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June 6, 1994
Project No. 107.20

Ron Goode Toyota
1825 Park Avenue
Alameda, CA 94501

Attn: Mr. Len Goode

Re: **Site Soil and Groundwater Bioremediation Workplan**
Site at 1825 Park Avenue, Alameda, CA

Dear Mr. Goode,

This workplan has been prepared for the above referenced site. As you know, several phases of work have been performed at this site. The proposed work is in response to a request from the Alameda County Department of Environmental Health (ACHD) for site cleanup.

Background

Two underground storage tanks were removed from the site on December 27, 1990 by Zaccor Corporation. One 300 gallon waste oil tank was located in the main building and the second 550 gallon gasoline tank was located outside the building. Soil sample and analysis showed detectable levels of oil and grease and Total Petroleum Hydrocarbons as Gasoline (TPHG) were below detection limits. Subsequent investigations be Zaccor and ACC Environmental Consultants (ACC) defined the soil and groundwater contamination. The results of those investigation show that TPHG and Benzene, Toluene, Ethylbenzene and Xylenes (BTEX) were the major contaminants and the plume had spread under the site in broad front to the north. Minor amounts of oil and grease are also present. Currently the leading edge of the plume is just offsite under Clement Avenue.

One groundwater monitoring well (MW-3) has consistently yielded inconsistent data, and is not a reliable monitoring point. Two upgradient services stations (located about 350 feet away) are known to have TPHG and BTEX contamination. The sites are prepared for cleanup however conflicts have delayed the startup apparently due to concerns of deleteriously spreading the plumes by pumping for capture and hydraulic containment.

The site owner intends to demolish part of the existing structure for future automobile retailing purposes. The remedial action will coincide with the site renovation since the owner is under time pressures to complete this project with limited financial resources.

Technical Approach

The technical approach to this project is to build upon the previous work. The flat groundwater gradient and sandy sediment under the site are favorable for an excavation and bioremediation approach. The shallow groundwater, TPHG and BTEX contaminants and sandy sediment should allow the biologic action to occur in this semi-passive cleanup. The upgradient sites mentioned above are apparently reluctant to start active pumping because of possible unwanted plume movement at, or off their property. Active pumping at 1825 Park Avenue could induce similar problems. Hence the approach will be to excavate the soil contamination and upper portion of the aquifer to a depth of about 8- to 10-feet. The excavated soil will be stockpiled for aeration and biologic treatment on the surface, and the water will be recirculated in the excavation (pit).

Soil will be excavated to a depth of about 8-feet, or advancing the excavation about 2-feet into groudnwater. During the excavation, the five hydraulic lifts and spotty contamination by oil and grease will be excavated and segregated in soil stockpiles. One monitoring well, MW-4 will be abandoned by digging it out. Excavation will continue, removing obvious or suspected contamination to the limits accessible (the edge of the office/showroom and Clement Avenue).

The groundwater flooding the pit by natural recharge will be inoculated with a commercial biologic product to enhance the hydrocarbon degradation. This approach has been used in portions of the United States for years and material is attached regarding the biodegradation process. The groundwater will be recirculated and sprayed back into the pit to oxygenate the water since oxygen is necessary for the process to allow the microbes to respire. This will continue for 7- to 14- days, when samples would be collected to determine contaminant concentration. When the water was determined to be none-detected, it would be pumped out into a surface holding tank for ultimate proper off-site disposal or approved recycled use.

The groundwater will be allowed to reflood the pit carrying dissolved contaminants into the pit where the process will be repeated. The water in the holding tank will be tested for TPHG and BTEX and when those contaminants are not detected, the water will be recycled ? (as for construction use). The approach is "passive" in that the natural recharging of aquifer water into the pit will flush contaminants at the perimeter into the pit. It is currently anticipated that the biologic process will be completed in two or three cycles (three to five weeks). Following water treatment, the site will be backfilled an paved.

Data collected from existing well MW-3 has consistently displayed anomalous patterns of results. Therefore, Well MW-3 will be abandoned following the excavation and water treatment, and replaced. The replacement location will be moved to a point across Clement Avenue. Site perimeter monitoring wells will be regularly monitored. Additional monitoring wells may be installed at the owners discretion since plumes are known to be upgradient and could be migrating toward 1825 Park Avenue.

It is anticipated that the biologic cleanup will be effective, however, a "complete" site cleanup is not envisioned. That is, as with all cleanup technologies and approaches, some low levels of contaminants are anticipated to remain following the work. It is estimated that at this site, these concentrations could be in the range of one part per million TPHG, and less that 25 parts per billion BTEX, or lower. However both TPHG and BTEX components usually decline to not detectable levels since the biologic process continues following active work. Consequently, at least four quarters of periodic groundwater monitoring well monitoring is envisioned following the soil and groundwater treatment.

Scope of Work

Field Activities and Methods - Excavation and Aeration

Soil will be excavated from the area of the former tank locations, under the building to the edge of the office building and the northern side of the site along Clement Avenue. Shoring is currently anticipated to be used to hold the excavation walls. The need for shoring requirements will be made following a review by a registered civil engineer. Excavation will continue to a depth of 8- to 10- feet below existing grade. The depth variance will be selected during excavation if areas of contaminants are observed. Currently, it is anticipated that about 2,500 cubic yards of soil will be excavated following building and pavement removal. Contaminated soil will be aerated on-site under permits of the Bay Area Air Quality Management Board. The excavated soil will be spread out in thin (about 12- to 18-inch lifts) on a plastic liner, and periodically turned to allow aeration and biologic activity to degrade the petroleum contaminants. Biologic products will be added to enhance the biologic activity and soil samples will be collected to ascertain contaminant levels.

The stockpiled aerating soil will be tested by collecting soil stockpile samples which were analyzed by a certified analytical laboratory. Soil samples will be collected by pressing a clean brass liner into the soil to a depth of about one foot, completely filling the liner. The liner will then be removed, sealed, labeled, logged onto a chain-of-custody forms and packed in a chilled ice chest for transport to the laboratory. Once soils were tested as not containing contaminants, the soil will be properly disposed off site.

Biologic Groundwater Treatment (Bio-Treatment Pond)

The area of the Bio-Treatment Pond (Pond) will the extent excavated to a depth of 8- to 10-feet, penetrating 2- to 4-feet into the aquifer. If free-phase product is encountered, it will be collected and properly disposed. A sump will be excavated in the middle of the pit, and lined with a geotextile. A pump will be installed for the water recirculation and directed back into the pond by sprinklers. This will operate continuously for water oxygenation. The water will be inoculated with a commercial biologic product (InterBio L-104), which are naturally occurring microbes adapted to use hydrocarbons as a "food" source. This will be done to the manufacturer specification. As part of the process, the water will also be treated with nitrogen and monitored with a kit the manufacturer supplies for this process.

Prior to initiating the water treatment process, the water will be tested to ascertain the concentrations of TPHG and BTEX using a State certified laboratory using the methods discussed below. As water is treated, it will be tested again following a "degradation cycle" which is estimated to take 7- to 10-days. When analytical testing shows that contaminants are not detected, then the water will be pumped out and the pond will be allowed to recharge, and the process will be repeated. It is anticipated that two to three cycles of groundwater recharge, treatment, recirculation and proper off-site disposal or use will be performed.

Monitoring Well Destructions and Replacement

Two monitoring Wells are anticipated to be abandoned, Wells MW-4 in the area of proposed excavation and Well MW-3. Well MW-4 will be destroyed by excavating out the well during the soil excavation phase. Existing Well MW-3 will be destroyed by completely drilling it out and backfilling the borehole with neat cement grout from the bottom to top. The abandonment will be done under required permits of Alameda County Flood Control and Water Conservation District (ACFCWCD). Monitoring Wells MW-1 and MW-2 will be left in-place.

One exploratory boring for well MW-3 replacement will be drilled at the location shown on Figure 1. Drilling and well installation permits will be secured from the ACFCWCD prior to doing the field work. The borehole will be converted to a groundwater monitoring well. All drilling equipment and sampling tools will be cleaned prior to arriving, and before leaving the site. The augers will be advanced to the desired sampling depth interval, and a drive split spoon sampler will be driven ahead of the drill bit. The sampler will then be retrieved and dissembled, and the soil filled brass liner will be sealed with Teflon® paper and plastic endcaps, labeled, logged onto chain-of-custody forms and place in a chilled ice chest.

The borehole will be logged using the Unified Soil Classification System under the supervision of a registered geologist using the attached GTE Sampling Protocol. Additional lithologic information will be collected to describe the subsurface geology. Soil samples for logging will be collected at five-foot intervals, at intervals of obvious contamination and at stratigraphic features of interest.

One monitoring well will be installed. The well will be cased with Sch. 40 PVC casing, threaded together; glues will not be used. The slotted interval will be a 0.020 inch slot and the annular space around the slots will be backfilled with a 2/12 size sand. Previous experience has shown this to be a reliable well design in fine grained and stratified depositional environments. Final well design will be modified to the site specific conditions encountered in the borehole during drilling. Once the aquifer strata has been defined, the casing will be lowered to the bottom of the borehole, leaving a slotted interval above the occurrence of groundwater to observe for floating product. The sand pack will be placed to a point about two feet above the slots. A bentonite seal will be placed atop the sand pack, and a cement grout seal placed atop the bentonite using a tremie line, filling from the bottom to top of the borehole. A traffic rated well head access box and security device will complete the well.

Well Head Survey

All wells will be surveyed to mean sea level using a known datum and tied into the existing survey for the site. This will allow accurate measurements and groundwater gradient to be calculated.

Monitoring Well Development and Sampling

All monitoring wells will be developed to remove the drilling muck, grade the sand pack and provide a more complete hydraulic connection to the aquifer. The well volume will be calculated and a number of those volumes will be removed until the water becomes clear and the amount of sand pumped is minimal. The well will be allowed to recover for at least 72 hours prior to sampling. A log of the development will be kept for each well.

Each monitoring well will be purged using calculated well volumes based upon the depth to water in each casing. Depth to groundwater measurements will be made to the nearest one-one hundredth of one foot, and also checked for the presence of separate phase product. As each purge volume is removed, measurements of pH, electrical conductivity and temperature will be taken until these parameters stabilize, which is interpreted to be aquifer water entering the casing. The sample will be carefully collected with a clean bailer and poured into the appropriate laboratory prepared container with minimum cavitation. Each water sample will be labeled, logged onto a chain-of-custody form, and placed in a chilled ice chest. Upon completion of the borehole sampling, the borehole will be sealed. Upon completion of well sampling, the well will be closed and locked and the sampler will move to the next sampling point.

Chemical Analysis

Soil and groundwater samples will be analyzed at a State certified analytical laboratory. The ultimate number of samples cannot be estimated at this time, however the number will be the minimum to meet the regulatory requirements. Samples will tested for the following; Total Petroleum hydrocarbons as Gasoline (TPHG), Benzene (B), Toluene (T), Ethylbenzene (E), Xylene (X) and Oil and Grease (OG) using EPA Methods 3550, 3510/8015, 5030, 5520, and 8020. + Chlorinateds!

Soil and Groundwater Disposal

Treated soil and groundwater will be stored on-site until the levels of contaminants have declined to not-detected. At that time, they will be properly disposed off-site, or recycled. The Regional Water Quality Control Board encourages water reuse or recycling as long as the appropriate documentation and reuse are present and proposed. Documentation of haulers and correspondence and manifests will be attached to the final report.

Report

A report of the findings of this site investigation will be prepared. The report will include the field methods, permits, excavation observations, well abandonment procedures and observations, exploratory boring log, monitoring well construction details, soil and water treatment length, chemical analytical data and report narrative. The narrative will include site observations, soil and water disposal and documentation and estimates of effectiveness of cleanup.

Quarterly Reporting

ACC currently is performing the site quarterly monitoring. It is anticipated that ACC will continue quarterly sampling and reporting until the request for site closure is made.

If you have any questions, please call.

Sincerely,
Gen Tech Environmental, Inc.

Stuart Solomon
Principal

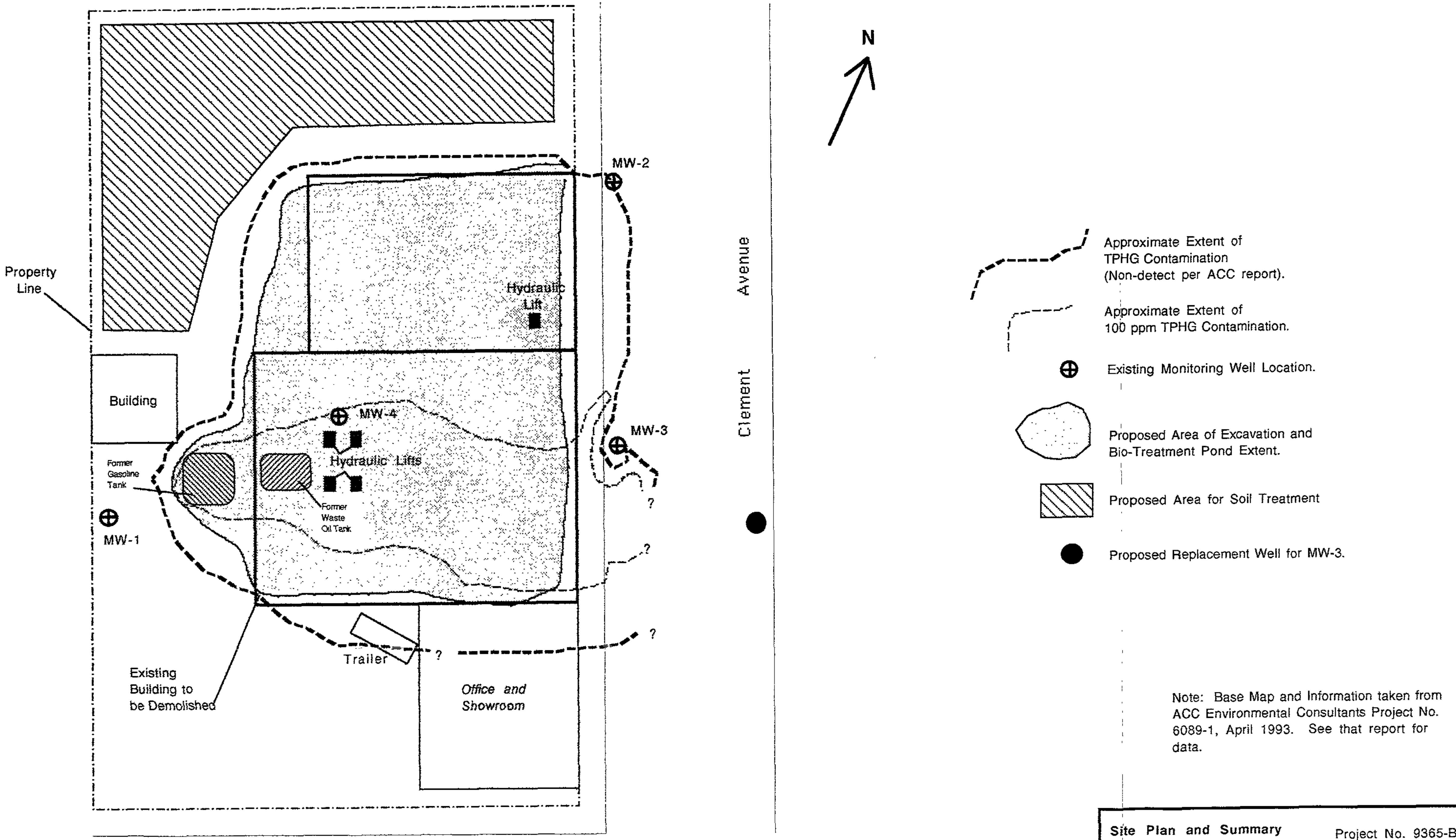
Christopher M. Palmer
C. E. G. 1262

REGISTERED GEOLOGIST
CHRISTOPHER M. PALMER
№ 1262
CERTIFIED ENGINEERING GEOLOGIST
STATE OF CALIFORNIA

attachments: Figure 1. Proposed Excavation and Biologic Treatment Pond Location
GTE Sampling and Well Construction Protocol
InterBio (Solmar) Hydrocarbon Degrading Microorganisms Information

Reference

ACC Environmental Consultants report dated June, 1993 entitled, "Soil and Groundwater Investigation Ron Goode Toyota 1825 Park Avenue Alameda, CA," Project No. 6089-1, 11 pages with attachments.



Gen Tech Environmental, Inc.
San Jose, CA

Site Plan and Summary Contamination Map; Proposed Excavation and Bio-Treatment Pond Site at 1825 Park Avenue Alameda, CA	Project No. 9365-B Scale: 1" = 40' Date: June, 1994 **Figure 1**

APPENDIX 1

BIO-TREATMENT PROTOCOL

BIO-TREATMENT PONDS

Bio-Pond Groundwater Treatment For Migration Control and Mitigation of The Contaminated Plume:

The area of plume - as depicted in previous studies - will be excavated to approximately 8 to 10 ft. BGS. According to previous reports, groundwater in the area is at a depth of 6 ft. to 8 ft. below grade. During the excavation, free product (if encountered) will be skimmed from the ground water and appropriately disposed of. In effect, at this point, we will have created a large pool - representative of the majority of the area of groundwater contamination - and the groundwater in the pool will be exposed and available for direct treatment.

Method of Initial Groundwater Treatment:

(In most cases) a sump will be dug into the center of the pit and lined with geotextile fabric. A pump will be installed and plumbed such that water pumped from the pool will be circulated through a sprinkler system and directed back into the pond. The sprinklers will be located within the excavation pit. During the treatment of the water, these sprinklers will be operated continuously, providing oxygenation and circulation of the water. The groundwater in the pond will be inoculated with Microorganisms (bacteria) specifically designed for hydrocarbon degradation. This inoculation will be made to an exact standard deemed appropriate through proper studies of the water at the subject site. During the inoculation - as part of the inoculation process, the water will be treated with nitrogen and checked for a proper balance of nutrients necessary to support active growth of the bacteria.

Water Treatment System Operation:

Prior to initiating the treatment process, water samples will be obtained from the pond and tested at a State Certified Analytical Laboratory for the presence of TPH and BTEX. Additional field tests will be performed periodically to determine the effectiveness of the process. After inoculation, the circulation system will be permitted to operate through the treatment cycle. Our estimate is that each degradation cycle in this case will be 7 to 14 days under reasonable conditions. Samples of the water will be taken and lab tested after each cycle to prove successful degradation of the contaminants. Once this is determined, the clean water will be pumped from the treatment pit into water trucks where the cleaned water can be used for purposes of irrigation (or other approved alternate uses).

Disposal of the water will be done under sanction of a waiver from the Regional Water Quality Control Board. Each pumping of the pit will create a temporary cone of depression in the natural ground water level. As the water in the pit redevelops, contaminates from outlying areas will be drawn into the pit. The cycle of decontamination will again be initiated, and then repeated as many times as is necessary to produce consistently clean groundwater.

Decontamination of Affected Soils:

Soils taken from the excavation pit and found to be contaminated will be handled in the following manner:

The soil will be run through a Screen-All system to provide uniform breakdown and aeration of the soil. The soil will be fertilized and treated with microorganisms and growth enhancers as it is run through the screening system. The contaminated soil will be spread throughout the treatment and tilled regularly. Field tests of the soil will be performed regularly. When the soil is successfully decontaminated, soil samples will be taken and submitted for certified laboratory testing to prove the decontamination success (less than 10 PPM).

Backfill, Compaction, Resurfacing:

The cleaned soil will be used along with the overburden soil to backfill the excavation areas. Soil will be placed in 1 ft. lifts and compacted to at least 90% ASTM up to sub-grade. If desired, compaction tests will be performed to verify geotechnical engineering specifications. The surface areas where concrete was previously removed will be re-poured with reinforced concrete of similar thickness to what was originally removed. Asphalt areas will be resurfaced with thickness alike to what was removed. A minimum of 6 inches of base rock will be used to form a base for the asphalt.

Reports and Documentation:

All phases of work included herein will be professionally documented. A professional Technical Report will be written by a Certified Engineering Geologist for submittal to the Alameda County Water District and Regional Water Quality Control Board.

Site Restoration:

The site will be thoroughly cleaned and restored after the project is completed.

APPENDIX 2

SOLMAR HYDROCARBON DEGRADING MICROORGANISMS

ENHANCED BIODEGRADATION OF ALIPHATIC AND AROMATIC HYDROCARBONS THROUGH BIOAUGMENTATION

BY

R.B. "Jones" Grubbs

AS PRESENTED TO THE 4TH ANNUAL
HAZARDOUS MATERIALS MANAGEMENT
CONFERENCE/EXHIBIT

June 2-4, 1986
Atlantic City, New Jersey

INTRODUCTION

The use of biotechnology to alleviate many of the current environmental problems is here. There has certainly been no shortage of articles, promises and hype predictions of the huge potential of biotechnology, particularly in medicine and agriculture. It can spur the imagination in all areas from solving the world's hunger, health and pollution problems to creating the dreaded andromeda strain. Sometimes though, the media seems so caught up in dreaming of the future that we neglect to look at all the good work going on now. This is the area which I wish to address today; particularly of how bioaugmentation is being successfully used in the treatment of hydrocarbon and chemical wastes.

HISTORY

Commercial bacterial formulations have been used with petroleum wastes for several years. In 1968 the Queen Mary was brought into Long Beach Harbor to be converted to a museum and convention center.[8] Some 8,000 tons of machinery had to removed. The 800,000 gallons of oily bilge water posed a real fire hazard. It contained nearly 20% lubricating oil and waste fuel. Through the use of bioaugmentation the flash point of the bilge water was raised sufficiently that the Long Beach Fire Department approved the use of acetylene torches within 18 hours after bacterial innoculation. Within six weeks the bilge water was so purified that it could be discharged into the harbor. The program removed 160,000 gallons of hydrocarbons.

In 1969 several programs were undertaken to clean up various oil-production sites stretching from Ventura, California to Santa Maria, California. At one site of the Home Stake Production Company, 55,556 gallons of heavy, tar-like oil were digested by bacterial supplements in a pond system.[14] Other disposal methods were tried without success. The oily waste was so viscous that vacuum trucks could not be used. There was insufficient volatile matter to allow incineration. They had also attempted to till the waste into the soil, but solar warmth caused the oil to bleed back to the surface.

In another successful project with Getty Oil Company, bioaugmentation was used in a 3,700 barrel sump of heavy, tarry crude oil.[17] Some 2,000 barrels were biodegraded and the remainder was converted to a low viscosity, pumpable crude of economic value.

Caswell reported in 1971 the use of specially selected cultures in improving the efficiency of an activated sludge facility handling wastewater from a bulk oil handling terminal.[3] The terminal stored and transhipped vegetable oils, animal fats and greases as well as petroleum products and petrochemicals. The cultures consumed oils and helped alleviate shock loadings.

Deutsch in 1979 reported the improvement in the performance of activated sludge systems through bioaugmentation.[4] In a controlled test at an Exxon refinery in Benicia, California a 32% improvement in total organic carbon removal was noted after innoculation with a commercial formulation. This plant had the advantage of parallel trains so comparisons between the bioaugmented train and the other, operated in routine fashion, could be made. A switch of the innoculation led to a reversal in the performance of the two trains, showing that bioaugmentation was responsible for the performance improvement.

Currently bioaugmentation is used in a wide variety of hydrocarbon and petrochemical uses from activated sludge units to land farming applications and spill cleanups. Lets look at some of the principles involved in the biological treatment programs.

HYDROCARBON STRUCTURE AND BIODEGRADATION

Of the various petroleum components, n-alkanes and N-alkylaromatics, of the C_{10}-

STRAIGHT-CHAIN SATURATED HYDROCARBONS

Name	Boiling Point	Formula	Structural Formula
Methane	−161°	CH_4	CH_4
Ethane	−88	C_2H_6	CH_3CH_3
Propane	−45	C_3H_8	$CH_3CH_2CH_3$
Butane	0	C_4H_{10}	$CH_3CH_2CH_2CH_3$
Pentane	36	C_5H_{12}	$CH_3CH_2CH_2CH_2CH_3$
Hexane	69	C_6H_{14}	$CH_3CH_2CH_2CH_2CH_2CH_3$
Heptane	98	C_7H_{16}	$CH_3CH_2CH_2CH_2CH_2CH_2CH_3$
Octane	125	C_8H_{18}	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_3$
Nonane	151	C_9H_{20}	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$
Decane	174	$C_{10}H_{22}$	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$

FIGURE 1

C_{22} range are generally considered the least toxic and most biodegradable.[2] Counterparts in the C_5 - C_9 range may have high solvent-type membrane toxicity. At low concentrations they are biodegradable. In waters and impermeable soils probably more is lost by volatilization than by biodegradation. Gaseous alkanes in the C_1 - C_4 range are biodegradable, but again this usually isn't the removal mechanism. Aromatic hydrocarbons are also degraded, but less readily as the number of condensed aromatic rings increases.[3] The alkanes, alkylaromatics, and aromatics above C_{22} have low toxicity, but their physical characteristics, including low water solubility and their solid state at the ideal physiological temperature of 35°C, disfavor biodegradation.

ALKYL AROMATIC STRUCTURES

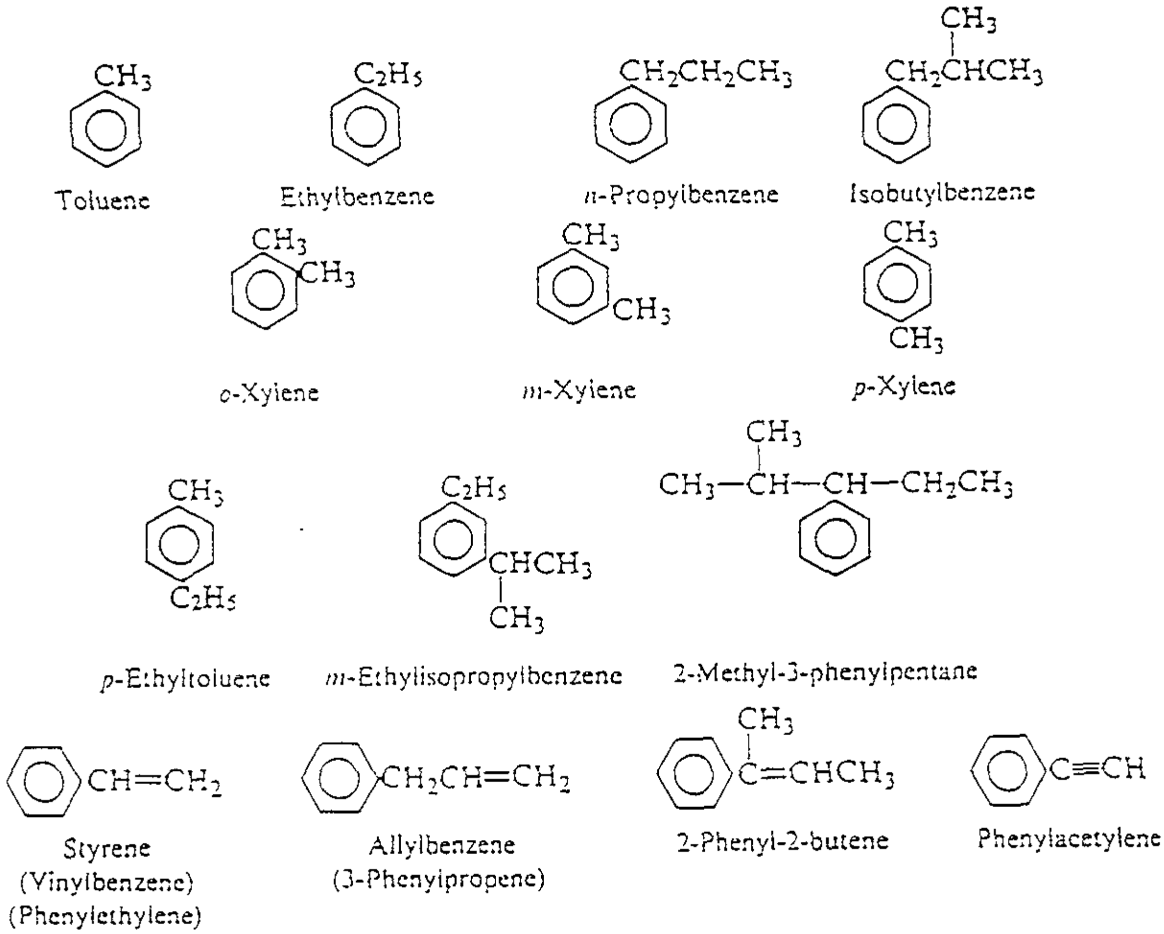


FIGURE 2

AROMATIC STRUCTURES

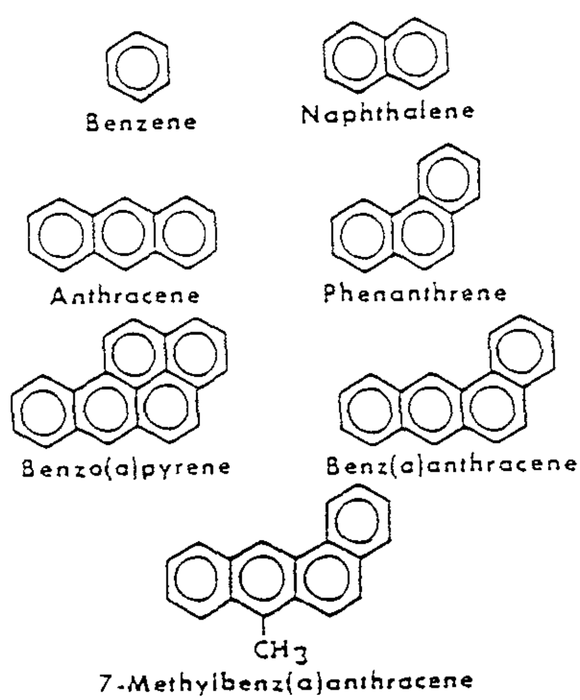
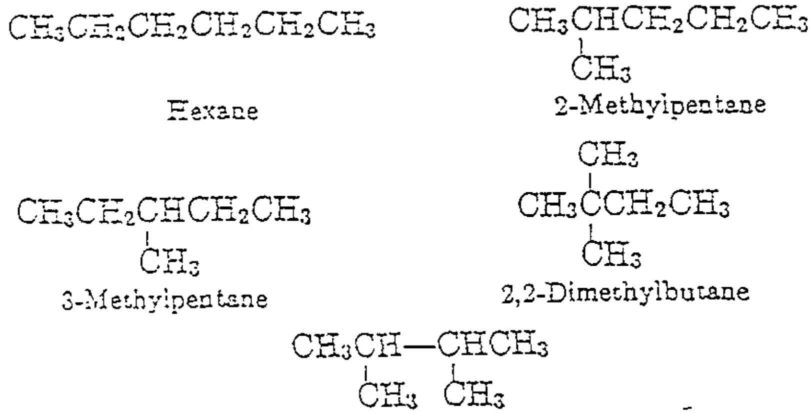


FIGURE 3

Branched compounds are considered less biodegradable. This makes sense inasmuch as the tertiary and quaternary carbon atoms present hinder beta oxidation.

N-HEXANE AND BRANCHED FORMS OF HEXANE

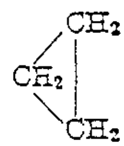
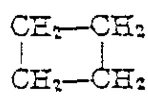
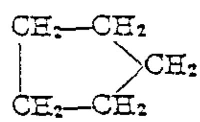
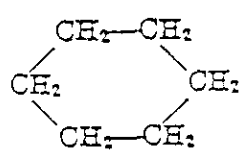


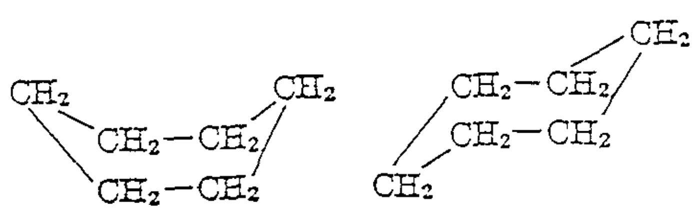
Hexane; 2-Methylpentane; 3-Methylpentane; 2,2-Dimethylbutane; 2,3-Dimethylbutane

FIGURE 4

The biodegradation of cycloalkanes of C_{10} and below exhibit high solvent type membrane toxicity. Highly condensed aromatic and cycloparaffinic systems, parti-

CYCLOALKANES

Name	Structural Formula	Boiling Point
Cyclopropane		−34.4°
Cyclobutane		13
Cyclopentane		49.5
Cyclohexane		81.4



Boat form   Chair form

Cyclohexane models

FIGURE 5

cularly those with four or more rings such as Benzo(a) pyrene and Benz(a) anthracene and other components of tar, bitument, and asphalts are considered the most resistant to biodegradation[2].

The saturated n-alkanes are considered to be the most readily degraded compounds of petroleum[3]. The presence of double bonds adds greater resistance to complete

UNSATURATED STRUCTURES

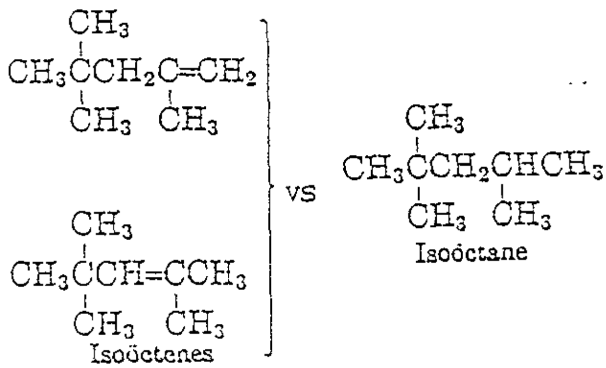


FIGURE 6

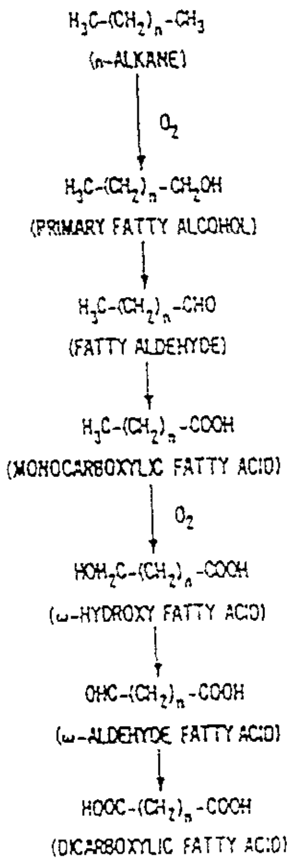
degradation[3]. A typical crude oil contains tens of thousands of compounds that differ markedly in volatility, solubility and susceptability to biodegradation. A refined product such as kerosene may contain 5,000 - 10,000 different compounds. Crude oils from different fields and even parts of the same field differ in their proportions of components as well as classification of compounds. Obviously different petroleums will have different biological fates. The engineer's dream of a steady state treatment system is just that, a dream.

MEASURING BIODEGRADATION POTENTIAL

There is no accurate way to measure petroleum degradation[3]. In situ estimates of rates are subect to so many variables. We've already discussed the chemical composition differences. Environmental changes have a great effect. And there are analytical limitations, not the least of which is the phase system one ordinarily deals with in aquatic systems. In the field there can be emulsification, sinking of the oil, freeing up of oil from the bottom, etc., all which complicate the situation in aqueous systems.

Soil situations aren't any easier. Biodegradation is commonly measured by periodic extraction with solvents such as diethyl ether or methylene chloride, where the solvent is then evaporated and the hydrocarbon determined as the residue. The problem here is that the solvent extract contains not only polluting hydrocarbons, but also lipids, waxes, and hydrocarbons of non-fossil origin. This may only be 1-5% of mineral soils, but it can be as high as 20% in peat soils. In dealing with soils one needs to find an uncontaminated control sample for a correction factor.

Something else to be considered is that biodegradation intermediates on the way to mineralization contain oxygen. Depending on the substrate as well as the particular organism, there are a variety of ways to go. The literature contains all sorts of pathways. For example with alkanes there is the diterminal oxidation



Pathway of diterminal alkane oxidation.

FIGURE 7

pathway wherein the alkane is oxidized to a fatty acid, which is subsequently oxidized to the hydroxy fatty acid, and then to a dicarboxylic acid. Most studies have been done with single organisms, which isn't typical of the real world. So in essence scientists don't understand fully what is going on. But the point is that the conversion products, be they fatty acids, alcohols, ketones or whatever, have a higher molecular weight than "pure" hydrocarbons, so that weight losses underestimate the hydrocarbon loss.

Spectrophotometric methods in the UV or IR range have been used for quantitative assessments. Gas chromotography can determine the fate of individual components. High performance liquid chromatography is generally required when one gets above the C_{35} range.

Mineralization or total degradation can be monitored by CO_2 emissions, which can reach improved precision and specificity through ^{14}C techniques. They do not measure the conversion to metabolities or cell mass, however.

Laboratory studies cannot duplicate the chemical and photochemical degradation that normally occurs in the field. Generally in the field autooxidation of organic compounds occurs through free radical formation or photochemical excitation.[2] This may be aided by light-absorbing photosensitizers. Metal ions and some organosulfur compounds can act as free radical generating catalysts. These mechanisms obviously play a bigger part in aquatic situations than in soil environments, where penetration becomes a significant factor.

Laboratory methods are also inhibited by scale effects. Microbial life like all life seems to compensate for its environment. A gold fish remains small in the fish bowl at home, but can become quite a large fish if put into a pond. This is not the only factor. In small scale batch studies bacterial waste products accumulate, greatly decreasing the efficiency of the biomass.

Other means of measuring oil degradation are used but each has shortcomings. For example the disappearance of a visible oil film is very relevant to the effects on birds and other wildlife in a marine environment, but it gives little information on what happened to the oil. Was it emulsified, mineralized, modified, or whatever? Solvent extraction has many of the same problems as outlined before.

Loss of total organic carbon measures mineralization but nothing more (as does CO_2 generation).

Oxygen uptake does take in both mineralization as well as partial oxidation, but one doesn't know how far the oxidation has gone or what's been converted to biomass.

One can measure the properties of the oil which may or may not affect the ultimate pollution hazard, and is highly subjective!

Bacterial numbers can provide a qualitative indication of the organisms present, which may or may not give a quantitative assessment of the extent of degradation.

Is all of this saying it's so confusing there is no hope? No it isn't. It's simply saying that there are no pat answers. It's much like the medical field. There too, no one has all the answers. There are too many variables. But like medicine, through experience and a thorough understanding of the knowledge that is available, expert professionals can cure many of the chronically ill "environmental patients." What are some of the techniques used for enhancing the biological treatments?

FACTORS AFFECTING MICROBIAL ACTIVITY

Before we address successes in bioaugmentation, we need to consider the various factors involved in biodegradation. As you must be aware there is far more involved with these programs than simply adding cultures. Other factors need to be considered.

Oxygen Levels

Hydrocarbons, being highly reduced substrates, require an electron acceptor with oxygen, nitrate and sulfate being the most common. Oxygen pathways are the most important in nature since the initial steps of hydrocarbon biodegradation are oxygen dependent. Sporadic reports of anaerobic degradation in vitro remain controversial and convincing proof of significant anaerobic hydrocarbon biodegradation is still outstanding.[2] A pathway for alkanes has been suggested wherein terminal carbons are dehydrogenated to alkenes which are in turn hydrated to secondary alcohols, but at best this plays a minor role in nature.[6] The intermediates of aerobic hydrocarbon biodegradation can be metabolized further under anaerobic conditions. Sulfates are a potential electron acceptor, but are not abundant in soils. Nitrate is not energetically favorable for this purpose in soils.[9]

In soils aeration depends on the total amount of air filled pore space. Elimination of air-filled pore space by waterlogging or compaction reduces oxygen transfer. Large amounts of biodegradable organics in the top layers will deplete oxygen reserves in the soil and slow down oxygen diffusion rates to the deeper layers. No wonder tilling is so valuable in land farming operations. Tilling also distributes the hydrocarbons more evenly in the soil.

Oxygen can become a limiting factor in all types of petroleum degradation, so aeration is required in most applications. In aqueous systems aeration and agitation also provide more surface area of hydrocarbons to the bacteria which live only in the aqueous phase of the system and work at the oil to water interface.

Moisture

Moisture is essential to active life processes. Bacteria rely on water to exchange everything through the cell. At 100% moisture in soils, however, all pore spaces are filled with water. At only 10% moisture osmotic and matric forces reduce metabolic activity to marginal levels. Moisture levels in the range of 20% to 80% generally allow suitable biodegradation in soils.[2]

Nutrients

The addition of large quantities of hydrocarbons in a system usually creates a nutritional imbalance which needs to be corrected by the application of inorganic fertilizers containing nitrogen and phosphorous. Biosludges from refinery and petrochemical treatment facilities normally contain enough nitrogen and phosphorous.

For land farming operations the American Petroleum Institute recommends a C:N ratio of 160:1. Laboratory experiments by Dibble and Bartha showed C:N ratio of 60:1 and a C:P ratio of 800:1 to be optimum.[5] The expense of fertilizer and the potential for groundwater contamination encourage more conservative application rates. Most agricultural fertilizers contain excessive P and K for microbial use. Urea and ammonium compounds can be added to such fertilizer to bring up the nitrogen levels. Nitrates can pose leaching problems and encourage denitrification under anaerobic conditions. The ammonium ion being positively charged binds to the negatively charged soil particles. But in well aerated soils with neutral pH values above 50°F the ammonium ion is nitrified to nitrates in one to two weeks after application.[18]

In cleanup situations, one frequently can not do any sort of mass balance of pollutants. In such cases sufficient nitrogen and phosphorous must be present to start off microbial activity and both be monitored continually to assure they don't become too low by being used up for cell mass, leaching, nitrification, or volatilization. We've found that N levels should exceed 5 ppm and phosphorous exceed 1 ppm at all times to maintain adequate microbial activity.

Oil contaminants that percolate deep into the soil and to the water table are difficult to clean up. Deep infiltration can occur with low viscosity oils, gasoline, kerosene, light crudes and light heating oils as well as a variety of petrochemicals on porous sandy or gravelly soils. Subsoil cleaning can be achieved by installing drainage systems or wells and pumping the groundwater.

Oils tend to cling tenaciously to soils and their limited solubility in water minimizes the effectiveness of flushing. The metabolites are more water soluble and can break down anaerobically, helping the oxygen availability limitations. Nitrogen compounds are relatively mobile in soils but phosphates bind up readily so that their transport can be a problem. Aeration can be provided by direct air injection or oxygenated water.

In running a treatment plant the accepted BOD:N:P ratio of 100:5:1 must be maintained to assure adequate treatment.

Oil soluble fertilizers such as paraffinized urea and octyl phosphate have shown enhanced degradation in the sea.[15]

Temperature

Temperature affects the rates of microbial metabolism as well as the physical state of hydrocarbons. It also affects the solubility of the substrates. Some small alkanes and aromatics are more soluble at 0°C than at 25°C.[16] Elevated temperatures can influence nonbiological losses, mainly evaporation. In some cases the decreased evaporation of toxic compenents at lower temperatures has been reported to have inhibited degradation.[6] In general most mesophiles perform best at about 35°C but their performance can be affected by these other factors. Consequently scientists have reported different optimums and considerable variance in activity at different temperatures, little change in activity over given temperature ranges, and other superficial contradictions. Hundleston and Cresswell (1976) reported petroleum degradation in soils as low as -1.1°C as long as the soil solution remained liquid.[10] Degradation rates were quite slow. In natural habitants, shifts in microbial populations due to temperature changes have been reported.[21] As one might suspect from such shifts, as well as changes in solubilities, there are reports showing that the types of hydrocarbons being degraded may vary with temperature. My own firm has been involved with a number of projects that went through cold weather. Although visually everything seemed to be put on hold, as soon as warm weather returned very rapid breakdowns occurred, indicating that some activity had continued through the colder weather.

pH Effects

While the pH of the marine environment is uniform, steady, and slightly alkaline, the pH of various soils covers a wide range. The marine environment is well buffered. In soils and poorly buffered treatment situations, organic acids and mineral acids from the various metabolic processes can significantly lower the pH. The overall biodegradation rate of hydrocarbons generally is higher under slightly alkaline conditions. So appropriate monitoring and adjustments should be made to keep such systems in the 7-7.5 pH range. Variations or swings in pH in treatment systems can have a very deleterious effect on the performance of the biomass.

Emulsions

Since oils and most petroleum hydrocarbons are only sparingly soluble in water, the relatively small interfacial area of oil in contact with water can limit the microbial degradation of oil. Microbes colonize the surfaces of oil droplets and the undersides of slicks. Many hydrocarbon using microorganisms produce emulsifying agents which greatly enhances their effectiveness in handling the oil. In my own firm's treatability studies, this is one of the things we look for. It is widely held that emulsifiers can be involved in the entry of hydrocarbons into the cells, but degradation can occur without emulsification. Emulsifiers have proven useful in some cleanup operations,[8] but various sources indicate that not all dispersants enhance biodegradation.[13,18]

Weathering

The so called weathering of organic products is sort of a two edged sword. Weathering is the collective term for solubilization, volatilization, photochemical reactions, emulsification, oxidation and microbial breakdown of the organics after introduction to an ecosphere. Most of the biodegradation compounds are more water soluble. This may promote leaching in soils or make them more available to microorganims which require water for their life processes. On the other hand many of the oxygenated compounds exhibit greater toxicity to microbiota. Some of the metabolities might even crosslink or "cure." It is believed that Alberta's heavy oils are of biodegradative origin as the light weight components were degraded leaving heavy tars.[20] There have been reports of decreases in bacterial and algae growth in natural habitants with the weathering of crude oil, possibly as the more degradable components were lost.[5]

BIOAUGMENTATION

Bioaugmentation has proven to be beneficial in many petroleum and petrochemical applications. But it needs to be understood that the mere additions of bacteria of any type is not the answer. Those added must be able to utilize the waste present and to successfully survive and compete in the environment to which they are being added. Jobson et al (1974) detected no significant stimulation of biodegradation in soil following innoculation with oil degrading cultures.[11] Lehtomaki and Niemela on the other hand reported positive results with a brewery waste (1975)[12] Dibble and Bartha (1979) found that the addition of yeast extract failed to stimulate oil biodegradation in soil, and that sewage sludge actually inhibited it.[5] Mechanisms for such supression could be competition for the oxygen or actual repression of hydrocarbon degrading enzymes by the availability of more readily utilizable substrates.

Results can be obtained using suitable bacterial formulations for the intended application. To put this in perspective, the Gas Division of the City of Clearwater, Florida working through Biological Consultants, Inc. of Trussville, Alabama and Wastewater Engineers of Hermitage, Tennessee utilized bioaugmentation for the cleanup of 610,000 gallons of coal tar left over from the old days of manufacturing producers gas in 1982 and 1983. These types of wastes are usually considered as being quite biorefractory. Within a nine month period the overall volume was reduced some 87%. The COD on a mass basis was reduced 96%. Final oil and grease levels were only 0.3% of the original on a mass basis. Most importantly the phenolic content was virtually eliminated. Whereas the original content was 1,660 mg/l the final concentration was only 0.01 mg/l. Whereas they initially had some 10,000 pounds of phenol to contend with, only 0.008 pounds remained. This is an outstanding example of detoxification. Clearwater was originally faced with disposing of 610,000 gallons of hazardous wastes with an unlimited future liability hanging over their heads. Through bioaugmentation techniques they were able to obtain permission to bleed the treated residual contents into the sewer system. Disposal costs and future liability were eliminated.

TABLE I

CLEARWATER GAS BIOAUGMENTATION PERFORMANCE SUMMARY

	Start 08/04/82	8.8 mos 04/23/83
Volume of Tar	610,000 gals	80,000 gals
Flash Point	29°C	100°C
COD	3,750,000 mg/kg	1,238,000 mg/kg
Oil & Grease (O & G)	286,900 mg/kg	5,788 mg/kg
Phenol	1,660 mg/kg (approx. 10,100 lbs)	0.01 mg/kg (0.008 lbs)

OVERAL VOLUME REDUCTION	86.9%
OVERALL COD REDUCTION (ON A MASS BASIS)	95.7%
OVERALL O & G REDUCTION (ON A MASS BASIS)	99.7%
OVERAL PHENOL REDUCTION (ON A MASS BASIS)	Approx. 100%

Similar results were obtained in treating three collection ponds in 1978 from a pilot coal conversion facility. BOD_5's initially were 300 to 495 mg/l. Bioaugmentation brought these down to 4 to 8 mg/l levels. Phenols had been originally at 28 to 33 mg/l and were reduced to 0.057 to 0.094 mg/l levels.

At one of the superfund sites phenol reductions were required. Roughly 40% of the phenolics present were substituted alkyl, chloro and nitro phenols. Initial phenolic content from four quadrants of the dumpsite was 516 mg/l. Within 40 days of bioaugmentation this was brought down to 262 mg/l, when cold weather set in. After warm weather resumed, the phenol content was again checked and found to be less than 100 mg/l, indicating degradation during the winter months.[8]

In 1984 my firm and Loma Industries were involved in the cleanup from a ruptured transfer line which ran under a railroad track of a national petro chemical company. Coincidentally, a jumbo tank car was moving on the track as solvents were being pumped through the line. The resulting rupture allowed 300 to 400 gallons of mixed solvent to penetrate the ground 38 inches below grade and to contaminate the aquafer for 120 feet along the track bed.

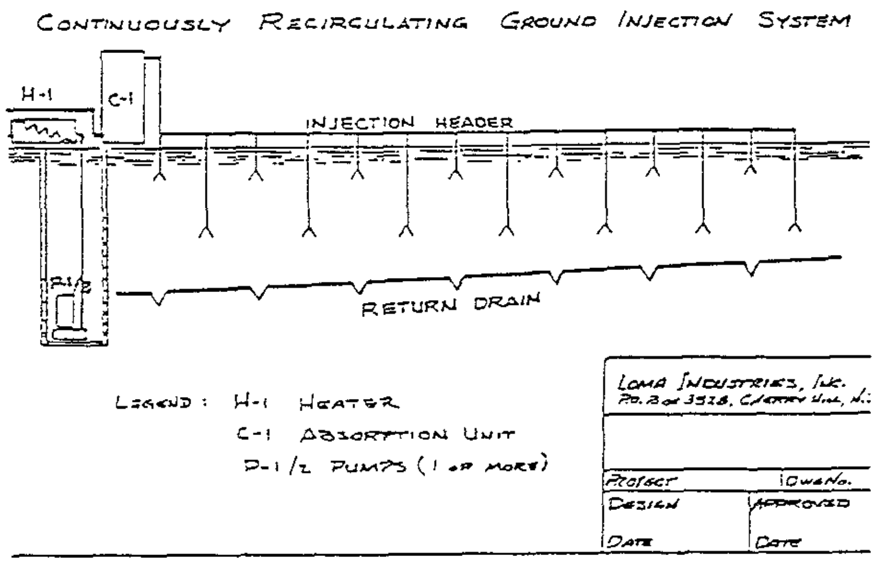


FIGURE 8

Following a cleanup program of nine months with a biological recirculating ground injection system, a 99.5 % decomposition of contaminants was achieved (see Table 2)

TABLE 2

COMPONENT	09/24/84 (ppb)	10/31/84 (ppb)	04/04/85 (ppb)	% Reduction
Benzene	N/A	96	31	67.7
Carbon Tetrachloride	N/A	65	nil	99.9
Chlorobenzene	9,050	227	37	99.6
1,1 Dichloroethane	N/A	508	341	32.9
Ethyl Benzene	154,000	1,119	382	99.8
Toluene	31,000	1,276	526	98.3
1,1,1 Trichloroethane	N/A	82	nil	99.9
Xylene	1,249,840	16,825	1,979	99.8

N/A - not analyzed for

Last year Biological Consultants and Haz Tech of Decatur, Georgia were involved with my firm in the disposal of printing ink wastewaters of a major container corporation. The wastewater had been temporarily stored in tanks. A biological system using portable tanks was installed to employ bioaugmentation. Initially the COD was 5,500 mg/l. Individual components are shown in Table 3.

TABLE 3

COMPONENT	INITIAL	FINAL
Acetone	143 mg/l	<1 mg/l
Hexane	2,650 mg/l	<1 mg/l
Isopropyl Acetate	205 mg/l	<1 mg/l
Methyl Ethyl Ketone	257 mg/l	<1 mg/l
Toluene	17,000 mg/l	<1 mg/l

Within a matter of days the concentration of all of these compounds were brought below 1 mg/l.

These are just a few examples of the potential bioaugmentation offers.

CHOOSING A SOURCE

Bioaugmentation obviously involves more than the simple innoculation of some biological additive. Technical service backup is crucial to the success of any program, and you must satisfy your own requirements as to who can do this best for your own organization.

Formulations vary widely. One should be particularly cautious of would be suppliers who have only one all purpose formulation or who make recommendations without the benefit of laboratory treatability studies. Using the medical analogy again, most hydrocarbon situations require prescription rather than over the counter drugs. Prescription type drugs require a physician who can take a multitude of factors into account for the patient's needs. Simple directions as given with over the counter drugs will not suffice.

There should be caution in dealing with firms that have limited documented similar case studies and who cannot refer prospective customers to several satisfied clients with recent success in similar systems. Gimmicks in the promotion of products should also lead to caution. Advertising the storage of stock cultures in a bank vault, emphasizing the remoteness of the earth from which certain cultures were isolated or additions of trace amounts of mutagenic agents, unique biological additive so that the resulting formulation is implied to have unproven uniqueness or undemonstrated value should all be suspect.

Many firms boast of high plate counts. But unless the formulation is carefully balanced and adequately tested for the intended application, sheer numbers are virtually worthless, and may as Dibble and Bartha found be counterproductive.

Bioaugmentation is simply another tool that can be used in your efforts to make this world a better place to live. Hopefully you have a better appreciation of how you might use it.

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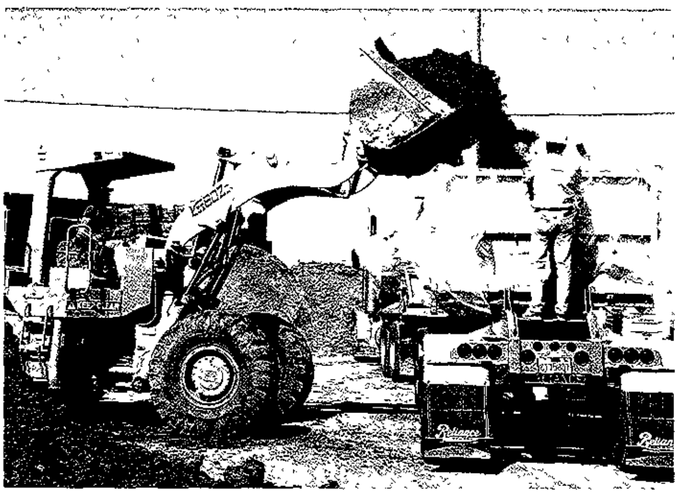

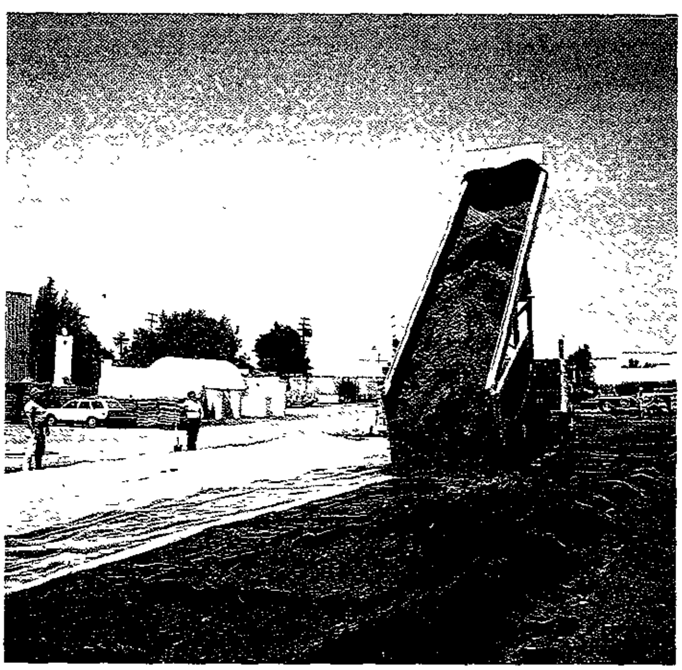

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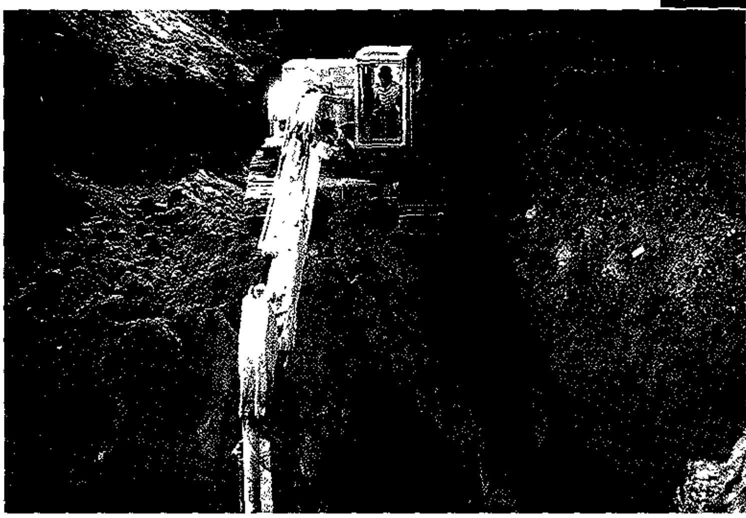

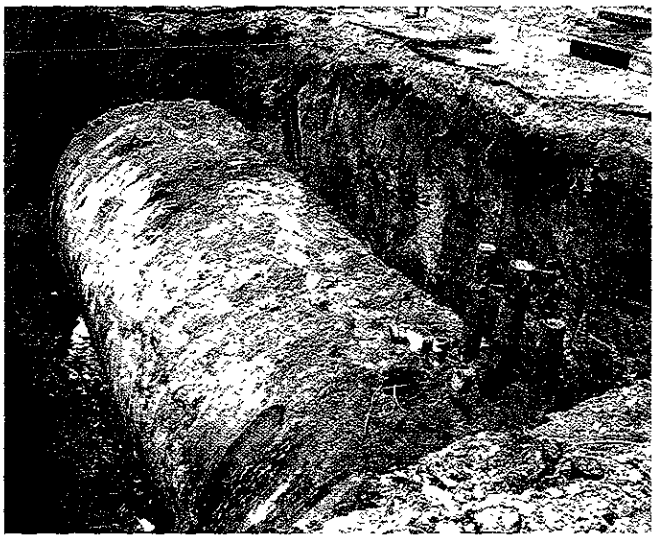

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