CytoCulture
INTERNATIONAL INC.
Biotechnology Research, Scale-Up and Marketing

OPERATIONAL PLAN

Supplement to Proposal for

IN SITU SITE REMEDIATION OF SOIL AND GROUNDWATER HYDROCARBON CONTAMINATION BY AUGMENTED BIORECLAMATION USING LABORATORY SELECTED BACTERIAL CULTURES [P.I.E. NATIONWIDE EMERYVILLE SITE]

submitted to

Alternative Technology and Policy Development Section
Toxic Substances Control Divsion
State of California Department of Health Services

by

CytoCulture International, Inc.

in a Joint Venture with

Sybron Chemicals, Inc.

in Collaboration with

Alton Geoscience

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Introduction

This Operational Plan describes in detail the conceptual design for the P.I.E. Nationwide Emeryville Site Bioreclamation project proposed by the joint venture of CytoCulture International and Sybron Chemicals. This Plan is intended to assist the regulatory agencies involved in permitting this project and to serve as a supplement to the original proposal (drafted July 29, 1987, updated October 1, 1987) submitted to the client.

Specific concerns about the reinfiltration of treated water and the possibility of spreading the product plume are addressed directly. On-site and off-site well studies, contaminated water extraction techniques, bioreactor system designs, monitoring plans, reinfiltration strategies, potential problems and alternative approaches will all be discussed in the context of a **phased** program for proceeding initially with the treatment of groundwater followed by the in situ bioreclamation of the soil. Permitting of the soil treatment by the Regional Water Quality Control Board will be dependent on the active participation of the Alternative Technology Office (DHS) in monitoring this site.

Monitoring Well Drilling

The original proposal called for drilling eleven monitoring wells to establish groundwater flow and the extent of the product plume on site. The Site Characterization Study by Alton Geoscience (November 3, 1987) then recommended adding five more monitoring wells, three of which were to be off site to the south and east (see Figure 2 of Site Characterization Report). Of the thirteen on-site wells, three along the southern property line and three along the western property line were to be drilled with 4 inch casings to permit their use for continuous extraction of water for treatment should it be necessary. The remaining eight on-site wells and three off-site wells were to be drilled with less expensive 2 inch casings for monitoring purposes only.

To improve off-site monitoring of the plume, two additional wells will be drilled: a third well off-site to the south and a second well off-site to the east, for a total of 18 wells (13 on site and 5 off site). Both additional wells will have two inch casings. The updated positions of all 18 monitoring wells are indicated on the attached Site Plan modified from the original Figure 2 of the Site Characterization Report. Positions may vary slightly to accomodate existing or anticipated structures on or off site. Drilling of these wells is scheduled for Janaury 1988. See specific sections below on the intended uses of these wells.

Groundwater Characterization and Product Plume Definition

The observed inconsistencies in soil and hydrogeological data of past geotechnical and site characterization reports underscore the need for better groundwater studies before proceeding with any bioremediation plans. It is quite apparent that petroleum hydrocarbons hot spots are showing up at considerable distances from the original leaking underground tanks. In particular, repeated observations and laboratory analyses during storm sewer installations suggested contamination and some free product in the soils below 6 feet along the southern property line. These observations have been partially substantiated by previous and recent borings; there have also been undocumented observations of apparent hydrocarbon contamination in the soils of the Judson Steel property to the south. These worrisome indications of an moving free product plume can be explained by at least three phenomena:

1. Motor oil or fuel spill/leak contamination from truck servicing activities or above ground tanks around the site over the last 30 years. Although unlikely, some of this hydrocarbon could have penetrated through the original fill (which has since been removed) into the saturated zone of the underlying soil.

2. Actual plume migration southward from the leaking tanks and/or motor oil or fuel spill contaminated areas. Migration could be influenced by underground tributaries of an old creekbed located to the south. Alternatively, tidal flow could be distorted by other subterranean features such that local groundwater flow is southward in this area.

3. Hydrocarbon contamination detected sporadically or along the southern boundary could be moving in from off-site sources. The Pfizer Chemical plant to the east and the former Judson Steel plant to the south are two obvious potential sources.

Some, all or none of the above phenomena could be contributing to the high levels of hydrocarbon found along the southern boundary and intermittently near buildings B, C and D. The lack of a defined product plume is a major problem for this project as it precludes any detailed planning for the locations of the extraction trenches, the bioreactor systems or the infiltration galleries. The, as yet, undefined extent of contamination also means it will be difficult to really assess the level of clean-up desired vs. actually attained at this site. It is therefore imperative that we proceed with careful measurement and sampling of the 18 monitoring wells in an effort to define the expanse, composition and migration of the targeted hydrocarbon plume(s).

Accurate measurements of the groundwater flow will be difficult at this site on account of the proximity (1/10th mile) of the Bay and the resulting strong tidal effects on the water's movement. Up to three feet of rise and fall have been reported. This tidal motion alone could explain the dispersion of the plume(s) beyond original point sources. Nonetheless, it will be important to establish baseline measurements for later detection of depression zones after extraction of contaminated water begins (see sections below).

Optional Water Extraction from Four Inch Wells

As indicated above, six of the eighteen monitoring wells will have 4 inch casings to permit the extraction of contaminated groundwater, should this prove necessary. Three of the wells will be drilled in a line along the southern boundary of the site and are likely to be integrated with the french drain extraction trenches in the same area (see below). This entire southern edge of the property may be used to create a depression zone to pull contaminated water from under buildings B-D while at the same time cutting off the apparent southward migration of the plume into the Judson Steel property. The depression zone along this boundary might also draw contaminated water over from the former Judson Steel property.

The three 4 inch wells along the western boundary (near the fence along the Eastshore Freeway) could serve as a partial containment "fence" should it ever be necessary to recover contaminated water in this area in the event that the plume did accidentally migrate westward instead of towards the depression zone created by the extraction trenches along the southern property line. The extent to which these three wells could really contain or recover contaminated water themselves is unclear. Alternatively, a long gravel trench or other containment barrier could be erected (at a high cost) along the freeway to more effectively block accidental migration of the plume towards the bay, but we consider this unrealistic for the site.

Design and Excavation of French Drain Trenches

Contaminated groundwater will be extracted from a series of french drain trenches installed along the southern boundary of the site with the intention of creating a long depression zone downstream of the apparent plume migration. Exact location and orientation of the trenches must await plume definition and more extensive groundwater characterization data expected from the upcoming drilling and monitoring of the 18 wells.

The extraction trenches will be excavated 18 inches wide and eleven feet deep to gain full access to the groundwater surface even at extreme low tide. The trenches will be at least 50 feet in length (up to 200 feet) depending on the interference of existing storm sewers, pipes, utilities and structures. Maximal coverage of the southern and western boundaries of the site will be attained if trenches are installed along the respective property lines along the parking lot behind building D. Long stretches of trenching could then extend along the property lines from the southwest corner to effectively catch the migrating plume. This entrapment of the plume could be enhanced by the careful infiltration of treated water, nutrients and bacteria along the sides of building D, up to approximately 150 - 200 feet to the north and east of the trenches.

The french drains will be lined with a synthetic drainage fabric (e.g., Mirafi) which will serve as a sediment control barrier. Bay mud sediments are considered to be a potential problem if much silting occurs in the clarifiers or tanks of the bioreactor systems. The trenches will then be packed with class I graded pea gravel to a depth of five feet for optimal flow of contaminated groundwater at any tide level. The drainage fabric will then be folded over to enclose the graded gravel and engineered fill removed during the excavation will be returned to fill in the remaining troughs. Landscaping or paving will be restored. These trenches will have 4 inch perforated PVC pipes installed to enhance the flow of contaminated water to the groundwater depression pumps (pneumatically operated PVC pumps).

The rise and fall of the tidal groundwater presents an interesting challenge for the continuous pumping of contaminated water into the bioreactor systems. If the pump inlets are placed only in the low tide zone, floating hydrocarbon product will only be recovered at low tide (the rest of the time, only water would be extracted). Similarly, if placed in the high tide zone, pump inlets would only recover product at high tides (sucking air the rest of the time). Therefore, we have devised an automated dual pumping system that permits continuous recovery of water and product on the vertically moving tidal groundwater surface. Two separate pump systems will be installed at the french drains, one with access to the high tide water zone, the other placed to recover hydrocarbon from the low tide water zone. Level sensors will activate the appropriate pumping system to ensure that the maximal recovery of free product and dissolved phase hydrocarbon occurs, irrespective of the tide level. Alternatively, floating pump inlets may be employed to siphon floating free product and contaminated water to the surface for treatment, but these devices are expensive and prone to failure.

Design and Installation of Bioreactor Systems

Two permanent bioreactor systems will be constructed on site for the biodegradation of petroleum hydrocarbons in contaminated water extracted from the french drain trenches and 4 inch wells along the southern and western property lines. These 2,000 gallon bioreactors will then serve as continuous flow chemostats to grow up additional bacterial cultures for reinfiltration with nutrients back into the contaminated soil "upstream" of the product plume. The process of biodegrading hydrocarbons in the extracted groundwater and the contaminated soil will therefore occur in two distinct phases, as described below.

Each bioreactor system will consist of an initial clarifier tank equipped with an oil/water separator to recover free product (if present) and up to four 500 gallon bioreactor vessels for the continuous biodegradation of the dissolved phase hydrocarbon. Excess free product will be collected for recycling by a commercial oil recovery service.

By establishing a concentration gradient through the four vessels of each bioreactor, the biodegradation process can readily achieve parts per billion levels of hydrocarbon from starting material that was in the parts per million level of initial contamination. Long retention times and moderately slow flow rates through the system (e.g., 1-2 gal/min) will ensure compliance with the November 26, 1986 NPDES permit variance issued by the San Francisco Bay Regional Water Quality Control Board to P.I.E. Nationwide for the discharge of treated water brought to the surface at this site (see copy of this letter in the October 1 version of the original proposal).

An additional single 1,000 gallon mobile BATCH culture bioreactor will be used to infiltrate oxygenated water, nutrients and high densities of the "diesel blend" bacteria into contaminated soil areas around buildings B and C (southeast corner). This batch bioreactor will use tap water rather than extracted groundwater to periodically grow up bacterial cultures. The batch bioreactor will also be employed to grow up bacterial cultures for treating excavated contaminated soil from the trenching operations using augmented soil farming techniques.

CytoCulture bioreactor proprietary designs used on this site include efficient sparging and mixing systems, automated nutrient dispensing systems, automated tracer (Lithium chloride) dispensers, continuous seeding of bacteria from dry cultures and process control systems which minimize the maintenance to weekly visits by technical personnel.

Precautionary measures for the containment of the bacteria and contaminated water include the construction of 18 inch walls around the concrete slab on which the bioreactor systems are built in case of spills. These continuous systems will also process residual contaminated water from the monitoring wells. Should silting of bay mud sediments occur, the tanks will have to be pumped out by commercial sanitary service companies.

"Pump and Treat" Phase of Operation with Discharge to Sewer

The three bioreactor systems described above will be put into operation as soon as permitted to begin treatment of hydrocarbon contaminated groundwater pumped out of the french drain trenches and the 4 inch extraction wells along the southern property line of the site. The flow rates, nutrient levels, pH and dissolved oxygen levels, and retention times will be optimized to achieve the most rapid biodegradation possible. Treated water which meets the current RWQCB levels acceptable for surface discharge (as per the November 26, 1986 NPDES permit variance letter) will be discharged temporarily into the storm sewers along the southern edge of the site. Acceptable levels means no more than 100 ug/liter of total petroleum hydrocarbon in the water. Once some evidence of hydraulic control of mounding int the groundwater has been established with the monitoring wells, and the concerns for spreading the plume have been addressed with an adequate soil treatment stategy, the treated water will be mixed with nutrients and fresh cultures of bacteria for reinfiltration into the unsaturated zones of the soil upstream of the plume (see sections below). If any delays are encountered in starting up the reinfiltration phase, the bioreactors will continue operating at maximum capacity to clean-up contaminated groundwater with discharge of the treated water into the storm sewer.

Verification of Compliance with NPDES Permit Variance Letter

Compliance with the San Francisco Regional Water Quality Control Board NPDES variance letter of November 26, 1986 will require extensive monitoring of the treated water in the bioreactors prior to discharge. The exact schedule has been spelled out in the variance letter, requiring daily, then weekly sampling and analysis. Total hydrocarbon, phenol and polynuclear aromatic analyses will be performed by local laboratories whereas CytoCulture/Sybron will monitor the bacterial counts. This information will be used to fine tune the biokinetics of hydrocarbon substrate utilization in terms of flow rates, nutrient addition, pH control, dissolved oxygen levels and retention times within the four tanks of each system.

The treated water does not need to meet drinking water quality standards, although our experience at other bioreclamation sites in the State indicate this approach can lower hydrocarbons and polynuclear aromatics to the parts per billion range. Ironically, the water is clean enough for discharge into storm sewers (direct to the Bay) but it cannot be returned to the contaminated soil from where it came.

Concerns over Reinfiltration of Treated Water

The most pressing issue which threatens the permitting of our plans to reinfiltrate treated water and bacteria back into the soil on this site is the possibility of dispersing rather than containing the product plume during treatment. If the plume were to migrate westward directly to the bay (or southward to the nearby creek), the hydrocarbon would foul the seawater and render all of our attempts at cleaning this site futile. There is no threat to any drinking water supplies in the area; all the groundwater is tidal brackish water. Furthermore, it is widely held that the Emeryville area's aquifers have been substantially polluted from years of heavy industrial and chemical manufacturing activity.

Nonetheless a bad precedent was set in the central valley by an attempt to reinject treated water at a site. In spite of apparent precautions to monitor the process, the infiltration of treated water at that site caused a major dispersion of the original plume into previously clean adjacent areas.

We believe the situation will be very different at this site. The hydrogeologists tell us that whatever flow rates for reinfiltration are achieved (e.g., 2 gals/min X 3 bioreactors), the total amount of water introduced into the soil will contribute minimally to the natural tidal and surface drainage effects on the plume migration, particularly during the winter season. Every effort is being made to contain all migrating free or dissolved phase product from the original underground tank pit area under building D by installing 400 linear feet of extraction trenches to the west and south of this area. This area will be extensively surveyed for possible migration using the monitoring well system proposed in our Initial Site Characterization Study.

"Reinfiltration" Phase of the Bioreclamation Project

The first step in this phase of the project is to attempt to attain some hydraulic control over the area around building D where the underground storage tanks had been located. We plan to routinely sample at least six of the 18 proposed moniotring

wells. However, the hydrogeologists at Alton Geoscience have already expressed strong doubts about accurately measuring groundwater migration in the presence of such strong tidal effects. It may be impossible to establish true hydraulic control given a three foot rise and fall of the tide, in which case we will have to rely on extensive trenching to adequately control any possible migration of free or dissolved phase product. The monitoring wells will however continue to provide baseline and subsequent operating values for levels of petroleum vs. natural hydrocarbons, bacterial counts, and lithium chloride tracer concentrations once the reinfiltration process begins.

Reinfiltration will involve blending expanded cultures of the BTX strains of Sybron bacteria with nutrients and treated water (that already meets the NPDES permit variance levels for discharge to sewer) in the chemostat bioreactor. At optimal cell densities, the slurry will be mixed automatically with lithium chloride tracer and pumped through an array of perforated pipes known as the "Infiltration Gallery" (see sections below).

Design and Construction of Infiltration Galleries

The distribution of bacterial cultures and nutrients suspended in reclaimed water from the site will require a series of perforated pipe leach fields referred to as infiltration galleries. The galleries consist of 2 inch PVC pipe manifolds connected to parallel perforated 2 inch PVC pipe (up to 20 foot lengths) packed in gravel beds over or upstream of the contaminated zones of soil. At this site, much of the contaminated ground is now under buildings, so the only choice left is to place the infiltration piping upstream (north) of the presumed groundwater gradient established by a combination of natural flow and the depression zone created by the pumping water from extraction trenches along the southern property line. One likely location would be beneath the newly installed landscaping "islands" in the shopping center parking lot.

Leach field piping is to be packed in graded pea gravel at a depth of about three feet, well above the highest tide level for the groundwater. Each bioreactor system will have at least two infiltration galleries corresponding to extraction trenches downstream along the southern boundary. Plumbing connections from the bioreactors to the leach fields may utilize existing 2 inch PVC piping now running under some of the buildings. In cases were this is not available or practical, above ground hose lines may have to be installed around the buildings and buried in troughs across the parking lots.

Optimization of Bacterial Culture Infiltration

Once the infiltration galleries have been constructed and the permits for start-up of soil treatment have been issued, the reclaimed water from the bioreactors (assuming the treated water is in compliance with NPDES discharge regulations) will be further cultured with laboratory selected bacteria capable of emulsifying and degrading hydrocarbons absorbed to the soil.

Expansion cultures will be optimized for maximal growth kinetics by adjusting hydrocarbon substrate levels, the addition of nitrogen (diammonium phosphate) and phospate nuturients, pH and aeration (oxygen). Continuous flow of organisms and nutrients out of the bioreactors and into the soil should prevent overgrowth or clogging of the pipes by keeping the organism culture densities under control.

Part of the original infiltration plan was to take advantage of seasonal high tides and heavy rains to disperse the bacteria vertically into unsaturated zones above the normal tide levels.

Product Plume Monitoring by Hydrocarbon Analyses

Given the great concern over the possible spreading of the (as yet undefined) hydrocarbon product plume, samples will be taken weekly, then monthly from eight pre-selected monitoring wells for measuring total hydrocarbon levels around the plume. Total petroleum hydrocarbon (TPHC) chemistries will be performed locally using modified EPA method 8015 to distinguish gasoline, diesel fuel and motor oil fractions as was done with the most recent borings (B1-B4) in the Site Characterization Report.

Water Infiltration Monitoring by Tracer Analysis

Lithium chloride solutions will be dispensed automatically into the bioreactor effluent containing the bacterial cultures and nutrients on their way to the infiltration galleries. A concentrated stock solution of lithium chloride will be diluted into the bioreactor effluent on its way to the infiltration galleries. Since this inert, non-toxic salt is rarely found in natural seawater or groundwater, even a few ppm levels will be easily detected by routine sampling analyses of the same eight monitoring wells. Laboratory analyses will be performed simultaneously with the bacterial characterization studies on the same eight monitoring well samples (weekly at first, then monthly) at Sybron's research facility in Virginia. Dr. Douglas Goldsmith, Director of Research at Sybron Biochemical, has had considerable experience with this method and will assist us on this project.

Monitoring of Exogenous Bacterial Cultures in Groundwater

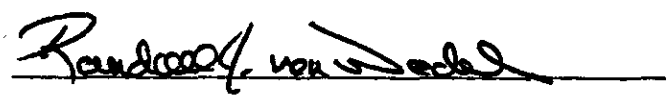
The same eight monitoring wells which will be routinely sampled for hydrocarbon chemistries and tracer analysis will also be assayed for total bacterial plate counts. Periodic assays for phenotypic enzyme activities will confirm the identity of the infiltrating bacteria which have diffused away from the infiltration galleries. Typically the augmented bacterial cultures can attain densities in bioreactors of several orders of magnitude greater than the natural indigenous strains. However limitations of nutrients, and especially of oxygen, will most likely suppress the densities of added bacteria found in soil.

Routine Reporting of Monitoring Data and Clean-Up Results

As per our NPDES permit variance letter, reporting of daily, weekly and monthly results for total petroleum hydrocarbons will proceed on schedule. These reports will be augmented with data on tracer levels, bacterial counts and hydrogeology monitoring of the plume and groundwater on and off site.

Next Report Due: Phase II Report on Hydrogeology and Site Characterization Studies (Alton Geoscience and CytoCulture)

For further information or updates for this Operational Plan, please call the Project Director at CytoCulture (Tel. 564-1516).



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