The *CytoSol* Process:

Cleaning Oiled Shorelines With a Biosolvent

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ABSTRACT
A novel shoreline cleanup process has been developed to aid in the removal of crude or fuel oil from shorelines using the highly effective CytoSol "biosolvent" formulation based on methyl esters in combination with bioremediation enhancers. The CytoSol Process uses the proprietary biosolvent to extract oil from shoreline surfaces during spills that impact harbors, waterways and coastlines. After the CytoSol dissolves and floats the oil, the oil/ biosolvent mixture is rinsed off with ambient temperature water for collection as a consolidated layer with skimmers. The collected oil mixture can be recycled. Nutrient enhancers in the formulation then stimulate the natural biodegradation of the remaining residual hydrocarbons. This new approach minimizes physical and chemical impacts to marine organisms, cleans oiled surfaces effectively, and allows the oiled ecosystem to recover with less mortality than conventional methods involving hot water, detergents or other chemical cleaners. The CytoSol Process is ideally suited for port facilities and waterfronts dealing with occasional small oil spills, or addressing large catastrophic shoreline oil contamination from oil spills..

The CytoSol biosolvent is on the U.S. Environmental Protection Agency's National Contingency Plan (NCP) Product Schedule. This listing does NOT mean that the EPA approves, recommends, licenses, certifies, or authorizes the use of CytoSol biosolvent on an oil discharge. This listing means only that the data have been submitted to EPA as required by subpart J of the National Contingency Plan, 300.915 (Source 40 CFR section 300.920 (e)).

Advantages of the CytoSol Biosolvent

The CytoSol biosolvent can extract heavy petroleum (crude, fuel oils) off shoreline habitats, mussel-encrusted breakwaters or pilings, and estuary vegetation without major disruption to these ecosystems. Although the CytoSol is an aggressive methyl ester solvent, it is produced from renewable oil feed stock and is virtually non-toxic. CytoSol biosolvent presents no threat or discomfort to workers, and has less effect on vegetation and delicate sea life than cleaning solvents derived from petroleum solvents or acidic citrus oils. The product has a low specific gravity (0.87), tends to consolidate oil, and is practically immiscible with water, so it facilitates the recovery of spilled oil with conventional skimming and absorbent boom technologies. The recovered floating oil/biosolvent mixture can be recycled as burner fuel. Since the biosolvent is an excellent carbon/energy source for hydrocarbon-degrading bacteria, residual biosolvent and petroleum hydrocarbons remaining on the shoreline biodegrade more rapidly than untreated, weathered oil.

CytoSol Biosolvent Actions

The CytoSol Biosolvent expedites the removal of spilled oil through specific physical and biochemical changes it imparts on the petroleum:
1. Dissolves the petroleum and decreases its viscosity by dilution
2. Extracts the oil and decreases its adhesion to shoreline/structure surfaces
3. Reduces the density of weathered oil, allowing recovery by floatation
4. Breaks oil emulsions and mousse, separating oil as a floating organic phase
5. Consolidates the oil into floating globules and patches for easier recovery
6. Enhances the biodegradation of the residual petroleum hydrocarbons

First Step: Oil Extraction with CytoSol

The CytoSol Process is a two-step cleanup technology for removing spilled oil accumulated on shorelines, breakwaters, piers, river banks and other waterway structures. In the first step, the CytoSol biosolvent is sprayed directly onto the oiled shoreline to dissolve and release the oil adhering to surfaces or trapped in mussel beds, vegetation, gravel and rocks. After a few hours, or at the end of a tide cycle (the biosolvent is applied as the tide recedes), the dissolved oil is floated off the shoreline or washed off vegetation and shellfish-encrusted structures using ambient temperature, low pressure water spray to minimize damage to the affected ecosystems. Pressure washers can be used to hasten the removal of the oil/biosolvent mixture from oiled surfaces and structures lacking viable marine life.

In laboratory efficacy tests and pilot field trials, the CytoSol biosolvent released 50 to 98% of the original oil adhering to or trapped in various shoreline sediments ranging from oiled coral beach sand collected at the San Juan, Puerto Rico spill (1994) to coarse California beach sand and intertidal gravel from Prince Williams Sound. This first step requires only a single CytoSol application followed by extensive, passive deluges with ambient temperature fresh or sea-water to flush out the floating oil/biosolvent mixture.

The process is amenable to rapid response applications involving oiled shellfish and vegetation. In recent field trials, the CytoSol Process proved effective at removing oil from sensitive marsh plants and mussel beds with much less mortality or injury to the intertidal organisms than has been documented with earlier methods employing hot water, steam, detergents, or mechanical removal of the oiled communities.

Conventional booms and skimmers (weir type or vacuum) are deployed in advance of the biosolvent application to collect and recover the consolidated, floating layer of oil/ biosolvent mixture for recycling as a burner fuel or reprocessing at a oil refinery.

Second Step: Bioremediation

In the second step, the remaining CytoSol and residual petroleum hydrocarbons are biodegraded through a proactive bioremediation program focused on the nutrient enhancement of indigenous, hydrocarbon-degrading bacteria already present at the site. This second step generally takes 6 weeks (at warmer temperatures, e.g., Puerto Rico spill) to several months in cooler weather for the residual petroleum to break down to levels acceptable for site closure. Biochemical monitoring of the shoreline residual oil biodegradation process by CytoCulture's in-house laboratory is important to establish levels of nutrients, pH and bacteria populations capable of degrading the residual hydrocarbons. Biodegradation rates can be substantially improved along gravel or sand shorelines by tilling to improve mixing and oxygen transfer.
Bioremediation now has widespread acceptance as a cost-effective process for reducing petroleum hydrocarbon concentrations in the environment over time without major impact to sensitive ecosystems relative to previously damaging chemical treatments, dispersants and detergents (R.A. Bayless*, Port Technology International* (5): 197-199). The biodegradation of residual hydrocarbons in shoreline and estuarine environments can be accelerated through controlled nutrient addition, particularly through the use of oleophilic nitrogen and phosphorus fertilizers that support acclimated hydrocarbon-degrading bacteria living at the oil-water interface (R.M. Atlas & R. Bartha, *Microbial Ecology,* 4th Ed., Benjamin/Cummings Publishing Co., Inc., Redwood City, CA, 1997). The vegetable oil methyl esters of the CytoSol biosolvent have been shown in laboratory studies and field trials to further boost the populations of bacteria that break down petroleum. In bioremediation studies of oiled sand and slurries of oiled sediments, the bacteria that acclimated to the biosolvent as a sole food source were at least as active at degrading crude oil as bacteria grown up on the crude oil alone.

Applications for the CytoSol Process

The following shorelines would be good application candidates for CytoSol Process:

- Breakwaters, bulkheads and piers with marine life that trapped or adsorbed oil
- Coarse sand beaches where the petroleum has penetrated into subsurface
- Marsh areas, wetlands and mangroves where oil is trapped in vegetation
- Gravel and rocky shorelines, where stranded oil has become inaccessible
- Public beaches, fisheries, hatcheries, estuaries and other ecologically sensitive areas

A major goal of the CytoSol Process is to eliminate the "re-oiling" that often occurs as trapped oil is continually released after conventional washes and treatments, particularly in sensitive ecosystems that would otherwise have to be destroyed to recover the oil. The CytoSol biosolvent may be applied with a variety of spraying or washing equipment depending on the scale and type of surface to be cleaned. The product is shipped to the site in bulk containers or 55-gallon drums for direct application to the oil. In small-scale applications, the biosolvent may be applied with hand sprayers, or with portable pumps, hoses and nozzles to spray the product directly onto oiled surfaces and shorelines.

Dose rates for application of the CytoSol biosolvent will vary with the type and amount of petroleum spilled, the extent of weathering, and other site-specific conditions. In general, the ratio of applied CytoSol to crude oil is between 0.5:1 and 1:1 parts of CytoSol to immobilized oil, although it could be used at higher dose rates for direct application to release inaccessible, trapped or weathered oil.

Recovery of Oil/Biosolvent Mixture

Shoreline washing with ambient temperature sea water (e.g., header flooding systems or moderate pressure hoses) is essential for releasing the extracted oil/biosolvent mixture. The CytoSol facilitates the collection of adherent oil by reducing the oil's adhesion to surfaces and consolidating the floating mixture into discrete patches. As a result, weir or disc skimmers and vacuum recovery systems work more effectively in collecting the consolidated oil. The oil/biosolvent mixture's lower viscosity and greater cohesion dramatically increases the extracted oil penetration into absorbent pads and booms compared to results obtained with dispersed oil and sheen.

The CytoSol biosolvent also serves as a non-toxic, non-irritating solvent to use in the decontamination of oiled boats, pumps, tanks and remediation equipment without the use of water (no waste water disposal problems).

Low Impact on the Environment: Marine Toxicity and Biodegradation Studies

The Biosolvent fatty acid esters are readily biodegraded and can provide an easy food source to build up populations of hydrocarbon-degrading bacteria capable of breaking down the more complex petroleum hydrocarbons remaining in the environment after the initial treatment. In laboratory simulations conducted at CytoCulture using California Department of Fish & Wildlife protocols, the dissolved phase CytoSol esters (saturation = 14 ppm at 17 Deg. C.) biodegraded with naturally occurring bacteria in San Francisco Bay water with a half-life of approximately 4 days.

Acute toxicity bioassay studies were conducted on two marine larval species as required by US EPA protocols for testing shoreline washing agents to be listed in the NCP schedule of products used at oil spills. *Mysidopsis bahia* shrimp larvae and *Menidia beryllina* fish larvae were exposed to increasing concentrations of the CytoSol added as an emulsion to agitated sea water during a 48-hour survival test. A third test species, abalone larvae (*Haliotis rufescens*), was used to evaluate the CytoSol in a leachate bioassay test required for licensing in the State of California by the Department of Fish & Game. Marine toxicity levels for emulsified CytoSol biosolvent were found to be 15-20 times lower than for reference fuel oil.

Subsequent studies determined that the biosolvent's apparent effects on sensitive marine life, although low and within acceptable limits by these test protocols, were due to the emulsion of the product as required by the test procedure. The actual chemical toxicity of the dissolved phase (less than 14 ppm in sea water) would, in practice, be much less than suggested by these laboratory results.

Pilot Demonstrations of the CytoSol Process

CytoCulture has performed a series of pilot demonstrations of the CytoSol Process since the first demonstration at the *Berman* barge fuel oil spill in San Juan, Puerto Rico in January, 1994. These demonstrations have been conducted for the US Coast Guard, NOAA, the US Environmental Protection Agency, and the US Department of Agriculture, as well as for local regulatory agencies in California, Washington and Alaska. A similar, larger pilot demonstration was performed for Japanese authorities on the west coast of Japan (Kaga) in April, 1997 as they cleaned heavy oil from a major tanker spill.

In the demonstrations, heavily oiled (saturated) sand is treated in confined pools with the CytoSol biosolvent and then rinsed with cold water to float off the consolidated petroleum. The floating oil/biosolvent mixture is collected by a weir skimmer and recycled as burner fuel. Over 80% of the fuel oil is recovered from the sand by a single CytoSol biosolvent application and water rinse in the first step of the process.

For step two, the treated sand is fertilized with time release and liquid nutrients, and then inoculated with cultures of hydrocarbon-degrading bacteria grown up on site in sea water. The sand is tilled and irrigated on a weekly basis to promote the biodegradation of remaining hydrocarbons. In the Puerto Rico demonstration, the total hydrocarbon concentrations had dropped by over 90% to less than 200 ppm after 6 weeks of tilling. In oiled sediments obtained from a crude oil production field in Mexico, the population of hydrocarbon-degrading bacteria in CytoSol treated samples increased 2-3 logs to over 10^7 colony forming units per gram.

CytoSol Process Field Tests

In a field test conducted on San Francisco Bay in 1995, the CytoSol Process succeeded where conventional washing had failed to release weathered fuel oil trapped in the intertidal gravel. A `single CytoSol application followed by a passive water deluge released over 60% of the trapped oil near the high tide zone as measured by samples collected and analyzed by the California Department of Fish & Game, Office of Oil Spill Prevention and Response. Full-scale shoreline cleanup applications would probably release 50-90% of accumulated oil, depending on the temperature and the condition of the spilled oil, and other site parameters such as the volume of CytoSol applied, the time allowed for contact with the oil, and the amount of rinse water applied.

Subsequent field tests of the CytoSol Process on oiled creek beds (Santa Barbara) and oiled mussel beds and pilings (Long Beach Harbor) have confirmed that the CytoSol can effectively remove oil from shoreline communities without major disruption to these sensitive ecosystems.

The CytoSol Process has been undergoing research and development with private funds and the support of the United Soybean Board and the US Department of Agriculture since 1994.

The CytoSol biosolvent is now commercially available worldwide with distribution points in North America, Europe and Japan. CytoCulture provides laboratory and field support services to assist in oil spill remediation efforts as needed.

About the Author

Dr. von Wedel is a biochemist and the Director of Research at CytoCulture International, Inc., which he founded in 1986. CytoCulture is an environmental biotechnology firm based in the San Francisco Bay area offering microbiological laboratory and field consulting services for oil spill cleanup efforts. The CytoSol biosolvent and process are proprietary technologies developed in 1994 as an extension of Dr. von Wedel's experience with designing continuous flow bioreactors and *in situ* bioremediation technologies for the cleanup of petroleum hydrocarbon contamination in soil and groundwater.

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