

**HC-2000 Bioremediation Accelerator
& Surface Cleaner**
A Green Sustainable Technology



*Restoring Our World
for
Future Generations*

for
Petroleum Hydrocarbon & Solvent Cleanups
in
Soil, Railroad Ballast, Gravel
Groundwater, Surfacewater, Wastewater
Concrete, Asphalt and Other Surfaces



HC-2000 BIODEGRADATION & CLEANING APPLICATIONS ON PETROLEUM HYDROCARBON & SOLVENT CLEANUPS (A GREEN SUSTAINABLE TECHNOLOGY) REMTECH ENGINEERS ©, 2016

Introduction

HC-2000 (**HC2**) is a biostimulation bioremediation accelerator with biosurfactants (developed by Remtech in 1998) that degrades, cleans, desorbs, and reduces odors from fuels, oils, lubricants, and chlorinated and non-chlorinated solvents in soils, ballast, gravel, surface water, groundwater, and other media.

HC-2000 is a *Green Sustainable Technology*. HC2 restores the environment by accelerating natural systems (heterotrophic bacteria) to degrade petroleum and solvent based contaminants. HC2 is non-toxic and removes contaminants with minimal economic disruptions to business and generally cost less than other remedial technologies. When treatment is complete, native conditions are restored.

HC-2000 has been used successfully on over 400 cleanup sites during the past 17 years. HC-2000 is designed to work in freshwater applications on refined fuels and solvents not addressed by EPA's NPL List of saltwater approved crude oil remediation and cleaning products. HC-2000 is ten times less toxic to aquatic organisms than most of the NPL products.

HC-2000 is particularly cost-effective where: service or business interruptions need to be minimized; access is limited; areas are environmentally sensitive; or sites are geotechnically, hydrologically or structurally sensitive or unstable, and where contamination exists around grounding grids at power plants or substations.

HC-2000 Multi-Media Applications

Soil Treatment - treats surface soil passively (via topical or gravity feed systems for soil depths up to 3 to 5 ft) or actively (in combination with biovent/injection & biosparge/multiphase extraction systems for depths exceeding 5 ft).

Ballast, Gravel & Soil Treatment (Railroad Ballast, Power Plants, Substations & Tank Farms) - Gravel, soil and/or ballast can be treated with HC-2000 at railroad mainline and siding tracks, power plants, substations, and in above ground storage tank secondary containment areas. HC-2000 is particularly effective in railroad ballast (that functions like a trickling filter with elevated baseline heterotrophic bacteria plate counts). In place treatment minimizes rail or power service interruptions and disturbance of grounding and cathodic protection systems.

Groundwater Treatment - treats groundwater insitu passively (infiltration galleries, biofences) or actively (reactor trenches, sparge wells, and total fluids extraction wells).

Surface Water Treatment - removes sheens by lowering surface tension and allowing petroleum hydrocarbons to come in contact with particulate born bacteria for degradation. Removes light sheens and odors almost immediately and desorbs fuel from banks, leaves, limbs, and other organic matter. Degrades solution phase contaminants in aerobic, facultative, and anaerobic environments.

Pavement & Surface Cleaning - cleans/removes oils and solvents from asphalt, concrete, metal surfaces, parts, and other surfaces. Accelerates degradation rates of rinsates.



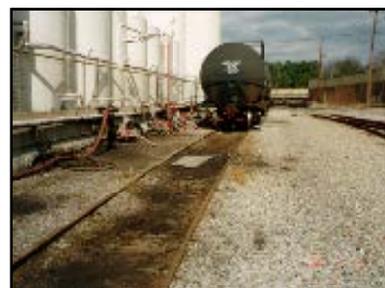
HC2 Gasoline Contamination
Wetland Remediation



HC2 New York Solvent
Remediation Under Building



HC2 Removes Diesel Fuel
in 10^{-6} cm/sec Conductivity
Aquifer under Building



Motor Oil Contaminated Ballast
Before HC2 Treatment



Ballast Staining & Oil
Concentrations Reduced by 70%+
after 4 months of HC2 Treatment

Sediment Treatment - desorbs flammable/combustible fuels and solvents from sediment in sewers, retention ponds, stream/river beds, and storage tanks. Flushing systems with HC-2000 accelerates solvent removal/desorption, degradation, and lowers flammable vapor concentrations. Insitu treatment methods minimizes erosion.

Solids & Sludge Breakup - breaks up sludge in digesters, sludge pits, septic tanks, and solids buildup in ASTs and USTs.

Forrest Canopy, Vegetation, River Banks, & Wildlife - elutriates fuels and solvents from leaves, foliage, sod, etc., to minimize interference with photosynthesis and minimizes loss of foliage. Rehabilitates flora & fauna from oil exposures. Accelerates degradation of wash residues.

Wastewater Treatment - accelerates biological degradation in oil/water separators, truck and car washes, parts washing, septic tank systems, and conventional biological wastewater treatment systems.

Odor Control - HC-2000 kills pathogenic and odor causing bacteria, reduces vapor pressure of volatile compounds, and activates bacterial odor degrading microorganisms.

Technology Selection Factors

Environmentally Sensitive Areas - food quality natural components in HC-2000 accelerate bioremediation in environmentally fragile areas such as wetlands, marshes, beaches, national parks, dunes, nature preserves, and forests without adverse environmental impacts on flora or fauna. HC-2000 synergistically accelerates bioremediation in the rhysosphere (root zone), and organically enriched and microbial diverse environments.

Structurally Sensitive/Restrictive Areas - treats soils and groundwater under buildings, around footings & building foundations, utilities, old fragile sewer systems, and grounding & cathodic protection systems.

Geotechnically Unstable or Erosion Sensitive Areas - treats drainage ditches, stream beds, river banks, and highway right-of-ways while minimizing erosion and adverse environmental impacts.

Security Sensitive Facilities - in access restricted areas, applications can be made quickly with minimal disruptions at airports, military and penal installations.

How Does HC-2000 Work?

Native heterotrophs are ubiquitous in the environment and are found in soil, water, groundwater, and waste in aerobic, facultative, methogenic, and anaerobic zones. Indigenous (insitu) bacteria are the predominant degraders in soil, groundwater, surface water, railroad ballast, gravel, and saltwater.

Numerous publications conclude that very little scientific data demonstrates that enhanced degradation (microbial addition or bioaugmentation) works on full-scale cleanups. According to peer-reviewed literature, bioaugmentation appears to have little benefit for the treatment of spilled petroleum hydrocarbons in the environment. Microbial addition has not been shown to work better than nutrient addition alone in many field trials (10). Biostimulation has been proven to be a promising tool to accelerate and enhance the natural attenuation process.

Native soil populations capable of degrading petroleum hydrocarbons have been reported to range from 10 to 20% of all soil heterotrophic bacteria. Soil particles containing heterotrophs are found in lower concentrations in groundwater and surface water (2×10^6 cells/ml) than in soil (2×10^7 cells/gm) (11), albeit provide the metabolic and synthetic activity to digest petroleum hydrocarbons. Approximately 67% of all bacteria in groundwater sediment are attached to the silt and clay fraction, though this sediment fraction may account for a small fraction of the total sediment by weight.



International Airport JP4 HC2 Remediation in Restricted Area



HC2 Degrades Diesel Fuel & Odors in Railroad Ballast w/o Track Time



HC-2000 Desorbs & Degrades Lube Oil in Railroad Ballast

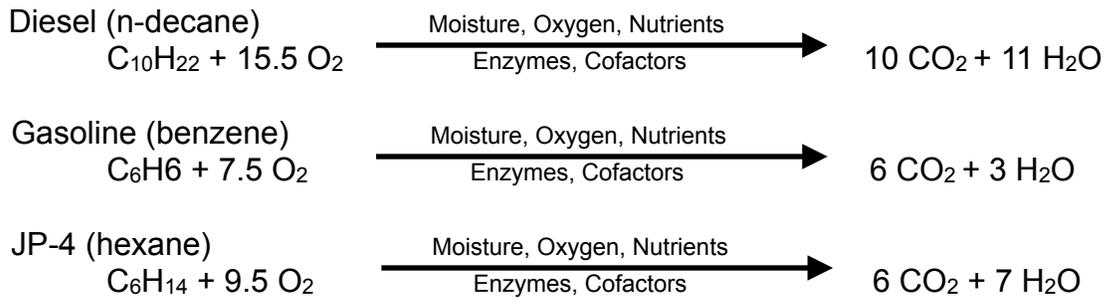


Bulk Diesel Removed prior to HC-2000 Treatment



HC-2000 Removes Sheens, Treats Surface Water & Fuel Contaminated Sediment

SIMPLIFIED MICROBIAL AEROBIC WEATHERING/MINERALIZATION OF PETROLEUM HYDROCARBONS



Complete mineralization results from a consortium of native microorganisms working together synergistically. Cometary activity (enzymes generated by an organism growing at the expense of one substrate) can also transform a different substrate that is not associated with that organism's energy production, carbon assimilation, or any other growth process - degradation of a compound only in the presence of other organic material that serves as the primary energy source (11). Multiple bacteria populations found in nature provide the mechanisms to break down petroleum compounds to CO₂ and water that is not possible with a single bacterial strain.

Many organic contaminants will serve as a substrate. However, for some hazardous chemicals, another substrate is necessary. The compound targeted for degradation may be at concentrations below that necessary for good biological response for bioremediation. The addition of a primary substrate (contained in HC-2000) supplies additional energy. When another energy and carbon source is made available to stimulate biological growth, it becomes the primary substrate and the target compound to be degraded is the secondary substrate.

Heterotrophs have been demonstrated to be effective degraders of a variety of hazardous and non-hazardous wastes including petroleum hydrocarbons, chlorinated solvents, and other pollutants. A myriad of degradation data was generated in the 1980s during research on land farming of hazardous wastes (1). Ross McKinney in his book *Microbiology for Sanitary Engineers* states ... *the best source of microorganisms is soil. The soil can furnish all the microorganisms ever needed in waste disposal. My advice to all sanitary bacteriologists who seek a special culture is to look under their feet; the supply is inexhaustible* (2). HC-2000 targets native heterotrophs that control the bioremediation process.

Heterotrophic degradation is limited by enzymes, co-factors, nutrients, electron donors, biosurfactants, and the proper environmental conditions that accelerate biochemical reactions. Natural attenuation can take years and generally reaches asymptotic concentrations due to insufficient nutrients or inadequate environmental conditions. HC-2000 supplies the limiting factors outlined above and reduces degradation times to months or longer periods for more resilient compounds.

HC-2000 is a non-toxic aerobically fermented natural organic product that is readily assimilated and eliminates long lag reaction/acclimation periods. HC-2000 can be applied under various environmental conditions (aerobic, facultative, methanogenic, anaerobic).



HC2 Riverbank Treatment at JP-8 Contaminated Golf Course Prevents Erosion Claims



HC-2000/BioSparge Injection Well at Gasoline GUST Trust Fund Reimbursed Site



Transformer Oil Removed from Containment Structure at Power Plant

What's In HC-2000?

HC-2000 is an organic food quality indigenous soil bacteria biostimulation agent that accelerates and improves natural attenuation of crude oil and refined petroleum and solvent products when compared to EPA's synthetic inorganic fertilizer blend and non-spiked controls. HC-2000 contains aerobically fermented organic nutrients including Kjeldahl nitrogen, proteins, enzymes, vitamins, and biosurfactants. Pulp present in HC-2000 acts as a *slow release* mechanism that provides *extended release* of active ingredients over time.

Organic Versus Inorganic Nutrients

Due to toxicity of some synthetic oleophilic fertilizers, natural organic origin fertilizers in urea, uric acid, and HC-2000 are considerably less toxic. Organic fertilizers have low solubility in water (contribute to slow release); organic nutrients in HC-2000 bind to petroleum hydrocarbons and are therefore available to bacteria which grow at the hydrocarbon-water interface.

The addition of organic carbon sources such as surfactants and organic nutrients will increase the bacterial respiration of petroleum degrading bacteria and enhance n-alkane biodegradation (12).

Role of Biosurfactants

Biosurfactants (a key ingredient of HC-2000) have been used widely to break down and/or desorb contaminant matrix size to allow greater contact with native bacterial degraders. Native bacteria are already distributed in environmental media and rapidly acclimate to spilled material. Biosurfactants are used to increase mass transfer rates, and desorb/mobilize contaminants for greater contact with soil microbes.

Biosurfactants assist in petroleum hydrocarbon availability either by chemical or biological means. Microbial attachment takes place mainly at the oil-water interface and increased mixing of petroleum hydrocarbons results in dramatic increases of the surface area for microbial colonization. Biosurfactants are amphiphilic compounds of microbial origin with considerable environmental advantages over chemical surfactants in terms of biodegradability and effectiveness at extreme temperatures or pH and have a much lower toxicity (12). Biosurfactants facilitate the bioremediation of hydrocarbons having limited water solubility by increasing bacterial cell surface hydrophobicity, via fatty acid moieties, which promotes their adsorption to hydrocarbons.

Biostimulation with organic nutrients and biosurfactants enables naturally occurring microbes to adapt better and faster to the contaminated environment resulting in shorter lag phase and faster petroleum hydrocarbon degradation.

Fate of Degradation Products in the Environment

HC-2000 desorbs and degrades contaminants. Biostimulation bioremediation projects require adequate reaction zones and retention periods to complete degradation. Offsite migration can be minimized by removing as much of the mobile free product phase or grossly contaminated matrix as possible at the beginning of the project and monitoring downgradient receptors to determine if additional downgradient HC2 injection points and/or containment/leachate recovery systems are required. Controlled addition of HC2 can also enhance removal of bulk fuels or solvents by *washing and desorbing* for recovery by conventional mechanical recovery systems.

On soil or ballast applications, the desorption properties of HC-2000 accelerates the removal of mobile phase constituents and brings residual contaminants in contact with an increased number of soil bound degraders.

On water applications, the mobile phase is removed first by conventional methods. Non-recoverable sheens are removed with HC-2000 by temporarily lowering surface tension (not emulsifying) and bringing contaminants in contact with particulate born degraders. Oil skimmers, oil/water separators, and other conventional free product recovery devices are not adversely impacted by HC-2000 since HC2 does not emulsify contaminants.

Examples of leachate containment systems that may be used to provide adequate treatment periods include: filtration/adsorption dams, containment booms, interceptor trenches, cut-off walls, perimeter monitoring wells, downgradient air curtains or sparging, or groundwater depression/vacuum extraction systems.

Overcoming Mass Transfer Challenges

In situ treatment is limited by mass transfer. Treatment technologies must come in contact or mix with contaminants and the environment to be effective. Native heterotrophic bacteria are already dispersed and rapidly acclimate to contaminants around them.

The ability to deliver treatment agents uniformly to heterogeneous media (such as soils and groundwater) is required to achieve complete site treatment. HC-2000 supplies wetting agents and biosurfactants that improve mass transfer. Bound or adsorbed contaminants in soil pores (frequently account for up to 60% of the total contamination) restricts bioavailability. Biosurfactants in HC-2000 *desorbs or washes* contaminants moving them towards microbial degraders. This same desorption mechanism is a major factor that facilitates cleaning surfaces.

HC-2000 enhances both passive and aggressive delivery methods. Passive in situ technologies rely on advection (bulk groundwater flow) and dispersion to disseminate reactants. Aggressive pulsed (air and/or reagent) injection enhances mixing and mass transport and generally produces more rapid and uniform results.

HC-2000 Delivery Systems

For bioremediation or cleaning, HC-2000 and water mixture may be applied by a diaphragm, roller, or centrifugal pump with a coarse fire nozzle. Trace quantities of pulp may clog finer nozzles and piston pumps (especially pressure washers). HC-2000 may be injected past pressure washer pumps and applied with chemical feed nozzles followed up with a high pressure water chase (cold or hot - <120 °F) to drive HC2 into the media. Best results are achieved when surface solid media (soils, gravel, ballast, etc.) are mixed (tilled) or agitated with the initial application and several times during the treatment period. Agitation of hard surfaces (manual or power brooms, pressure or hot water washers), followed by a reaction period (approximately 5 to 20 minutes) and water rinse enhances surface cleaning effectiveness.

For subsurface soil applications, HC-2000 may be applied through infiltration galleries, horizontal or vertical biovent systems or reaction trenches/fences. HC-2000 may be applied as foam to enhance movement through and contact with soil. Soil moisture should be maintained at 70% of field holding capacity (or 20% moisture).

For subsurface groundwater applications, HC-2000 may be applied through horizontal or vertical biosparge systems, reaction trenches/fences, or in conjunction with total fluids vacuum extraction systems. HC-2000 may be injected as a liquid or foam to increase contact with pollutants.

For in situ soil and groundwater applications, reaction zones are generally defined by areas where 10% of the applied air pressure is observed at response points. Radius of influence or reaction zone tests should be performed to determine injection grid layouts and spacing. Application of HC-2000 during high water table conditions may avoid contamination desorption from the smear zone.

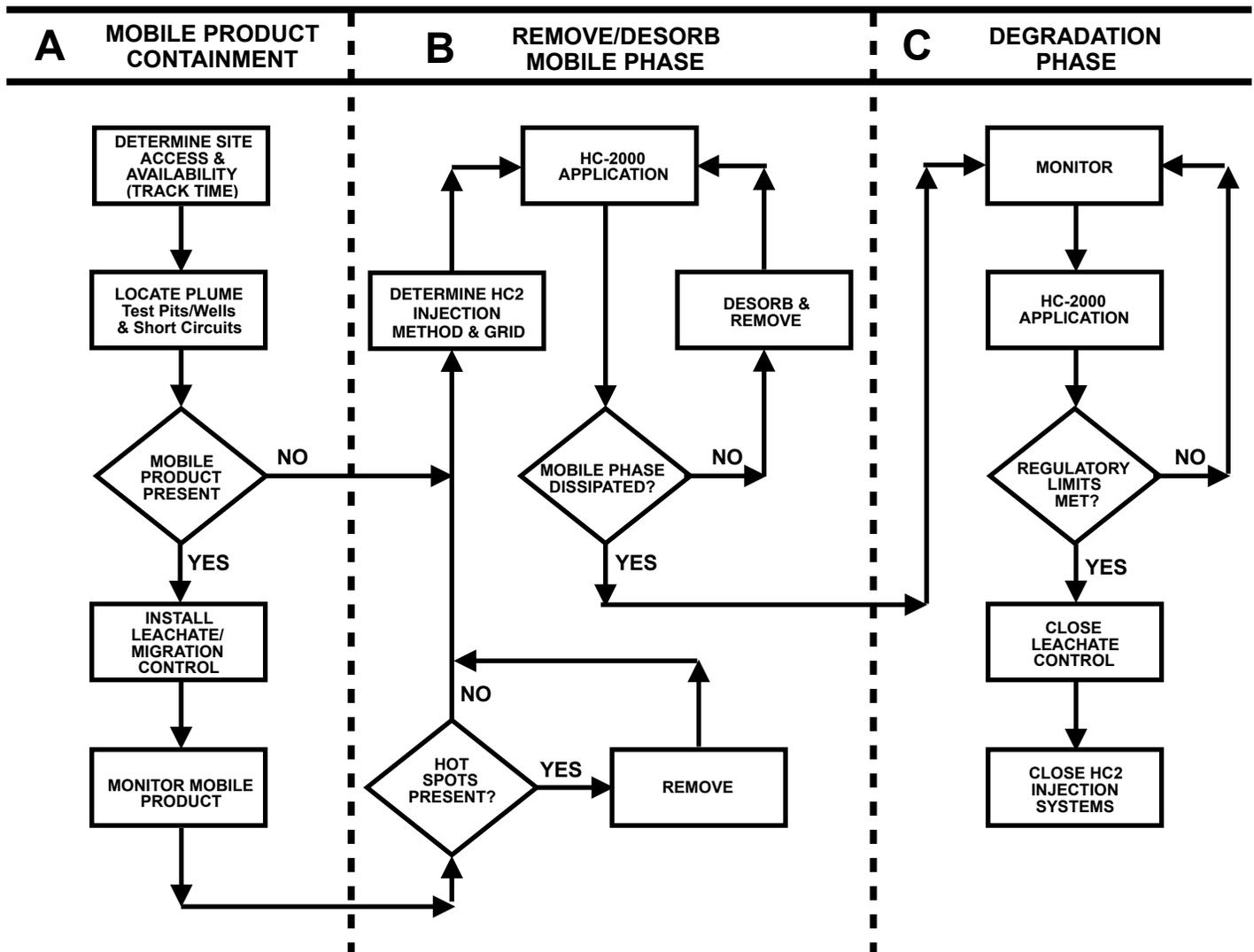
Suggested Monitoring & Dosage Rates

Optimal degradation conditions are present when total heterotrophic plate concentrations are elevated and maintained during the treatment period. Elevated plate counts indicate that sufficient nutrients, moisture, and environmental conditions are present. Secondary parameters that may be monitored include; respiration by-products, moisture, dissolved oxygen, pH, and redox potential. Monitoring the reduction of the contaminant(s) of concern determines when treatment is complete and/or when natural attenuation can complete the degradation process.

Dosage rates are site and contaminate(s) specific. Dosage rules of thumb range from three (3) to ten (10) cubic yards of contaminated media (soil, groundwater, railroad ballast, etc.) per gallon of concentrate over a five-week treatment period. Concentrate dilution ratios of one part of HC-2000 to sixteen parts of water is recommended for soil, ballast or gravel applications.

Groundwater dosage rates vary from 1:16 to 1:30 (HC-2000 to water mixture). Always add HC-2000 concentrate to water to avoid excessive foaming. For example, to treat 10 cubic yards of contaminated media (assuming one gallon of concentrate treats 10 cy), weekly dosages would be 0.2 gallons in 3.2 gallons of water. Assuming a 16-week treatment period, then 16 x 0.2 gallons or 8 gallons of concentrate would be required. For surface cleaning applications a 1:5 to 1:10 ratio may be used. For pressure and/or hot pressure washers chemical feed ratios of 1:2 to 1:5 are recommended.

HC-2000 Treatment Steps



A. Phase 1: Locate, Contain, and Monitor Mobile phase

- Determine site access and available site service interruption time, i.e., track time, business interruptions, environmental receptors, sensitive environments and structures, and cleanup and risk based targets
- Locate contamination and mobile phase pathways. Look for short circuits, i.e., sewers, swales, drains, backfill, utilities, streams using test pits, monitoring wells, geoprobe points
- If mobile phase present, install containment or free product migration monitoring system. Install leachate/migration control systems (straw bale filtration dams, collection trenches, pits, drainage tiles & sumps, or temporary detention pools). For track applications, install leachate control near toe of ballast and/or in drainage swales. Apply HC-2000 followed by oxygen saturated water to increase penetration, moisture, and oxygen.
 - ♣ Protect structural integrity of track, grounding, and cathodic systems - Do not disturb ballast below ties or within a 45% slope of tie edges.
 - ♣ Monitor and contain free product movement.

B. Phase 2: Remove/Desorb Mobile Phase

- Remove mobile phase or saturated soil/solids hot spots and HC2 injection method and grid.
- Apply HC-2000 (followed by water chase) to accelerate removal of mobile phase and assist with product desorption. Bound contaminants account for over 60% of contamination. Fuels may become mobile as they are desorbed and broken down into shorter hydrocarbon chains.
- Ballast Treatment – Apply HC-2000 topically followed by a water chase or through injection probes. Fouled ballast - mixing or agitation enhances HC2 delivery and treatment. Pressurized water (3,000 to 5,000 psi) or a cribbing bucket loosens ballast next to ties.
- Repeat HC-2000 applications until mobile phase dissipated.

C. Phase 3: Degradation Phase

- Monitor leachates and soil degradation targets (visual observation of stormwater runoff - no sheens), sample ballast fines and downgradient soil and leachate for total petroleum hydrocarbons. Supplemental water may be required to maintain moisture levels at 70% of field holding capacity.
- Apply HC-2000 per label instructions and adjust for site conditions.
- Repeat until regulatory limits, risk based closure, or until natural attenuation can reach regulatory limits.
- Remove/close leachate control systems.
- Close HC2 injection systems.

Activation & Stability of HC-2000

HC-2000 has a shelf life that exceeds five (5) years (as long as it is stored in its concentrated state and at temperatures below 120°F). HC-2000 is activated when it is mixed with water according to the contaminated matrix and applicable dilution ratios. Diluted mixtures of HC-2000 have a shelf life of a couple of months and should be utilized as soon as possible after mixing with water.

Regulatory Approvals

Regulatory authorities frequently favor (and generally quickly approve) the acceleration of natural degradation processes with a *Green Sustainable Technology* as apposed to addition of foreign microbes or toxic materials into the environment. Stimulating natural biochemical processes reduces the possibility of toxic by-product formation and allows multiple native species (operating under a variety of environmental conditions) to reduce contaminants to minimum levels. When treatment is complete, native conditions are restored.

HC-2000 has been approved by Georgia, Florida, and other states for the treatment of soil and groundwater on a case-by-case basis. No specific approval is required for soil applications in Georgia. Georgia frequently only requires three applications of HC-2000 to soil without performance sampling to complete a cleanup.

HC-2000 Benefits

HC-2000 is a *Green Sustainable Technology*. HC2 restores the environment by accelerating natural systems (heterotrophic bacteria) to degrade petroleum and solvent based contaminants. HC2 is non-toxic and removes toxic contaminants with minimal economic disruptions to business and generally cost less than other remedial technologies. When treatment is complete, native conditions are restored.

Other remedial technologies may leave residual contaminants that require additional treatment. These technologies include; pump and treat, soil venting and air sparging, and total fluids extraction. Why not use a technology that can finish the job?

Site remediation costs with HC-2000 typically range from \$15 to \$225/cy of contaminated media. Costs are site specific and are affected by the type and amount of contamination, local geology, volume of contaminated media, and contaminant location. HC-2000 performs best in formations where adequate communication and mass transfer are present or can be established.

HC-2000 is non-toxic, non-allergenic, and contains food quality ingredients. Accelerating the natural degradation process (Biostimulation) with HC-2000 is generally received favorably by regulatory authorities and the general public. Native heterotrophs are already acclimated and distributed in the environment. All that is required is to deliver HC-2000 to the degraders and provide adequate environmental conditions. When treatment is complete, native conditions are restored.

HC-2000 goes right to work by energizing native heterotrophs. Chemical oxidization with permanganate, peroxide, and ozone frequently oxidize materials other than target contaminants, i.e., a significant mass of reagents are wasted. Chemical oxidization and bioaugmentation may form toxic by-products that are not normally associated with cometabolic native biochemical reactions.

Oxygen and hydrogen release compounds singularly introduced generally rely on passive slow release mechanisms and depend on advection and dispersion to transport the reagent to the contaminant. Limiting nutrient deficiencies, enzymes, and co-factors are not addressed. Adsorbed and soil pore bound contaminants are only addressed by sufficient concentration gradients to draw reactants to contaminants. HC-2000 provides contaminant desorption with biosurfactants that is generally more effective than concentration gradients. Aggressive pulsed reagent injection (used with HC-2000) generally provides better mass transfer and mixing.

HC-2000 is easily assimilated by native bacteria without a lag time. Commercial fertilizers and synthetic surfactants may initially inhibit microbial degradation. Nutrient and carbon sources such as molasses, sugars, and vegetable oil need to be broken down further prior to assimilation by microbes. Organic nitrogen and proteins (contained in HC-2000) are a preferred source of nitrogen over nitrates, ammonia, and other compounds containing inorganic nitrogen.

References

1. *Hazardous Waste Land Treatment*, Environmental Protection Agency, Solid Waste & Hazardous Waste Research Division, Cincinnati, Ohio, April, 1983.
2. McKinney, Ross, *Microbiology for Sanitary Engineers*, McGraw-Hill Book Company, Inc., 1962.
3. Suthersan, Suthan S., *Natural and Enhanced Remediation Systems*, Lewis Publishers, 2002.
4. Suthersan, Suthan S., *Remediation Engineering*, CRC net Base, 1999.
5. Ryckman, Mark D., et. al., *Enzyme helps Remediate at Lightning Speed*, Soil and Groundwater Cleanup Magazine, February - March 1997.
6. Ryckman, Mark D., et.al., *Cut Project Life Cycle Costs*, Soil and Groundwater Cleanup Magazine, January - February 1996.
7. *Enhanced Bioremediation with HC-2000*, Remtech Engineers News Letter, Vol. 6, December, 2002.
8. *HC-2000 Multi-Media Applications*, Remtech Engineers News Letter, Vol. 9., November, 2004
9. Remtech Engineers, Confidential Client Project Report Files, 1997- 2016.
10. Venosa, A., *Literature Review on the Use of Commercial Bioremediation Agents for Cleanup of Oil-Contaminated Estuarine Environments*, USEPA, National Risk Management Research Laboratory, Cincinnati, OH, July, 2004.
11. Cookson, John T., *Bioremediation Engineering - Design & Application*, McGraw-Hill, Inc., 1995.
12. Nikolopoulou, M., Kalogerakis, N., *Enhanced Bioremediation of Crude Oil Utilizing Lipophilic Fertilizers Combined with Biosurfactants and Molasses*, Marine Pollution Bulletin 56 (2008) 1855-1861., Elsevier Ltd, 2008.

HC-2000 Petroleum Hydrocarbon Efficacy & Aquatic Toxicity Testing in Freshwater & Soil Environments

Remtech Engineers ©, 2016



Soil Slurry Bioremediation
Efficacy Tests



Powdered Clay Slurry
Efficacy Tests

Purpose

Range finding aquatic toxicities and biostimulation efficacy testing of HC-2000 was conducted to provide guidance on the appropriate selection and application of HC-2000 in soil and freshwater environments. Parallel efficacy and toxicity tests were also conducted on select NCP listed agents including Microblaze, Biosolve, and F-500 (Fuel Buster) for comparison purposes.

Current NCP List testing protocols address bioremediation agents efficacy of crude oil degradation in saltwater/ocean environments. Over 60% of fuel spills occur in inland freshwater environments. Microbial populations in inland environments differ from marine environments. In addition, refined products have different ratios of alkanes and aromatic compounds than crude oil. EPA is currently proposing revisions to test protocols to include freshwater and freshwater organisms (10), albeit do not specifically address biostimulation of indigenous soil microorganisms that vary at each site.

Remtech's range finding bioremediation efficacy testing was modified from a *Test Protocol (soil slurry reactors) for Evaluating the Capability of Indigenous Microorganisms to Degrade Target Compounds* cited in John T. Cookson, *Bioremediation Engineering - Design & Application*, McGraw-Hill, Inc., 1995 (1). Range finding aquatic toxicity screens were performed using fathead minnows and an abbreviated ASTM E729-96 method.

Range Finding Bioassays

Remtech conducted range finding aquatic toxicity tests (abbreviated ASTM E729-96 method) using freshwater Fathead minnows and an untreated control for HC-2000 and Microblaze. Test concentrations were prepared from 6% solutions of both products. Organism fatalities were recorded during a 96-hour exposure period. LC_{50s} were calculated using Trimmed Spearman-Kärber Method (4).

The LC₅₀ for HC-2000 was determined to be 5,764 ppm (11,528 ppm at 3%) and Microblaze 1,414 ppm. (8) Published aquatic 48-hour toxicity tests for Microblaze by Aqualabs (LC₅₀ - 1,390 ppm for Silverside), (11) compared favorably to Remtech's range finding test results considering variation in species toxicity and the additional exposure time.

Biostimulation Efficacy Tests

Indigenous bacteria bioremediation tests were conducted to provide relative comparisons for select listed compounds on the NCP List (Microblaze, F-500, and BioSolve) and HC-2000. Total Petroleum Hydrocarbons (TPH) concentrations were measured with EPA Method 9071B at independent testing labs. The test protocol was a modified version of the Test Protocol for Evaluating the Capability of Indigenous Microorganisms (1) and using EPA inorganic nutrient preparation [4.3.3.1, 40 CFR, Part 300 Appendix C, (5)] and an untreated control.

This CSTR (Continuously Stirred Reactor) method facilitates non-sacrificial reactor sampling at increasing time intervals (especially in view of the fact that the biosurfactants present in HC-2000 minimizes adsorption of petroleum hydrocarbons to glass reactor sidewalls). Each reactor was charged with distilled water and either diesel fuel or crude oil. Crude oil was obtained from EPA (ANS521 Crude, recd. 20 BEREC 4/27/93, 9 April 2010 ELH, outside canister 485380, ATCC 1081 Univ. Blvd., Manassas, VA 20110 (6)). EPAs inorganic nutrient control and an untreated control were run in parallel with each agent.

Results

Percent biostimulation efficacy tests were conducted in glass erlenmeyer flasks with magnetic stirrers covered with foil tops or stoppers. Native soil heterotrophs were obtained from sandy CLAY soil or powdered clay. Nutrient concentrations were not checked in source soils. Crude oil degradation tests with the EPA nutrient and non-treated controls had a tendency to adsorb on reactor sidewalls due to the lack of surfactants. Dawn detergent was injected to these controls in an effort to prevent adsorption, albeit was unsuccessful. The degradation efficacy percentages of the controls are therefore elevated (to high) and infers that HC-2000 degradation performance compared to controls are higher than reported.

HC-2000 bioremediation degradation efficacy outperformed Microblaze, F-500, BioSolve, and EPA's synthetic inorganic fertilizer control for the petroleum hydrocarbons tested. In addition, HC-2000 proved to be less toxic than that the other products tested by having the largest increase in total heterotrophic bacteria counts following inoculation. Plate counts decreased following inoculation with Biosolve and F-500. HC-2000 has a freshwater 96-Hr aquatic toxicity that is 4.1 times less than Microblaze.

Soil Slurry Reactors

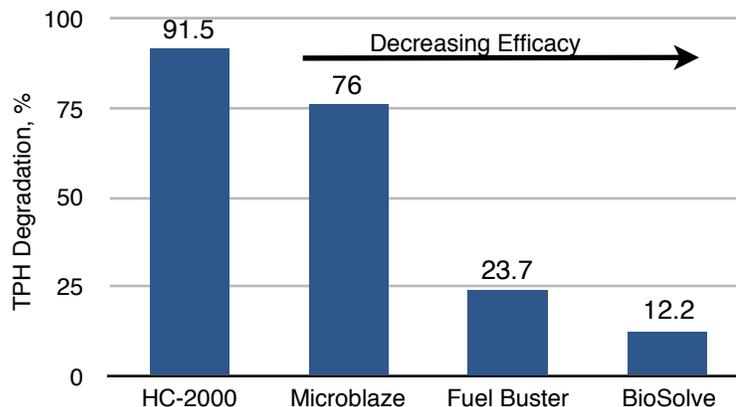
	Days	
<u>Diesel Fuel Efficacy (a), %</u>	<u>5</u>	<u>7</u>
HC-2000, 3% Solution	60.52	
Microblaze, 3% Solution	43.37	
EPA Nutrient Control		34.4
Control		35.9

Powdered Clay Slurry Reactors

	Days	
<u>Diesel Fuel Efficacy (a), %</u>	<u>4</u>	<u>23</u>
HC-2000, 3% Solution	84.62	92.45
Microblaze, 3% Solution	76.00	88.67
EPA Nutrient Control	44.62	70.77
Control	52.52	70.00
<u>Crude Oil (b) Efficacy (a,c), %</u>	<u>7</u>	<u>28</u>
HC-2000, 6% Solution	42.86	78.75
EPA Nutrient Control	32.43 (d)	51.35 (d)
Control	84.00 (d)	88.10 (d)

Soil Slurry Reactors (7)

	Days
<u>Diesel Fuel Efficacy (b), %</u>	
HC-2000	91.5%, after 4 days
F-500 (Fuel Buster)	23.7%, after 4 days
BioSolve	12.2%, after 7 days
(a) EPA TPH Method 9017B	
(b) EPA TPH Method 418.1	
(c) Crude Oil - ANS521 Crude, recd 20 BERC 4/2/93, 9 April 2010 ELH, outside canister 485380, ATCC 1081 Univ. Bld., Manassas, VA 20110	
(d) Excessive Adsorption to Glass Reactor Sidewalls, Results void	



4-Day Bench-Scale Soil Slurry Diesel (TPH) Degradation Product Comparisons, (Fuel Buster - 7 days).

HC-2000 Range Finding Aquatic Toxicities

Remtech Engineers ©. 2016



Range Finding Bioassay Tests

Introduction

Toxicity screens were conducted on HC-2000 and Microblaze (agent in water only) between March 12 and March 15, 2010. Fathead minnows (*Pimephales promelas*) were exposed to five concentrations of Microblaze and six concentrations of HC-2000 in freshwater. Tests were conducted at Remtech's research lab in Marietta, Georgia. Both bioremediation agents suggest a dosage rate of 3 to 6 percent. Acute, static, range-finding aquatic bioassays were conducted over a 96-hour period. Dissolved oxygen, pH and temperature were monitored throughout the assay period. Two liter test solutions were made up in one-gallon glass test chambers.

HC-2000 applied at 3% is 50% less toxic to fathead minnows than at 6%. Test results are summarized below:

HC-2000, 6% solution

Fathead Minnows 96-hr, LC₅₀ - 5,764 ppm (11,528 ppm at 3%)

Microblaze, 6% solution

Fathead Minnows 96-hr, LC₅₀ - 1,414 ppm

Method

Fathead minnows were subjected to a 96-hour, acute, static, range-finding aquatic toxicity test (using an abbreviated ASTM E729-96 method without duplicates and smaller sample size). The LC₅₀ data was calculated using the Trimmed Spearman-Kärber Method and the results are given in parts per million (ppm) (2). Test fish were obtained from Davis Fish Farm located in Leesburg, Alabama.

Toxicity screening on Remtech's HC-2000 (Biostimulation Agent) and Verde Environmental's Microblaze (Enhanced Bioremediation Agent) was conducted at Remtech's research lab in Marietta, Georgia to determine the expected median lethal concentration (LC₅₀) using the Fathead Minnow (*Pimephales promelas*) as the test organism. Five concentrations of Microblaze and six test concentrations of HC-2000 were tested with a total of 7 to 12 minnows in each reactor. During the 96-hour experiment, dissolved oxygen concentration, pH and temperature were monitored to assure optimal water quality conditions. LC₅₀ (median lethal concentration) were calculated using the Trimmed Spearman-Kärber Method and the results are given in parts per million (ppm).

Microblaze concentration ranges tested were: 600, 1000, 2000, 3000, and 6000 ppm.

HC-2000 concentration ranges tested were: 600, 1000, 3000, 6000, 8000, and 10000 ppm.

Fathead minnows were as uniform as possible in age and size. Dissolved oxygen levels were maintained between 60 and 100% of saturation and the fish did not incur a temperature fluctuation of more than 1 deg C in any 12-hour period. 7 to 12 fish were placed in each 2 liter chamber and were exposed to varying concentrations of each agent.

Discussion

Remtech bioassay test results for Microblaze toxicity compared favorably to those reported on their website and FAA document respectively:

Coastal Bioanalysts for Verde Environmental, 1998

Microblaze 6% solution

Mysidopsis bahia 48-hr, LC₅₀ - 1,230 ppm
estuarine mysid shrimp

Menidia beryllina 96-hr, LC₅₀ - 1,390 ppm
inland silverside (coastal and freshwater habits)

Federal Aviation Administration, *Aquatic Toxicity Screening of Fire Fighting Agents*, 2003
(Applied Research Associates, Inc.), Tyndall AFB, FL.

Microblaze Out, 6% solution

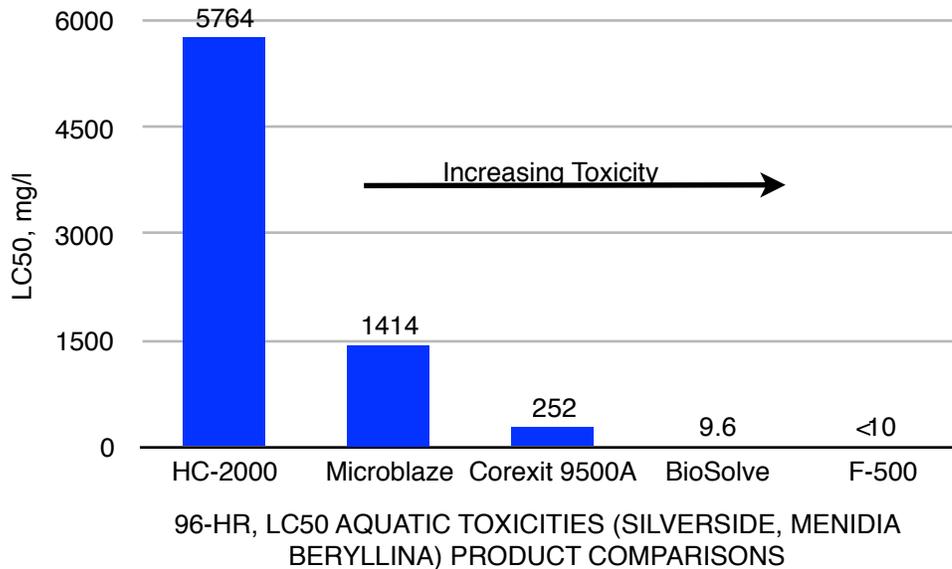
Fathead Minnows 48-hr, LC₅₀ - 949 mg/l

Results

Microblaze is approximately 4.1 times more acutely toxic to fathead minnows in freshwater than HC-2000. When compared to EPA NCP 96-hr LC₅₀ aquatic toxicity tests on the inland silverside, *Menidia beryllina*, (1), HC-2000 appears to be over an order of magnitude less toxic than the dispersant (Corexit 9500A) used on the recent Gulf oil spill (Deepwater Horizon oil exploration platform on April 20, 2010):

Agent	LC ₅₀ mg/l	
	This Study	NCP Schedule
HC-2000	5,764*	
Microblaze	1,414*	1,230
BioSolve		6.4
F-500		<10
Dispersit SPC 1000	2.9	3.5
Nokomis 3-F4	19	29.8
Nokomis 3-AA	19	34.2
ZI-400	21	31.8
Saf-Ron Gold	44	29.4
Sea Brat #4	55	30
Corexit 9500A	130	252
JD-2000	>5,600	407

* Remtech Range Finding Aquatic Toxicity Tests

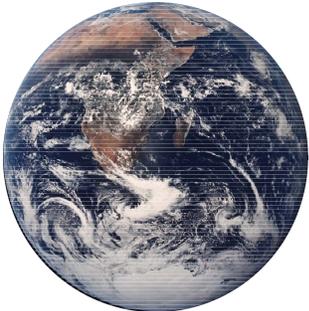


References

1. *Comparative Toxicity of Eight Oil Dispersant Products on Two Gulf of Mexico Aquatic Test Species*, EPA, U.S. Environmental Protection Agency Office of Research and Development, June 30, 2010.
2. *Trimmed Spearman-Kärber Method. Version 1.5* for HC-2000 presented below:

HC-2000 Product Label

**HC-2000 Bioremediation Accelerator
& Surface Cleaner**
A Green Sustainable Technology



for
**Petroleum Hydrocarbon &
Solvent Cleanups**

in
**Soil, Railroad Ballast, Gravel
Groundwater, Surfacewater,
Wastewater
Concrete, Asphalt
and Other Surfaces**

- non-hazardous
- hypo-allergenic
- non-abrasive

Mix Well Before Using

*Restoring Our World
for Future Generations*

Remtech Engineers™
Remediation Specialists

200 North Cobb Parkway, Suite 208, Marietta, Georgia 30062, Phone: 770-427-7766 or 800-377-3648

HC-2000 Activation & Stability

HC-2000 (HC2) is activated when mixed with water. It is stable when in its concentrated form and may be stored for over 5 years. HC-2000 concentrate is sold with vented caps. It is also preferable to loosen caps and store in areas less than 120°F to avoid pressure buildup. Be sure to use up all diluted mixtures when cut with water. Shelf life of diluted solutions is a couple of months.

Soil, Ballast & Gravel Treatment

Each gallon of HC-2000 concentrate will treat between 6 and 10 cubic yards of petroleum hydrocarbon contaminated soil, ballast, or gravel. For example, 100 cubic yards of contaminated soil requires 10 to 16.7 gallons of concentrate. Over a five-week treatment period, apply 1/5th of the total dosage or 2 to 3.34 gallons of concentrate in 16 volumes of water or 32 to 53.4 gallons of mix each week. Note that actual treatment periods may extend to six months or longer depending on type, location, and permeability of soil; type of petroleum hydrocarbon, and cleanup level required.

Each site may require different application rates. Soils less than three feet in depth, may be treated topically and should be tilled prior to the first application and at the treatment mid point. For deeper soils, deep tilling methods may be employed, providing soil stability and geotechnical requirements are not violated.

Note that it is best to remove mobile bulk fuels prior to treating with HC-2000 with leachate collection or drainage trenches. HC-2000 may be used to “wash” bulk fuels out of soil pours or gravel voids to allow collection and removal via pumping.

HC2 applications can be made through injection wells or trenches. Optimal degradation rates generally occur under aerobic conditions at 20% moisture or 80% of the field holding capacity of soil. See Remtech’s Technical Application Package for further instructions.

Groundwater Treatment

Each gallon of HC-2000 concentrate will treat between 6 and 10 cubic yards of petroleum hydrocarbon contaminated groundwater. For example, if you have 100 cubic yards of contaminated groundwater, 10 to 16.7 gallons of concentrate are required. Over a five-week treatment period, apply 1/5th of the total dosage or 2 to 3.34 gallons of concentrate to 30 volumes of water or 60 to 100 gallons each week.

Note that actual treatment periods may extend to six months or longer depending on location, permeability of the saturated zone, type of petroleum hydrocarbon, and cleanup level required. It is best to remove bulk mobile fuels prior to treating with HC-2000. HC-2000 may be used to “wash” or accelerate the removal of bulk fuels. See Remtech’s Technical Application Package for further instructions.

Asphalt, Concrete & Surface Cleaning

Mix one part of concentrate in five to 10 parts of water. Apply cleaning solution to surface and agitate with broom or power broom. Let stand for 20 minutes and rinse surface with water or pressure washer. Collect rinsates for disposal. Repeat if necessary. For pressure washer applications - **DO NOT INJECT THROUGH PUMP - ONLY INJECT PAST PUMP.** Mix one part of concentration with three parts of enzyme concentrate and educt with chemical nozzle. Let enzyme set for 20 minutes then wash off with high pressure. See Remtech’s Technical Application Package for further instructions.

HC-2000 Application Systems

HC-2000 may be applied with with the following pumps: diaphragm, centrifugal or roller. Use coarse fire nozzles as finer spray systems may be clogged by pulp in concentrate. Do not inject HC2 through pressure washers (post pump injection only). See Remtech’s Technical Application Package for further instructions.



200 North Cobb Parkway, Suite 208, Marietta, Georgia 30062
Phone: 770-427-7766 or 800-377-3648, website: www.remtech-eng.com

HC-2000 Safety Data Sheet

January 1, 2016

Section 1. Identification HC-2000

- Chemical Name & Synonyms: Enzyme Blend Formula: Proprietary Blend
- DOT Proper Shipping Name: Non-Hazardous Liquid, DOT Hazardous Class: Non-Hazardous
- Manufacturer - Remtech Engineers, 200 Cobb Parkway North, Suite 208, Marietta, GA 30062, Phone 770-427-7766, Fax 770-427-7001.
- Use - Biostimulation agent for cleaning and biodegradation of petroleum hydrocarbons and solvents.

Section 2. Hazardous Ingredients None

Section 3. Composition/Information on Ingredients Enzymes, biosurfactants, nutrients

Section 4. First-aid Measures

- Eye Contact: May have slight irritation affect, wash with water
- Skin Contact: None, wash with water
- Inhalation: None
- Ingestion: May cause slight laxative condition
- Non-carcinogenic

Section 5. Fire and Explosion Data Treat same as water

Section 6. Accidental Release Measures

Material is viscus and sticky. Solidify with oil dry or sand. Material may be moped up with water.

Section 7. Precautions for Safe Handling and Storage

No special gloves, ventilation, exhaust, protective clothing, special equipment or respiratory protection required. Store at temperatures less than 120°F. Store with vent cap or loose cap to avoid pressure buildup.

- Incompatibility: None. Product is compatible, will not polymerize nor create hazardous by-products. There are no specific conditions to avoid.
- Hazardous Polymerization: Will not occur.

Section 8. Exposure Controls/Personal Protection

No special gloves, ventilation, exhaust, protective clothing, special equipment or respiratory protection required.

Section 9. Physical/Chemical Properties

- Specific Gravity: (H₂O) 1.17 , Boiling Point: 212°F, Freezing Point: 32°F, Vapor Pressure: N/A, pH: 3.1 to 3.5
- Evaporation Rate: Same as Water
- Solubility in Water: Complete
- Appearance & Odor: Brown Liquid with Slight Sweet Odor
- Bacterial Information: Contains no pathogens, no fecal coliform, and no salmonella
- Environmental Information: Contains no nitrates or sulfites. Potassium, sodium, sulfates, and organic nitrogen - <1% each. Calcium, iron, magnesium, phosphorus - <0.1% each. Water - > 50%. Enzymes, cofactors, biosurfactants – proprietary

Section 10. Stability & Reactivity Data

- Stability: Stable. Avoid high temperatures (>120°F) as this will neutralize the enzymes.
- Avoid low or high pH substances (i.e., acids, caustics).
- Incompatibility: None. Product is compatible, will not polymerize nor create hazardous by-products.
- There are no specific conditions to avoid.
- Hazardous Polymerization: Will not occur.

Section 11. Toxicological Information Material is non-toxic

Section 12. Ecological Information Use or apply according to label instruction should have no adverse environmental impact

Section 13. Disposal Considerations Dispose of according to local, state and federal regulations

Section 14. Transport Information DOT Proper Shipping Name: Non-Hazardous Liquid, DOT Hazardous Class: Non-Hazardous

Section 15. Regulatory Information N/A

Section 16. Other information SDS prepared 12/2015



200 North Cobb Parkway, Suite 208, Marietta, Georgia 30062
Phone: 770-427-7766 or 800-377-3648, website: www.remtech-eng.com

Georgia Department of Natural Resources

7 Martin Luther King, Jr., Drive-Room 643, Atlanta, Georgia 30334

Environmental Protection Division

Lonice C. Barrett, Commissioner

Harold F. Reheis, Director

404/656-6905

Mr. Mark D. Ryckman, P.E., D.E.E.
Remtech Engineers
200 North Cobb Parkway
Suite 124
Marietta, GA 30062

May 22, 2002

Dear Mr. Ryckman;

I have reviewed the information you forwarded regarding the use of your proprietary bioremediation product HC-2000. Based on this information, this product may be used for hydrocarbon spill response and remediation in Georgia, with the following restrictions.

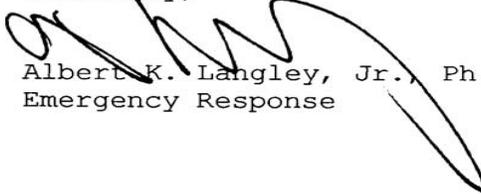
No product may be applied to waters of the state of Georgia for purposes of spill remediation without the case-by-case approval of the Georgia Environmental Protection Division, and the Regional Response Team. Approval can be obtained by calling my office at the above number during working hours or our Emergency Operations Center after hours at (404) 656-4863 or 1-800-241-4113. Approval can usually be granted within an hour.

The use of any spill control or bioremediation product on land-based spills is subject to the same requirements as any other industrial operation. Any material applied must be contained and properly disposed. Likewise, water containing the control agent may not be discharged to state waters or onto the land surface without direct approval by EPD. This material may be discharged into a sanitary sewer system, with the approval of the sewer authority.

We encourage the use of bioremediation in appropriate situations. Your HC-2000 material does not contain any hazardous chemicals and in general should be useful in many remediation applications. However, use of it in remediation of insitu groundwater contamination will require specific case-by-case approval due to Georgia's specific rules for underground injection. Use of the product for land-based remediation however does not require any specific approval.

I hope the above is of use to you. Please feel free to call with any further questions.

Sincerely,



Albert K. Langley, Jr., Ph.D.
Emergency Response



Jeb Bush
Governor

Department of Environmental Protection

Twin Towers Office Building
2600 Blair Stone Road
Tallahassee, Florida 32399-2400

David B. Struss
Secretary

May 6, 1999

Mr. Mark Ryckman
Remtech Engineers
200 North Cobb Parkway, Suite 208
Marietta, Georgia 30068

Re: HC-2000

Dear Mr. Ryckman:

The Bureau of Petroleum Storage Systems hereby accepts HC-2000 as a product for both in situ and exsitu bioremediation of soil and groundwater at petroleum and other suitable hazardous waste contaminated sites in Florida. As indicated in Remtech's submittal of information, the product is a non-toxic, non-allergenic, biodegradable mixture of proteins, enzymes, micronutrients, and emulsifiers. It does not contain bacteria or other microorganisms but rather stimulates the activity of the indigenous contaminant-degrading microorganisms already present at a remediation site. Enclosure 1 is a proprietary chemical analysis voucher for the product.

For vadose remediation where the underlying groundwater will not be affected by leaching of this product, there are no special concerns beyond those which would normally need to be addressed in preparing a Remedial Action Plan and conducting a cleanup in accordance with the petroleum cleanup requirements of Chapter 62-770, Florida Administrative Code (F.A.C.). For ex situ groundwater treatment, where an aboveground treatment system produces effluent meeting the petroleum cleanup criteria of Chapter 62-770, F.A.C., and the drinking water standards of Chapter 62-550, F.A.C., for disposal via recharge gallery or NPDES permit, there are no special concerns. But for in situ groundwater remediation, via injection of products into an aquifer, there are underground injection control (UIC) regulations that must be observed. Since in situ aquifer remediation via injection is likely to be the most common application of this product, the bulk of the regulatory requirements discussed herein will be directed to that topic.

The bureau recognizes HC-2000 as a viable product for the bioremediation of petroleum contaminated sites in Florida. There are no objections to its use provided: (a) the considerations of this letter are taken into account; (b) a Remedial Action Plan is approved by the Department; and (c) applicable and appropriate underground injection control regulations are observed when the product is used for in situ remediation.

While the Department of Environmental Protection does not provide endorsement of specific or brand name remediation products or processes, it does recognize the need to determine their acceptability from an environmental standpoint with respect to applicable rules and regulations, and the interests of public health, safety, and welfare. Vendor's must then market the products and processes on their own merits regarding performance, cost, and safety in comparison to competing alternatives in the marketplace. For HC-2000, the major environmental and regulatory considerations are set forth in enclosure 2.

Preparers of Remedial Action Plans are advised to include a copy of this letter in the appendix of plans they submit, and call attention to it in the text of their document. In this way, technical reviewers throughout the state will be informed that you have contacted the Department of Environmental Protection in regard to HC-2000. To aid those reviewers, the Bureau of Petroleum Storage Systems provides supplemental information as enclosure 3.

The Department reserves the right to revoke its acceptance of any product or process if the nature or composition of either or any of its principal and proprietary ingredients, or its performance has been falsely represented. Additionally, Department acceptance of any product or process does not imply it has been deemed applicable for all cleanup situations, or that it is preferred over other treatment or cleanup techniques in any particular case. A site specific evaluation of applicability and cost-effectiveness must be considered for any product or process, whether conventional or innovative, and adequate site specific design details must be provided in Remedial Action Plans prescribing the product or process. You may contact me at 850/487-3299 if there are any questions.

Sincerely,

Rick Ruscito, P.E.
Bureau of Petroleum Storage Systems

c: W. Evans - FDEP/Tallahassee
T. Conrardy - FDEP/Tallahassee



200 North Cobb Parkway, Suite 208, Marietta, Georgia 30062
Phone: 770-427-7766 or 800-377-3648, website: www.remtech-eng.com