DRAFT BIOREMEDIATION PLAN SUBMITTAL
670 AND 692 98TH AVENUE
98TH AND EDES AVENUES
OAKLAND, CALIFORNIA
SCI 272.016

Sep 1990

James P. Bowers, PE
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September 21, 1990
SCI 272.016

Mr. Dan Lau
City of Oakland, Construction
7101 Edgewater Drive
Oakland, CA 94621

Draft Bioremediation Plan Submittal
670 and 692 98th Avenue
98th and Edes Avenues
Oakland, CA

Dear Mr. Lau:

This letter transmits a draft remediation plan and a groundwater monitoring study for the cleanup of contaminated groundwater and soil at the subject site. The plan was prepared by CytoCulture, Inc. under subcontract to Subsurface Consultants, Inc. (SCI).

In summary, it is our opinion that bioremediation of the contaminated groundwater and soil at the site is the most cost effective alternative to mitigate hydrocarbon contamination beneath 98th Avenue near the site. Based on our modeling study, an extraction trench located along 98th Avenue should capture the contaminants in groundwater originating from the former tank sites. Initially, the water will be treated using biotechnology, and disposed into a sanitary sewer. Later, after obtaining an NPDES permit, the treated water will be loaded with bacteria and nutrients and pumped into infiltration trenches and wells located upgradient from the former tank sites. This technique should hasten remediation of the contaminated groundwater and soil at the site.

This plan does not consider remediation of contaminated groundwater and soil that may exist downgradient from 98th Avenue. We understand that this is to be addressed in a later phase.

Subsurface Consultants, Inc.
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We trust we have provided the information required at this time. If you have questions, please call.

Yours very truly,

Subsurface Consultants, Inc.

William K. Wikander
Geotechnical Engineer 892 (expires 12/31/92)

WKW:RWR:clh

Copies: Addressee (6)

CytoCulture
ENVIRONMENTAL
BIOTECHNOLOGY
A Division Of CytoCulture International Inc.

REMEDIAL ACTION PLAN

FOR

AUGMENTED BIOREMEDIATION

AT

98TH AND EDES AVENUE SITE

CITY OF OAKLAND

Prepared for

Subsurface Consultants, Inc.

171 12th Street Suite 201
Oakland, CA 94607

DRAFT VERSION
September 7, 1990

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EXECUTIVE SUMMARY

This Remedial Action Plan outlines the steps for the City of Oakland to proceed with the bioremediation of hydrocarbon contaminated groundwater and soil encountered during the widening of 98th Avenue north of the intersection with Edes Avenue. This Plan summarizes CytoCulture's proposal to design, construct and operate an Augmented Bioremediation system using hydrocarbon degrading bacteria to clean the contamination. The Plan is divided into 2 construction and 2 remediation phases designed to integrate into the City's street construction schedule:

Project Summary

PHASE	TASK	MONTHS
I	CONSTRUCTION OF TRENCH IN 98TH AVENUE	1
II	CONSTRUCTION OF BIOREMEDIATION SYSTEM	1
III	GROUNDWATER BIOREMEDIATION ABOVE-GROUND (+ OPTIONAL EXCAVATED SOIL BIOREMEDIATION)	6
IV	REINFILTRATION FOR <u>IN SITU</u> TREATMENT	6

In Phase I, a groundwater extraction trench will be constructed along the west side of the widened section of 98th Avenue to recover residual free product (if encountered) and contaminated water. This first phase is limited to street construction work which must be performed concurrently with widening operations in order to minimize costs and avoid future street excavations. In Phase II, an infiltration trench will be constructed on the adjacent corner lot to the east for returning treated groundwater laden with bacteria and nutrients into the aquifer to promote *in situ* degradation. A bioreactor treatment system will be installed on the same lot, formerly a service station with underground storage tanks and now City owned property. In addition, two infiltration wells will be installed near MW-2.

After Phase II construction of the treatment system, Phase III groundwater bioremediation will be proceed using laboratory selected bacterial cultures supplied by Sybron Chemicals, Inc. (Salem, VA) to accelerate the natural biodegradation of gasoline and other petroleum hydrocarbons found on this site. By continually pumping contaminated groundwater from the extraction trench which intercepts the down-gradient flow of contamination from former underground storage tank locations, a local cone of depression will be created in the aquifer under the City street to accelerate the removal of any residual free product (if encountered) and pull contaminated groundwater from the private properties to the west of 98th Avenue. If substantial amounts of free product are encountered, it will be processed for recycling by an optional oil/water separator in the bioreactor system. The contaminated groundwater will be treated in CytoCulture proprietary suspension culture bioreactors supplemented with the hydrocarbon degrading bacterial cultures.

In Phase IV, the treated groundwater, containing the bacteria and nutrients, will be returned to the contaminated aquifer via the infiltration trench up-gradient of the original contaminated excavation tank areas east of 98th Ave. Treated water will also be returned to the contaminated aquifer via the two infiltration wells installed near MW-2. However, until NPDES and local permits for this in situ treatment are obtained, CytoCulture will temporarily discharge the clean, treated groundwater to the sanitary sewer.

Process monitoring and studies of the surrounding monitoring wells will confirm the recovery and treatment of contamination as well as verify that satisfactory hydraulic control will have been established as required to obtain the permits for reinfiltration. Mounding of infiltrated water may reach contamination adsorbed to soil in the saturated zones immediately above the water table. The groundwater bioremediation project (Phases III + IV) is planned and budgeted for one year of continuous treatment once the system is operational. CytoCulture will work jointly with its strategic business partners, Erickson Environmental and Sybron Chemicals to achieve site remediation to the maximum extent possible based on economic and technical feasibility. Site plans, regulatory considerations and a hydrogeological model of groundwater extraction/infiltration are included in this Plan.

INTRODUCTION

The purpose of the proposed remedial action is to enable the City of Oakland to properly address the immediate groundwater and soil contamination problems encountered during excavations for the widening of 98th Avenue, while having a minimal impact on the schedule for ongoing street construction. This Plan describes a bioremediation approach designed to decontaminate City property under and to the northeast of the intersection of 98th and Edes Avenues (referred to as "the site"). The approach will remove residual fuels (if encountered) and clean the extracted groundwater in bioreactors to near drinking water standards for temporary discharge into the sanitary sewer. During the above-ground cleaning of extracted groundwater, permits will be obtained to proceed with an <u>in situ</u> approach for treating contaminated soils in the saturated zone immediately above the water table by reinfiltration of treated groundwater containing hydrocarbon degrading bacteria and nutrients. Reinfiltration will be directed via a trench to the contaminated aquifer up-gradient of former leaking underground storage tank areas in the corner lot and via two infiltration wells to contaminated areas under and along 98th Avenue across from Walter Avenue.

SITE DESCRIPTION AND HISTORY

The contamination on site consists of petroleum hydrocarbons, predominantly gasoline, associated with former underground fuel and waste oil storage tanks from service stations which used to be located along 98th Avenue. The underground storage tanks of the corner lot along Edes Avenue were removed in 1983. This Remedial Action Plan addresses the former service station corner lot (now City property) immediately southeast of the intersection of 98th and Edes Avenue, and to a limited extent, the second former service station site where US Rentals in now located (next lot north of the corner lot, along 98th Avenue).

A detailed description and history of the Oakland site may be found in the Phase 2 Soil and Groundwater Assessment Report prepared by Subsurface Consultants on April 10, 1990. Briefly, during an excavation in 1989 to install a new water line as part of the City of Oakland's construction project to widen 98th Avenue, hydrocarbon contamination was discovered in the soil immediately above the water table just northeast of Edes Avenue. The preliminary and subsequent Phase 2 contamination assessments of the site by Subsurface Consultants revealed extensive hydrocarbon contamination, predominantly gasoline, in a plume migrating southwest (across 98th Avenue) from the former underground storage tanks of the service station which had occupied the site from 1947 through 1983. The approximate locations of the tanks are indicated in the detailed Site Plan.

Groundwater samples from the site also contain purgeable hydrocarbons at concentrations less than 70 parts per billion. The presence of purgeable hydrocarbons in monitoring wells up gradient from the former tank locations suggests that they originate from an off-premises source.

In the process of constructing utility joint trenches for the street intersection, another contractor (IT Corporation) excavated contaminated soil down-gradient from the former tank pits of the corner lot in July. No free product was observed in the groundwater, estimated to be at about 10-12 feet, but a sheen was observed. The excavated soil was distributed in long windrows along 98th Avenue to promote aeration of the volatile fractions of the gasoline. Some contaminated soil remains stockpiled at the Pippin site (at 98th and San Leandro Street) awaiting additional treatment. CytoCulture has offered to treat the residual waste oil and heavy fractions of petroleum with an augmented bioremediation approach using excess cultures generated by the groundwater treatment program described in this Plan.

The relatively flat groundwater gradient is southwesterly, as indicated in the Site Plan and favors good hydraulic control for the proposed reinfiltration program. Please refer to the supporting documents provided by Subsurface Consultants in Appendix 1.

Further east along 98th Avenue, the adjacent lot was occupied by another service station from 1949 to 1963. Previous underground fuel tanks from the second station were removed in 1970, but the exact former locations are unknown today. Contamination levels in this area are high enough to warrant immediate remedial action, but much of the contamination may exist on private land east of the City's new sidewalk along 98th Avenue.

Taken together, the implied and direct evidence for downstream migration of the hydrocarbon contamination prompted the need for remedial action for migration control and groundwater treatment.

The City and Subsurface Consultants have asked CytoCulture to prepare this Remedial Action Plan, complete with a detailed Project Budget (submitted under separate cover), in order to immediately address hydrocarbon contamination on City owned property and adjacent private land. The first priority is to design an extraction trench for construction during the current street widening operations at the site.

PROPOSED REMEDIAL ACTION

AUGMENTED BIOREMEDIATION

CytoCulture is proposing to design, construct and operate an augmented bioremediation system using laboratory-selected bacterial cultures to degrade the hydrocarbon contamination in the groundwater and soil. After Phase I and II construction, implementation of this action will occur in two consecutive phases of remediation:

Phase III: Groundwater bioremediation using extraction trenches and above-ground bioreactors to decontaminate any free product (if encountered) and dissolved-phase hydrocarbons

Phase IV: In situ bioremediation of adsorbed-phase hydrocarbons in the aquifer soil by reinfiltration

The objectives of this remedial action are to:

* Intercept the migration of petroleum hydrocarbons and prevent further movement of contaminated groundwater onto private land.
* Extract contaminated groundwater from BOTH former service station sites for continual bioremediation above-ground.
* Extract contaminated groundwater from under the roadbed of 98th Avenue for continual bioremediation above-ground.
* Extract additional contamination from under the buildings and parking lots (private properties) to the west of 98th Avenue

* Treat extracted groundwater to non-detectable levels of petroleum hydrocarbon in an above-ground bioreactor system for discharge to the East Bay Municipal Utility District sanitary sewer.
* Reinfiltrate treated groundwater, laden with bacteria and nutrients, to the contaminated zones to facilitate the _in situ_ biodegradation of hydrocarbons in the groundwater and soil.

CytoCulture is proposing to install 240 linear feet of extraction trench along the west side of 98th Avenue to capture any migrating product and hydrocarbon-contaminated groundwater. The trench will be excavated to 16 feet, well into the sandy native soil to enable well pumps in the trench to draw down a local cone of depression in the water table (groundwater now at about 10 feet). The extraction trench has been positioned parallel to existing water, sewer and storm sewer lines in the street so as to maximize the recovery of migrating contamination down gradient. The hydraulic gradient at this site is notably flat, which should facilitate the hydraulic control needed to capture contamination.
Zone of capture modelling data provided by Subsurface Consultants (Appendix 1) support the designated location of the trench in order to be effective in recovering migrating contamination, as well as drawing some contamination upfield from inaccessible areas under 98th Avenue and under the adjacent structures to the west. The recovery of contamination from the former service station areas will be enhanced by subsequent flushing and mounding of reinfiltrated (treated) groundwater in Phase IV of this project.

PROJECT DESCRIPTION BY TASK

The following task descriptions summarize the scope of work for the implementation of the bioremediation project. The project has been scheduled and budgeted for one year of continuous groundwater treatment after the construction phases, with 6 months of discharge to the sanitary sewer, plus 6 months of reinfiltration under an NPDES permit. Impossible Excavators will be subcontracted through Gallagher and Burk for excavation and earthmoving work in Phase I to accommodate previously arranged construction/insurance agreements. CytoCulture will remain the prime contractor for all subsequent construction work.

PHASE I: CONSTRUCTION OF TRENCHES IN 98TH AVENUE

Permitting

CytoCulture, Impossible Excavators and CytoCulture's other subcontractors will submit applications for permits pertaining to trenching, construction and treatment operations on the site. These applications will be filed within a week of the contract authorization to minimize any delays on street construction. CytoCulture will also begin the permitting process for discharge of treated water to the East Bay Municipal Utility District (EBMUD) sanitary sewer. The Phase I extraction trench construction itself will not require special permits beyond those normally required of a licensed excavation subcontractor.

Extraction Trench Construction in 98th Avenue

In order to prevent further migration of free product (if encountered) and contaminated groundwater beyond 98th Avenue, CytoCulture will provide design work, excavation supervision and technical service (limited labor/materials) for the construction of 240 linear feet of groundwater extraction trench. The trench will be excavated by Impossible Excavators along the west side of 98th Avenue, parallel and very close to the existing street curb from about the center of Edes Avenue to the storm sewer drain on the south corner of the intersection with Walter Avenue.

Concept: The extraction trench will be roughly perpendicular to the natural flow of groundwater in order to maximize the capture of contaminated groundwater and any free product (if encountered) across the span of the former service station tank locations along 98th Avenue. For ease of construction and local control of groundwater flow, the extraction trench will be installed in 2 sections, 120 feet each. Each section will be equipped with three well risers for access to the trench bottom, providing a total of 6 well pumps. The trench will be located in the street adjacent to the old curb along the west side of 98th Avenue, parallel to the telephone and gas lines running under the street. As shown in the Site Plan, the extraction trench will extend from the Edes Avenue intersection NE along 98th Avenue to just before the Walter Avenue intersection, so as draw contamination down-gradient and away from both former service stations.

Utility Boxes/Conduit: The 6 trench well risers will be accessed from the street surface with truck duty utility boxes (12" x 22") for the later installation and operation of pneumatic groundwater pumps. The well boxes will be interconnected with 4 inch cast iron pipe conduit which will later carry the pneumatic air lines, air control lines and groundwater discharge lines. A separate 4 inch cast iron pipe conduit spur will then lead from the extraction trench back to the bioreactor treatment area as shown in the site plan (Figure 1). A 17" x 30" utility box will be set in the concrete curb or sidewalk at the junction of the two trench sections to accommodate a pneumatic well pump controller. The cast iron pipe in the street will be trenched to at least 18" in depth and capped with a 10" concrete 2-sack slurry to support the new roadbed. Clearly, the trench construction and installation of the cast iron pipe conduit is designed to be completed before the installation of new storm sewer drains or the final surfacing and paving of the west side of 98th Avenue. The 4" conduit trench leading to the bioreactor work area from the extraction trench and infiltration wells will have been installed during the reconstruction of the east side of 98th Ave.

CytoCulture will provide materials and labor for installing the perforated piping, risers, pneumatic lines, well control lines and groundwater discharge lines inside the 4" cast iron conduit constructed by Impossible Excavators or other contractors.

Trench Spoils: Excavation of the extraction trench will generate clean fill (expected from the upper six feet) and contaminated soil (expected from a depth of six feet below the surface down into the water table). This lower 10 feet of trench soil (less than 300 cubic yards) will be stockpiled on the corner lot (south east of Edes and 98th) or transported to the Pippin Site for bioremediation treatment (if appropriate) once the complete bioremediation system has been installed (Phase II). A soil bioremediation proposal is included in this Plan (Appendix 5).

Trench Detail: The two 120 foot sections of two foot wide trench will be excavated and lined with drainage filter fabric which will serve as a sediment control barrier without interfering with the transport of hydrocarbons. Four inch plastic perforated piping will be installed along the bottom of the trench and connect to vertical risers for access to utility boxes on the ground surface (see above). The lower five feet of the trench will be back-filled with washed drain gravel to act, in effect, as a french drain. The remaining trench space above the gravel will be back-filled and compacted with clean trench spoils. Imported clean fill will be used to supplement the backfill as needed. The backfill will be compacted and capped with a 10" concrete 2-sack slurry to support the new roadbed and concrete utility boxes according to the City's specifications. See Figure 2 for cross section profiles of the proposed extraction trench.

Time Frame: The construction of the extraction trench is expected to take 6 to 8 working days in October, assuming no unforseen difficulties or obstacles during the excavation. Delays may be necessary to integrate trench construction with other street building operations, such as the installation of new storm sewer drains in the immediate vicinity of the extraction trench. The 4" cast iron pipe conduit spurs for the trench and future wells will take at least a day to install during the reconstruction of the east side of 98th Avenue in September.

PHASE II: Construction of the Bioremediation System

As the extraction trench in 98th Avenue is completed and the City's street reconstruction project moves on, CytoCulture will be prepared to proceed immediately with the installation of a complete groundwater bioremediation system. The first task in this second phase of construction will be to build a bioreactor work area in the corner lot formerly occupied by the Union 76 service station. Electric power will be installed underground to the bioreactor work area by a subcontractor, followed by the trenching and installation of the sanitary sewer discharge line leading to Edes Avenue. Impossible Excavators will be subcontracted to construct an 80 foot infiltration trench perpendicular to Edes Avenue on the same lot for reinfiltration up-gradient of the former waste oil tank. Exceltech will be subcontracted to install two 4" infiltration wells flanking monitoring well MW-2 west of the US Rental property along the east gutter of 98th Avenue. These wells will be connected to the bioreactor system by underground 4" piping (cast iron and PVC).

The wells will be equipped to also function as extraction wells.

Construction of the Bioreactor System Work Area

CytoCulture will subcontract the construction of a 18 ft x 22 ft concrete pad for the bioremediation system equipment on the corner lot accessed from Edes. The concrete pad (5" thick with No. 3 rebar on 16 inch centers) will be surrounded by a 17 inch cement block wall to create a double containment barrier for the system processing of free product (if encountered) and contaminated groundwater. The work area will be enclosed by an eight foot chain link security fence with a man gate, trimmed with redwood slats to improve its appearance from the street. Security in this area warrants the additional installation of barbed wire on the fence.

The work area will be serviced with 440 volt power (3 phase) and city water (see below). A temporary roof structure will be built to protect the electrical equipment (compressor, blowers, pumps). The exact placement of the work area will be determined, in part, by the City's future plans for the corner lot. As at other CytoCulture sites, the fenced, concrete work pad could be useful to the city in the future as an equipment storage area. The bioreactor work area should take about 3 weeks to build, including the installation of power, water and discharge lines.

Electric Power Installation to Bioreactor Work Area

The electrical requirement for the compressor and blower is 480 volt, 3 phase power, which is presumed available from PGE via existing underground utilities in Edes Avenue (estimated at 100 feet from the bioreactor work area). An electrical contractor will be subcontracted by CytoCulture to install underground (trenched conduit) wiring from an available PGE utility box in Edes Avenue to the bioreactor work area. Additional 220 volt and 110 volt power subpanels will be installed in the work area off the main power panel. All electrical panels will be mounted on a plywood wall under the protection of a roof within the fenced work area. This electrical work will be performed immediately after completion of the work area facility and will take less than one week to complete.

Construction of Sanitary Sewer Discharge Line

During Phase III groundwater bioremediation all treated effluent will be discharged to the EBMUD sanitary sewer, and then during Phase IV, the majority of treated water will be discharged to the infiltration trenches to accelerate in situ biodegradation. A subcontractor, probably Impossible Excavators, will install the sewer connection to the EBMUD sewer line in Edes Avenue via an existing manhole access for a clean-out (located in the sidewalk along the edge of the corner lot). The effluent discharge line will be installed inside 3 inch cast iron pipe leading down to the manhole from the bioreactor work area (estimated at about 100 feet) and will be buried in a trench about 18 inches deep, or as specified by the City. This work will be performed immediately after the construction of the bioreactor work area and will take less than one week to complete. Upon completion of all trench and facility construction, the site will be ready for the installation of CytoCulture's bioremediation equipment.

Installation of CytoCulture's Bioremediation System

The bioremediation equipment consists of a pneumatic groundwater well pump system (6 pumps + controller + compressor), an optional oil/water separator to recover free product and a 5,000 gallon modular bioreactor system for continuous flow treatment of contaminated groundwater at up to 9 gallons per minute.

Groundwater Pumping System

As with current groundwater treatment systems in operation at the

CytoCulture facility in Emeryville, automated pneumatic well pumps will be used to continuously collect groundwater from the extraction trenches. The six well pumps can be adjusted individually, but are regulated by an automated pneumatic controller installed in a 17" x 30" utility box to be set in the concrete of the west sidewalk gutter along 98th Avenue. Three pumps will be installed per 120 feet of groundwater extraction trench, approximately 40 feet apart and 20 feet from the trench ends. Each well pump will be connected to the pressurized air delivery and groundwater discharge lines installed in the subsurface 4" conduit leading from the junction between the two trench sections back to the controller in the sidewalk gutter.

The entire groundwater pumping and reinfiltration systems will be powered by a single 125 psi/23 cfm compressor installed in the bioreactor work area. The pneumatic well pumps and controllers are designed to continuously recover groundwater at a average rate of 1-1.5 gpm each. A 6 well, 240 foot extraction trench should therefore yield 6-9 gpm when abundant groundwater is available. The pumps will be equipped with skimmers to simultaneously pump floating free product if it is drawn into the trench. The pumps, discharge lines and bioreactors are all made of PVC, polyethylene, nylon or teflon to prevent electrolysis, corrosion and to avoid the danger of spark from metals. There are no metal or electrical components in direct contact with free product (if encountered) or contaminated groundwater anywhere in the treatment process, from well to bioreactor, except for the storage of recovered product in EPA approved tight lid cast iron drums if they are really needed.

Free Product Recovery System (Optional)
Although modest amounts of free product can be processed by the bioreactors, an optional oil-water separator would have to be

installed if significant amounts of free product were extracted from the trench. CytoCulture fabricates its own separators to integrate into the bioreactor system and permit skimming of free product for recycling. The treatment system would have a storage capacity of 200 gallons of free product in EPA-approved tight drums to be recycled within 90 day time periods. An oil recycling firm would be sub-contracted to manifest accumulated free product.

Modular Bioreactor System

Groundwater extracted through the trenches will be processed through a series of aerated bioreactors. A modular **4** unit bioreactor treatment system will be leased to the City and installed for continuous flow treatment of contaminated groundwater pumped from the trench at flow rates up to **9** gpm. CytoCulture's bioreactor system will have a **5,000** gallon working volume capacity and a nine hour hydraulic retention time, similar to the system in use at our successful Emeryville truck terminal clean-up site. The three 1,000 gallon high cell density suspended culture bioreactor units can efficiently process hydrocarbon-saturated groundwater in continuous flow to yield clean effluent water meeting drinking water standards with respect to total petroleum hydrocarbons TPH (< 0.5 ppm) and BTXE (< 1 ppb). Treatment data from our Emeryville site (saturated with diesel and gasoline) is available on request, and some examples of influent vs. effluent analytic data are included.

Construction of an Up-Gradient Infiltration Trench

In situ bioremediation of the former service station underground storage tank areas (essentially spanning the width of the corner lot) will require an infiltration trench on the up gradient side of the contamination. As shown in the Site Plan, CytoCulture has designed an 80 foot infiltration trench (two sections of 40 feet) to be located up gradient of the former waste oil tank location. This infiltration trench will be two feet wide and 16 feet deep to provide direct access to the sandy subsurface soil in the groundwater table (see Figure 2 cross section profiles). The trench will have perforated piping installed along the bottom and will be back-filled with 3 feet of washed drain gravel, as with the infiltration trench planned for the west side of 98th Avenue. As currently envisioned, the infiltration trench would run parallel to the fence along the east side of the corner lot, but it could also be angled across the lot to line up better with the apparent groundwater gradient contours. MW-4 will serve as the proximal monitoring well for observing changes in the water table and groundwater composition over the course of reinfiltration.

Utility Boxes/Conduit: The trench piping will connect to two risers, accessed from the surface with 12" diameter utility boxes (set in concrete) and will be interconnected with 4" cast iron pipe serving as conduit for infiltration lines leading down from the bioreactor work area. CytoCulture will install the infiltration lines which run inside the cast iron pipe conduit to the risers.

Trench Spoils: Excavation of the trench is expected to generate less than 100 cubic yards of contaminated soil from the lower two feet of soil in the saturated zone. Any contaminated soil will be stockpiled on 10 mil plastic liner in the immediate area (within the bounds of the corner lot) or transported to the Pippin site for bioremediation treatment during Phase III. The trench will be back-filled and compacted as described for the other trenches, but the scope of work does not include resurfacing and paving over the trench.

Time Frame: The up gradient infiltration trench will take about one week to construct, including the installation of conduit pipe and utility boxes.

Infiltration Wells: MW-2 area

Since there is currently no access to the private land (US Rentals) at the former ARCO service station site for building an infiltration trench to flush contamination towards the extraction trench in 98th Avenue, CytoCulture and Subsurface Consultants have proposed the installation of a pair of 4" infiltration wells flanking monitoring well MW-2. This area is known to be contaminated with petroleum hydrocarbons, and the monitoring well (MW-18) directly down-gradient has shown the highest recorded contamination levels of the entire site. Short of installing an entire infiltration trench along the new sidewalk, the most prudent action would be to use infiltration wells to "chase" the contamination under 98th Avenue directly into the extraction trench close to MW-18 (see Site Plan, Figure 1 and cross section profiles, Figure 2).

Exceltech, Inc. will be subcontracted by CytoCulture to install two 4" infiltration wells approximately twenty-five feet apart, one on each side of monitoring well MW-2 in the street, and opposite the intersection of Walter Avenue and 98th. These wells will be drilled to twenty feet to gain clear access to the sandy soils in the saturated zone of the aquifer (see cross section profile). These wells could also serve as alternate extraction wells to recover contamination migrating down-gradient from the US Rentals property to the east. Underground 4" conduit pipe and infiltration/extraction lines will have to be installed prior to the resurfacing of the east side of 98th Avenue in mid-September.

PHASE III: GROUNDWATER BIOREMEDIATION

Establishing a Groundwater Depression Zone

The groundwater extraction trenches, now equipped with pneumatic well pumps, will recover free product (if encountered) and contaminated ground water 24 hours/day. The continuous extraction of contaminated groundwater will draw down the water table, creating a local groundwater depression zone in the immediate vicinity of the extraction trench, both up gradient pulling from both former service stations locations and down gradient from under buildings. This groundwater depression zone will maintain a constant flow of contaminated water into the extraction trenches. Once the depression zone is established, any free product (if encountered) floating on the groundwater within the zone of influence will be selectively recovered by skimmers mounted on the well pumps. Flow rates are anticipated to average 1 gpm/well (6 gpm total). Seasonal winter rainfall may increase the flow of water and dilute the concentration of contaminants entering the extraction trench. The hydrogeological model supports these predictions.

The groundwater depression zone will have 3 main effects:

(A) It will effectively block further migration of floating product (if any) and contaminated groundwater off City property onto private property to the southwest.

(B) It will capture floating product (if any) and contaminated groundwater migrating down gradient from the most contaminated areas of both former service station underground storage tank areas. For the corner lot, this extraction will be accelerated by the flushing action of treated groundwater laden with bacteria and nutrients introduced via the infiltration trench (Phase IV).

(C) It should pull contaminated groundwater (and any free product) up-gradient from under the buildings along the west side of 98th Avenue, to the north of the intersection with Edes Avenue. This upfield recovery of contamination has been predicted by computer modelling (see Appendix 1) of the extraction trench's zone of capture and should be evident by the lowering of the water table in monitoring wells MW-18 and MW-3. Monthly measurement by Subsurface Consultants of the water level in the current and proposed additional surrounding monitoring wells should demonstrate the effects of the groundwater extraction in depressing the local water table.

Free Product Recovery for Recycling (if encountered)

By continually pumping groundwater from the six wells in the extraction trench, the down-gradient flow of contamination into the cone of depression around each well should greatly facilitate the recovery of any floating free product, particularly since the saturated zone (below 12 feet) appears to be porous, sandy soil.

Treatment System Start-up of Bioreactors

CytoCulture will inoculate and activate the bioreactor system once the EBMUD discharge permit has been approved. The bacterial cultures will be acclimated to gasoline and waste oil for rapid biodegradation of contaminated influent before influent from the extraction trenches is pumped into the system.

Sybron Biochemical will supply bacterial cultures, soluble nutrients and laboratory service support on a monthly basis for the duration of the 12 month groundwater treatment program.

Treated groundwater generated in the first 24 hours at a reduced flow will be stored in a 2,000 gallon aerated holding tank, and tested before final approval for discharge to the sanitary sewer.

Continuous Groundwater Bioremediation

The suspension cell bioreactor system will operate 24 hours a day with automated process control and a hydraulic retention time of about 9 hours. The oil/water separator, pumps, discharge lines and bioreactors and all components in contact with the contaminated water are made of high density polyethylene, polyvinyl chloride, or nylon. All-plastic components minimize problems associated with electrolysis, corrosion and the handling of volatile, explosive vapors.

There are no metal or electrical components in direct contact with contaminated groundwater anywhere in the treatment process, from the wells to system discharge. If required by the Air Pollution Control Board, the bioreactors will be fitted with closed tops and vents equipped with carbon canisters (spent carbon processed in bioreactors) to minimize escape of volatile organic compounds (VOC's).

Augmented Biodegradation Process

The biological treatment program will utilize laboratory-selected bacterial cultures developed by Sybron Biochemical, a division of Sybron Chemicals, Inc. (Salem, VA) to augment the natural biodegradation of dissolved phase petroleum hydrocarbons. These cultures are sole-carbon enrichment isolates, meaning that specialized strains have been selected in a laboratory to use specific hydrocarbon fractions (e.g., benzene, toluene, xylene, ethylbenzene, aliphatics, etc.) as their only carbon and energy food source.

There are no chemical by-products or residual chemicals resulting from the biological degradation process. Unlike carbon adsorption processes, there will be no hazardous materials to be hauled for disposal. Effluent from the bioreactor system will be clean, drinking quality water (with respect to hydrocarbons), laden with bacteria and nutrients. The bioreactor system design incorporates automated nutrient addition systems, continuous seeding of bacteria from dry cultures and process control systems which minimize the time spent on operational maintenance.

CytoCulture's technical service includes the periodic reinoculation of the bioreactors with fresh cultures of bacteria to guarantee a healthy population of hydrocarbon degraders. Technical information and publications related to the augmented bioremediation process are available.

Discharge of Treated Groundwater to Sanitary Sewer

During the first 6 months (Phase III) of the bioremediation program, treated water will be discharged to the EBMUD sanitary sewer system via an access point in Edes Avenue. Details for connecting our system to the sanitary sewer system have yet to be worked out with the City, but making the appropriate underground connection to the available EBMUD easy-out in the sidewalk along Edes Avenue is presumed to be straight forward.

As required during the start-up of the bioreactor system, treated groundwater will be temporarily retained in a 2,000 gallon aerated holding tank for sampling and immediate analysis prior to direct discharge to the sanitary sewer.

Sanitary Sewer Discharge Permit Self-Monitoring

Discharge of treated groundwater under permit requires strict adherence to specified sampling and analysis requirements as outlined in EBMUD Guidelines for Discharge of Treated Water.

EBMUD discharge rates are charged according to volume, total suspended solids and filtered chemical oxygen demand (CODF).

From experience with our current treated groundwater discharge permit with EBMUD for the Emeryville site, CytoCulture estimates there will be approximately 24 bioreactor effluent samples and 8 groundwater influent samples over the first 6 months of operation. CytoCulture field technicians will collect the oil/water separator influent and bioreactor effluent samples for routine analysis by our affiliated analytical laboratory, Erickson Analytical (Richmond, CA), according to the guidelines specified by the EBMUD. The process monitoring analysis will employ the modified EPA method 8015 for extractable petroleum hydrocarbons in aqueous solutions using EPA extraction method 3510. Effluent samples will also be analyzed for BTXE by the EPA method 8020 with the extraction by EPA 5030 (purge and trap). Since there were some chlorinated (DCE) solvents on this site, CytoCulture will also test the treated effluent for purgeable halocarbons using the EPA method 8010.

In Phase IV, the EBMUD mandated self-monitoring program may be supplemented by additional tests required under an NPDES permit for <u>in situ</u> discharge of treated groundwater according to which regulatory group is designated the "lead agency" for this project.

<u>Biological Process Monitoring</u>
CytoCulture's in-house microbiology laboratory (Point Richmond) will conduct the biological monitoring from samples taken in the field for verification and optimization of the process. The biological monitoring includes pH tests, nutrient (ammonia, ortho-phosphate) analyses, and plate counts of total vs. hydrocarbon-degrading organisms. The biological monitoring will be scheduled to coincide with BTXE and TPH tests according to the specified "self monitoring" program specified by the EBMUD and, later, the NPDES discharge permits. On account of CytoCulture's

good track record over the past two years, the frequency of self-monitoring and the frequency of the District's own monitoring of groundwater treatment programs has been greatly reduced. During Phase IV, a substantial portion or all of this water will be diverted to the infiltration trench and infiltration wells.

PHASE IV: In Situ Reinfiltration of Treated Groundwater

Permitting

Within the six months of Phase III continuous bioremediation of contaminated groundwater, CytoCulture expects to receive the NPDES permit to divert treated effluent from the EBMUD sanitary sewer to the infiltration trench and two infiltration wells constructed on site. Details are discussed in the section on Regulatory Considerations.

Proceeding with Reinfiltration

The infiltration trench and two infiltration wells will have been constructed in Phases II for *in situ* treatment in Phase IV. The 80 foot up-gradient trench will flush nutrients and bacteria down the aquifer, across the contaminated underground storage tank area towards the extraction trench. The two infiltration wells to the north along 98th Avenue are designed to introduce bacteria and nutrients to the contaminated zones up-gradient of the heavily contaminated area detected by monitoring well MW-18 just beyond the edge of the City property (down-gradient). These two wells will introduce treatment water into the contaminated aquifer under 98th Avenue itself, moving the front of nutrients and bacteria down-gradient to the extraction trench in a line from MW-2 to MW-18.

The infiltration trench will be constructed down to 16 feet deep to gain direct access to the porous sandy aquifer for efficient transfer into the saturated zone of soil. Much of the adsorbed phase hydrocarbon contamination is presumed to reside at the interface of groundwater and soil at about 10-12 feet down.
The infiltration wells will be drilled deeper in the event that they may later serve as alternate extraction wells to recover contamination migrating down-gradient from the US Rentals property to the east. Ultimately, an infiltration trench should

be installed on this private land to fully treat the contaminated aquifer under the former ARCO service station. Treated groundwater (bioreactor treatment effluent), laden with hydrocarbon degrading bacteria and nutrients, will be reinfiltrated into the water table to promote the in situ degradation of gasoline and waste oil contaminants in the aquifer. As this water travels down gradient toward the extraction trench, it will distribute bacteria and nutrients into both the groundwater and the soil. Mounding infiltrated groundwater on the east side of 98th Avenue is expected to improve access to contamination in the unsaturated zones above the water table. Hydraulic control of this reinfiltrated water will be maintained by the constant groundwater depression zone along the 240 foot extraction trench positioned in 98th Avenue to intercept the gradient.

Comments on In Situ Biodegradation

Although the laboratory-selected cultures originate from naturally occurring organisms, they are 10-100 times more effective than the background native flora at degrading most hydrocarbons, and they are more resistant to toxic shock, salts and other potentially adverse growth conditions. Even under the anaerobic conditions expected in the groundwater, the facultative bacteria introduced with the nutrients in the treated groundwater will biodegrade most or all of the dissolved phase product over a period of months.

In-situ bioremediation of the soil contamination will also occur in saturated zones where groundwater migrates through the soil. In addition to the exogenous bacteria leached into the aquifer, the infiltration of nutrients will promote anaerobic respiration by indigenous hydrocarbon-bacteria in the saturated zones within the influence of the reinfiltration trench.

Tangible evidence of in situ bioremediation will be provided by monitoring: (a) sharp increases in the nutrient concentrations, (b) increased bacterial cell counts, particularly hydrocarbon degraders and (c) detection of tracers injected into the bioreactor effluent and measured downfield in the extraction trenches, and (d) a decrease in dissolved hydrocarbon levels. The entire reinfiltration process will be monitored by Subsurface Consultants using water table level and hydrocarbon readings in the surrounding monitoring wells.

REGULATORY CONSIDERATIONS

Regulatory Agencies Directly Involved

An integrated groundwater/soil remediation program will address both the short-term and long-term concerns of regulatory agencies, including the California Department of Health Services (DHS), the San Francisco Bay Regional Water Quality Control Board (RWQCB) and the Alameda County Environmental Health Department. The Regional Water Quality Control Board (RWQCB) will ultimately address the remedial action proposals presented in this document. CytoCulture expects this site will be reviewed at the Staff level, probably by Mr. Cecil Felix or his immediate supervisor, Mr. Tom Callaghan. The group leader, Mr. Lester Feldman, will probably review their findings and return specific questions for further consideration.

It is anticipated that the lead regulatory agency for this site clean-up program will be the Alameda County Department of Environmental Health. Mr. Ariu Levi, a Hazardous Materials Specialist familiar with the site, is likely to be actively involved in the review of this Remedial Action Plan, and will probably work together with RWQCB to set clean-up specifications to allow for site closure.

William Meckel, in the Source Control Division of the East Bay Municipal Utility District, has been informed of the project and has indicated that there should be no difficulty in obtaining a discharge permit to direct treated water to a City of Oakland sanitary sewer (accessed via Edes Avenue). The steady state discharge rate, and therefore, the monthly discharge fee, will be reduced when reinfiltration begins in Phase IV.

John Wesnousky, of the Toxic Substances Control Program, Alternative Technology Division, California Dept. of Health Services, recommends that local regulatory agencies take the lead during the first three phases. CytoCulture will present the entire project for review by Alternative Technology, in anticipation of their role as lead assessors of the Phase IV reinfiltration portion of the bioremediation program. Alameda County and the RWQCB will be presented with DHS's completed assessment before reinfiltration permitting is initiated. It is anticipated that an Operational Plan will be required before project initiation in order to address specific questions raised by regulatory agencies during the review of this Remedial Action Plan.

<u>Phase III System Start-Up Anticipated Sampling</u>

Before operation of the bioremediation system, a sampling protocol similar to this is likely to be required:

* Initial groundwater influent sample for extraction trench:
  EPA 624, EPA 625, priority pollutants, metals
* First 24 hours after system start-up before discharge to EBMUD sewer (discharge to 2000 gallon aerated holding tank)
* Test results on 24 hours laboratory turn around for EBMUD discharge approval after laboratory results reviewed

<u>Phase III - Groundwater Treatment Sampling for EBMUD Discharge</u>

Treated Groundwater Effluent Discharge
At 24 hours, 48 hours, 72 hours
Twice/week for the first 2 weeks
Once/week for next 2 weeks
Biweekly thereafter

Groundwater influent will be tested at least monthly.
Sampling frequency and analysis subject to change with new NPDES permit application for Phase IV reinfiltration.

<u>Reinfiltration of Treated Groundwater - Special Regulations</u>

The remedial action plan recommends extracting contaminated groundwater, treating it and discharging the treated groundwater to a reinfiltration gallery or trench. An NPDES permit for Waste Discharge from the RWQCB is required in order to reinfiltrate the treated groundwater. A Report of Waste Discharge is the application for such a permit. This report must include:

* Results of a hydrogeologic assessment which details:
    - -Site geology, including geologic cross-sections
    - -Aquifer properties, the groundwater gradient, depth to groundwater, and seasonal fluctuations
    - -Estimated surface infiltration rate, irrigation rate
* Results of a containment assessment which includes:
    - -Site map showing the extent of the groundwater contamination zone
    - -Existing concentrations of contaminants dissolved in the groundwater, including the history of and/or presence of free product (if encountered)
    - -Site map showing the vertical and horizontal extent of soil contamination
* Description of the proposed extraction/treatment/reinfiltration system including:
    - -Site map showing the location of monitoring wells, extraction wells and reinfiltration trenches (including dimensions and construction details)
    - -Daily average flow and maximum flow rates
    - -Description of the treatment system, including schematics and operating principles and parameters
    - -Identification of organisms and/or chemicals which are added to groundwater prior to discharge
    - -Potential impact of organisms and/or chemicals and their respective breakdown products on groundwater which may potentially be used as a drinking water

* A groundwater monitoring program designed to detect the migration or dispersion of contaminants which includes:
    - -Site map showing the locations of monitoring wells, including details of well construction
    - -Groundwater sampling frequency, including constituents for which the groundwater is analyzed, the analytical methods, and the detection limits
    - -Corrective action plan which describes the response to data which indicates the contamination is spreading

<u>Site Closure Certification</u>

The goal of the remediation plan is to clean groundwater, excavated soil and the <u>in situ</u> aquifer to the maximum extent practicable. This accepts the reality that most cleanup efforts conclude with some level of residual contamination. The presence of this contamination may not adversely impact the environment or public health. In order to obtain a site cleanup certification, the following topics will be addressed:

* Investigative methodology, including:
    - -Soil sampling
    - -Groundwater sampling, including monitoring well design, installation and development, including methods to measure groundwater elevation & free product thickness
    - -Sample chain of custody, preservation, preparation methods and detection limits
* Definition of vertical and lateral extent of contamination of:
    - -Groundwater (free product and dissolved constituents)
    - -Soil (adsorbed phase, water soluble fractions)
* Hydrogeological characteristics including:
    - -Nearby water bodies and/or wells which might be impacted
    - -Potential pollutant pathways and hydraulic connections

-Evaluation of local gradients, including seasonal fluctuations
-Characteristics of the aquifer(s)
-Permeability of the soil

* The effectiveness of Remedial Actions including:
  -Rationale for the selected remedial approach
  -Groundwater treatment system
  -Soil remediation method
  -Verification monitoring program, including sampling

PROJECT MANAGEMENT

CytoCulture Environmental Biotechnology (Pt. Richmond, CA) will act as prime contractor and manage the design, construction, and operational phases of the project on site. CytoCulture will manage the installation of groundwater extraction and reinfiltration trenches. CytoCulture will provide technical expertise in the design, construction, and operation of the groundwater extraction system, above ground groundwater treatment, and <u>in situ</u> groundwater and soil bioremediation.

CytoCulture is a State Licensed General Building Contractor (class B, No. 563665) specializing in bioremediation projects of this type. CytoCulture personnel involved in the project are:

Randall von Wedel, Ph.D. - President and Project Manager
Robert Greenwald - Assistant Project Manager
Grady Meadows - Construction Manager & RMO
David Virva - Fabricator and Field Mechanic
Karl Gillette - Director of Field Operations
Eugene Duckart, Ph.D. - Soil Scientist
James Yost - Soil Mechanics Engineer
Richard Ortega - Field Technician

Sybron Chemicals (Salem, VA), an affiliate of CytoCulture, will supply bacterial cultures, nutrients, and some technical support. Sybron has been producing specialized bacterial cultures for industrial and hazardous waste clean-up for over 40 years. CytoCulture and Sybron have been performing a similar groundwater bioremediation program at a former truck terminal project in Emeryville, CA over the past year and a half, in addition to several soil bioremediation projects in the past year.

CytoCulture has had active research collaborations with Sybron over the past 3 years and maintains a close working relationship with Director of Production, Lois Davis, and the Director of Research, Dr. Mike Griffith.

Impossible Excavators (Oakland) are general building and excavation contractors specializing in difficult trenching operations as required for this site (into groundwater).

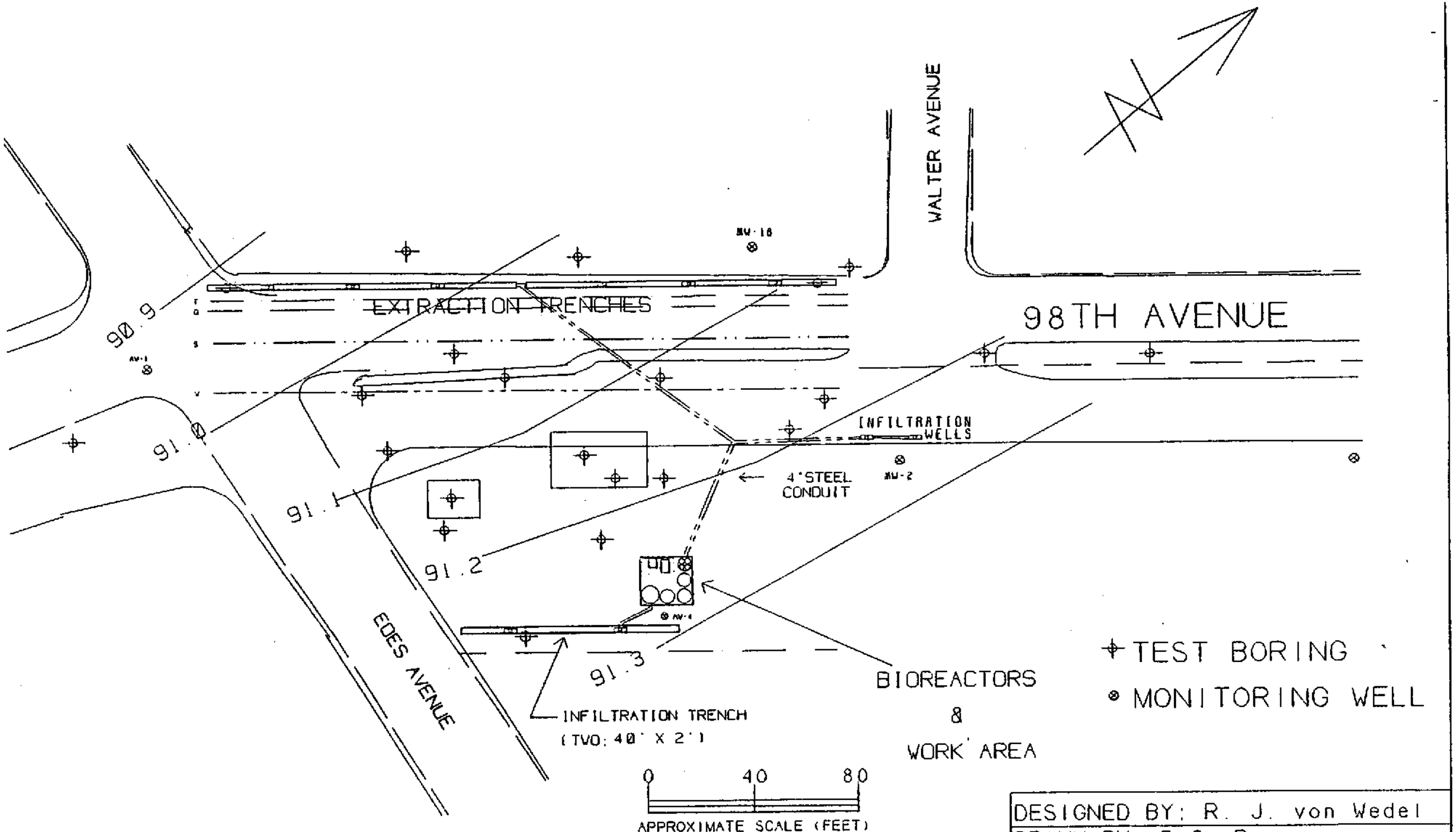
Exceltech, Inc. (Fremont) are experienced contractors in drilling and installing extraction wells and are working with CytoCulture at other groundwater bioremediation sites in the Bay Area.

Laboratory analytical services for contamination will be performed by Erickson Analytical (Richmond, CA). The Laboratory Director, Ms. Nancy Nelson, will oversee the EPA-certified analysis of samples.

Erickson Environmental (Benicia, CA) will participate as strategic business partners in performing this bioremediation project with CytoCulture. Erickson Environmental is currently negotiating a joint venture opportunity with CytoCulture, and is already actively participating in multiple bioremediation projects on a site by site basis.

The President of Erickson Environmental, Mr. Paul Taylor, and Vice President of Technical Development, Mr. Frank Straw, will both review all remedial action plans. An Erickson technical representative (designated site Health and Safety Officer) will be present during the Phase I and II construction operations. All contracts and insurance agreements will be reviewed for approval by our strategic business and joint venture partners at Erickson Enterprises, Inc.

FIGURE 1: SITE PLAN



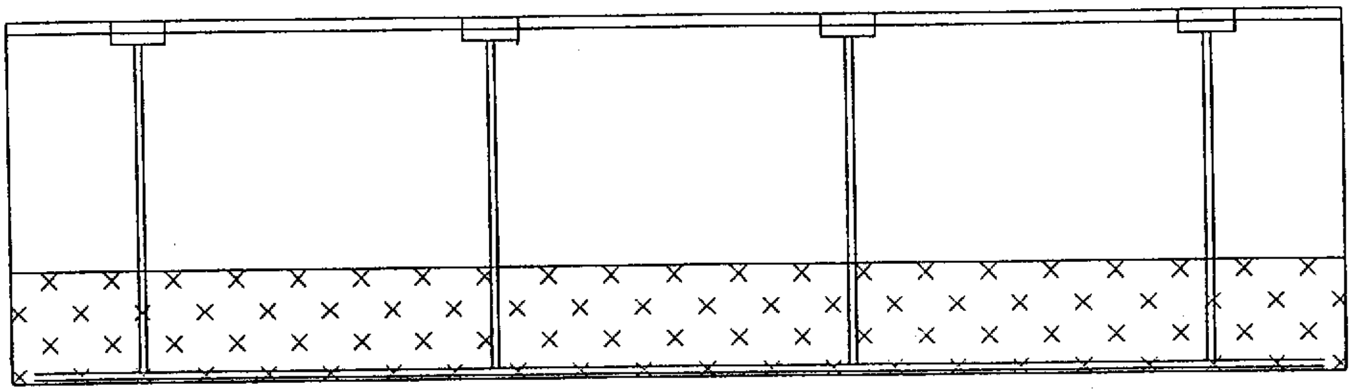
DESIGNED BY: R. J. von Wedel
DRAWN BY: D C. Rogers

Cyto Culture
Bioremediation

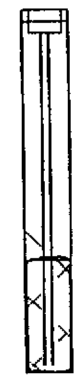
98TH & EDES AVE. / OAKLAND, CA

SEPT 7, 1990 | CODE: SCI 98TH

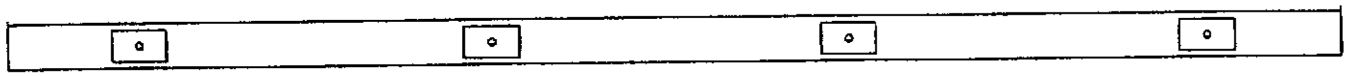
FIGURE 2: CROSS SECTION DETAILS



SIDE VIEW



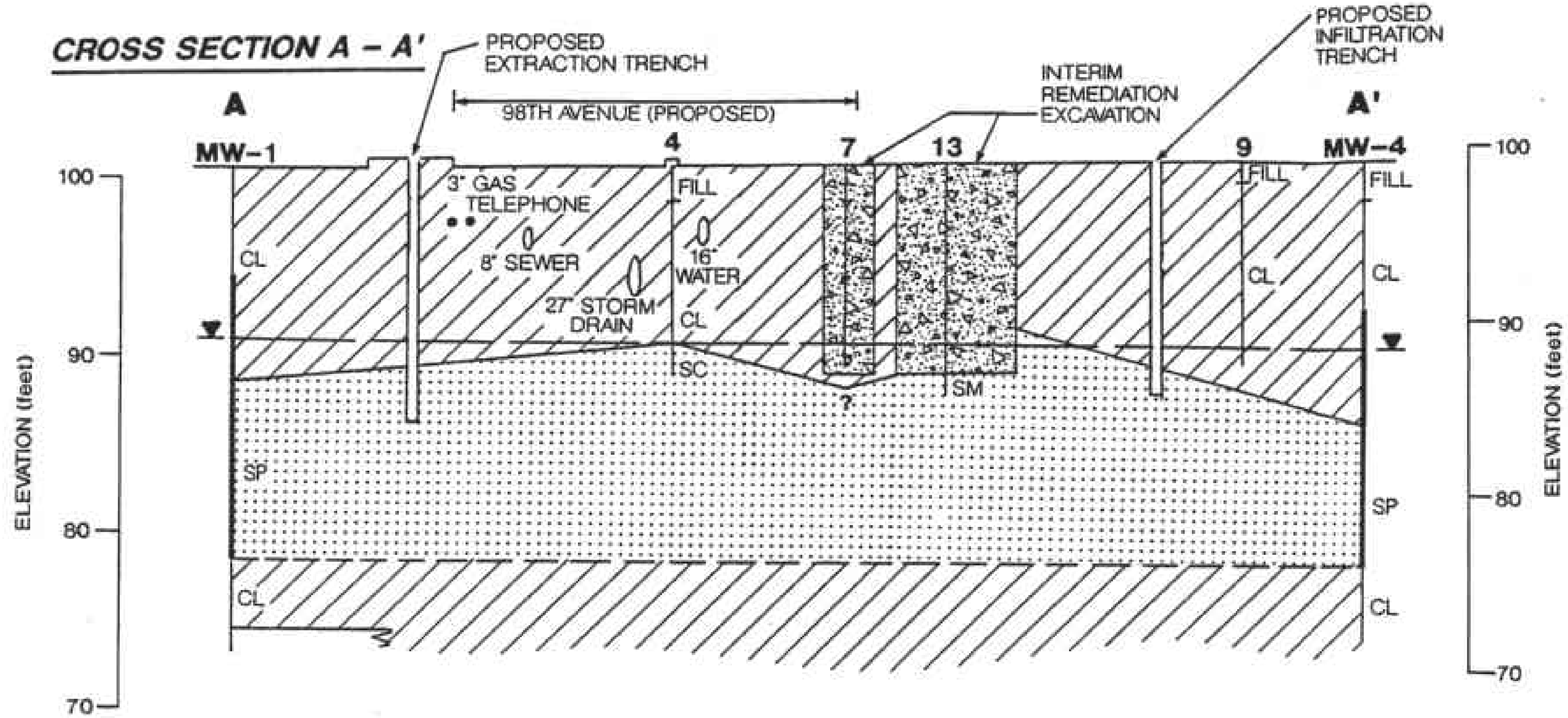
CROSS SECTION



TOP VIEW

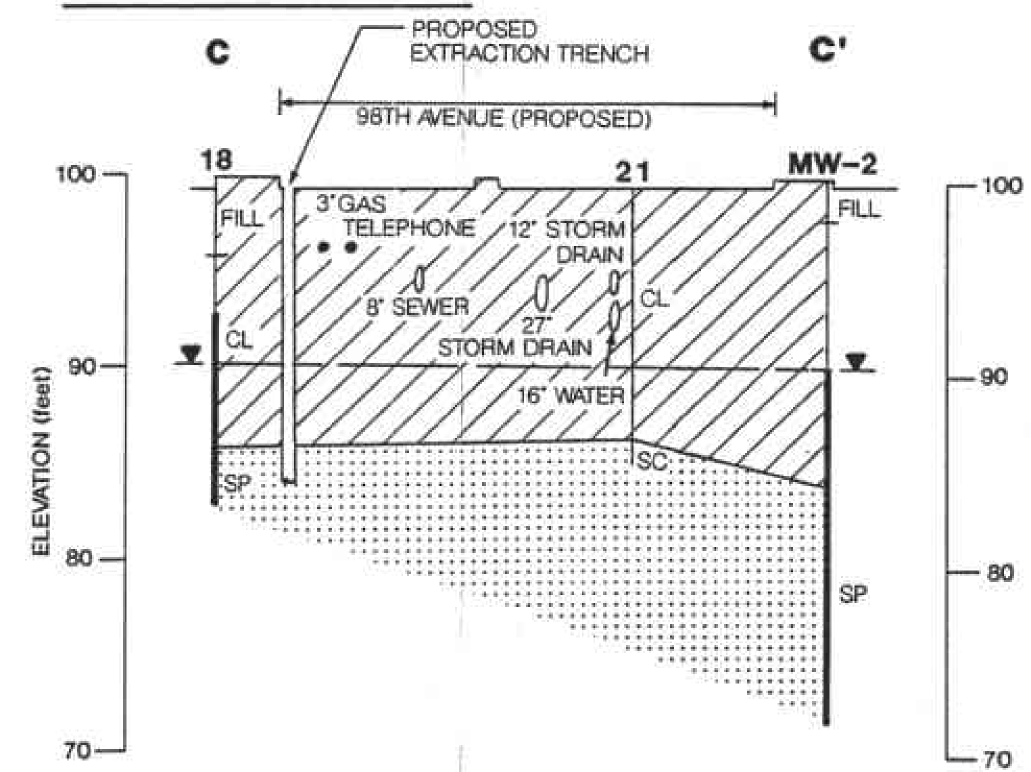
Cyto Culture BIOREMEDIATION	EXTRACTION TRENCH VIEWS	DATE: JULY 26. 1990
	DESIGNED BY: RANDALL von WEDEL / DRAWN BY: DANIEL C. ROGERS	CODE: SSC/98ST.

CROSS SECTION A – A'



NOTES:
1. Gas and telephone lines assumed to be 3 feet below street grade.
2. Elevation reference, see Site Plan.
3. The groundwater levels shown were measured on June 30, 1990.
4. The soil layer boundries shown are based upon linear interpolations between borings. The actual boundries could vary from those shown.
5. The screened interval of wells shown as a thick line.

CROSS SECTION C – C'



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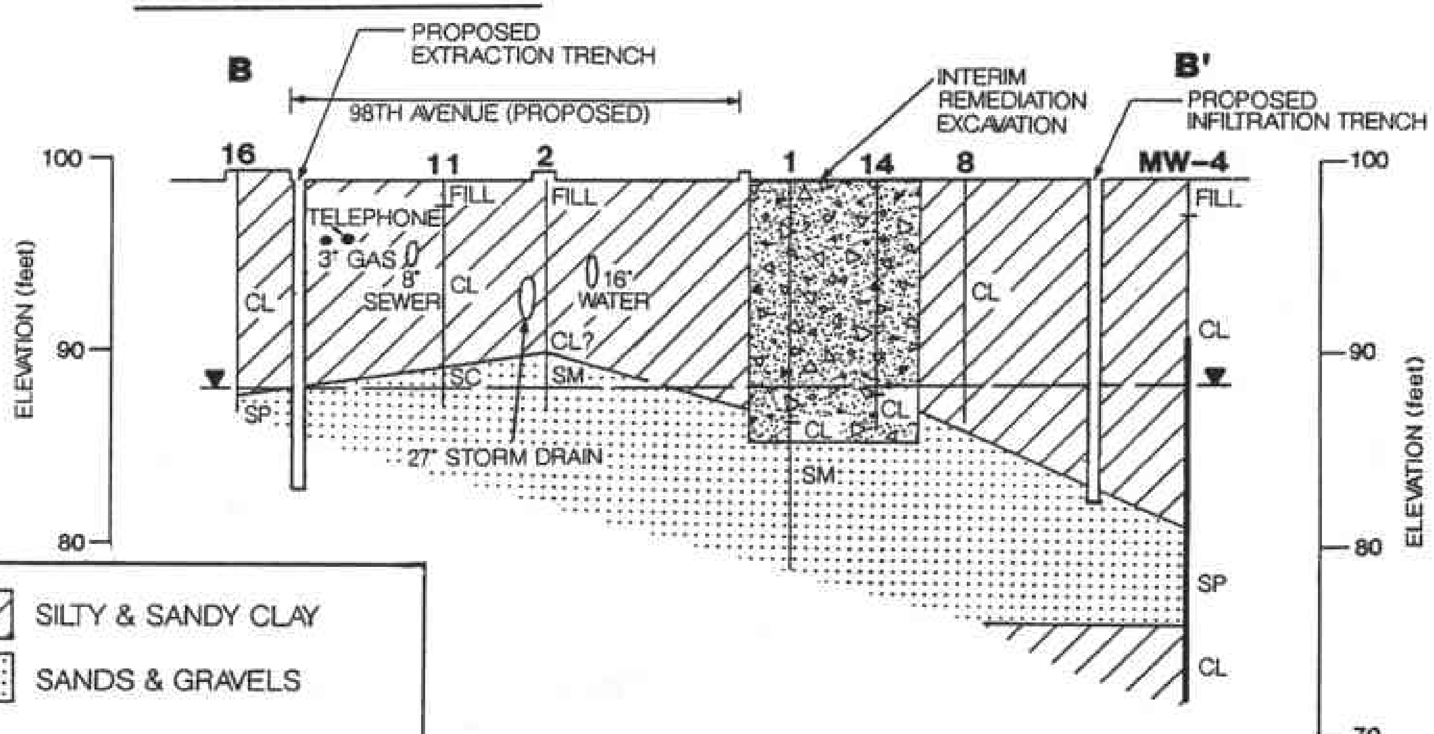


SILTY & SANDY CLAY

SANDS & GRAVELS

HORIZONTAL SCALE: 1" = 40'
VERTICAL SCALE: 1" = 10'

Subsurface Consultants	CROSS SECTIONS A – A' THROUGH C – C'		
	98TH & EDES – OAKLAND, CA		PLATE 2
	JOB NUMBER 272.016	DATE 8/30/90	APPROVED

APPENDIX 1

Hydrogeology Model for Zone of Capture

James P. Bowers, PE
R. William Rudolph, Jr., PE

September 18, 1990
SCI 272.016

Mr. Dan Lau
City of Oakland
Construction
7101 Edgewater Drive
Oakland, California 94621

Preliminary Groundwater Modeling Study
98th and Edes Avenues
Oakland, California

Dear Mr. Lau:

This letter presents results of our preliminary groundwater modeling study for the subject project. The purpose of the study was to estimate capture zone limits and drawdown potential using assumed aquifer characteristics. The results of our previous hydrogeologic investigations at the site were presented in reports dated July 17, 1989 and April 10, 1990.

Background

Our investigations to date have indicated that fuel related contaminants and purgeable halocarbons exist in groundwater beneath the site. The apparent origin of the fuel related contaminants was the underground storage tanks formerly located at 670 and 692 - 98th Avenue. Because purgeable halocarbons were encountered in upgradient wells from the underground tank locations, we judge that they originate from an off-premises source.

As previously discussed with representatives from the City of Oakland, the Alameda County Health Care Services Agency, and CytoCulture, we conclude that an extraction trench parallel to 98th Avenue, between Edes Avenue and Walter Avenue as shown on Plate 1, Site Plan, is the most cost effective way to collect the contaminated groundwater for treatment.

The groundwater pumped from the extraction trench will be treated on-site and released into the sanitary sewer. In the future, provided the necessary permits are obtained, the treated water will be mixed with bacteria and nutrients and reintroduced into an infiltration trench located upgradient from the former underground tank locations and the proposed extraction trench as shown on the Site Plan. This should reduce the time it takes to remediate the groundwater contamination as well as augment degradation of the hydrocarbon contamination in the soil just above groundwater.

Subsurface Consultants, Inc.
171 12th Street • Suite 201 • Oakland, California 94607 • Telephone 415-268-0461 • FAX 415-268-0137

Groundwater Model

The groundwater model developed for the project site was generated using FLOWPATH, an IBM-PC based, two dimensional, horizontal aquifer simulation model, available from Waterloo Hydrogeologic Software in Waterloo, Canada. This computer program utilizes a finite difference method to model two dimensional, steady state horizontal flow. An iterative technique is used to solve the algebraic equations generated by the finite difference grid.

For the purpose of the hydrogeologic model, it was assumed that the aquifer is unconfined and not influenced by evapotranspiration, infiltration or leakage from surface water bodies. In addition, it was assumed that the aquifer is homogeneous and has the same hydraulic conductivity in both the vertical and horizontal dimensions. The effects of pumped wells in the vicinity were neglected.

The proposed extraction trench location (shown on the Site Plan) was selected based upon hydrogeologic conditions and the locations of existing buildings and underground utilities. We assumed the extraction trench would be about 15 feet deep and 240 feet long.

Our interpretation of subsurface conditions at the site based on test borings drilled during our previous investigations, is shown on Plates 2 and 3, Cross Sections. The assumed hydrogeologic parameters for the aquifer and the rationale for each are presented on Table 1.

Table 1. Assumed Hydrogeologic Parameters

Parameter	Value	Rationale
Hydraulic Conductivity, K (ft/day)	50	Based upon grain size analysis
Effective Porosity, n (%)	0.3	Typical for silty sands
Saturated Aquifer Thickness, b (ft)	22	Test Borings

The average pump rate of 6 gpm from the trench represents typical flow rates for the groundwater extraction and treatment facility proposed by CytoCulture and resulted in drawdown depths that were within limits that can be reached by a 15-feet-deep backhoe excavated trench.

Results and Conclusions

The capture zone for the pump rate of 6 gpm, and the assumed hydraulic conductivity of 50 feet/per day is shown on the Site Plan. The model reveals that the contaminated groundwater will be intercepted by the extraction trench, and that significantly greater drawdown occurs with increased pump rates. The model also shows that the capture zone is smaller with greater hydraulic conductivity.

Based on our study, it appears that an extraction trench, with a trench pumping rate of about 6 gpm at the location shown on the Site Plan will be effective at capturing the contaminated groundwater below 98th Avenue, as well as a significant distance downgradient, assuming a hydraulic conductivity of 50 feet per day.

We recommend that after the extraction trench is installed, groundwater data should be obtained from the existing 5 monitoring wells. Once it appears that groundwater levels have stabilized, they should be compared to the results obtained from the model. The model should be refined to accurately simulate the actual drawdown in the monitoring wells. This refined model should then be used to evaluate future remedial actions, such as groundwater reinjection.

If you have questions, please call.

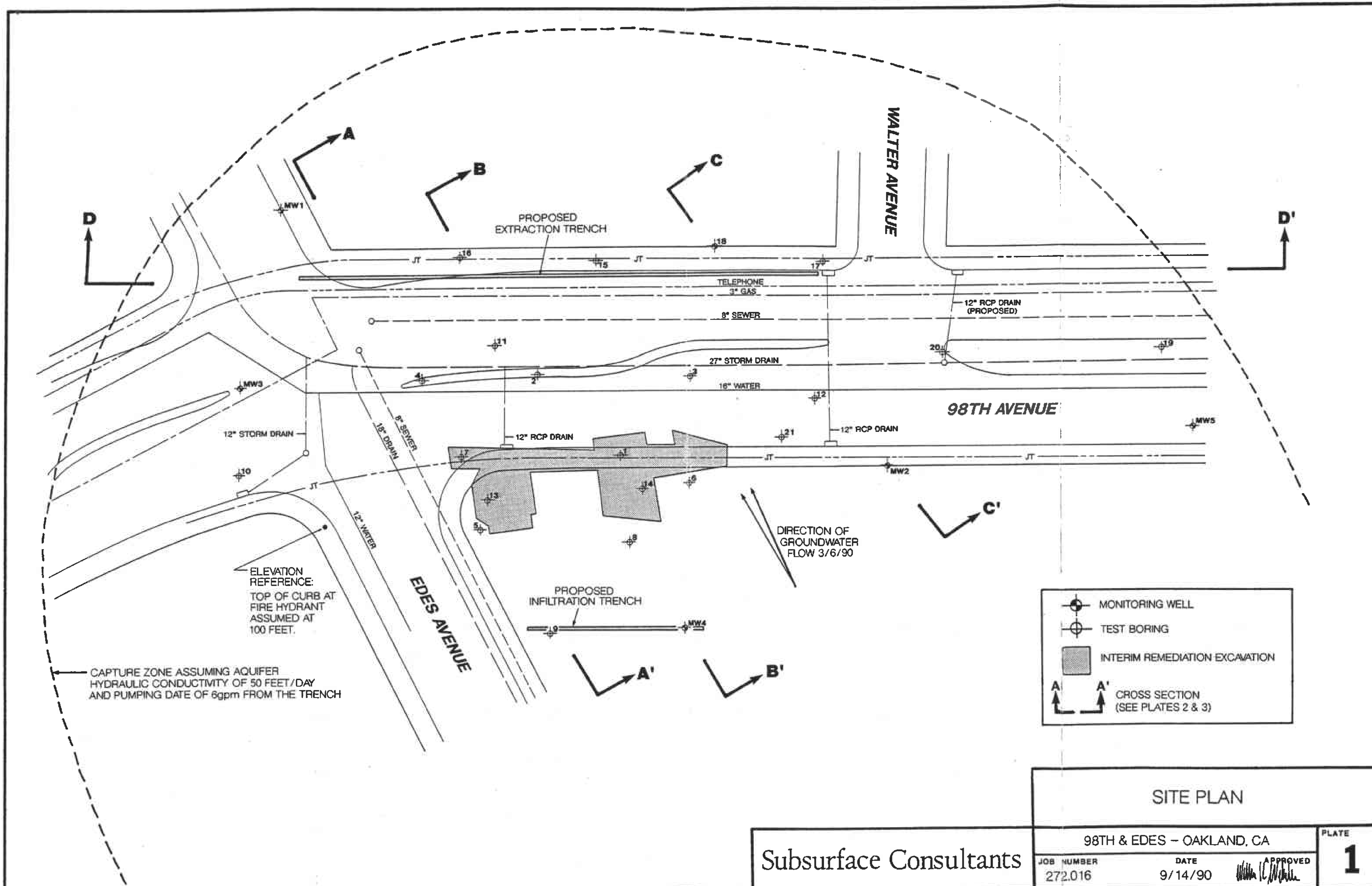
Yours very truly,

Subsurface Consultants, Inc.

R. William Rudolph
Geotechnical Engineer 741 (expires 12/31/92)

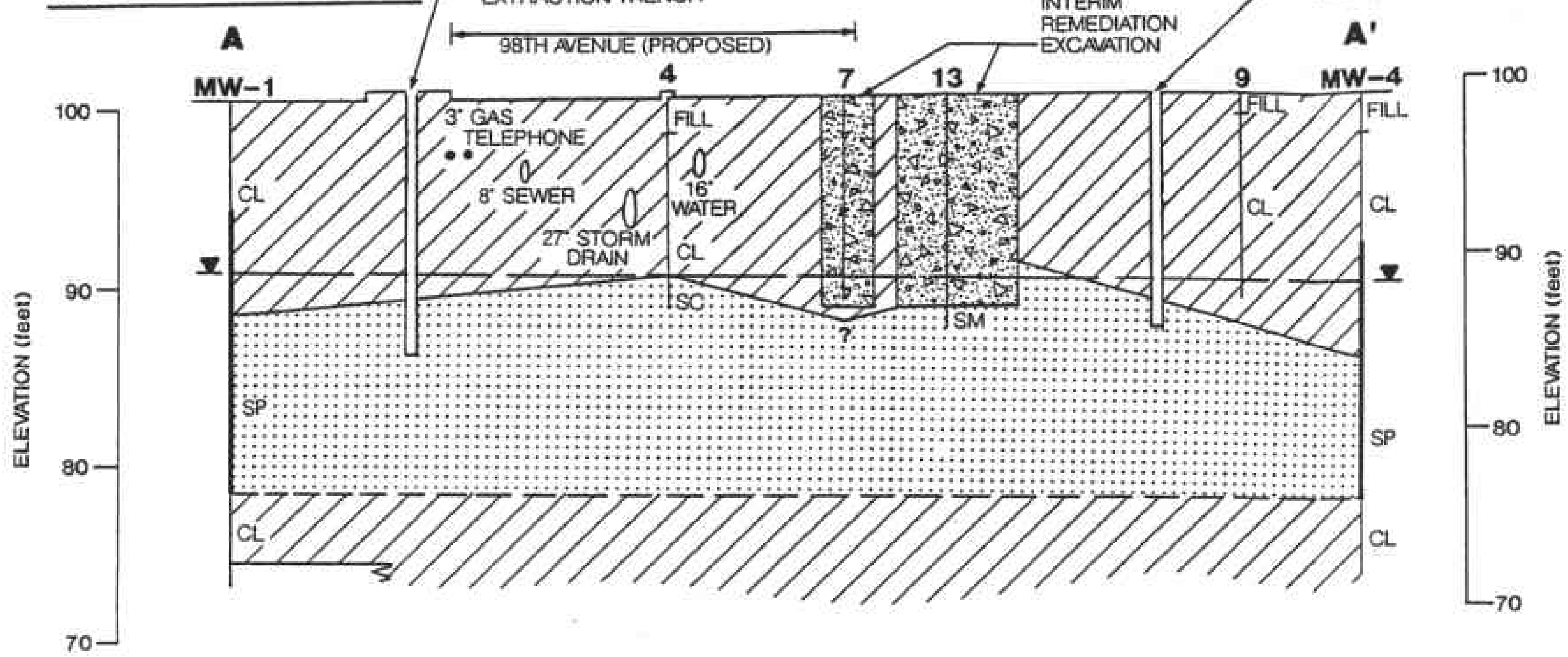
MK:RWR:WKW:ch

Attachments:	Plate 1	Site Plan
	Plates 2 and 3	Cross Sections



A
B
C
D
D'
PROPOSED EXTRACTION TRENCH
WALTER AVENUE
MW1
MW2
MW3
MW4
MW5
JT
TELEPHONE
3" GAS
8" SEWER
27" STORM DRAIN
16" WATER
12" RCP DRAIN (PROPOSED)
98TH AVENUE
12" STORM DRAIN
12" RCP DRAIN
8" SEWER
18" DRAIN
12" WATER
EDES AVENUE
DIRECTION OF GROUNDWATER FLOW 3/6/90
C'
ELEVATION REFERENCE:
TOP OF CURB AT FIRE HYDRANT ASSUMED AT 100 FEET.
PROPOSED INFILTRATION TRENCH
A'
B'
CAPTURE ZONE ASSUMING AQUIFER HYDRAULIC CONDUCTIVITY OF 50 FEET/DAY AND PUMPING DATE OF 6gpm FROM THE TRENCH
MONITORING WELL
TEST BORING
INTERIM REMEDIATION EXCAVATION
CROSS SECTION (SEE PLATES 2 & 3)
SITE PLAN
Subsurface Consultants
98TH & EDES – OAKLAND, CA
JOB NUMBER
272.016
DATE
9/14/90
APPROVED
PLATE
1

CROSS SECTION A – A'



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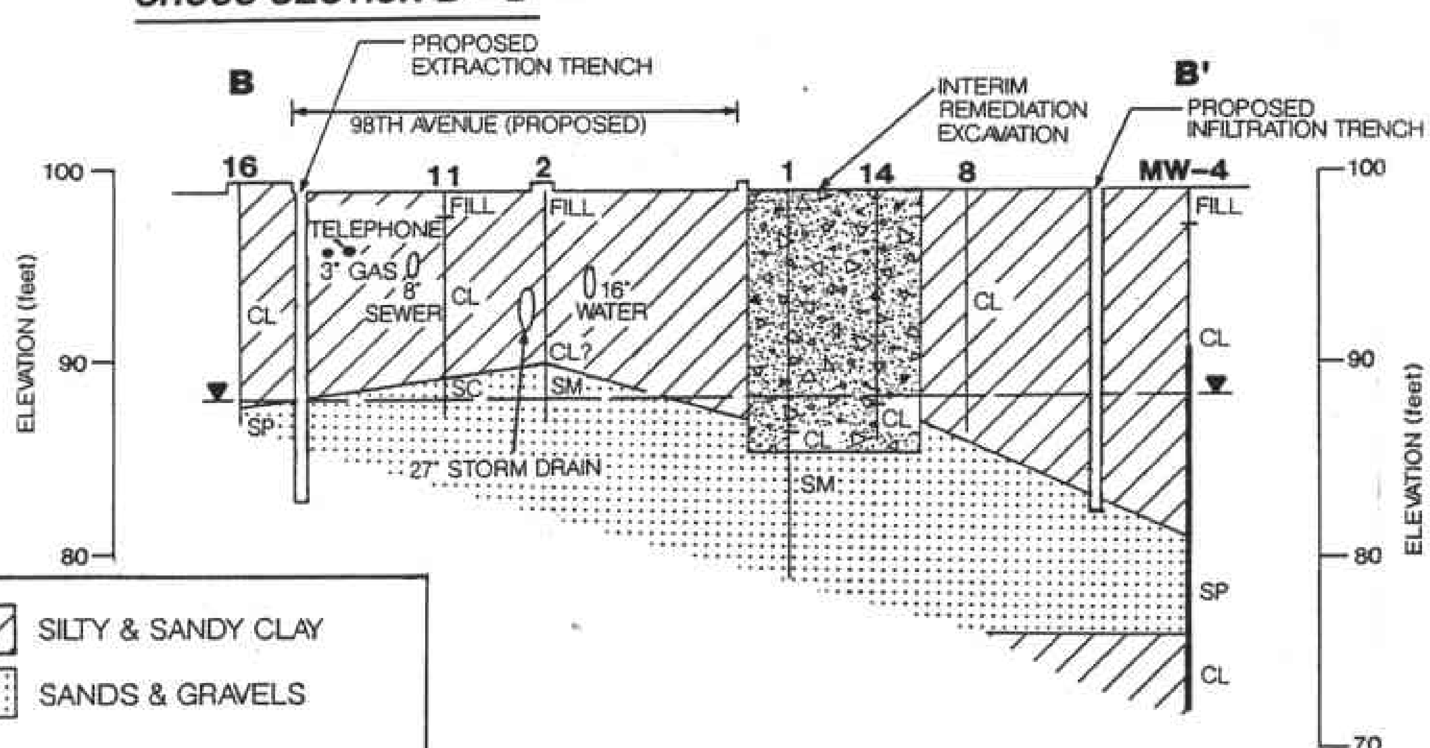

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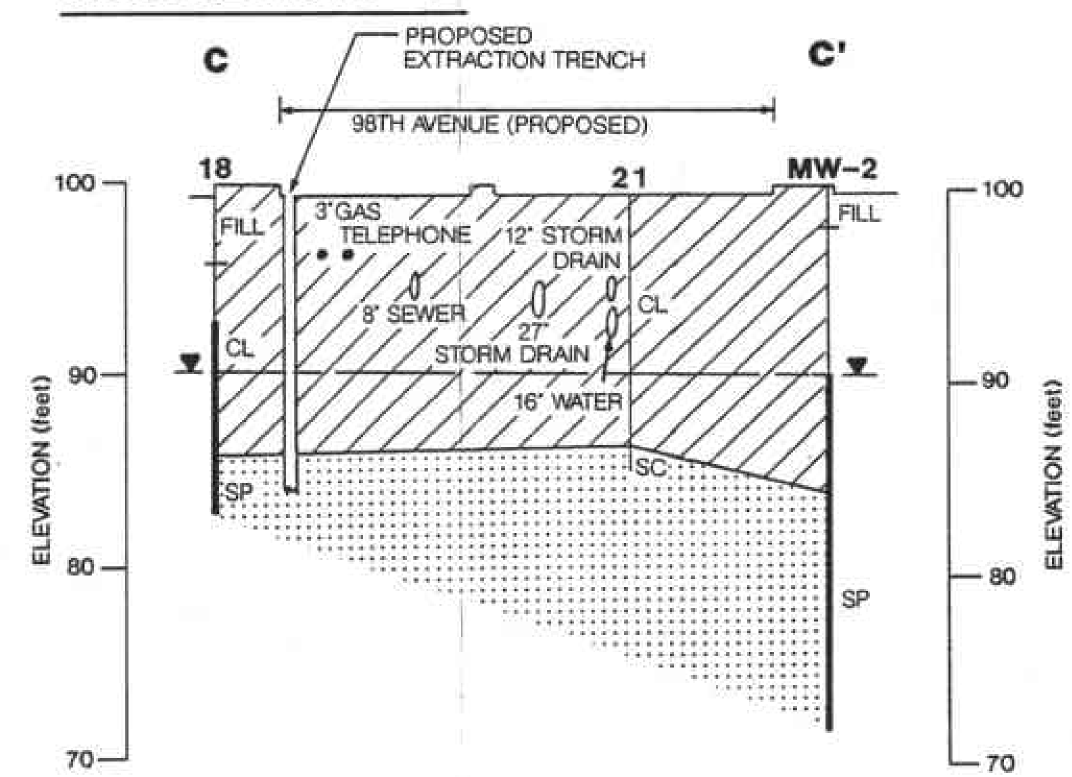

NOTES:

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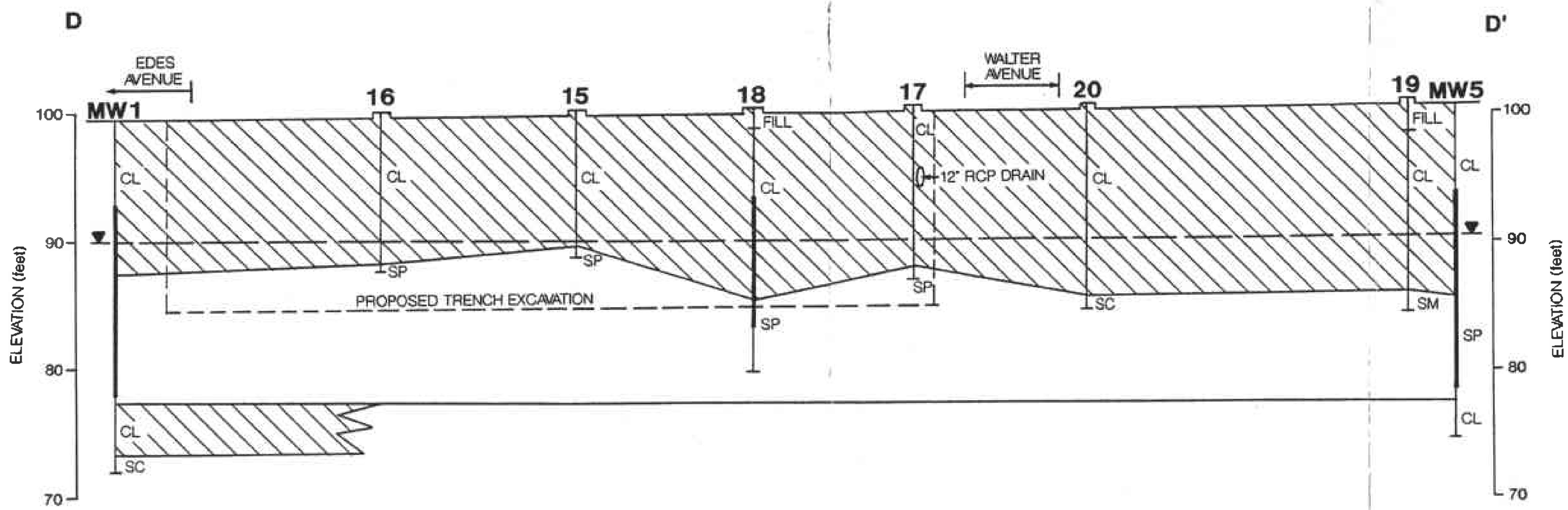
5. The screened interval of wells shown as a thick line.

SILTY & SANDY CLAY

SANDS & GRAVELS

HORIZONTAL SCALE: 1" = 40'
VERTICAL SCALE: 1" = 10'

Subsurface Consultants	CROSS SECTIONS A – A' THROUGH C – C'		PLATE
	98TH & EDES – OAKLAND, CA		2
	JOB NUMBER 272.016	DATE 8/30/90	APPROVED [signature]



HORIZONTAL SCALE: 1" = 40'
VERTICAL SCALE: 1" = 5'

GROUNDWATER LEVEL BASED ON 6/30/90 READING

Subsurface Consultants	CROSS SECTION D – D'		
	98TH & EDES – OAKLAND, CA		PLATE
	JOB NUMBER 272.016	DATE 9/10/90	APPROVED [signature]	3

APPENDIX 2

CYTOCULTURE TECHNICAL LITERATURE

Cyto Culture
ENVIRONMENTAL
BIOTECHNOLOGY
A Division Of CytoCulture International Inc.

September 1990

CytoCulture's Contract Bioremediation Services

CytoCulture is an environmental biotechnology firm based in Point Richmond, CA which performs contract bioremediation. The company uses laboratory-selected strains of naturally occuring bacteria and proprietary bioreactor technology to biodegrade a wide range of petroleum-based contaminants in soil and groundwater. As a California licensed general contractor (No. 563665) and through affiliated environmental engineering firms, CytoCulture manages bioremediation programs from site permitting to site closure by offering:

* Remedial Action Plan Preparation and Permitting
* Mobile Bioreactor Units for Rapid Response Clean-Ups
* Laboratory Scale Bio-treatability Studies
* Pilot-Scale Soil and Groundwater Decontamination Studies
* Fabrication of Bioreactor Systems (500-8,000 gallons)
* Free Product Recovery and Recycling
* Bioremediation of Contaminated Groundwater
* Vacuum Heap Bioremediation of Excavated Soil
* Leachfield Bioremediation of Excavated Soil
* In-Situ Soil and Groundwater Bioremediation
* Clean-Up Assessment and Site Closure

Permitting and Regulatory Affairs

An ongoing dialogue with regulatory agencies is essential to the success of a bioremediation project. Through previous project experience, the Department of Health Services (DHS), the San Francisco Bay Regional Water Quality Control Board (RWQCB), county Environmental Health Departments, and the East Bay Municipal Utility District are all familiar with CytoCulture's bioremediation technology. CytoCulture is collaborating with the DHS Office of Alternative Technology to promote the development and application of bioremediation.

CytoCulture Products

CytoCulture's major products are proprietary bioreactors utilized in many aspects of bioremediation. The bioreactors use intense aeration to maintain high density bacterial cultures, and are available with working capacities from 500 to 8,000 gallons.

124 WASHINGTON AVENUE POINT RICHMOND CA 94801
415 233-6660 FAX 415 233-3777

CytoCulture designs and builds complete bioremediation systems made of chemical and UV resistant plastics. CytoCulture bioreactors are inexpensive relative to conventional steel water treatment systems. Furthermore, the components are easy to ship and assemble into modular configurations for site-specific applications. A mobile bioreactor system, with a 1,000 gallon capacity, is available for emergency responses to surface and/or underground spills. Because many contamination problems result from hydrocarbon spills, CytoCulture's bioremediation systems can be fabricated as closed systems (carbon filter vented) to handle flammable solvents and fuels. Electrical components are kept to minimum and pumps are pneumatically operated. The systems are designed to ensure safety, reliability and low maintenance.

Current Projects

CytoCulture's longest ongoing project involves the bioremediation of hydrocarbon contaminated soil and groundwater in Emeryville, CA. This site, now a shopping center, was formerly a truck terminal. Phase one of the project was designed to decontaminate groundwater. Since the beginning of March '89, 2,000 to 4,000 gallons of contaminated groundwater have been processed through CytoCulture's bioreactor system each day. The hydraulic retention time of of the bioreactors is regulated to allow complete degradation of contamination before discharge (typically 8-12 hours). Influent contamination concentrations as high as 1,400 ppm Total Petroleum Hydrocarbons (TPH) have been reduced to non-detectable levels in the effluent. Aromatic components of the diesel contamination (BTXE) have been reduced from concentrations as high as 300 ppb to non-detectable levels.

In the second phase of this program, clean treated groundwater amended with bacteria and nutrients will be infiltrated into contaminated soil under pavement and buildings on the site. Infiltration of the bacteria upfield of the the contaminated areas should result in the in-situ biodegradation of contamination in the soil downfield. The degradation of hydrocarbon will be facilitated by surfactants produced by the bacteria. Tidal action is strong at the site, and is expected to enhance the distribution of bacteria and nutrients into less accessible areas. This project is being conducted with minimal impact on the appearance, and no alteration of operations, of the shopping center. A similar groundwater project will be started in October for diesel contamination at Santa Clara University.

In excavated soil applications where aggressive action is desired, a leachfield approach is designed for the continual recirculation of a treatment solution containing high density cultures of petroleum-selective aerobic bacteria, biodegradable emulsifiers and nutrients. This "soil washing" process hastens the desorption of heavier petroleum fractions into the aqueous phase and facilitates biodegradation of solubilized hydrocarbon.

The leachfield system may may also be used to extract toxic metals with a combination of dilute organic acids and chelating agents.

In Oakland, CA, CytoCulture was contracted to provide bioreactors and technical field service for the bioremediation of excavated diesel contaminated soil using the soil washing approach. In less than 10 weeks of operation, the average composite TPH decreased from 2,100 ppm to 85 ppm. Site closure was approved with residual hydrocarbon levels of less than 10 ppm.

In excavated soil applications where space is limited or where air pollution is a concern, CytoCulture employs a vacuum heap approach. Vacuum heap bioremediation is a process in which air is drawn through a pile of excavated soil to oxygenate bacteria inoculated into the soil pile. Continual introduction of oxygen, nutrients and water accelerates the biodegradation of the heavier fractions of petroleum contamination with minimal air stripping. As a result, blower exhaust filters may not be needed to trap volatile organics. The entire vacuum heap is covered with plastic to retain moisture and maintain compliance with air quality control laws.

In Oxnard, CA, CytoCulture was contracted by the city of Oxnard to construct a vacuum heap bioremediation system to decontaminate 2,400 cubic yards of soil at a pipeline spill site. As prime contractor, CytoCulture designed and built the soil treatment system to degrade diesel and crude oil. In the first month of treatment, the average TPH values were reduced from 6,700 ppm to 500 ppm. Site closure was approved after three months of operation, with average residual contamination levels of <2 ppm.

Using an augmented landfarming approach in Livermore, CytoCulture provided the bacteria, bioreactors and full technical service to bioremediate 800 cubic yards of soil contaminated with motor oil and diesel. In the first 3 weeks, TPH/TOH contamination was reduced by 33-82% (e.g. from 1900 ppm to 330 ppm). A similar landfarming project involving 2,000 cubic yards of crude oil contaminated clay soils was constructed last month in Oxnard.

CytoCulture is also experienced in the biological treatment of industrial hazardous waste streams. At the Naval Air Station in Alameda, CA, CytoCulture provides technical assistance in treating waste water from paint stripping operations. Using laboratory selected bacteria, phenol and methylene chloride concentrations have been reduced from 1,500 ppm and 1,000 ppm respectively, to less than 10 ppm.

New industrial biotreatment projects, now being developed as bench scale studies, will take CytoCulture to refineries later this year for on-site biodegradation of hydrocarbon and ammonia contaminated waste waters using 20,000 gallon bioreactors.

CytoCulture has also recently established a business alliance with Alpha Environmental (Austin, TX) to employ the petroleum degrading bacteria which were successfully applied to the clean up of the Texas Gulf oil spill in June.

Partial List of CytoCulture Bioremediation/Industrial Clients

PRIVATE SECTOR

Erickson Environmental, Inc. (Benicia, CA)
Staal, Gardner & Dunne, Inc. (Ventura, CA)
P.I.E. Nationwide, Inc. (former Emeryville terminal)
Blymyer & Sons Engineers, Inc. (Alameda, CA)
Chevron USA (Richmond, CA refinery)
Mobil Oil Co. (Torrance, CA refinery)
Certified Engineering, Inc. (San Francisco, CA)
Aquascience Engineers, Inc. (San Ramon, CA)
Transtech Consultants, Inc. (Santa Rosa, CA)
Kaldveer Associates, Inc. (Oakland, CA)
Subsurface Consultants, Inc. (Oakland, CA)
Brown & Caldwell, Inc. (Walnut Creek, CA)
Martin Properties, Inc. (Emeryville, CA)
Sequoia Station Developers, Inc. (Sausalito, CA)
Vintage Properties, Inc. (Alameda, CA)
Greyhound Lines, Inc. (Oakland terminal, CA)

PUBLIC SECTOR

U.S. Naval Air Station (Alameda, CA)
City of Oxnard, CA - Dept. Public Works & Engineering
City of Oakland, CA - Dept. Public Works

Corporate Affiliates

Erickson Environmental, Inc. (Benecia, CA)
Alpha Environmental, Inc. (Austin, TX)
Sybron Biochemical Division, Sybron Chemicals, Inc.(Salem,VA)

Insurance

CytoCulture is listed as "also insured" on Sybron Chemical's General Liability Insurance Policy (Certificate of Insurance indicating client by name) for standard $1 million coverage. Through a business relationship with Erickson Environmental, CytoCulture can also offer contractor's Pollution Liability Insurance coverage for an additional cost, listed as a line item in the project budget. CytoCulture carries standard auto and full Workman's Compensation insurances.

General Counsel

Fenwick, Davis & West (Palo Alto, CA)

CytoCulture
ENVIRONMENTAL BIOTECHNOLOGY
A Division Of CytoCulture International Inc.

EnSol'90 Conference: Santa Clara, CA September 12-14, 1990

IN SITU AUGMENTED BIOREMEDIATION OF HYDROCARBON CONTAMINATED GROUNDWATER USING LABORATORY SELECTED BACTERIA

Randall J. von Wedel, Ph.D.
CytoCulture Environmental Biotechnology
Point Richmond, CA

Lois Davis
Sybron Biochemical
Salem, Virginia

Laboratory selected strains of hydrocarbon degrading aerobic bacteria can significantly improve the effectiveness of soil and groundwater bioremediation. By culturing naturally occuring strains under rigorous survival conditions, enrichment isolates were selected to utilize specific hydrocarbon fractions as their sole carbon and energy sources. These acclimated strains have proven to be more efficient at metabolizing petroleum hydrocarbons, more resistant to toxic levels of contaminants and more likely to grow to high cell densities than indigenous strains often found on clean-up sites. Further, the facultative organisms selected for _in situ_ projects continue to degrade hydrocarbons in the low oxygen conditions typical of aquifers.

CytoCulture has developed high density, airlift suspension culture bioreactors for continuous flow treatment of diesel and gasoline contaminated groundwater on site. These bioreactor systems maintain flow rates of 2-5 gpm per 1,000 gallon modular unit, cleaning hydrocarbon saturated water to drinking water standards (non-detectable levels) with respect to BTXE and TPH. Routine re-inoculation with laboratory selected organisms and automated process control assures a maximum density of hydrocarbon degrading organisms over long periods of time.

In situ soil and groundwater projects employ trenches or extraction wells to recover floating free product and contaminated groundwater via pneumatically operated well pumps. Oil/water separators collect free product for recycling. Cleaned bioreactor discharge water, laden with nutrients and bacteria, is then re-infiltrated into the aquifer upfield of the contaminated zones. Hydraulic control of the reinfiltrated discharge water is maintained with the cone of depression generated by the extraction wells and trenches downfield. Surveillance of monitoring wells and treatment influent is used to confirm _in situ_ nutrient levels, bacterial culture densities and hydrocarbon degradation kinetics. A gasoline contaminated site in Riverside reached acceptable levels in less than 1 year.

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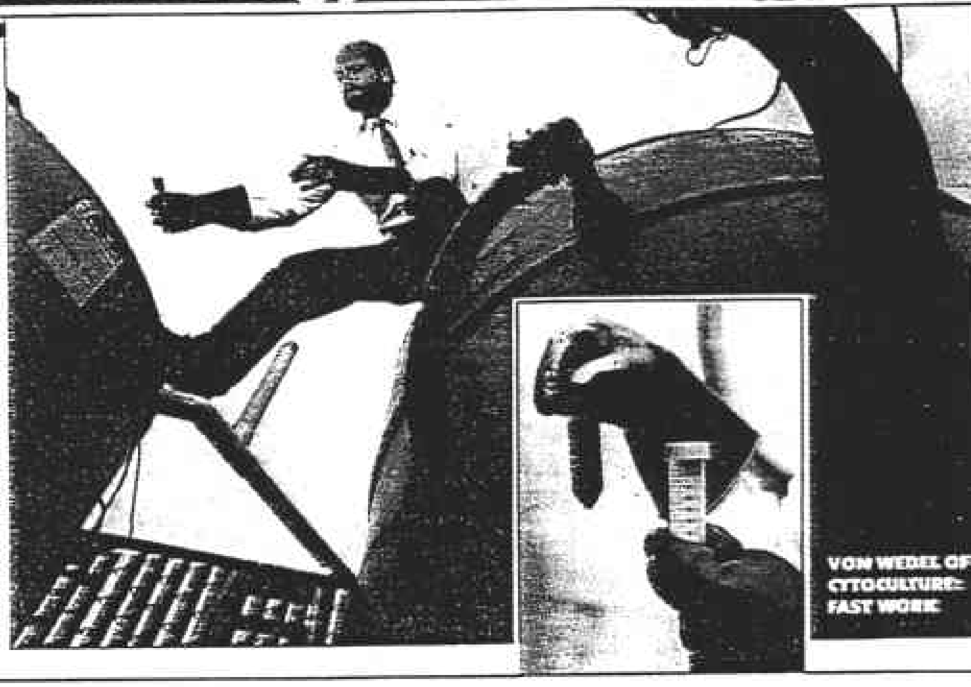
BusinessWeek

JUNE 4, 1990 A McGRAW-HILL PUBLICATION $2.00

Science & Technology

ENVIRONMENT

THE TINIEST TOXIC AVENGERS



VON WEDEL OF CYTOCULTURE: FAST WORK

More cleanup companies are using bacteria that gobble up wastes

Of the 1,100 miles of Alaskan shoreline fouled last year by the Exxon Valdez, few places suffered more than Passage Cove on Prince William Sound's Knight Island. Within days, oil seeped nearly two feet into its gravel-and-sand beaches. Even hot-water sprays didn't help much. By mid-July, says Robert L. Mastracchio, Exxon Corp.'s technical manager in Alaska, the shore was still "black and gooey."

That's when scientists decided to incite the natives—microorganisms that live in soil and water. They sprayed the beach with fertilizer, hoping that adding nutrients would stimulate the naturally occurring bacteria to feed on the gunk. Three weeks later, the fertilized areas were nearly clean of oil for a foot down, while untreated areas wore a sticky coat. And researchers found that the population of voracious bugs in the fertilized soil had increased a hundredfold.

'THIS WORKS.' The Valdez cleanup, while far from complete, is the biggest success yet for a technique called bioremediation, which uses nature's tiniest creatures to clean up mankind's biggest messes. "We've proven that this works," says scientist John A. Glaser of the Environmental Protection Agency. On May 2, Alaskan officials gave Exxon approval to fertilize 35 more miles of spoiled shoreline this summer.

Now, more than three dozen cleanup companies are turning to organisms that scarf up everything from diesel oil to highly toxic polychlorinated biphenyls (PCBs) and heavy metals, which were once thought to be impervious to decontamination. But it turns out that "there are bacteria that will eat anything," says Richard C. Cassin, founder of San Diego startup Bioremediation Inc.

This year, the market for bioremediation products and services is only about $30 million, says Concord (N. H.) environmental consultant William T. Lorenz. But it may be ready to bust loose. Some companies are netting contracts of more than $1 million, far higher than the $250,000 or so that was common just two years ago. Venture capitalists are beginning to fund a few startups, and even traditional waste-treaters such as IT Corp. are using more bioremediation.

The trend comes just in time. The EPA estimates that conventional cleanups of some 1,200 U. S. Superfund sites, areas of extreme contamination, would cost $24 billion. Some methods, such as incinerating contaminated soil, can run up to $1,000 per ton—and are under attack from both regulators and the public as potentially unsafe. Bioremediation, by contrast, typically costs less than $100 a ton. It also offers a big advantage: Instead of simply relocating the problem, bacteria eliminate it. And bioremediation may be safer: It has been used in wastewater-treatment plants—and even outhouses—for half a century.

EAT AND DIE. To obtain the right cleanup bugs, scientists typically take soil or water samples from a toxic site—Cassin has even scraped oil deposits off his driveway—and grow the microorganisms they contain in a lab. Some of these bacteria feed on the carbon atoms that make up organic chemicals, usually breaking the chemicals down to carbon dioxide and water. Researchers can then breed strains that depend on a contaminant to live and that will die off once the food source is gone. That way, there's little risk that they'll run amok.

The quickest way to treat contaminated soil is to excavate it, mix it up with water, nutrients, and bacteria on a plastic sheet, and pump air through it. In early 1989, Groundwater Technology Inc. in Norwood, Mass., used this method to clean up an oily mess at a Texas oil-storage facility in eight weeks. Contaminated groundwater is usually treated in a "bioreactor"—a tank containing specially selected bugs. Randall J. von Wedel, president of Cytoculture International Inc. in Point Richmond, Calif., hopes his company can shave months off a two-year project by pumping bacteria-laden water back into ground that has been contaminated with diesel fuel.

For all the recent success, bioremediation faces huge obstacles before it becomes the preferred pollution treatment. It usually takes longer for bacteria to work than for soil to be hauled away or incinerated, and bugs often stop munching before the contaminant is gone. One problem: They need nutrients and oxygen. "You can't just take a bag of bacteria and throw it on the ground," says Roger J. Colley, president of Envirogen Inc., a Lawrenceville (N. J.) startup.

Now, scientists are finding anaerobic bacteria, which can survive without oxygen. For instance, Woods Hole Oceanographic Institution in Massachusetts has discovered anaerobic bacteria living near warm water vents 6,000 feet deep in the Gulf of California that can degrade naphthalene, a stubborn hydrocarbon. And General Electric Co. has found both anaerobic and oxygen-dependent bugs that could help clean up 500,000 pounds of PCBs in a 40-mile stretch of the Hudson River in upstate New York.

HELPFUL FUNGUS. Another limitation for today's tiny toxic avengers: Bugs usually attack only one contaminant. So they may be useless in some of the worst waste sites, which contain many different toxins. One idea is to use genetically engineered organisms for these. Envirogen is exploring the insertion of several remediation genes into *Escherichia coli* bacteria, perhaps the most common around. But regulators must approve the use of any genetically engineered organisms. The Electric Power Research Institute, looking to clean up power-plant waste, hopes to avoid that by adjusting environmental factors, such as nutrients and oxygen, to get organisms to exchange genetic material naturally.

The search is also on for bioremediation bugs to tackle even tougher problems. Bacteria found at the Energy Dept.'s Hanford Reservation nuclear facility in Washington State keep radioactive materials such as cesium and uranium attached to rocks and soil—and out of groundwater. And researchers at the University of California at Riverside have found a fungus that detoxifies selenium, a metal that causes birth defects in migrating birds in California's Central Valley. Last month, a University of Illinois professor even described a bacteria-produced detergent that could be sprayed on beaches before a spill arrives, to prevent oil from sticking.

Some environmentalists still have reservations: In Alaska, they fear that fertilizers used to stimulate bugs may harm wildlife. They also worry that business may simply see bioremediation as a way to avoid more thorough cleanups. But GE scientist Daniel A. Abramowicz disagrees: His studies show that natural bacteria are already slowly eating some PCBs in the Hudson river. The challenge now is to help them along.

By Robert D. Hof in San Francisco

BIOREMEDIATION: A BARGAIN FOR CLEANING UP SOIL

$50-$100 a ton
BIOREMEDIATION

$50-$100 a ton
CHEMICAL STABILIZATION

$200 a ton
THERMAL DESORPTION

$300-$400 a ton
INCINERATION (ON-SITE)

$1,000 a ton
INCINERATION (OFF-SITE)

DATA: REMEDIATION TECHNOLOGIES INC.

BIOTECHNOLOGY AND HAZARDOUS WASTE, pp. 1-12

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GENETIC ENGINEERING GEN NEWS

THE SOURCE OF BIOPROCESS/BIOTECHNOLOGY NEWS
Volume 8 Number 9 October 1988

CytoCulture Cleans Up Contaminated Sites with Selected Bacterial Strains

By Fred Gebhart

Some forms of pollution don't have to be forever. Recent developments in bioremediation now offer new hope for the application of biotechnology to hazardous waste treatment and the cleanup of contaminated soil and groundwater. Just as bacteria and other microorganisms have adapted to decompose natural wastes in soil, laboratory selected bacteria can metabolize petroleum products and a wide variety of organic compounds into carbon dioxide and water.

Randall J. von Wedel, Ph.D., a biochemist and the founder of CytoCulture International, Inc. (San Francisco, CA), has developed pollution cleanup strategies that combine the latest advances in aerated bioreactor designs with blends of bacterial strains selected to biodegrade specific organic compounds. The technology shows promise of being able to clean up a wide range of carbon-based liquid pollutants.

Dr. von Wedel's biotech startup firm performs cleanup contracts and hazardous waste treatment for industrial clients using a process called "Augmented Bioreclamation" (ABR). The process is licensed from their strategic alliance partner, Sybron Chemicals, Inc. of Birmingham, NJ. Sybron also provides the commercial strains of bacteria for common pollutants such as petroleum hydrocarbons and some solvents. CytoCulture develops its own bacterial isolates to degrade substrates for which commercial strains are not yet available. The company is currently staffed with one other principal and four associates.

"An increasing amount of industrial waste or existing environmental pollution can be treated biologically," Dr. von Wedel explains. "We aren't even using particularly sophisticated technology," he continues. "We're implementing sophisticated ideas."

Bacterial digestion of waste is nothing new. Sewage treatment plants have been doing it on a huge scale for more than a century. Nor is the use of bacteria to degrade manmade pollutants new. Enhanced bioremediation, adding oxygen and nutrients to a contaminated site to foster the growth of naturally occuring bacteria, is a relatively established process.

Special Blends

CytoCulture has augmented this biological enhancement process by inoculating contaminated soil and water with specialized blends of bacteria. In addition to enhancing existing bacterial growth, augmented bioreclamation (APR) adds selected bacterial strains to the soil or water.

ABR is most frequently employed for cleaning up soil and groundwater contaminated with heavier fractions of petroleum hydrocarbons like diesel and fuel oil. Conventional treatments using air stripping and soil venting of volatile fuel simply don't work for long chain hydrocarbons.

Sand is a perfect soil type for decontamination—easily permeable to water, oxygen and, of course, the fuel-eating bugs. Real-world pollution more often occurs in denser soils where water, oxygen and bacteria penetrate more slowly.

In these conditions, the ABR technology is supplemented with biodegradable surfactants to help desorb the hydrocarbon into the aqueous phase.

Gasoline-contaminated groundwater in one southern California project showed a 99% decrease in specific and total hydrocarbon levels in 10 months. The traditional way to treat gasoline contamination is to haul the contaminated material to a hazardous waste dump—at a cost of up to $300 per cubic yard and involving up to 1,000 cubic yards per site. According to Dr. von Wedel, CytoCulture can do the job for less than $60 per cubic yard.

The bacteria that do the job are chosen from a library of dozens of strains that were selected and grown in commercial quantities by Sybron Biochemical, a Virginia-based subsidiary of Sybron Chemicals, Inc. "We're working with sole carbon-source enrichment isolates," Dr. von Wedel explains. Pre-treatment lab studies match specific mixture and concentration of pollutants to be detoxified. Treatment typically requires a mixture of two to five different strains of adapted aerobic bacteria (*e.g., Pseudomonas*).

Most of the strains were originally isolated from contaminated sites such as oil refineries and chemical plants. "From the perspective of bioremediation," Dr. von Wedel says enthusiastically, "bacteria are an endless treasure of degradative enzymes."

Enrichment Isolates

Individual strains are selected as enrichment isolates which can utilize specific compounds as their sole carbon source for metabolism. Examples of typical substrates degradable by commercial strains include benzene, toluene, xylene, anthracene, naphthalene, straight and branched chain hydrocarbons, phenols, cyanide and a variety of organic solvents. None of the strains has been altered by any means other than rigorous selection, according to Dr. von Wedel.

Starting with naturally-occuring bacteria has helped CytoCulture avoid the kind of questions and protests that have plagued researchers working with altered organisms. Once a potential biodegradent has been selected for tolerance to specific pollutants, a wide range of pH, temperature and salinity, and high background levels of heavy metals and other toxins, it is screened for sensitivity to 13 common antibiotics. Any strain that shows signs of resistance is destroyed.

Since bacteria used in augmented bioremediation are highly selected for specific carbon sources, adaptation to high concentrations of certain toxic compounds gives them enormous survival advantages over the background native soil populations. Once that preferred substrate has been depleted, the adapted strains lose their competitive edge and the less specialized forms dominate the culture.

There's more to augmented bioremediation than spreading a few hundred pounds of dried bacterial culture on contaminated soil. The detoxification process depends on the size and nature of the contamination.

Primary source pollution, usually an industrial wastestream, can be relatively easy to treat if a proper wastewater treatment facility is in place. CytoCulture currently assists in the pretreatment of industrial wastes ranging from methylene chloride and phenol at a military paint stripping facility to residual oils and ammonia at a major refinery along San Francisco Bay.

Typically, the first step in processing an industrial wastestream is to remove heavy metals and neutralize the pH. Residual organics and ammonia are then fed into fixed film, continuous flow bioreactors designed and built by CytoCulture.

The 1,000-gallon modular units are made of cross-linked polyethylene and designed for automatic operation and remote monitoring. Dr. von Wedel built up his experience in bioreactor design and process control from his three years of managing a consulting business in cell culture scale-up technologies for the biomedical community. CytoCulture still maintains a strong consulting role in assisting other biotechnology companies engaged in bioreactor scale-up processes for mammalian cells, from initial design modifications for manufacturers to selection of equipment for end-users. About half of their clients in the biomedical field are overseas. The firm also provides consulting

CytoCulture's in situ bioremediation site for diesel contaminated soil and groundwater at a former truck terminal. Dr. von Wedel holds a modular aeration unit from one 2,000 gallon bioreactor.

service to the University of California (San Francisco) in bioreactor scale-up technology for mammalian cells.

Automated all-plastic bioreactors are a step up from the manually controlled steel batch reactors most bioremediation firms use. Oxygen transfer and uniform mixing have been optimized with a proprietary aeration system in combination with draught tubes. These wastestream bioreactors were first developed for decontaminating former industrial sites. Areas which have already been contaminated by prior use or pollutant spills present more complex problems. Remediation is typically divided into two stages, cleanup of groundwater and cleanup of soil.

Test Wells

Test wells are drilled around the site to determine the extent and nature of contamination. The most common contaminants are fuels or solvents, which float on groundwater. Contaminants also adhere to soil particles and diffuse into groundwater, providing a lasting pollutant source after a primary source such as a leaking underground tank has been removed. Once the contamination plume has been mapped, wells and trenches are used to draw off contaminated groundwater. The water is cleaned in bioreactors.

If contaminated soil can be excavated, it is detoxified in what CytoCulture calls a vacuum heap. The soil to be treated is removed and layered with perforated plastic piping atop an impermeable layer of plastic. The detoxified groundwater is enriched and inoculated with a concentrated bacterial culture and reinfiltrated back into the contaminated soil. Oxygen is supplied by applying a vacuum to the piping and sucking ambient air through the pile. Supplied with water, nutrients and oxygen, the bacteria metablolize the carbonaceous contaminants and detoxify the soil. Once contaminant levels have dropped to acceptable levels, the soil is returned to the excavated site.

But polluted soils can't always be excavated. Contaminant plumes tend to move with the groundwater flow, which is often under buildings, freeways and other structures which cannot be removed. The soil must be treated in place.

Detoxified groundwater is inoculated with the appropriate bacterial culture and nutrients, then returned to the groundwater flow upstream of the contaminated area via a leach field.

Dr. von Wedel has found that oxygen is usually the limiting factor in *in situ* remediation. CytoCulture is exploring alternatives to direct aeration for achieving oxidative biodegradation of contaminants in low permeability soils.

Refractory Hydrocarbons

Chlorinated hydrocarbons are a particularly refractory class of organic molecules. Highly chlorinated compounds such as insecticides, wood preservatives (*e.g.*, pentachlorophenol (PCP)) and PCBs are highly resistant to biological degradation because the chlorine residues block the active sites of most enzymes.

At least two bioremedial strategies have emerged to address this problem. Many bacteria can utilize a particular hydrocarbon but not its chlorinated derivatives. When the bacteria are supplied with the unsubstituted hydrocarbon as well as the chlorinated form, it uses both. Dr. Dennis Focht of the University of California (Riverside) has reportedly used a strain of *Acinetobacter* to metabolize PCB by providing unchlorinated biphenyl as a growth substrate.

Another alternative is to remove some or all of the attached chloride by chemical or physical means (such as exposure to strong ultraviolet light) before complete biological decontamination.

CytoCulture has contracted with an Australian firm to commercialize the "CytoLight Process" for the complete, on-site destruction of chlorinated hydrocarbons at Superfund sites. This technology will be licensed to a subsidiary to be owned 51% by CytoCulture. Other corporate equity partners and the Australian biotechnology company hold the remaining shares of the subsidiary. CytoCulture will establish the venture firm at a university corporate research center on the east coast. ■

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BACTERIAL BIODEGRADATION OF PETROLEUM HYDROCARBONS IN GROUNDWATER: *IN SITU* AUGMENTED BIORECLAMATION WITH ENRICHMENT ISOLATES IN CALIFORNIA

R. J. von Wedel*, J. F. Mosquera*,****, C. D. Goldsmith**, G. R. Hater**, A. Wong**, T. A. Fox***, W. T. Hunt***, M. S. Paules***, J. M. Quiros*** and J. W. Wiegand***

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Commercial blends of enrichment isolates of aerobic bacteria are being used for in situ biodegradation of petroleum hydrocarbons contaminating soil and groundwater at several industrial sites in California. In situ site bioremediation programs have been developed to continuously treat contaminated groundwater in above-ground chemostats maintained with high density cultures of selected strains of Pseudomonas bacteria. After treatment in the bioreactors removes free product and lower levels of dissolved phase hydrocarbon, the water is mixed with additional cultures of bacteria and reinfiltrated into the ground to augment the in situ biodegradation of petroleum fractions absorbed to the soil. Preliminary results of one bioreclamation project and plans for additional projects are presented to demonstrate the potential of this alternative technology for site remediation of aquifers contaminated with fuels, solvents, and other petroleum products for which specific bacterial cultures are being developed.

CULTURE DEVELOPMENT

Enrichment culturing techniques were used to isolate individual strains of bacteria from petroleum contaminated soils at other sites. The contaminated soils are used as an inoculum in aerated shaker flasks to grow out populations which survive on minimal salts media with a particular hydrocarbon (e.g., toluene) as a sole source of carbon. After three days, the culture is diluted into fresh minimal salts media with the specific sole carbon source hydrocarbon, grown up for 8-13 days and transferred again. After four transfers, the flask culture is streaked onto agar plates containing the sole carbon source hydrocarbon with minimal salts. Colonies which displayed rapid growth were then tested for their resistance to a panel of thirteen common antibiotics. Organisms which exhibited resistance to antibiotics were destroyed. Several substrate concentrations are monitored for biodegradation and growth rates in batch cultures. For example, the aromatic ("BTX") strains used in these field studies had maximum substrate utilization rates of 0.13 - 0.26 /hr, first order specific substrate utilization rates of 4.5 - 6.9 x 10^-3 L/mg-hr, maximum growth rates of 0.36 to 0.90/hr and Ks values ranging from 30 - 38 mg/L (SEE Poster Presentation by C.D. Goldsmith and R.K. Balderson, pp. 505-507). After further characterization and biokinetic studies with different hydrocarbon substrates, the antibiotic sensitive strains were expanded to commercial quantities at the Sybron Chemical production facility in Salem, VA.

These commercial petroleum strains, most of which have been identified as Pseudomonas aeruginosa, are blended to provide the capability of biodegrading various straight chained, branch chained, aromatic and polynuclear aromatic hydrocarbons in diesel, gasoline and motor oil. The blend of cultures used for these bioreclamation projects includes strains selected for the biodegradation of benzene, toluene, xylene, anthracene and napthalene. Other strains have been developed using similar enrichment techniques for the biodegradation of phenols, cyanides, and many solvents.

GROUNDWATER TREATMENT METHODS

Contaminated groundwater is collected through a network of french drains and treated in a series of chemostat bioreactors designed to maximize the residence time of the exogenous bacterial cultures. Free product, if any, is removed automatically by a continuous oil/water separator upstream of the bioreactor system. Flow rates of 2 to 10 gallons per minute are maintained for months through the bioreactors as bacteria multiply on the continual influx of hydrocarbon substrate. Supplemental nutrients (nitrogen and phosphorus sources) are dispensed automatically. Weekly samples are taken to check the culture chemistries (dissolved oxygen, nutrients) and monitor the levels of hydrocarbon passing through the system. Flow rates are controlled to optimize the biodegradation of hydrocarbons in accordance with the substrate utilization rates and growth rates established for the specific blends of bacteria. In addition to their preference for specific hydrocarbon substrates, the augmented bacterial populations can be maintained at up to two orders of magnitude greater densities than indigenous populations, thereby greatly improving the efficiency of the bioreclamation process.

IN SITU SOIL BIORECLAMATION

Reclaimed, treated water can be used to introduce bacterial cultures directly into contaminated soils and aquifers. Bioreactor effluent water which has attained low levels of dissolved hydrocarbon (permissible for discharge) is blended with additional bacterial cultures and nutrients. The culture slurry is then aerated and pumped through an infiltration system of leachfield pipes installed above the contaminated zones. This continuous infiltration of high concentrations of enriched bacterial cultures allows for extensive in situ biodegradation of petroleum hydrocarbons remaining in the soil and at the moving soil/groundwater interface. Dissolved oxygen appears to be a rate-limiting factor in some soils; experiments are underway to improve aeration or introduce dilute oxidizing agents with the reinfiltration water as a means of increasing available oxygen to the subsurface cultures.

PRELIMINARY GROUNDWATER RESULTS

A petroleum products distribution site with extensive gasoline leakage in southern California has recently undergone treatment using Sybron bacterial cultures. An augmented bioreclamation program was initiated so as to reinfiltrate the exogenous bacteria directly to the contaminated groundwater through a gravel bed. Soil and groundwater contamination levels of petroleum hydrocarbons and aromatics were decreased dramatically over a period of several months of continuous bioreactor operation and treated water recirculation. Preliminary results for the groundwater cleanup at this site are shown in Figures 1 - 4 and are summarized as follows:

Petroleum Fraction	% Decrease During Treatment
Benzene	85%
Toluene	98%
Xylene	33%
Total Hydrocarbons	93%

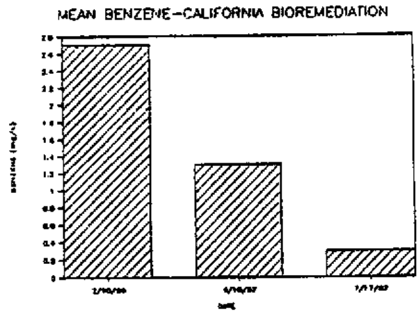


FIGURE 1

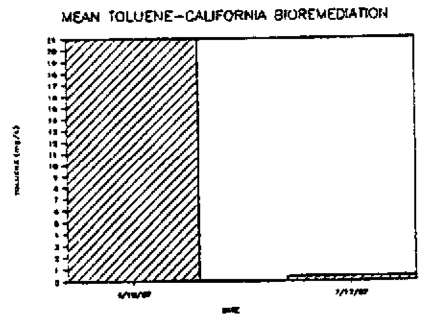


FIGURE 2

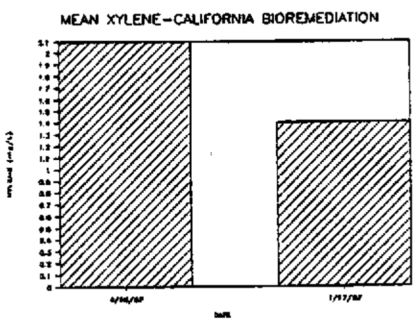


FIGURE 3

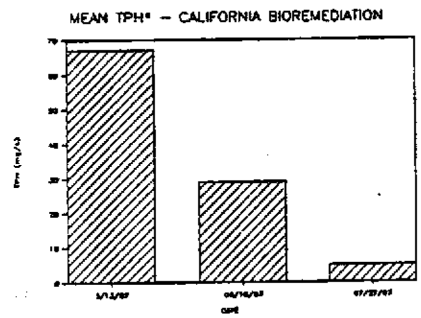


FIGURE 4
*TPH - Total Hydrocarbons.

NEW SOIL/GROUNDWATER PROJECT

A new augmented bioreclamation project using Sybron bacteria is getting underway at a former trucking terminal in Northern California. This site is located adjacent to the San Francisco Bay and involves extensive diesel contamination of the groundwater and soil. Free product has been reported on the brackish groundwater and it fluctuates with the rise and fall of the tides. Soil contamination within the saturated zone approaches 8,000 ppm total petroleum hydrocarbons.

Immediate site remediation plans call for removal of free product and continuous treatment of the extracted groundwater with enrichment isolates as described above. Free product will be collected automatically by oil/water separators at the depression wells. Three bioreactor systems, with a total water treatment capacity of 2,500 gallons each, will process contaminated water drawn from a series of french drain trenches installed downstream from the free product plume. Retention times and bioreactor conditions will be optimized to assure that levels of dissolved petroleum hydrocarbons in the discharge water fall below the required limit of 100 ug/l. Polynuclear aromatics will be controlled at less than 15 ug/l.

Plans for in situ treatment of the contaminated soil involve the reinfiltration of the treated discharge water with enriched cultures of bacteria and nutrients. A reinfiltration gallery of leachfield pipes has been designed to maximize the distribution of bacteria through the unsaturated zones of fill above the contaminated areas of soil and groundwater. A net circulation of 6 - 12 gal/min of treated water through the reinfiltration system should establish a gradient of bacteria over the contaminated areas. Extensive sampling of 16 surrounding monitoring wells will allow for an evaluation of the bacterial migration and biodegradation.

APPENDIX 3

Sybron Chemicals Technical Literature

Bioremediation Removes Gasoline Residues

In-place biological degradation is one way to clean up aromatic compounds in groundwater and soil.

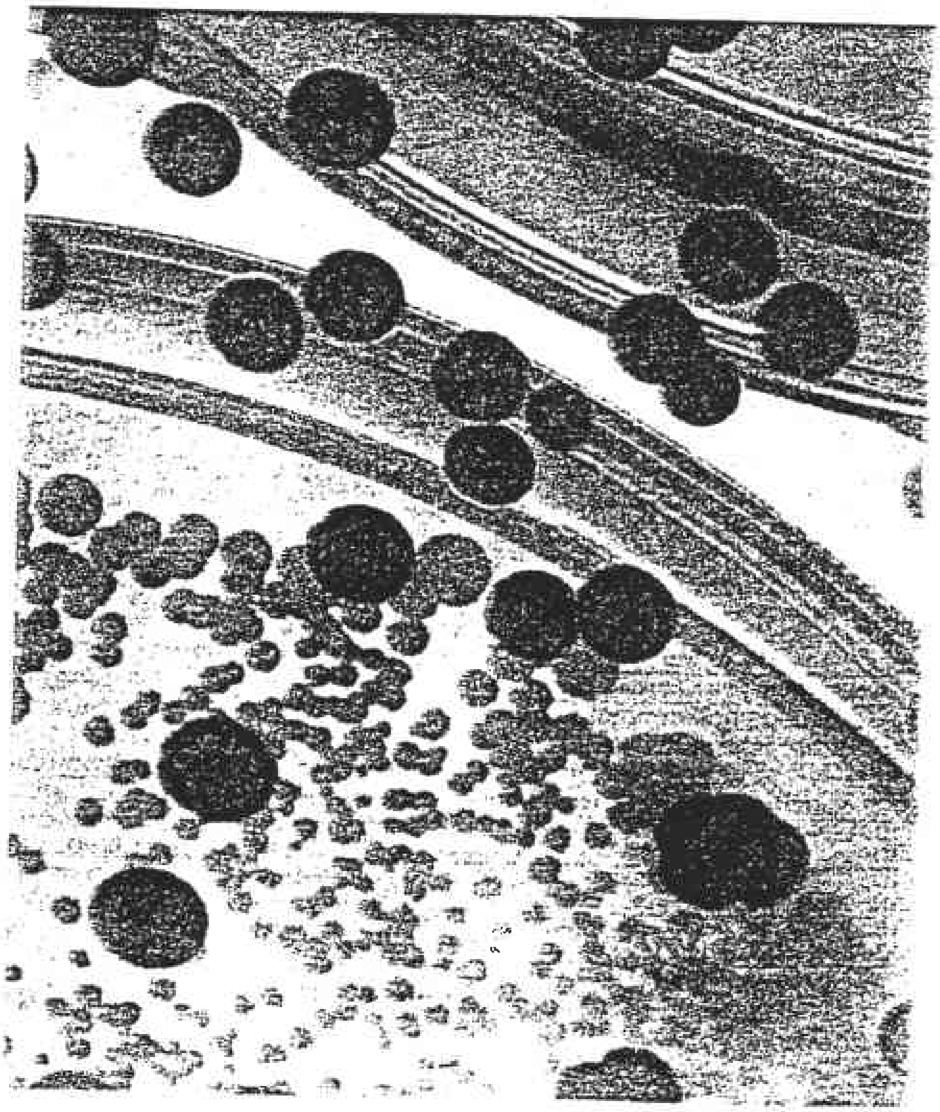
by Kevin G. Robinson, Kimyoung Kim, William S. Farmer and John T. Novak

Groundwater contamination has become a source of concern in the last several years because of its potential impact on sources of drinking water. More than 50 percent of the U.S. population draws on groundwater for its potable water supply, and total groundwater withdrawals are projected to increase in the future.

The presence of anthropogenic organic compounds in groundwater represents a serious public health problem. Sixty-five classes of such chemical compounds are considered hazardous and more than 100 organic compounds have been designated by the U.S. Environmental Protection Agency (EPA) as priority pollutants.

At least 33 toxic organic chemicals have been found in drinking water wells from 40 states and these numbers escalate as more data are obtained. Cleanup of these contaminants is difficult. Although pumping and above ground storage can be used to collect and treat high levels of contamination, residual contaminants can remain for years.

Much of this contamination, especially aromatic compounds, is sorbed to the soil matrix making physical re-



moval difficult. These same adsorbed compounds may desorb slowly, providing a long-term, low-level source of contamination. Recommended drinking water standards for some compounds are in the parts per billion range.

One possible method of cleanup or control of such contaminants is through enhanced, in-place biological degradation. Naturally occurring microbial populations may be capable of reducing the concentration of hazardous organic compounds. However, when naturally occurring organisms are absent or few in number, or when a more rapid cleanup is desired, acclimated organisms may be added to the subsurface environment.

For biological treatment systems to be successful, the proper microbial culture must be combined with the appropriate environmental conditions to promote degradation or conversion within a realistic time period.

Recent studies indicate such technol-

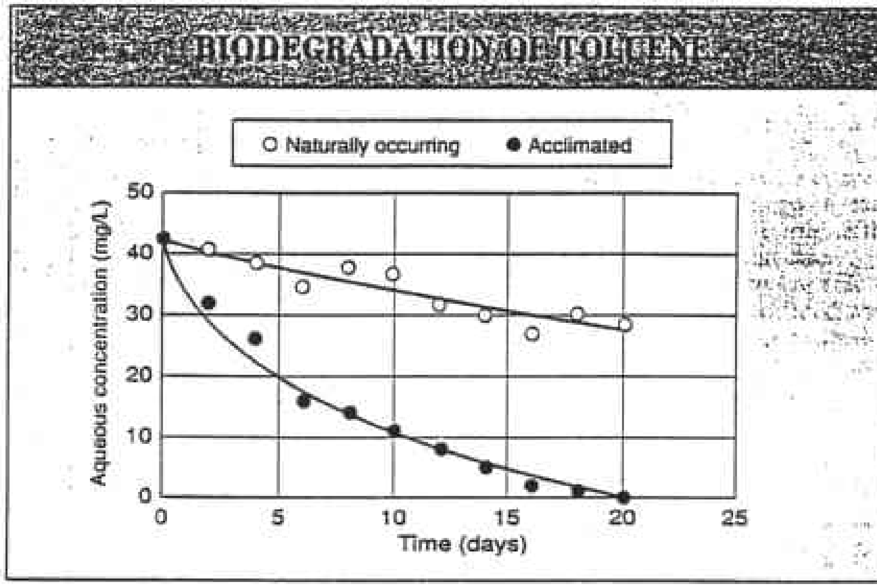
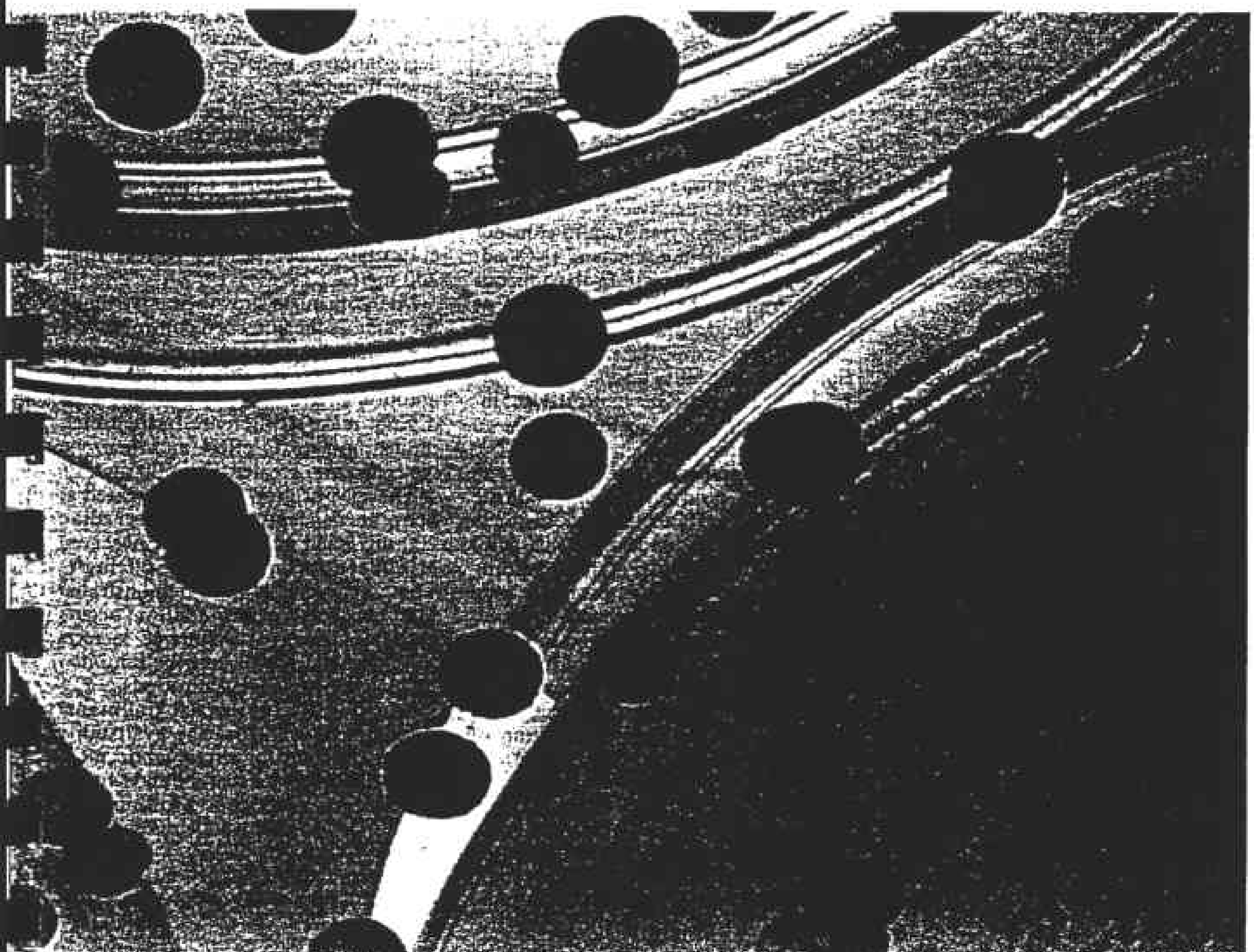


Figure 1. The addition of acclimated organisms increased the rate of degradation of toluene in soil.



Treatment of soil with H_2O_2 alters the sorption properties of the soil, increasing its capacity.

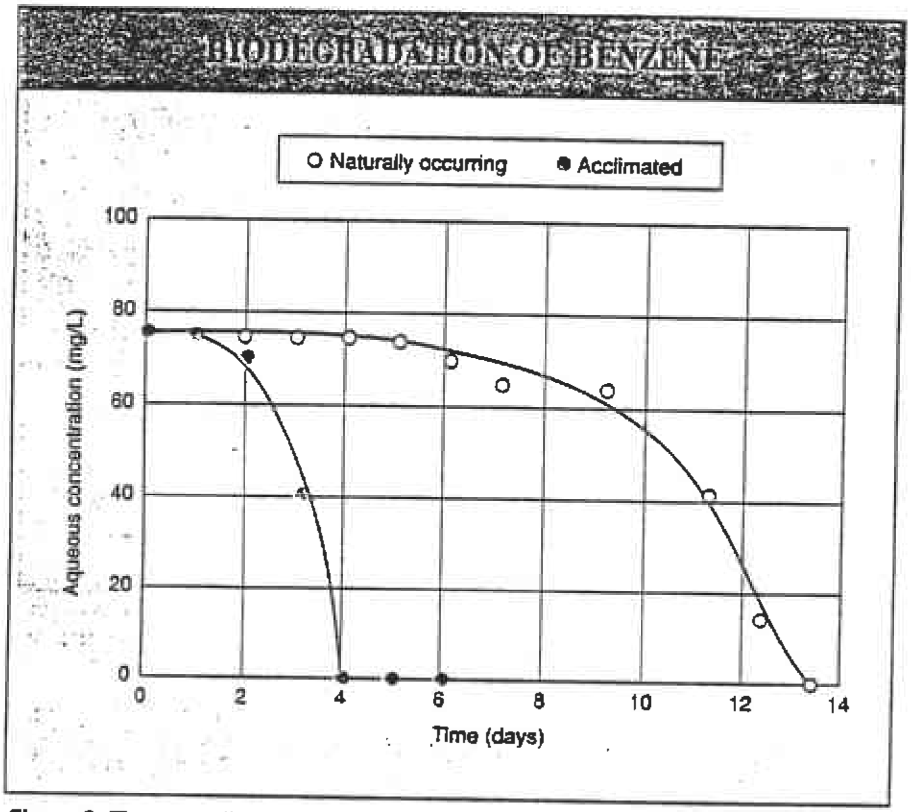


Figure 2. The naturally occurring organisms were able to degrade benzene rapidly.

ogy is feasible, but cost effective application requires better and more detailed information. The results of a laboratory study that addressed the effectiveness of soil inoculation to expedite biological degradation are reported in this article.

Methods

Batch soil microcosms containing benzene, toluene and m-xylene were used to model a contaminated groundwater system. These aromatic compounds are components of gasoline, are frequently found in contaminated subsurface environments, and are a health concern when found in potable groundwater supplies.

Aqueous toluene and benzene concentrations were measured by direct injection into a gas chromatograph. In addition, manometric BOD tests were used to evaluate biodegradation. BOD tests also were used to evaluate the toxicity of benzene to bacteria.

Results

The data indicate that all compounds rapidly and extensively sorbed to the soil, and both the treatment with hydrogen peroxide and addition of acclimated bacteria increased sorption. Apparently the treatment of soil with H_2O_2 alters the sorption properties of the soil, increasing its capacity.

This is an important fact to consider when evaluating the effects of H_2O_2 addition, since a decrease in the aqueous concentration could be interpreted as enhanced biodegradation, whereas the actual impact could be increased sorption onto the soil. The addition of organisms also can be expected to increase sorption due to the increase in organic matter.

Measurement of aqueous toluene concentrations in microcosms indicate the addition of acclimated bacteria greatly improves the use of this compound, as shown in Figure 1. Adding acclimated bacteria increased the rate of degradation and decreased the toluene concentration in solution to zero.

The biodegradation rate of the naturally occurring soil microorganism population was slow. Conversely, the naturally occurring soil population of organisms was able to degrade benzene rapidly, but only after a lag period and at a somewhat reduced rate, as seen in Figure 2.

To determine the effect of different microbial cultures on biodegradation, the degradation of benzene, toluene and m-xylene were compared using different types of microbial seeds. Figure 3 shows the biodegradation of the three compounds using a benzene acclimated culture and a mixed culture found in the stormwater collection pond.

The microorganisms from the pond were able to use all three aromatic hydrocarbons as a sole carbon and energy source. However, the benzene-acclimated microorganisms from the activated-sludge reactor could not oxidize m-xylene. It appears that organisms necessary to degrade m-xylene were initially present in the pond water but selectively removed from the benzene fed reactor.

The rate of biodegradation was found to be dependent on the amount of oxygen present in the microcosm. Higher concentrations of hydrogen peroxide increased the amount of oxygen in the microcosms, stimulating toluene biodegradation in microcosms containing toluene acclimated organisms. However, hydrogen peroxide is toxic to microorganisms at elevated concentrations. When no oxygen is available, biodegradation of toluene was found to be severely limited.

Biodegradation of benzene by the mixed microbial pond culture was inhibited by elevated benzene concentrations. Microorganism populations increased rapidly in microcosms where benzene was the sole source substrate at initial concentrations of less than 200 mg/ℓ.

Microorganism growth was virtually the same as the unseeded controls when the benzene concentration was more than 500 mg/ℓ as sole substrate. The addition of glucose and glutamic acid did not make a difference in the trend, indicating a toxic effect at a benzene concentration greater than 500 mg/ℓ.

The specific growth rate decreased at benzene concentrations greater than 200 mg/ℓ and no growth was observed at concentrations above 500 mg/ℓ. These experiments have demonstrated that enhanced biodegradation occurs with the addition of acclimated bacteria but microbial growth may be inhibited by high substrate concentrations.

Sorption of subsurface pollutants may directly influence the availability of contaminants for microbial degradation. The aromatic compounds found in gasoline tend to sorb to soil surfaces, thereby lowering the aqueous substrate concentration.

The availability of sorbed contaminants to acclimated microorganisms

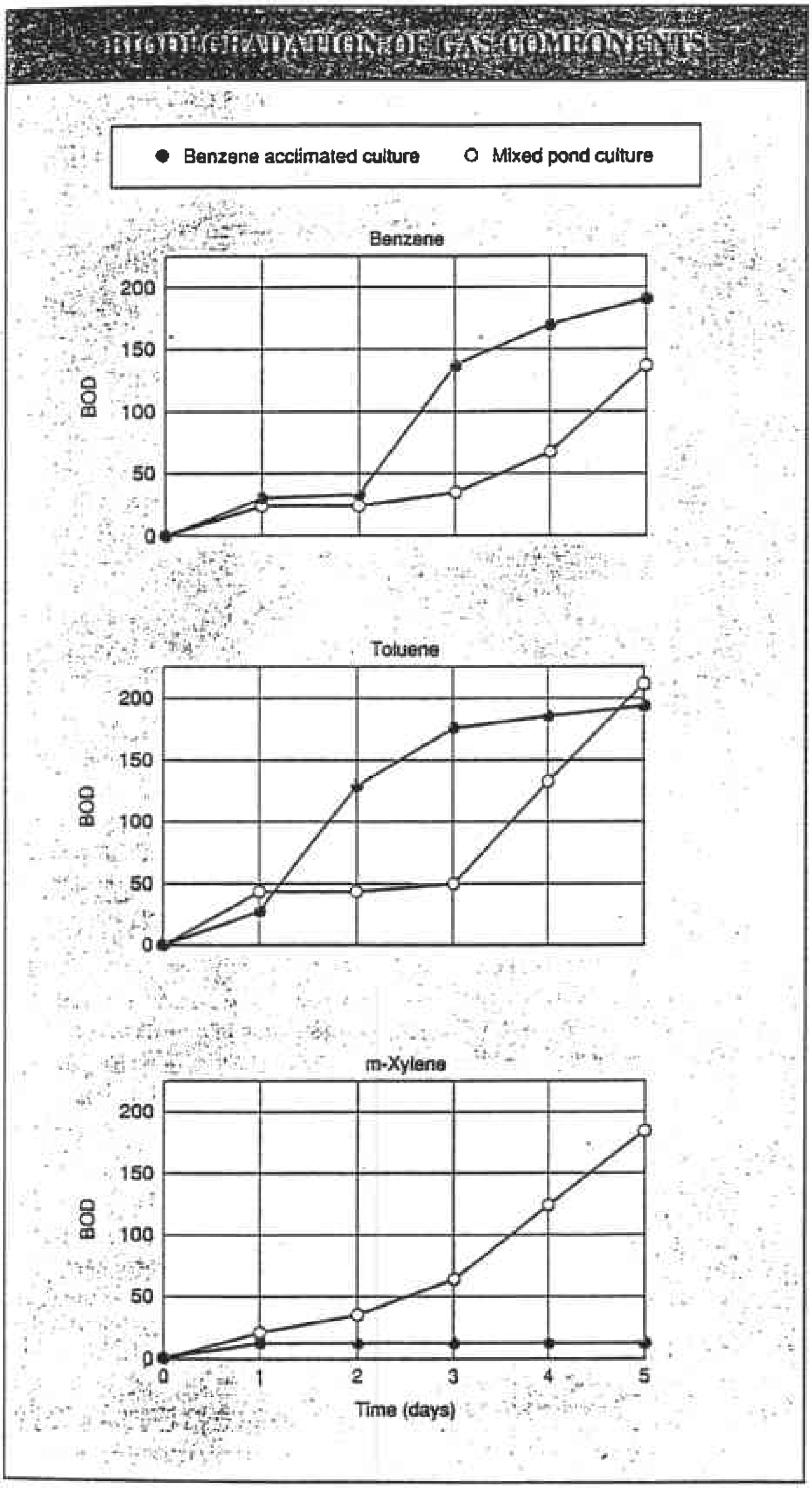


Figure 3. The biodegradation of benzene, toluene and m-xylene was measured by a manometric BOD test.

was addressed by first allowing toluene to equilibrate in sterilized microcosms for one month before adding acclimated bacteria and hydrogen peroxide. Soil-bound toluene was determined by solvent extraction.

In addition, a much greater concentration of bacteria was added to each microcosm (50 mg/ℓ) to determine the effect on the biodegradation rate.

The acclimated bacteria were able to rapidly use the toluene associated with the soil. It could not be determined if the organisms degraded the toluene while it was sorbed to the soil or if the toluene first desorbed before being acted on by the bacteria.

Summary

Adding acclimated bacteria substantially increases the biodegradation rate of toluene in soil microcosms. Also the total time needed to degrade benzene in microcosms can be shortened with the addition of acclimated bacteria by reducing the lag time needed by naturally occurring organisms.

Pure cultures may be capable of degrading individual compounds but may be unable to degrade multiple pollutants found at contaminated sites. Since the products produced by one organism may in turn provide energy yielding substrates for another species, the microbes in a mixed culture may use as sources of carbon and energy the compounds that cannot be degraded by a pure culture alone.

However, the concentration of the compounds of interest should be high enough to sustain growth, but they should not be so high as to be toxic to the organisms. In addition, nutrients and oxygen must be present at levels that are great enough to sustain aerobic degradation.

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IN SITU AND EX SITU BIOREMEDIATION OF CONTAMINATED SOILS

BY

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BACKGROUND

Since the early 1980's the Biochemical Division of Sybron Chemicals Inc. has developed several new commercial bacteria cultures for use in soil and groundwater decontamination. This culture series was designated Augmented Bioreclamation™ (ABR). These cultures were developed for the destruction of specific organic compounds. Furthermore, the biological kinetics of these cultures on specific substrates such as aromatic solvents (1), diesel fuel (2) and refinery petroleum waste (3) have been documented.

These cultures have been used extensively for reclaiming contaminated soils and groundwater. More than forty sites have been closed in the oil fields of Louisiana, while several diesel fuel and gasoline remediation projects are on going.

In situ and ex situ bioremediation of soil, sludges and groundwater has been accomplished using a variety of mechanical and biological techniques with these ABR cultures.

CULTURE DEVELOPMENT

Bacterial strains were isolated from previously contaminated areas by enrichment culturing. Specific organisms are obtained by slowly increasing the concentration of the organic contaminant substrate over many culture generations. This may require several months of laboratory work and eventually results in one or more strains for each contaminant or mixture of contaminants.

These strains are grown in a buffered media on the specific organic(s) during full-scale production. Subsequently, the liquid culture is coated onto an organic based solid media, dried, ground and packaged. The dry culture is supplied in the form of a patented Biosock™ for use as an inoculum in above ground biological reactors. Liquid cultures may also be supplied for inoculum upon request if there is sufficient lead time.

Three representatives of the ABR series are presented in Table 1 along with the target organics.

IN SITU AND EX SITU SOIL DECONTAMINATION

Gasoline and diesel fuel contaminated sites are treated using a combination of inplace and leachbed methods. Contaminated water is drawn from an interceptor trench or well as shown in Figure 1. Reactor design is site specific. Suspended culture, seqential batch and fixed film reactors are used most frequently. Some systems are designed to result in zero emissions to aid in the permitting process.

A closed suspended culture reactor system with a process scheme as shown in Figure 2 has yielded significant information. Removal of total petroleum hydrocarbons was in excess of 99.7%. Benzene and toluene concentrations were reduced from over 2 ppm to less than 4 and 2 parts per billion, respectively. Removal was in excess of 99%. All xylenes and ethylbenzene were totally degraded. These results were obtained from a gasoline contaminated site located at Buena Park, CA, when ambient air temperatures were at a minimum and the reactors were operating at maximum hydraulic capacity.

After eight months of treatment, six soil borings in the original tank cavity yielded five samples less that 10 ppm TPH and one of 200 ppm. Initially, the soil ranged from 60 to 600 ppm.

Similar data is being collected at a two acre diesel contaminated soil and groundwater site in Emeryville, CA. This system is performing both reinfiltration and pump and treat. Pump and treat effluent is being discharged to East Bay Municipal District, CA. Representative data are presented in Table 3. Removal efficiences approximate 100%. Suspended culture reators with diffused air draft tube aerators and a proprietary dispersion system are being used. Cytoculture International, the designer, has a patent pending. Analysis for the petroleum components, TPH/TEH and benzene, xylene, and toluene were performed by EPA methods 602 and modified 8015.

It should be noted that the project is under tidal influence and brackish conditions exist periodically.

Volatilization was negligible as calculated by the bacterial biomass generated (cell yield) and is supported by data from the local air pollution board.

IN SITU AND EX SITU BIOTREATMENT OF PETROLEULM HYDROCARBONS IN PRODUCTION PITS

Production pits vary from several hundred square feet to several acres. Their origin also varies including oil production, gas production, transmission pipelines, and refinery operations. Several factors are extremely important in the feasibility of a pit remediation. These are given in Table 4.

Over the last six years Sybron and L&A Contracting have closed approximately fifty pits using bioaugmentation coupled with good mechanical engineering. The basis for this type of augmented remediation is briefly summarized by a respirometric and oil and grease study conducted at SCI in Salem, VA. Several treatments were studied. These were a sterile control, non-sterile control without nutrients, non-sterile control with nutrients, ABR-Petroleum Blend and nutrients.

Residual oil and grease and total oxygen consumption were measured after 20 days. The sterile and non-sterile treatments showed little oxygen consumption. The need for on site acclimation of augmented cultures was demonstrated by superior oil and grease reduction with augmented treatments as given by Table 5.

The relationship between oxygen consumption and temperature is presented in Figure 3. The data in Figure 3 is a compilation of data from twenty cleaned pits.

Time to complete a remediation is generally under 150 days, but is directly dependent upon the initial oil and grease concentration. Figure 4 demonstrates the relationship between oil and grease reduction over time.

Seventeen of twenty-six pits, that were remediated and closed in 1987 and 1988, degraded oil and grease at a rate that was expressed in tons / month. This varied from 200 to 500 tons with an average value of 213 tons / month.

Augmented landfarming of some pits is still possible where air pollution is not a major concern and land is available on site. Properly operated landfarms can reduce oil and grease content in the manipulated soil from four percent to less than 0.3 percent in ninety days.

OTHER IN SITU AND EX SITU METHODS

As reported by Hater, 1988 the Bacterial Contamination Interceptor™ (patent pending) has been developed as an in situ vadose zone technology (4). The BCI system (Figure 5) allows for in situ bacterial destruction of hydrocarbon vapors using ABR cultures.

New residual hydrocarbon off-gas removal systems are under development (Figure 6). The intent of these systems is to replace and eliminate carbon useage completely.

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Table 1. ABR bacterial strains.

ABR Gasoline Blend	ABR Petroleum Blend	ABR Diesel Blend
Pseudomonas putida A	Bacillus subtilis	Pseudomonas putida
Pseudomonas putida B	Pseudomonas aeruginosa	Pseudomonas alcaligenes
Pseudomonas aeruginosa	Pseudomonas stutzeri	Pseudomonas aeruginosa
	Pseudomonas putida	Arthr. crystallopoietes
<u>Target Compounds</u>	<u>Target Compounds</u>	<u>Target Compounds</u>
Benzene	$>C_{20}$ aliphatics	Benzene
Toluene	Benzene	Toluene
Ethyl benzene	Toluene	Ethyl benzene
Xylene	Ethyl benzene	Xylene
C_5-C_{10} aliphatics	Xylene	Naphthalene
	Naphthalene	Methyl naphthalene
	Methyl naphthalene	Anthracene
	Anthracene	C_{10}-C_{17} aliphatics

Table 2. Buena Park, CA results.

DAY	BENZENE (ppb)	TOLUENE (ppb)	XYLENES (ppb)	ETHYL BENZENE (ppb)		TPH (ppm)
Effluent						
1	1.6	0.6	ND	ND		0.14
2	2.0	0.8	ND	ND		0.32
3	3.4	1.2	ND	ND		0.26
4	0.6	0.2	ND	ND		0.08
5	0.8	0.3	ND	ND		0.10
6	2.0	0.4	ND	ND		0.25
7	3.4	1.0	ND	ND		0.32
8	2.6	0.8	ND	ND		0.24
9	2.8	1.6	ND	ND		0.45
10	3.4	1.0	ND	ND		0.40
11	2.9	0.9	ND	ND		0.31
12	3.3	0.9	ND	ND		0.30
13	3.9	1.1	ND	ND		0.36
14	3.0	1.1	ND	ND		0.43
					AVG	0.28
INFLUENT						
6	2500	3000	4300	2750		149
8	2200	2360	3800	2300		97
13	1720	2160	4040	2300		103
					AVG	116.3
REMOVAL EFFICIENCY	99.8	99.4	100	100		99.7

Table 3. Emeryville, CA project.

SAMPLE	BENZENE (ug/L)	TOLUENE (ug/L)	XYLENE (ug/L)	TPH/TEH (mg/L)
Influent	450	13	4	2.9
Effluent	ND	ND	ND	ND

Table 4. Factors effecting successful pit bioremediation.

FACTOR	EFFECT
Temperature	2X difference in rate between 45 and 90 F
Salt Content	Salinity greater than sea water decreases rate dramatically
Residual Nutrients	Residual nitrogen and phosphorous must be above 1 ppm in order to maintain growth
Starting Oil Concentration	Initial concentrations below 5% F.O.G. often indicate the pit was burned and the remaining hydrocarbons are all asphalt like

Table 5. Oil and grease results for a batch treatment of oil pit waste.

TREATMENT	OIL & GREASE (mg/L) AFTER 30 DAYS	%REDUCTION
Oily Solids (pit bottom)	33500	-
Water Phase	204	-
BI-CHEM Treated Sample w/ 100g Sludge + 100mL Supernatant	2000	94.0
Indigenous Bacteria Sample w/ 100g Sludge + 100 mL Supernatant	17700	47.2

FIGURE 1
ABR - INSITU LEACHBED
(COLD WEATHER / AIR CONTAINMENT CONCEPTUAL)

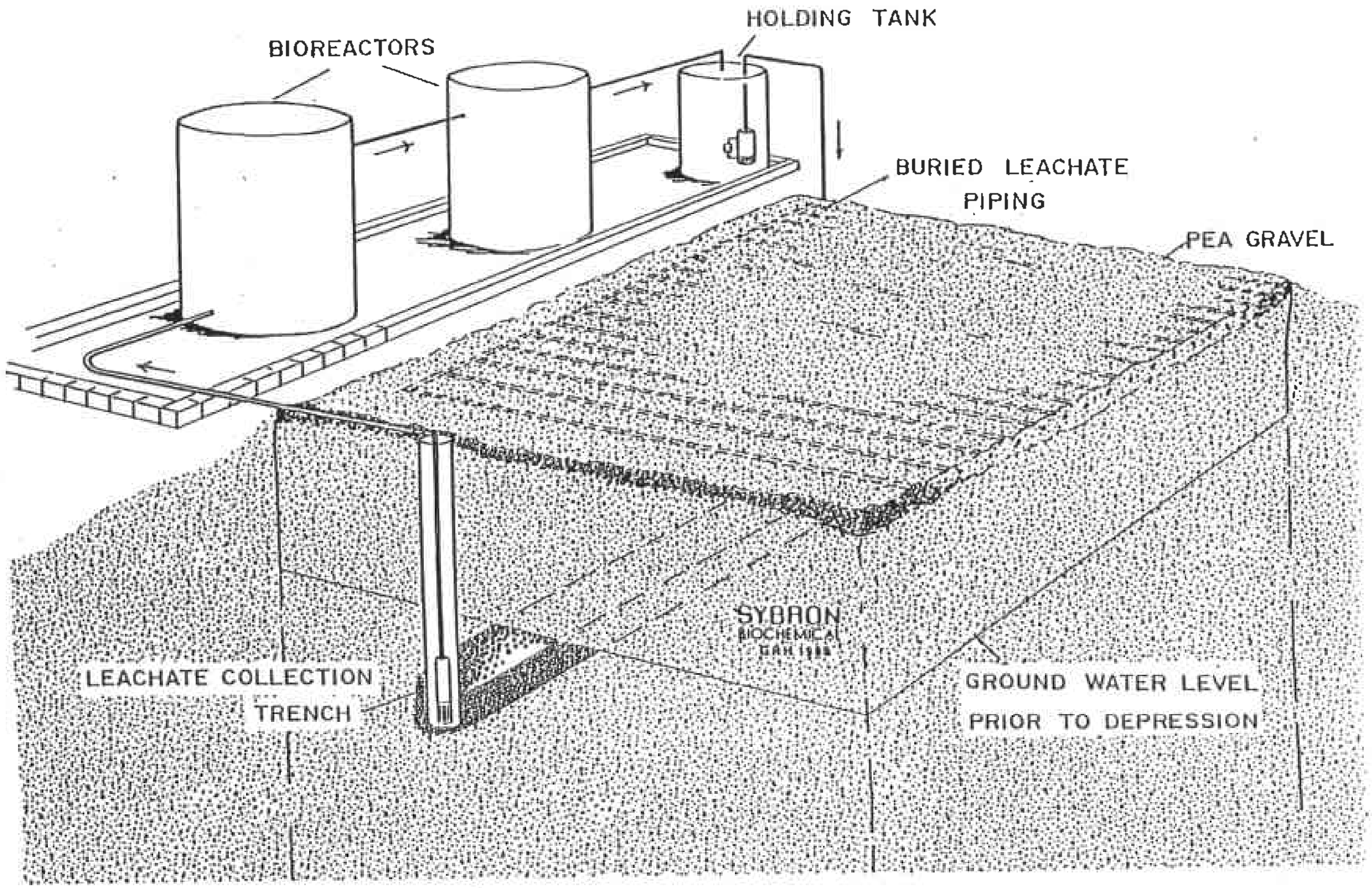
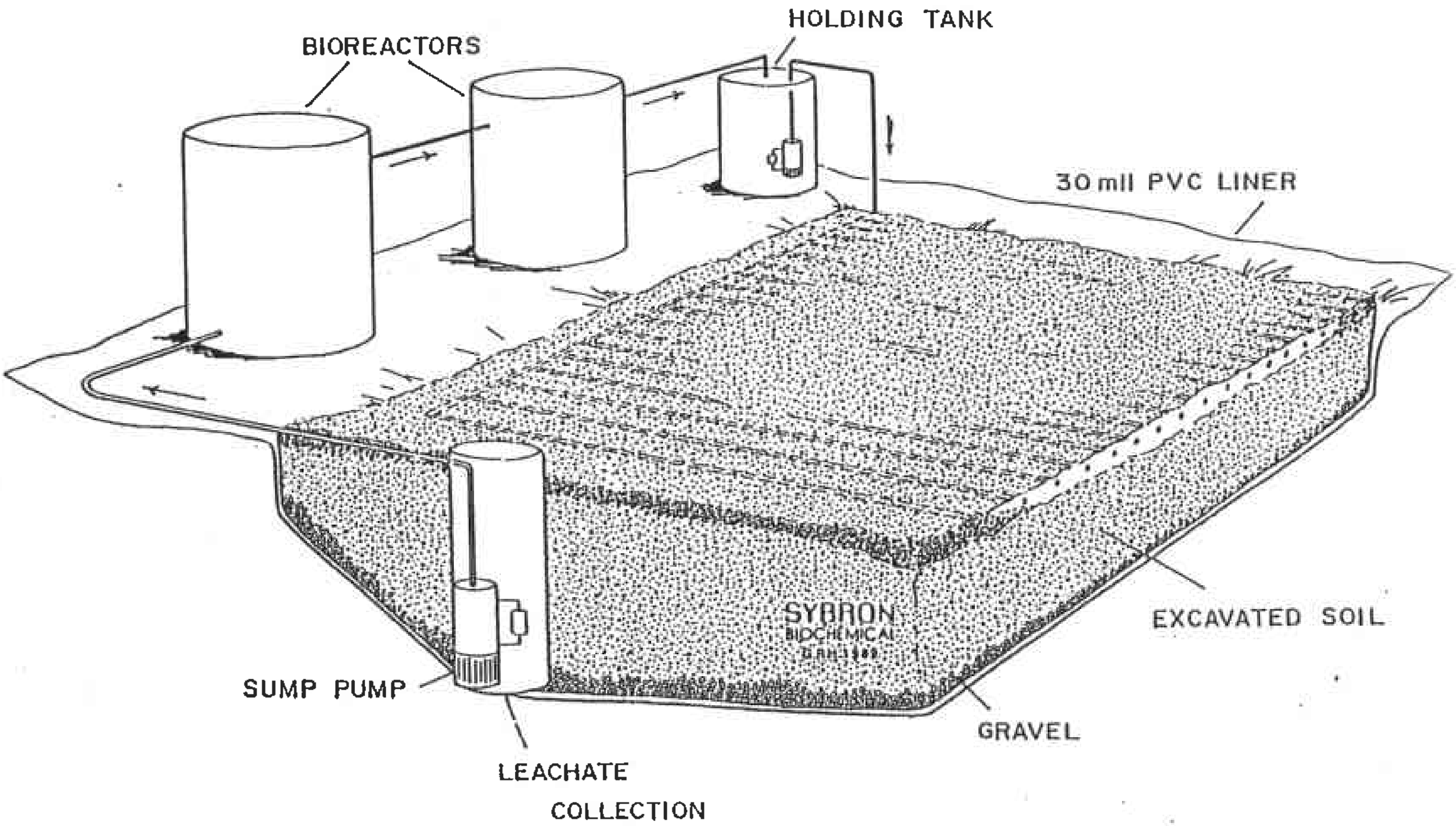
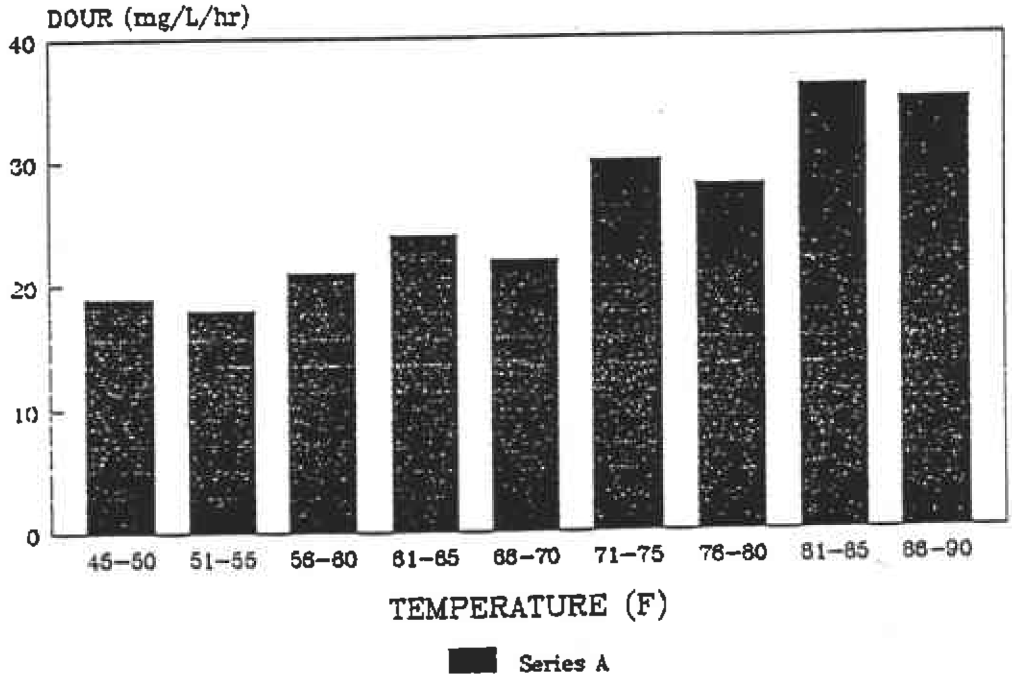


FIGURE 2
ABR-EXSITU LEACHBED
(COLD WEATHER & AIR CONTAINMENT CONCEPTUAL)



IMPACT OF TEMP ON THE DOUR

Figure 3



O&G REDUCTION OVER TIME

FIGURE 4

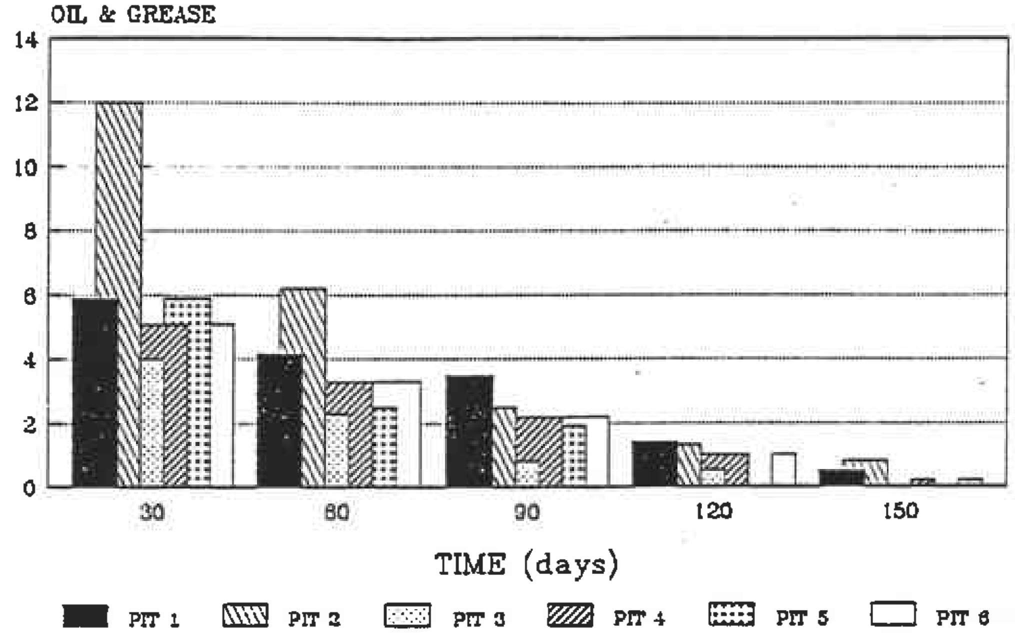


FIGURE 5

BACTERIAL CONTAMINANT INTERCEPTOR (BCI)

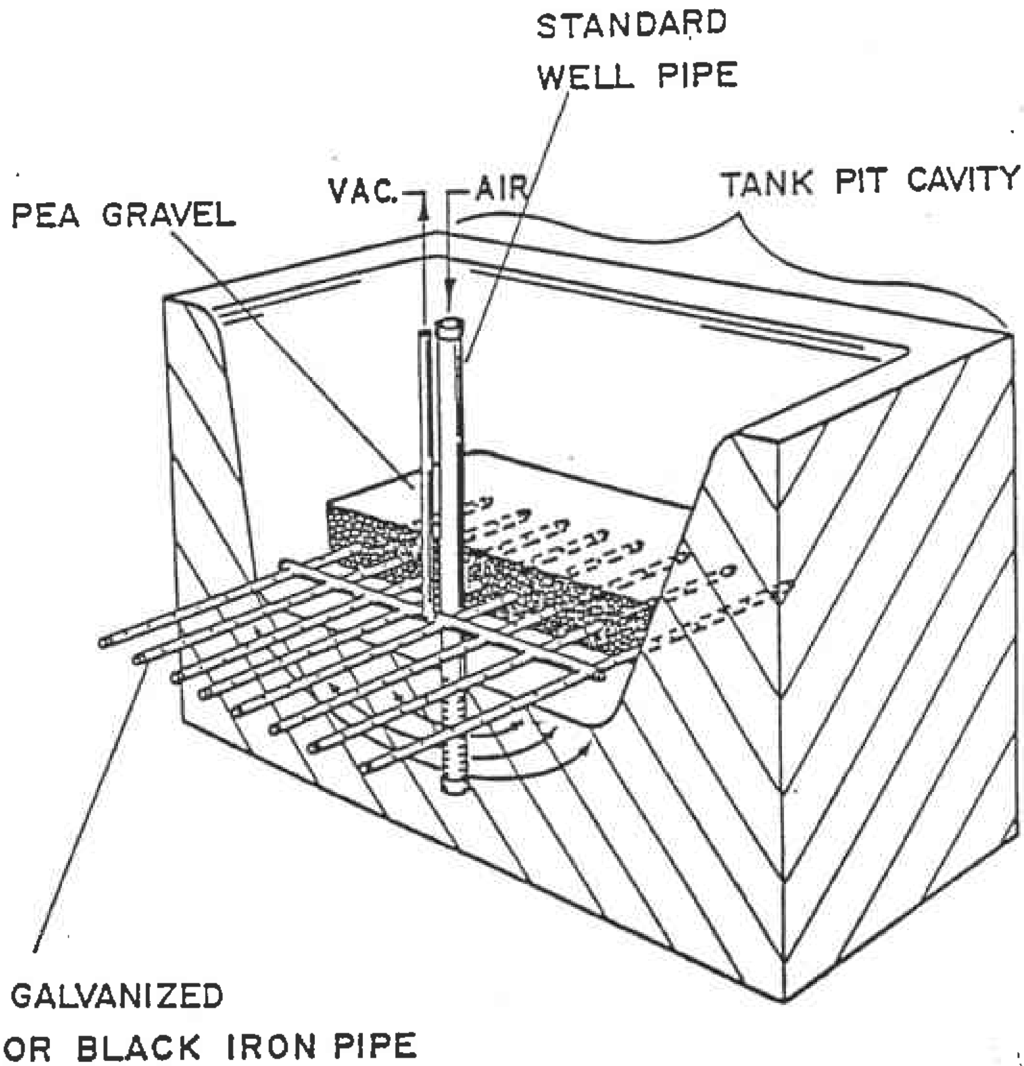


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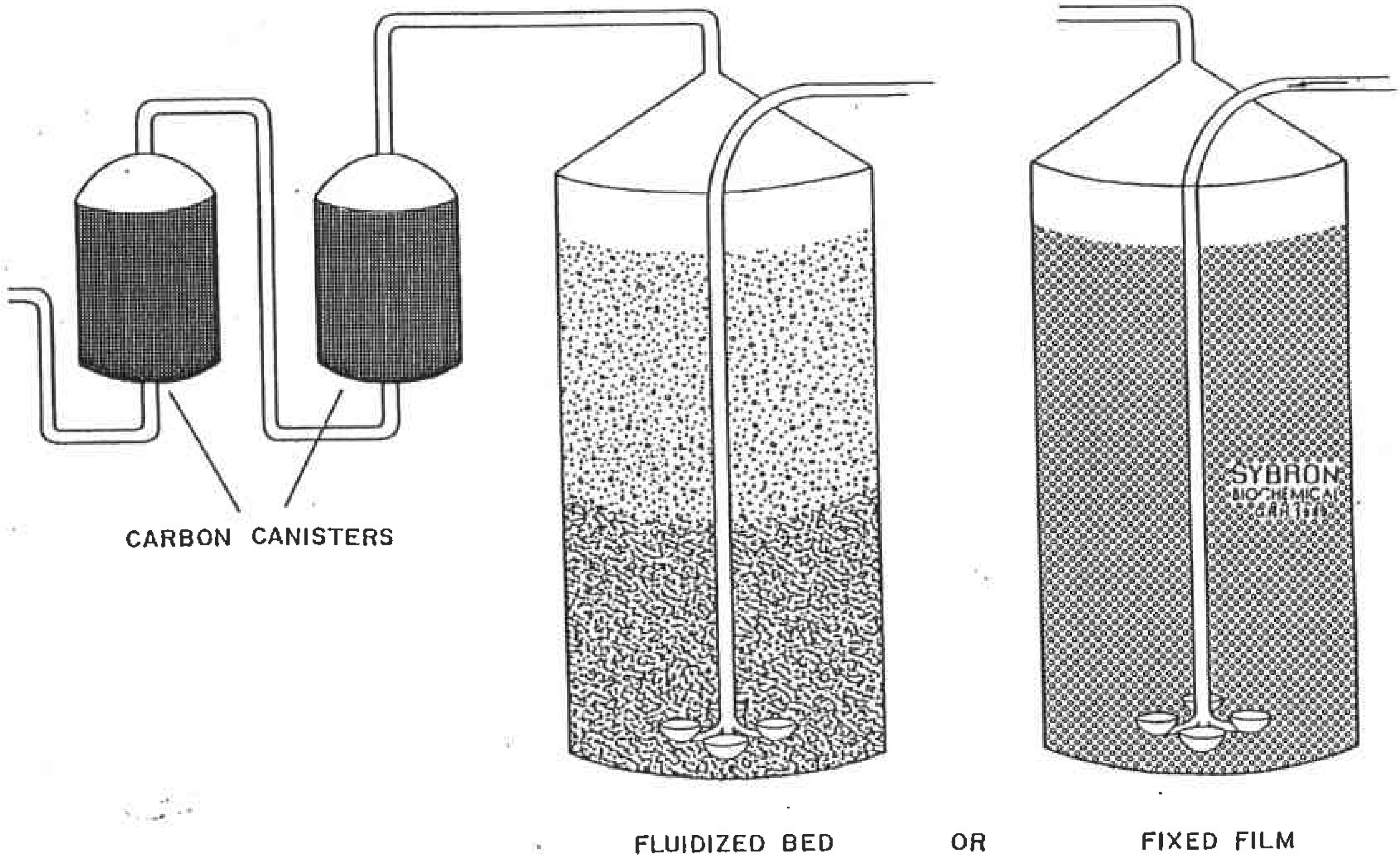
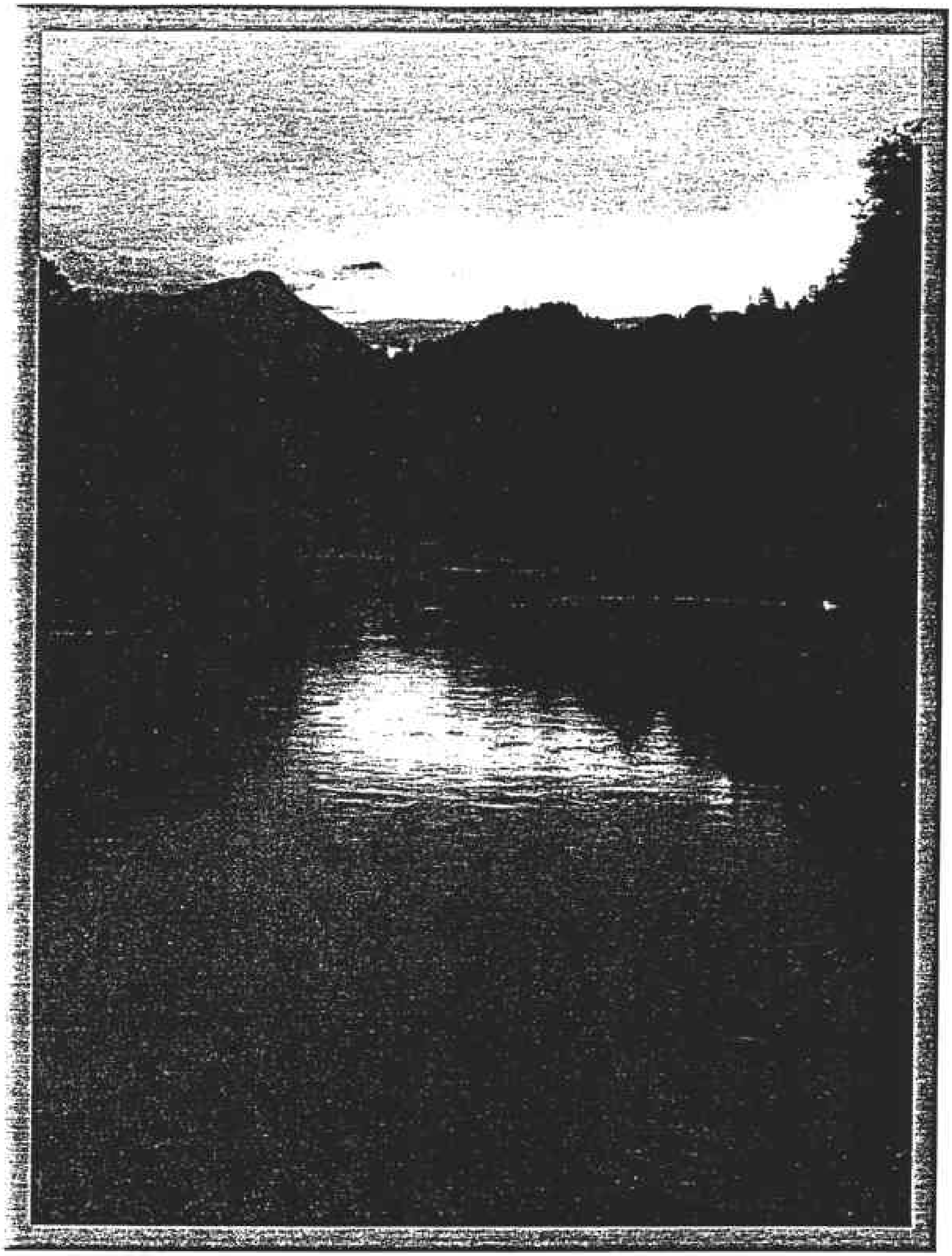
FIGURE 6
ABR-OFF GAS SYSTEM



APPENDIX 4

Erickson Environmental Technical Literature



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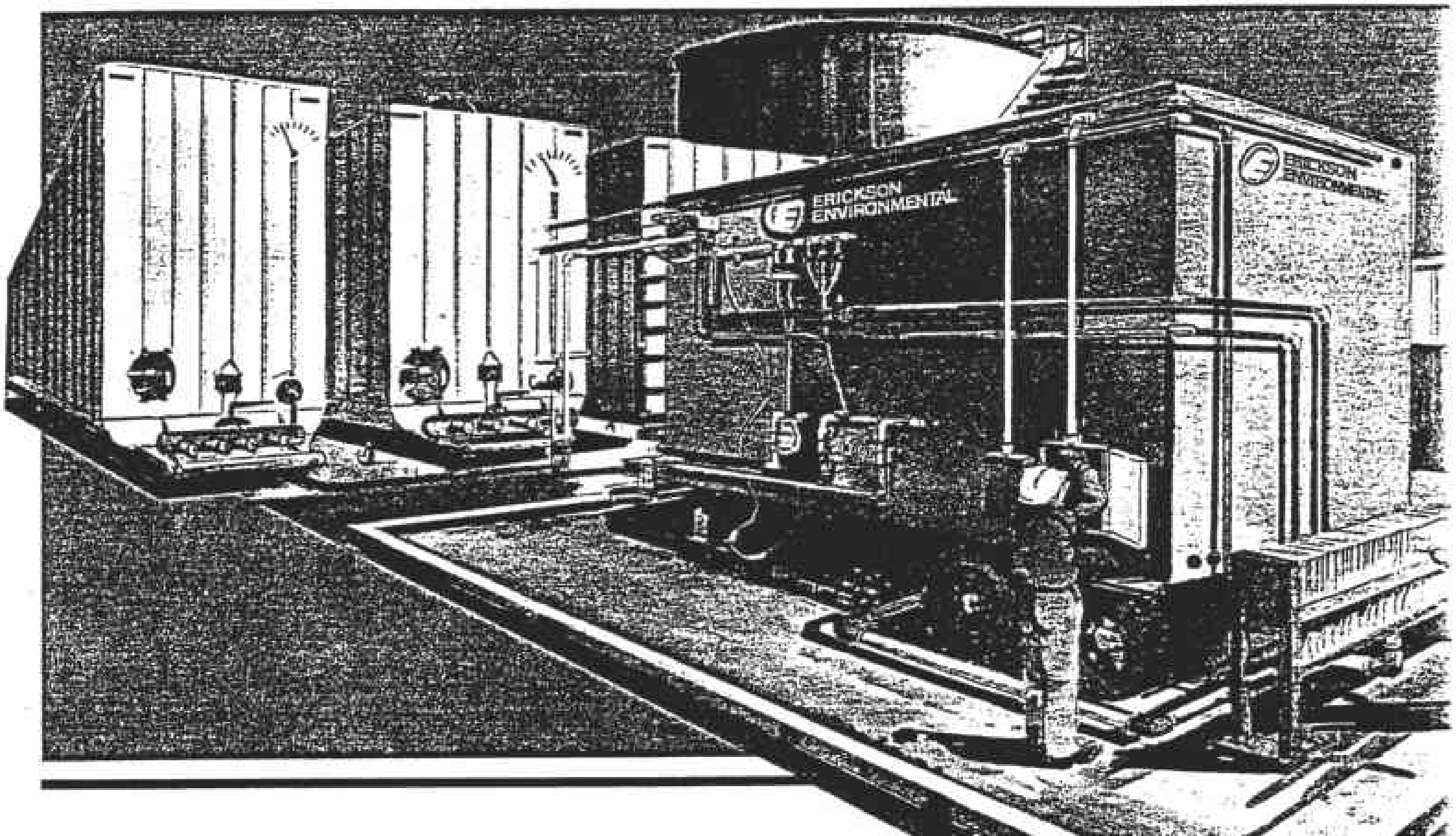

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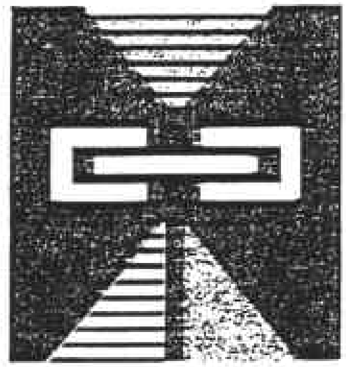

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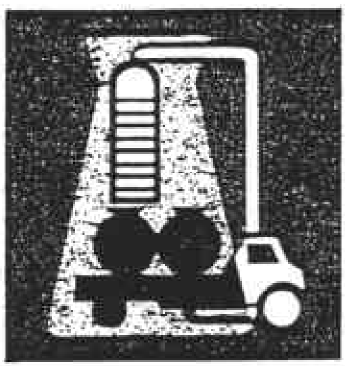

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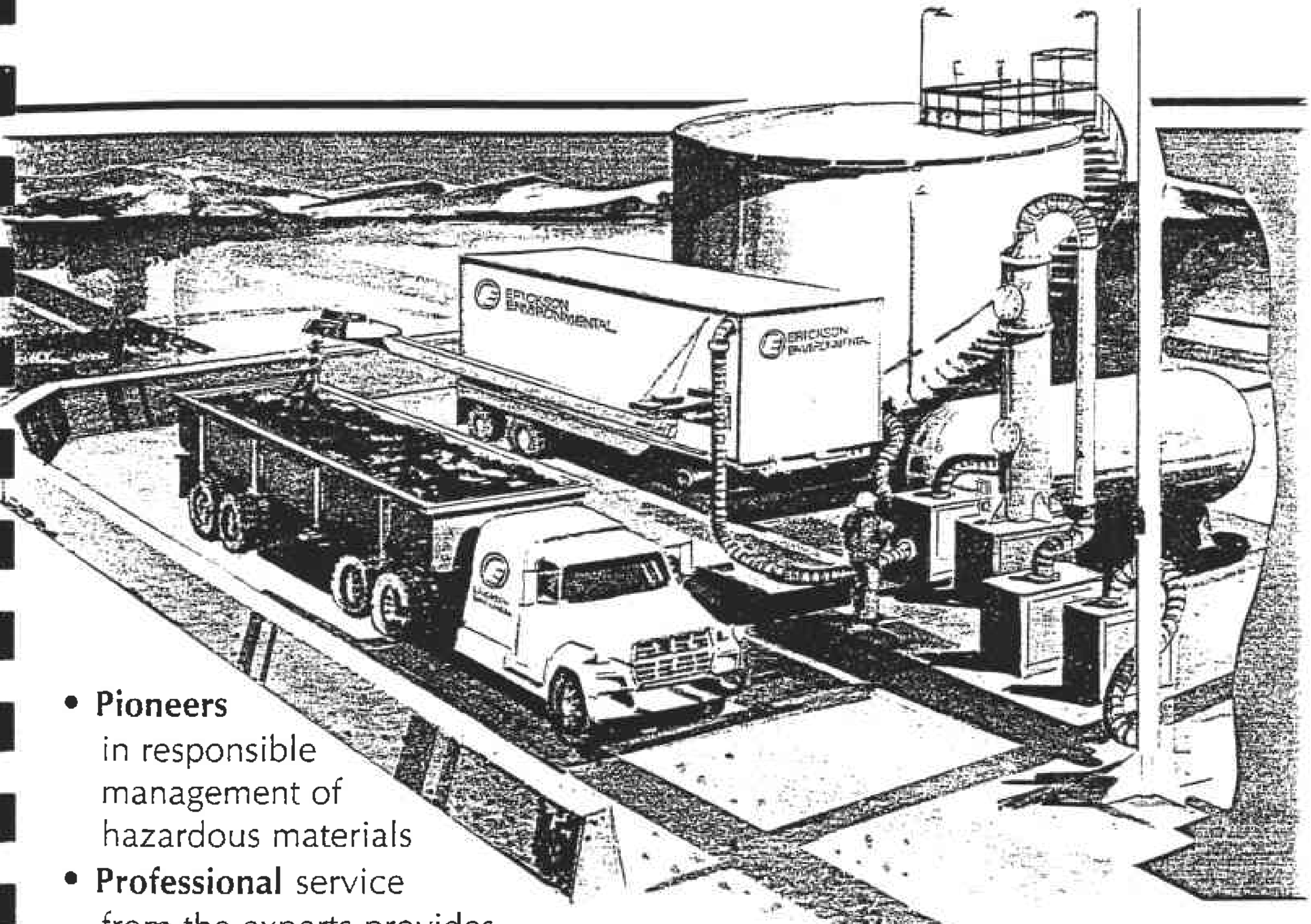

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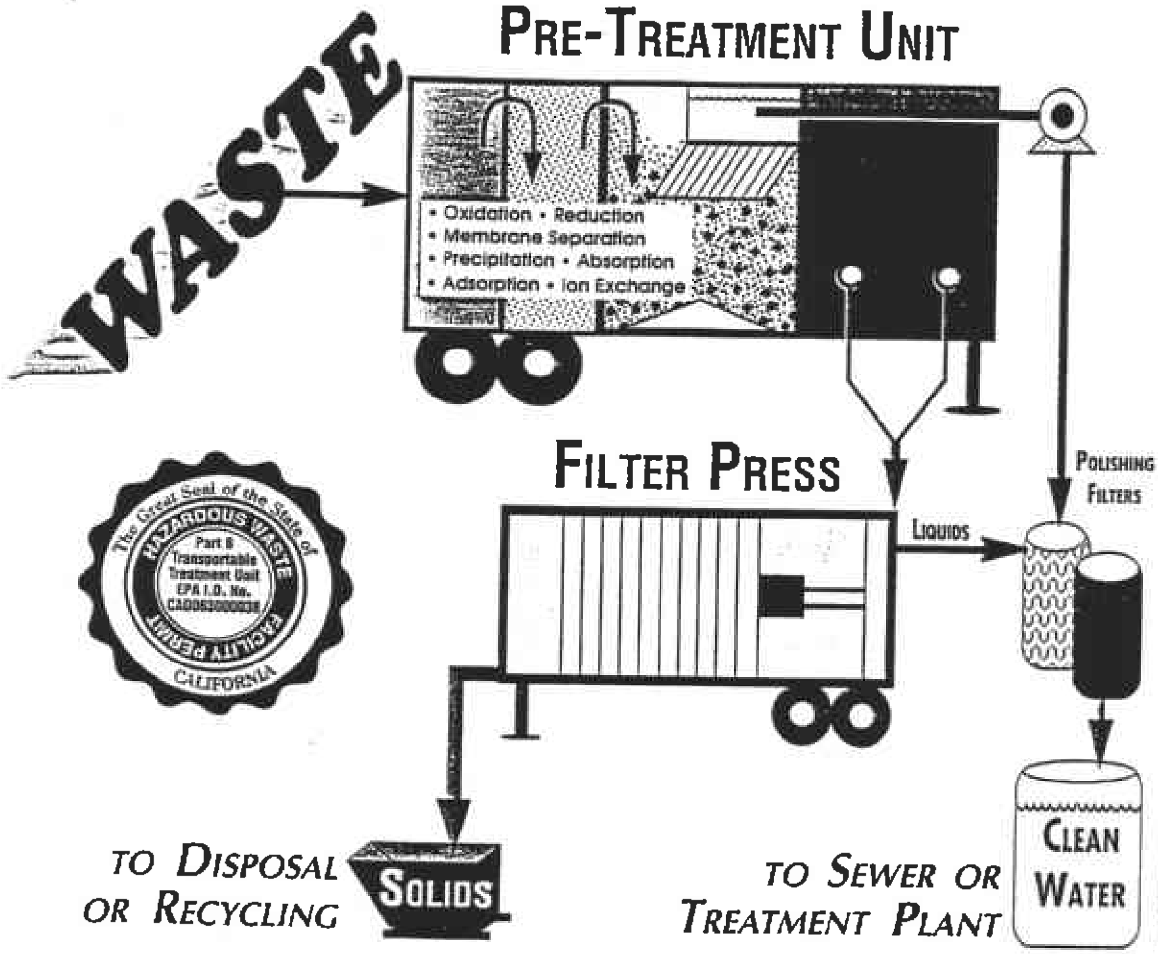

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APPENDIX 5

Optional Bioremediation for Excavated Soil

Vacuum Heap Bioremediation of Excavated Trench Spoils

The contaminated trench spoils (estimated at about 300 cubic yards) from the installation of the extraction and infiltration trenches, and the infiltration wells, could be treated along the eastern edge of the corner lot along the intersection of 98th and Edes Avenues. The trench spoils would have been stockpiled on a 10 mil plastic liner on the lot or would have to be transported back from the temporary stockpile area at the Pippin site.

CytoCulture proposes to simultaneously treat the estimated 300 cubic yards of hydrocarbon contaminated soil from the trenching along with the additional 800 cubic yards of stockpiled soil from the previous IT excavations for the utility trenches. By using the oil-degrading bacteria generated by the Phase III groundwater treatment process on site, the soil treatment will be very efficient and cost effective. The clay nature of the excavated soil and the presence of fairly heavy hydrocarbon fractions from the leaking waste oil tank will require an aggressive bioremediation program to achieve desired clean-up results in less than a year. CytoCulture proposes to design, build and operate an irrigated vacuum heap bioremediation system taking full advantage of the available bioreactor effluent from the groundwater treatment program which otherwise would be discharged to the sewer in Phase III.

Excavated soil, free of concrete debris, would first be mechanically broken up to reduce up the hard clay into fragments. The fragmented soil would have a much higher surface area, facilitating bacterial access to the diesel contamination and oxygenation of those cultures as they achieve high population densities. The processed soil would then be redistributed with a front loader into 3 foot lifts on an impermeable plastic liner equipped with a vacuum aeration piping designed and installed by CytoCulture. The 1,100 cubic yards of soil, with a net soil height of about 9 feet would occupy an area of 60 feet by 100 feet. Details on this soil remediation process are available directly from CytoCulture.

A plastic cover (2 ply nylon reinforced) will be used to protect the soil from rain runoff and minimize escape of volatile hydrocarbons to the atmosphere.