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

[redacted]

Mr. Lowell Miller
Senior Hazardous Materials Specialist
ALAMEDA COUNTY DEPARTMENT OF
ENVIRONMENTAL HEALTH
80 Swan Way
Oakland, CA 94621

Dear Mr. Miller:

I mailed you our **Phase II Report on the Hydrogeology and Site Characterization Studies** completed on June 3rd by ourselves and Alton Geoscience for the P.I.E. Nationwide bioreclamation project in Emeryville. This report included an overview and updated recommendations by CytoCulture for proceeding with the Phase III recovery of free diesel product and treatment of contaminated groundwater as originally outlined in the Operational Plan we submitted on December 18, 1987.

As of the end of July, we have completed the construction of the groundwater treatment basins and have installed two sixty-five foot trenches in the southwest corner of the site in accordance with our plans. Both trenches appear to have at least 18 inches of contaminated water and some free product, so we anticipate adequate flow rates to generate the desired depression zones.

As soon as we receive payments from P.I.E. Nationwide on our current and past due invoices for this project, we will proceed with the installations and start-up for the "pump and treat" Phase III program. I will keep you posted of our progress and look forward to discussing this program with you soon.

Thank you.

Sincerely,

Randall J. von Wedel

Randall J. von Wedel, Ph.D.
Project Director

cc: Chris Falbo, Blymyer & Sons Engineers for P.I.E. Nationwide

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

Phase II Report on

Hydrogeology and Site Characterization Studies

for

P.I.E. Nationwide Property
5500 Eastshore Freeway
Emeryville, CA

in preparation for

IN SITU SITE REMEDIATION OF SOIL AND GROUNDWATER
HYDROCARBON CONTAMINATION BY
AUGMENTED BIORECLAMATION USING
LABORATORY SELECTED BACTERIAL CULTURES

by

CytoCulture International, Inc.

in a Joint Venture with

Sybron Chemicals, Inc.

in Collaboration with

Alton Geoscience

June 3, 1988

CONFIDENTIAL INFORMATION

submitted to

Frederick A. Tornatore - DHS, Toxic Subst., Altern. Tech. Sect.
Janet Naito - DHS, Toxic Subst. Control, Emeryville
Mike Chee - RWQCB, Toxics Clean-Up, SF Bay Region
Lowell Miller - Alameda Dept. Health, Hazardous Materials Mgmt.

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Introduction

CytoCulture International, Inc. and Sybron Chemicals, Inc. have been contracted as a Joint Venture to attempt to reclaim soil and groundwater contaminated with diesel hydrocarbons at the former P.I.E. Nationwide truck terminal in Emeryville. A preliminary Site Characterization Study was completed by Alton Geoscience and submitted as a Phase I Report to the Department of Health Services (Toxic Substances Control Division, Alternative Technology and Policy Development Section) last November. An Operational Plan for the site remediation was then submitted on December 18 as a supplement to the original proposal of July 29.

In March of this year, Alton Geoscience was contracted to install and sample 18 monitoring wells at the site in order to better define the diesel contamination plume. Their results are presented here along with their detailed geological and hydrogeological characterization of the site as the Phase II Report for this remediation program. This information will guide the design of the bioreactor systems for treating contaminated groundwater drawn from extraction trenches and for reinfiltrating the treated water upfield so as to inoculate contaminated soil with additional bacteria and nutrients.

This remediation project has been structured into distinct phases beginning with the preliminary and final Site Characterization Studies (Phases I, II), followed by the Phase III decontamination of groundwater using Sybron bacteria in above ground bioreactors, and ending with the Phase IV reinfiltration of the treated water with additional bacterial cultures to inoculate the contaminated soil. This phased approach is described in the Operational Plan of December 18, 1987.

need WDR for reinjection

Results of Site Characterization Studies

Free Product Plume

In spite of the expected widespread presence of free diesel product in the groundwater along the southern and western boundaries of the property (see Initial Site Characterization Report by Alton Geoscience, November 3, 1987), free product was measured only at monitoring well 7, directly west of the former underground tank locations. Previous investigations in 1986 by Groundwater Technology (see p.4, op.cit.) reported free product in three wells drilled around the tank pit area where Building D of the new shopping center now stands. Directly to the south of this area, monitoring well 3 had no apparent free product in spite of an adsorbed phase hydrocarbon level of 17,000 ppm.

Figure 7 of the enclosed report shows the newly estimated extent of free product plume from the single data point obtained in the most recent Alton Geoscience investigation only. No explanation has been given for why free product has not appeared in other wells to the south in spite of the presence of high levels of adsorbed phase hydrocarbon, but it would appear that free product is migrating primarily in a westerly direction at this time. Tidal action may have dispersed free product into other areas which are now under buildings.

Dissolved Phase Hydrocarbon Levels in Groundwater

Dissolved phase hydrocarbon contamination in the groundwater is surprisingly low (< 2 ppm) in spite of previous studies conducted by Groundwater Technology suggesting total petroleum hydrocarbon levels from 3 to 600 ppm in wells drilled where Building D now stands. Figure 8 shows a 1 ppm concentration contour line for the site plan as determined in the most recent Alton study.

Adsorbed Phase Hydrocarbon Levels in Soil

The enclosed Report on Site Characterization Studies completed by Alton Geoscience on April 28, 1988 summarizes data collected from soil borings in October 1987 and March 1988. As expected from preliminary studies and observations at the site, the soil contamination on site is basically confined to the southwestern quadrant of the property in the vicinity of the locations for the former underground tanks.

Table 1 lists the laboratory results for soil samples analyzed from previous test borings and the recent monitoring well drillings. Contamination levels for the southwestern area of the site range from 5,400 ppm to 17,000 ppm in the subsurface soils in or above the groundwater table.

Figure 2 has a site plan depicting the approximate locations of the corresponding borings and wells. Figure 6 is the same site plan superimposed with concentration contour lines as estimates of the extent of the adsorbed phase product in the soil. The diesel hydrocarbon concentration values from Table 1 are shown by the corresponding borings and monitoring wells. The highest soil contamination (17,000 ppm) found to date came from a sample taken eight feet beneath the surface at monitoring well 3, directly south of where the underground tanks had been located (now under Building D). Although no free product was found in any of the monitoring wells along the southern boundary, there is no question that the soil in the saturated zone of this area is heavily contaminated, presumably from previous southward migration of free product through this area over the years.

Results of soil boring sample analysis by the 1986 Groundwater Technology investigation (see Table 1, Initial Alton Report) confirmed the presence of adsorbed phase product (e.g., 6,800 ppm at GT-6) immediately west of the former tank locations.

Figure 4 shows a representative cross section of the subsurface stratigraphy indicating where the samples were taken relative to the overlying artificial fill, bay mud sediments and prevailing tidal groundwater levels. Contamination is most apparent underneath the fill in the black bay mud sediments and mixed soils of the saturated zones.

Product Plume Definition

The FREE PRODUCT plume, from this recent Alton Geoscience study (Figure 7), appears to be confined in the vicinity of monitoring well 7 and the area eastward under Building D. Previous studies in 1986 by Groundwater Technology confirmed the presence of free product beneath the area now under Building D.

The DISSOLVED PHASE plume in the groundwater, although moderately low in concentration, follows a concentration contour line similar to that of the adsorbed phase hydrocarbon contamination, as shown in Figure 8. In the recent investigation reported here by Alton Geoscience, dissolved phase hydrocarbon levels never exceeded 2 ppm. In the 1986 study by Groundwater Technology, dissolved phase petroleum hydrocarbon levels of up to 600 ppm were reported for the area under Building D.

The extent of the ADSORBED PHASE contamination plume appears to be well defined as evidenced by the low levels of hydrocarbon found in the surrounding 11 monitoring wells to the north, east and south of the southwest quadrant of the property (Figure 6). From previous borings (see Initial Report) and the recent studies summarized here, the heavily contaminated soils lie under Building D and extend predominantly westward and southward all along the nearest bordering property lines. The anticipation of these findings led to the installation of six monitoring wells with four inch casings along the southern and western boundaries so they could later serve as extraction wells as described in the December 18, 1987 Operational Plan. Four of the six (not the most northerly nor the most easterly) four inch casing wells proved to have high adsorbed phase contamination levels, but ONLY ONE of the 4" wells had detectable free product (MW-7).

The contamination plume could not be defined beyond the western property line (Caltrans fence) along Interstate 80, but is presumed inaccessible and possibly stabilized on account of the freeway constructed there in 1953.

RECOMMENDATIONS

Based on results of the most recent Alton Geoscience Site Characterization and previous investigations, the following recommendations have been formulated to update our Operational Plan of December 18, 1987:

1. Install an extraction trench (approximately 200 feet) and complete bioreactor system (2,000 gallon, continuous flow) along the western property line across from Building D to recover free product and inhibit further western migration of diesel fuel in the area around Monitoring Well 7 (see Figure 7). Trench specifications have been described in the Operational Plan. Alternatively, larger extraction wells could be installed.

2. Install a second extraction trench (also about 200 feet) and bioreactor system just south of and parallel to the southern property line extending from near the southwest corner of the property eastward to beyond Monitoring Well 3. This depression zone will retrieve contaminated groundwater and may recover remaining free product present under or south of Building D. This trenching should also inhibit further southward migration of contaminated water or any undetected dissolved product.

3. The TWO continously operating bioreactor systems in the southwest corner of the property will treat contaminated groundwater extracted from their respective trenches along the southern and western boundaries. This system will consist of a 250 gallon clarifier and twin 1,000 gallon bioreactor tanks to maintain an anticipated flow rate of 1-2 gal per min and a retention time on the order of 8-12 hours. An optional oil/water separator will probably be available but probably not be necessary as the rate of bacterial biodegradation is expected to keep up with the influent levels of diesel product. The system will include a means of automatically dispensing and ionic tracer (e.g., sodium bromide or potassium thiocyanate) - see Operational Plan.

4. Pump and treat contaminated groundwater with both bioreactor systems to determine optimal operating parameters and to control product migration southward or westward in the area enclosed by the trenching system. Treated water, temporarily diverted to the sewer, will meet discharge limits set by the SF Bay RWQCB NPDES permit variance letter for this site of November 25, 1986. This pump and treat operation constitutes Phase III of the project.

5. Install reinfiltration trenches upfield from the extraction trenches to inoculate contaminated soil on both sides of Building D with treated water containing nutrients, an oxygen source and high densities of the diesel-specific cultures of bacteria.

6. Once product migration and mounding control has been demonstrated with the bioreactor/trench system, and RWQCB and DHS permission has been granted to proceed, initiate Phase IV reinfiltration with treated water, nutrients and bacterial cultures around Bldg D.

7. Additional reinfiltration galleries are planned for the contaminated soil south of buildings B and C in an attempt to treat the observed "hot spot" contaminations mentioned in the Operational Plan. Since groundwater contamination levels are so low (< 1 ppm in MW-1, MW-2 and MW-3), current plans now call for setting up a mobile 1,000 gallon BATCH bioreactor system which will periodically seed the soil in other contaminated areas of the site (such as around Buildings B and C) with batch cultures of diesel blend bacteria grown up on site using tap water.

In other words, this third batch bioreactor will not process groundwater but will periodically generate high density cultures for reinfiltration into long trenches running parallel to the Buildings and to the southern property line. Since the batch bioreactor will not be continuously oxygenating the soil, we intend to use facultative organisms capable of biodegrading diesel hydrocarbons under anaerobic conditions. In addition, we propose to introduce dilute hydrogen peroxide (< 500 ppm) to provide an oxygen source for the more efficient aerobic bacterial cultures. Specifications for this reinfiltration process will be presented in a forthcoming progress report and Operational Plan for Phase IV.

8. The Phase IV Reinfiltration Program will be monitored by testing selected monitoring wells for changes in petroleum hydrocarbon levels or the appearance of lithium chloride tracer and/or elevated bacterial counts. The proposed extraction trenching should adequately protect against any migration of free product or dissolved phase hydrocarbons south or west from the former tank pit areas under Building D.

It is important to note that in addition to the proposed extraction trenches, six 4" casing monitoring wells are now installed along the western property line (MW-7 and MW-15) and the entire southern property line (MW-1, MW-2, MW-3, MW-4) to create an additional "fence" for extracting contaminated water should any unexpected migration of the plume occur. See the discussion on optional water extraction methods in the Operational Plan.

Additional details on the operation, monitoring and verification of compliance for this soil/groundwater bioreclamation project are presented in the Operational Plan of December 18, 1987 or can be obtained from the Project Director at CytoCulture.

CytoCulture INTERNATIONAL

SYBRON Biochemical

INC

Bacterial Cultures for Wastewater Treatment

Randall J. von Wedel, Ph.D.
President and Director of Research

1208 Fourth Ave. San Francisco CA 94122 415/564•1516

Biodegradation
of Diesel and Aromatic Hydrocarbons

A TECHNICAL PRESENTATION BY

Sybron Biochemical and CytoCulture International

Industrial and Hazardous Waste Information Exposition
California Water Pollution Control Assoiciation
Wednesday June 8, 1988 Session B 2:40 PM

Biodegradation of Diesel and Aromatic Hydrocarbons

Sybron Biochemical / Sybron Chemicals, Inc. - New Jersey

C. Douglas Goldsmith, Jr., Ph.D. - Director of Research
Gary R. Hater, M.S. - Manager, Soil/Water Treatments

CytoCulture International, Inc. - San Francisco

Randall J. von Wedel, Ph.D. - Director of Research

Biodegradation of Diesel and Aromatic Hydrocarbons

Sybron Biochemical / CytoCulture International

- Bacterial Utilization of Diesel and Growth Kinetics
- Treatability Studies on Diesel Contaminated Clay
- Site Remediation Field Data for Dissolved Phase HCs
- Ongoing Augmented Bioreclamation Projects in California

Biodegradation of Diesel and Aromatic Hydrocarbons

Sybron Biochemical / CytoCulture International

- Bacterial Utilization of Diesel and Growth Kinetics
- **Laboratory Studies on a Model Diesel Fuel**

Biodegradation of a Model Diesel Fuel

Sybron Biochemical: Balderson & Goldsmith (1988)

EXPERIMENTAL APPROACH

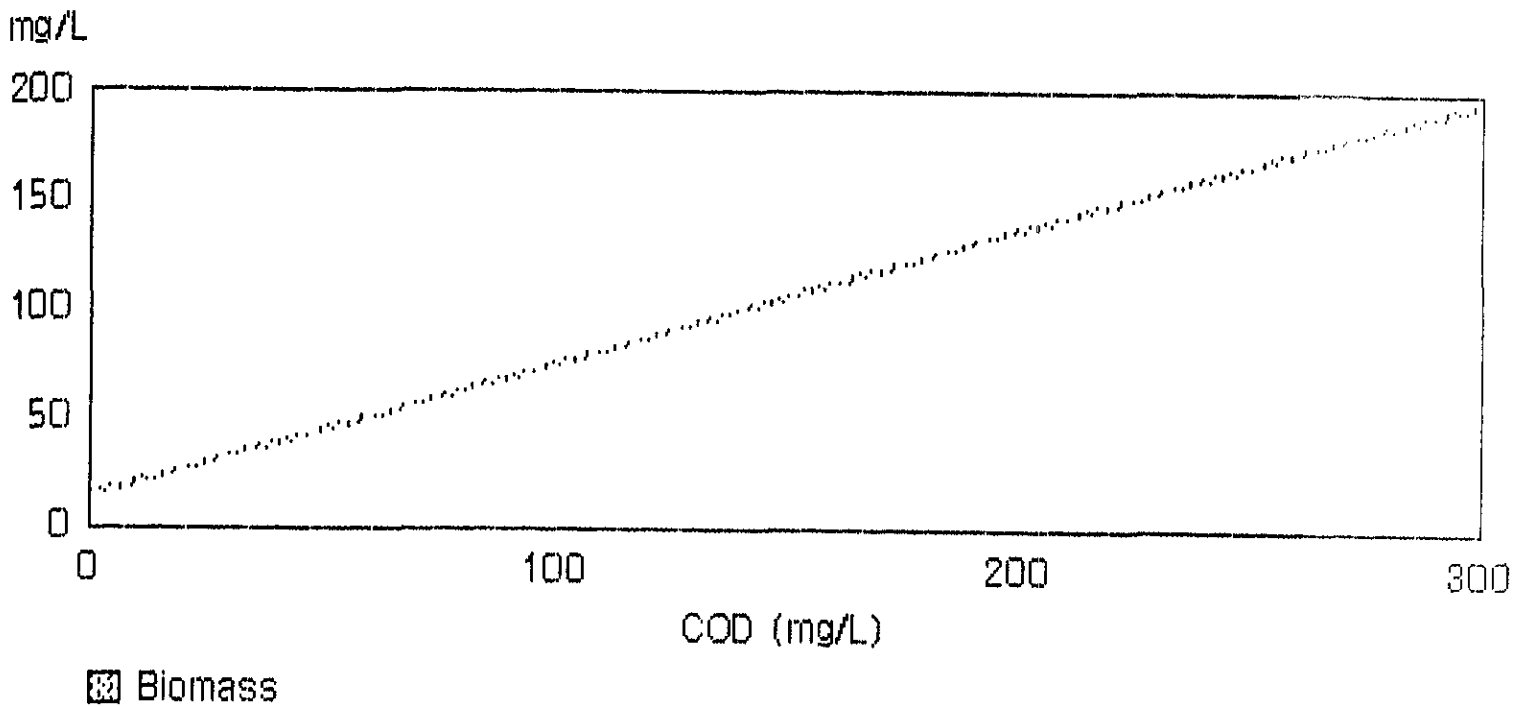
- Simulate Biodegradation in Groundwater
- Batch Microcosm: 500 ml Gas-tight Sampling Bulbs
- Synthetic Model Diesel Fuel as Substrate
- Commercial "Diesel Blend" of Aerobic Bacterial Strains
- Monitor Growth Rates, Utilization Rates, Cell Yield
- Establish Biokinetic Constants in Batch Cultures
- Predict Bioreactor Operating Parameters in Field

Biodegradation of Model Diesel Fuel

Sybron Biochemical: Balderson & Goldsmith (1988)

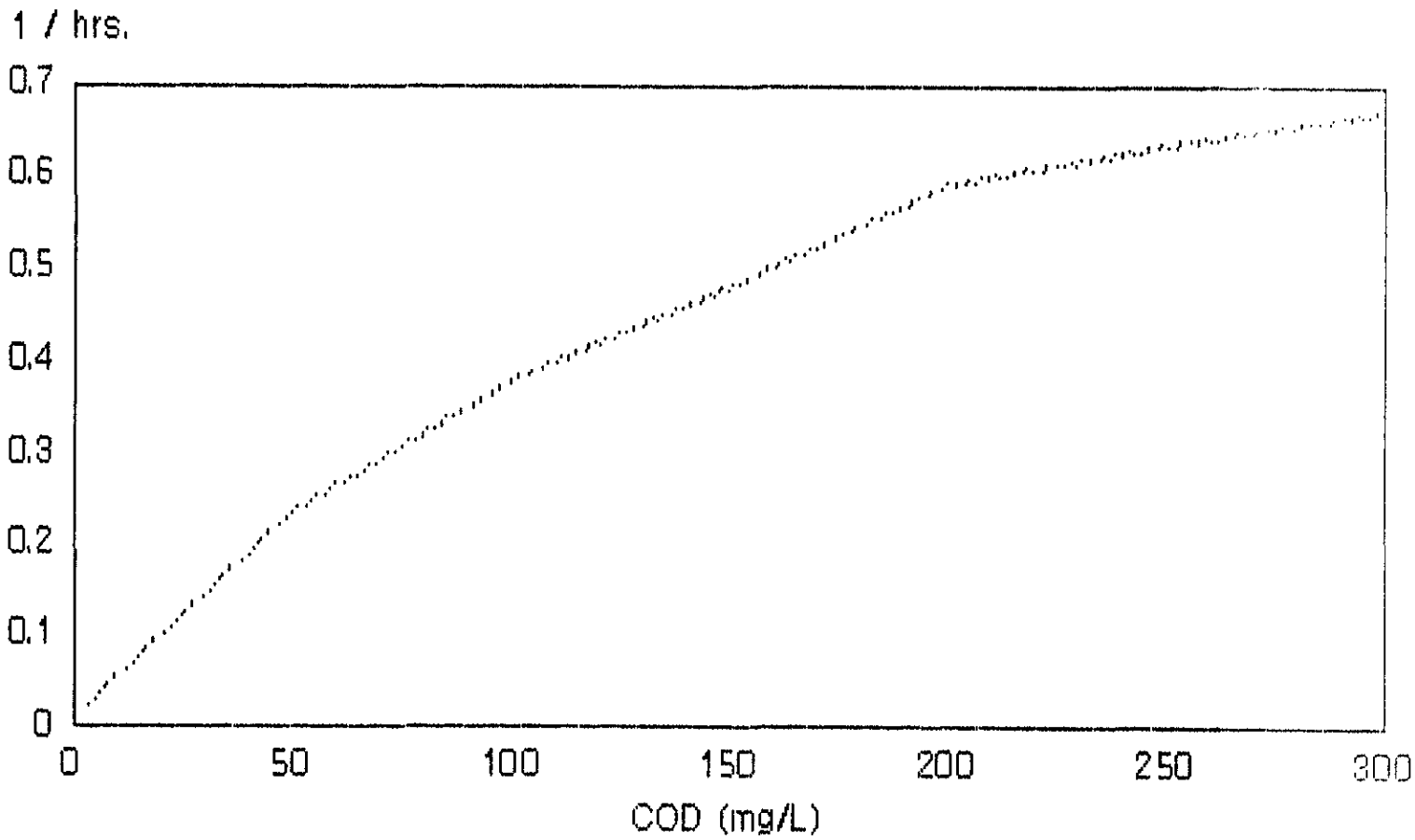
BIOMASS PRODUCTION VS. DIESEL SUBSTRATE CONSUMED

Cell Yield = 0.73 mg/L Biomass per mg/L Diesel utilized



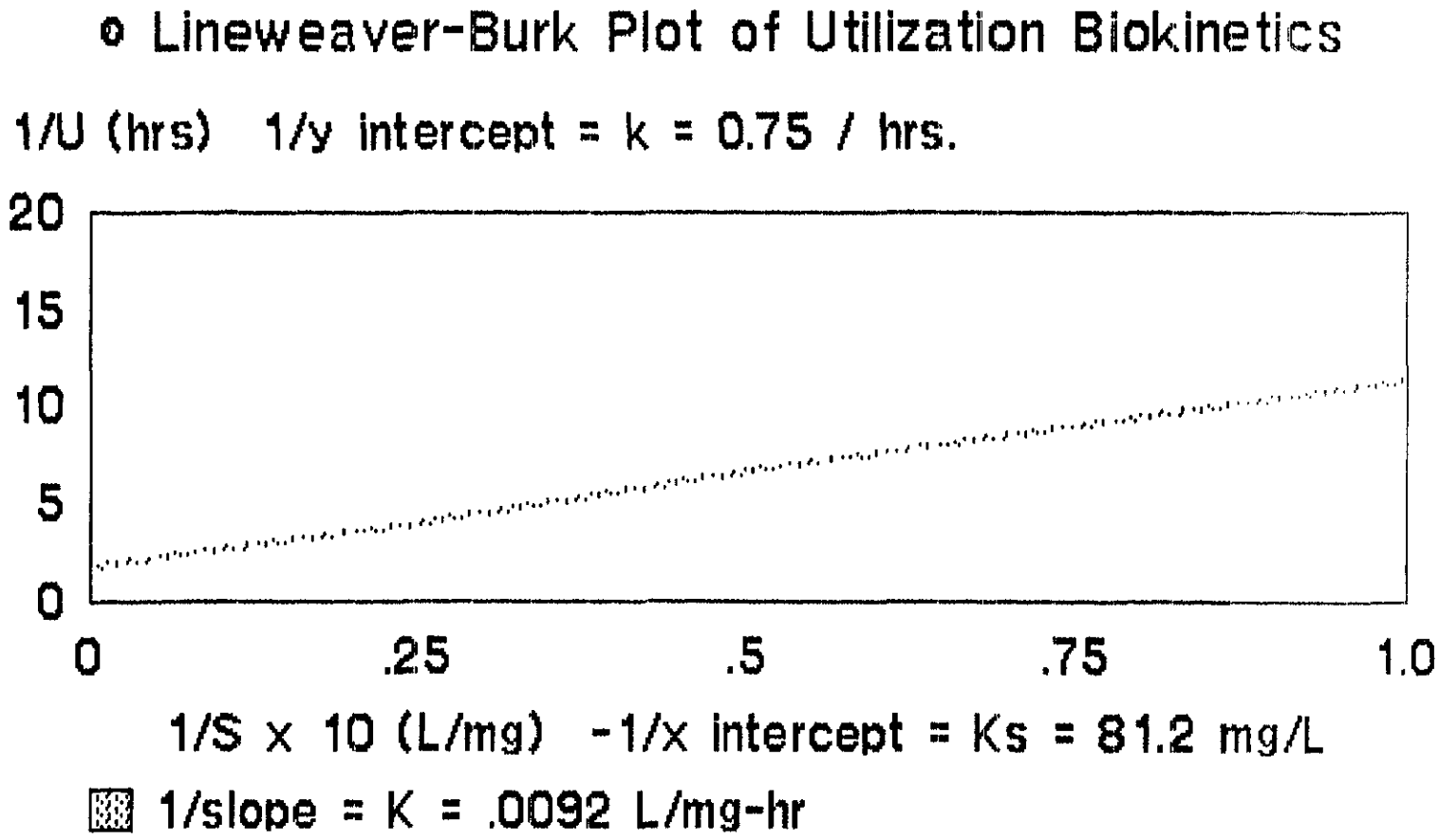
Sybron Biochemical: Balderson & Goldsmith (1988)

MONOD PLOT OF DIESEL SUBSTRATE UTILIZATION



Biodegradation of a Model Diesel Fuel

Sybron Biochemical: Balderson & Goldsmith (1988)



Biodegradation of a Model Diesel Fuel

Sybron Biochemical: Balderson & Goldsmith (1988)

- Biokinetic Constants

k = 0.75 / hrs. Ks = 81.2 mg/L

Umax = 0.55 / hrs. Y = 0.73 mg/L

Biodegradation of a Model Diesel Fuel

Sybron Biochemical: Balderson & Goldsmith (1988)

NAPHTHALENE - COMPARATIVE DEGRADATION RATES

As Sole Substrate vs. Mixed in Diesel

COD (mg/L)	Rate (mg/L/hr)	COD (mg/L)	Rate (mg/L/hr)
4.77	1.03	4.77	0.96
18.09	2.64	16.29	3.26
38.22	4.28	38.22	7.65

Biodegradation of a Model Diesel Fuel

Sybron Biochemical: Balderson & Goldsmith (1988)

PREDICTION OF BIOREACTOR OPERATING PARAMETERS

- Growth Rate, u = 0.693 x Batch Growth Rate
- Critical Dilution Rate, Dc = Umax (Sinf / Ks + Sinf)
- If Dilution Rate, D, is Constant, then Steady State will be reached where Growth Rate, u, equals D
- Steady State Biomass Conc., Se = Ks (D/Umax - D)
- Biomass in Bioreactor, X = Cell Yield (Sinf - Seff)

Biodegradation of a Model Diesel Fuel

Sybron Biochemical: Balderson & Goldsmith (1988)

CONCLUSIONS

- Diesel is readily degradable as a SOLE CARBON SOURCE
- Biokinetic Constants were determined for diesel components
- Direct correlation between Initial Diesel COD and Biomass
- Naphthalene degrades faster in a mixed substrate
- Bioreactor Operating Parameters in Field can be predicted for Biomass Production, Treatment Efficiency & HRTs

Biodegradation of Diesel and Aromatic Hydrocarbons

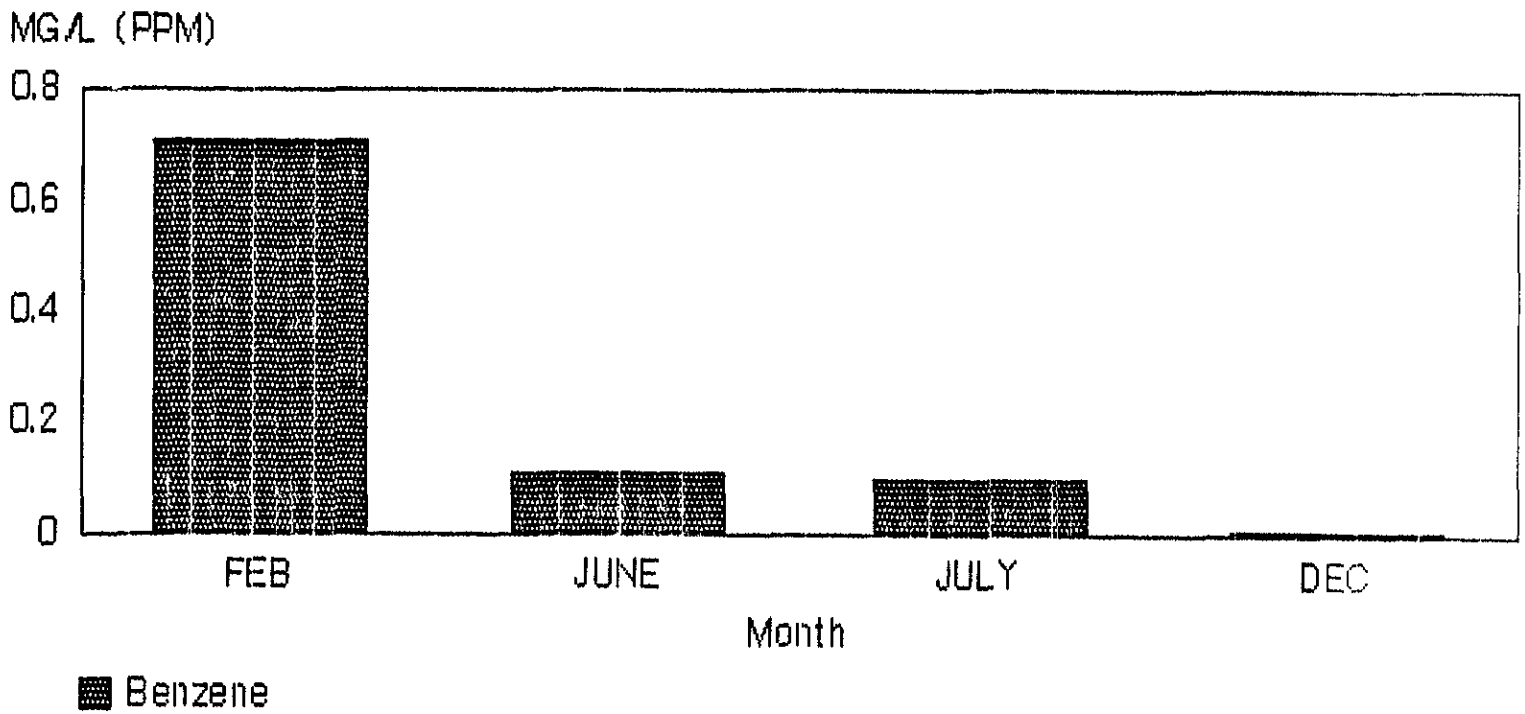
Sybron Biochemical / Alton Geoscience

- Site Remediation Field Data for Dissolved Phase HCs
- **Recent Results for Gasoline Contamination in California**

In Situ Biodegradation of Benzene in Gasoline-Contaminated Groundwater

Sybron Biochemical / Alton Geoscience

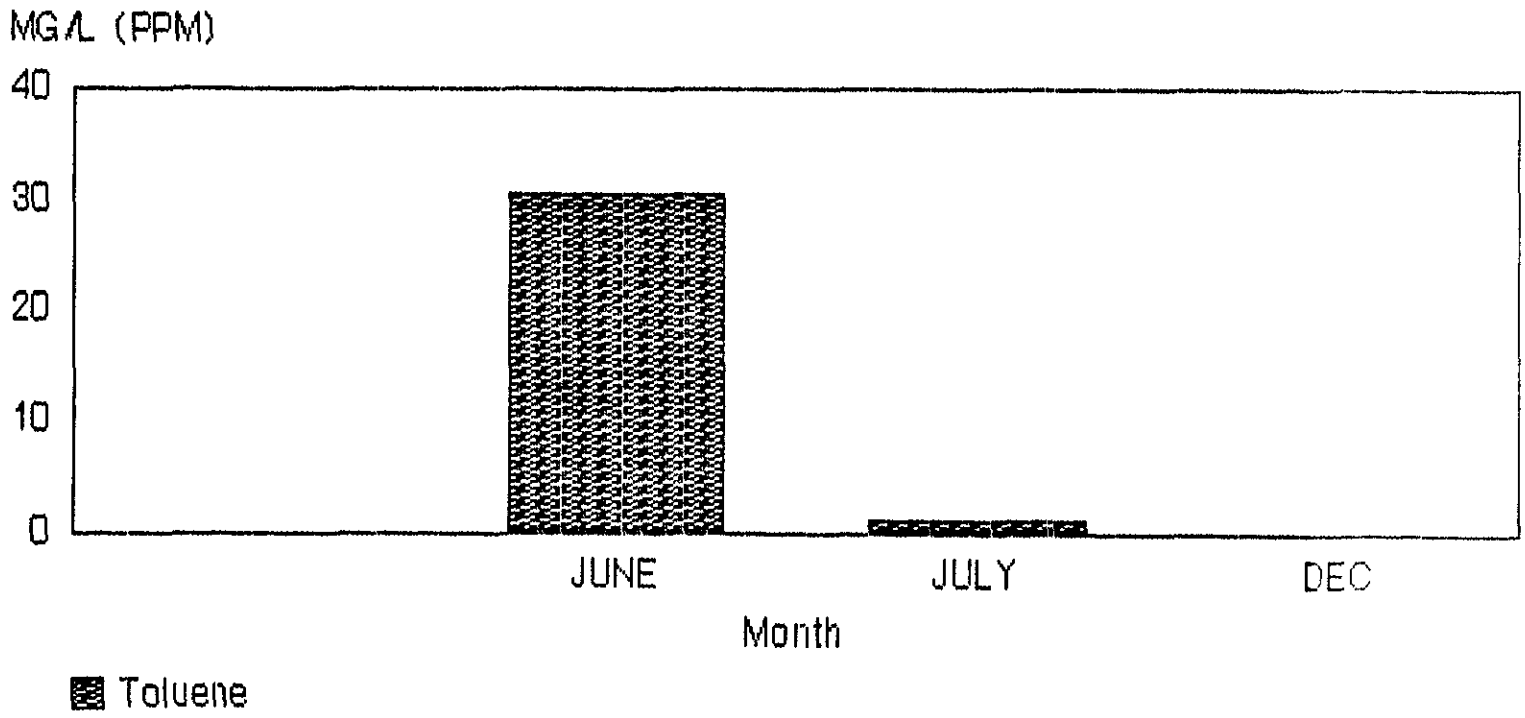
• Benzene Concentrations - Riverside 1987



In Situ Biodegradation of Toluene in Gasoline-Contaminated Groundwater

Sybron Biochemical / Alton Geoscience

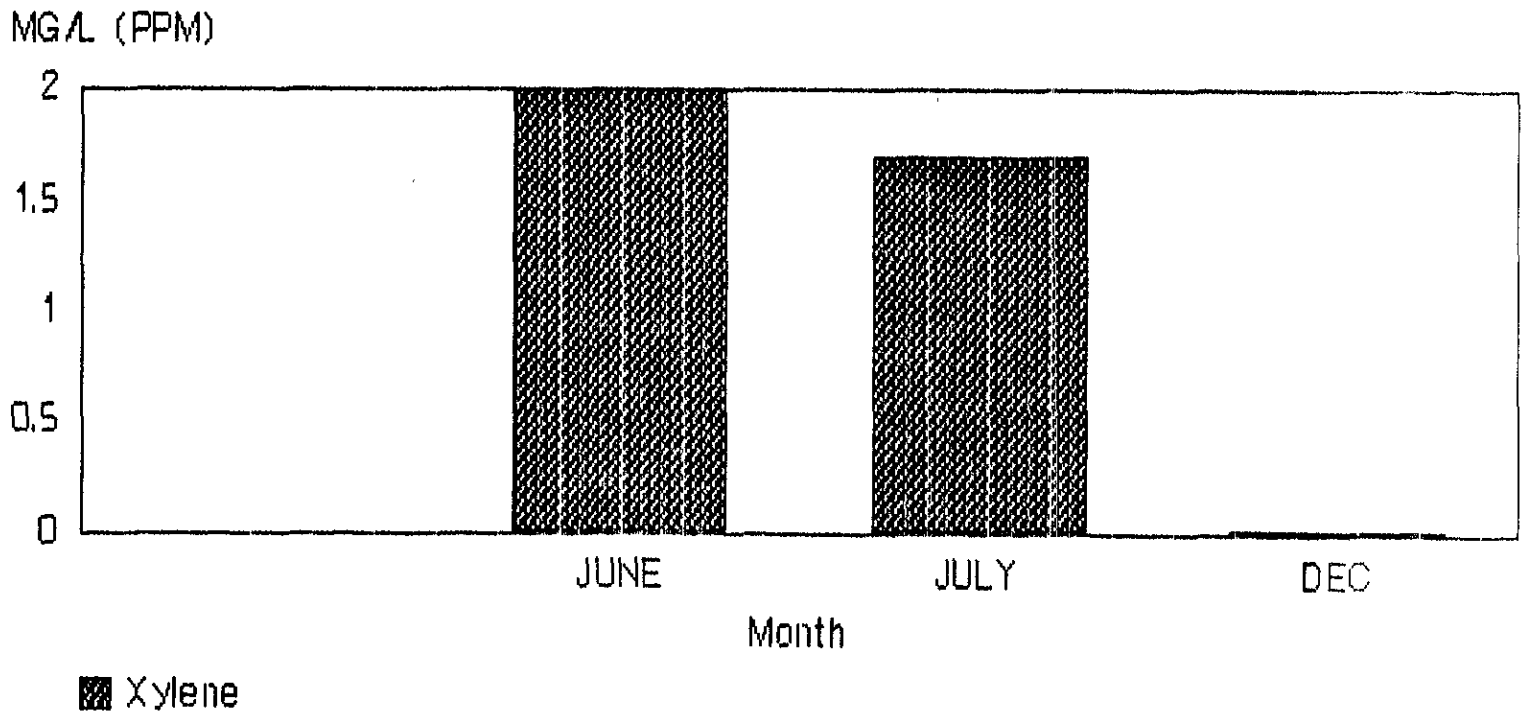
• Toluene Concentrations - Riverside 1987



In Situ Biodegradation of Xylene in Gasoline-Contaminated Groundwater

Sybron Biochemical / Alton Geoscience

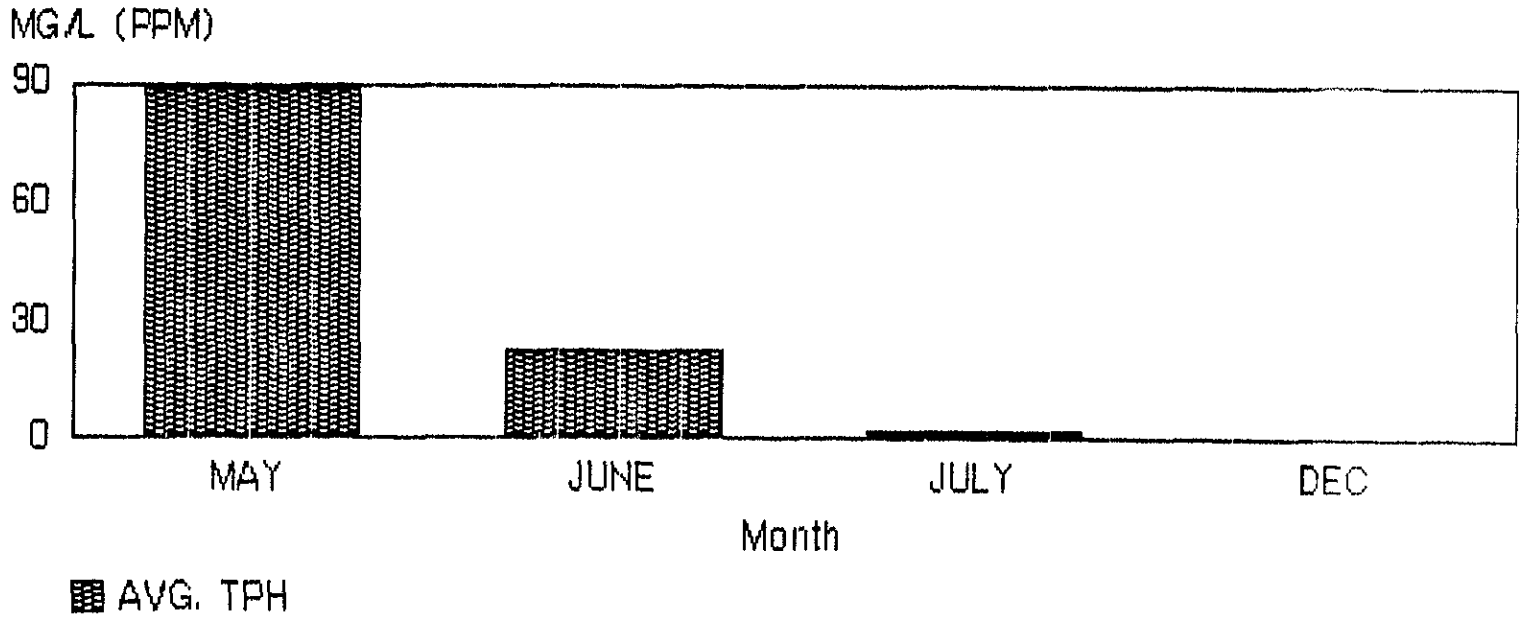
⊙ Xylene Concentrations - Riverside 1987



In Situ Biodegradation of Aromatics in Gasoline-Contaminated Groundwater

Sybron Biochemical / Alton Geoscience

⊙ Total Petroleum Hydrocarbons - Riverside 1987



In Situ Biodegradation of Aromatics in Gasoline-Contaminated Groundwater

Sybron Biochemical / Alton Geoscience

- Recent Tank Pit Monitoring Well Analysis
  MW-4 Riverside 5/88

Benzene	1.1 ppb
Toluene	5.0 ppb
Xylene	1.5 ppb
Ethyl Benzene	2.2 ppb
Total Petroleum HCs	1500 ppb

Biodegradation of Diesel and Aromatic Hydrocarbons

Sybron Biochemical / CytoCulture International

PRINCIPLES OF AUGMENTED BIORECLAMATION

- Trenching to Recover Free Product, Contaminated Water
- Above-Ground Treatment of Water in Bioreactors
- Specialized "Diesel Blend" Cultures of Aerobic Bacteria
- Reinfiltration of Bacteria & Nutrients Back Into Soil
- Continuous Treatment/Reinfiltration for up to 1 Year

In Situ Soil Bioreclamation Projects

CytoCulture International / Sybron Biochemical

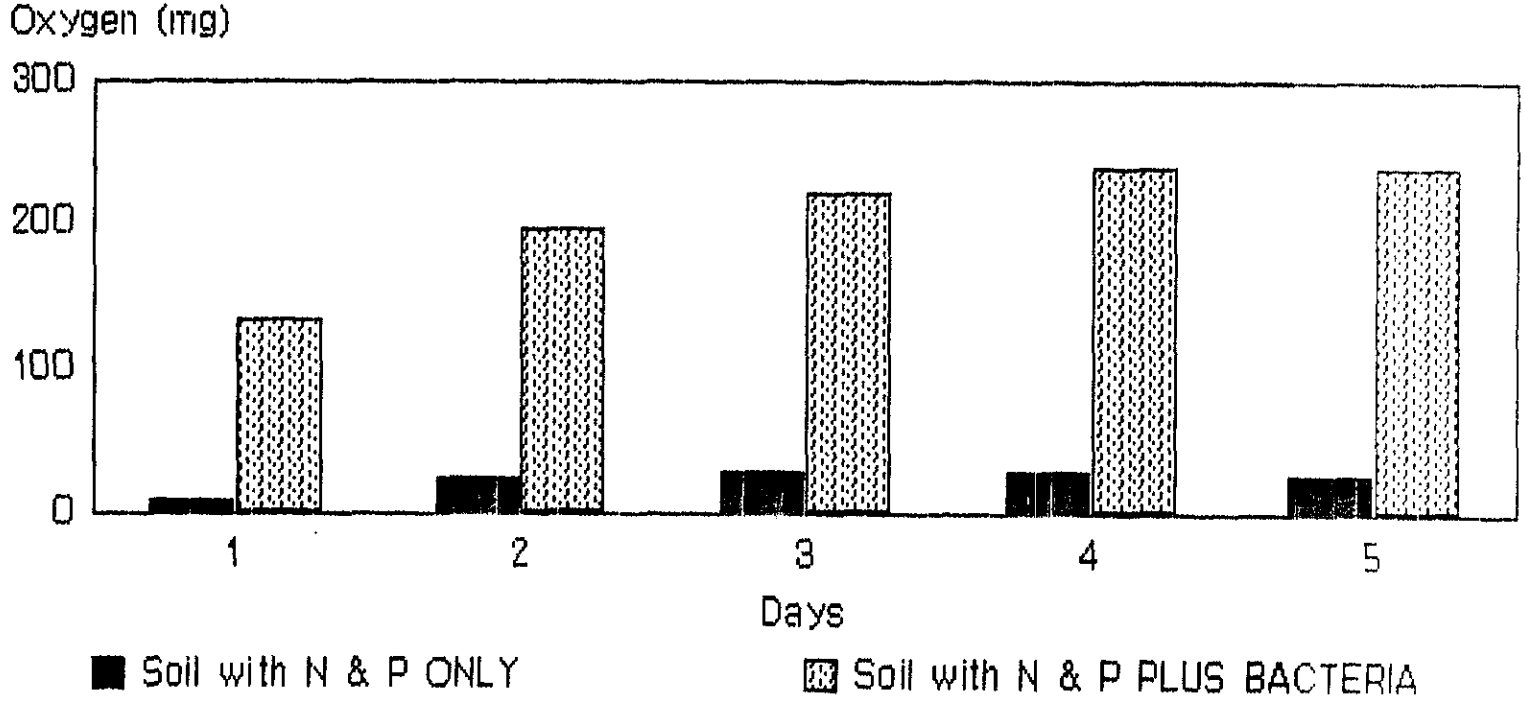
- Augmented Vacuum Heap Bioremediation

Biodegradation of Diesel Adsorbed to Clay Soil

Sybron Biochemical / CytoCulture International

o Results of Laboratory Treatability Study

COMPARATIVE OXYGEN CONSUMPTION IN RESPIROMETER



Biodegradation of Diesel and Aromatic Hydrocarbons

Sybron Biochemical / CytoCulture International

- Ongoing Augmented Bioreclamation Projects in California
  - **Diesel Contamination of Clay Soils and Tidal Groundwater**

NEW Airlift Suspension Bioreactor

introduced by CytoCulture International, Inc.

BENEFITS AND FEATURES

- High Cell Density / High Oxygen Transfer Rates
- Automated Solid State Process Controls
- Continuous Perfusion Culture / Long HRTs
- UV & Chemical Resistant Plastic Components
- Custom Orders / Local Fabrication & Service
- Competitively Priced for Industrial Applications