas). Oil spills in these areas are difficult to clean with conventional treatments developed for open ocean environments. Mushrooms do offer an 'all-in-one' treatment - the unique ability to sorb, disperse **and** remediate oil simultaneously with one treatment. This shows their potential as a multifunctional response to oil spills.