CytoCulture INTERNATIONAL INC.

Biotechnology Research, Scale-Up and Marketing

Abbreviated

Proposal for

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

submitted to

P.I.E. Nationwide, Inc. and Blymyer & Sons Engineers, Inc.

by

CytoCulture International, Inc.

as a Joint Venture with

Sybron Chemicals, Inc.

in Collaboration with

Alton Geoscience, Inc.

July 29, 1987
(Revised October 1, 1987)

Costs Deleted from this copy.

1208 Fourth Avenue San Francisco CA 94122 USA 415/564 1516

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

CytoCulture International, Inc., a local environmental biotechnology company, and Sybron Chemicals, Inc. have formed a joint venture to develop an in situ site remediation program for soil and groundwater contaminated with diesel fuel hydrocarbons at the former P.I.E. Nationwide trucking facility in Emeryville, CA. The remediation program is based on the use of individually selected strains of aerobic bacteria which biodegrade specific classes of hydrocarbons at much higher rates than naturally occuring microbes. A blend of seven pseudomonas strains has been formulated at Sybron Chemicals, Inc. to optimize the "Augmented Bioreclamation" of diesel and gasoline contaminated groundwater. The bacterial cultures will be used to treat contaminated brackish water which will be continuously drawn up from french drains placed in the tidal groundwater downstream of the contaminated areas. The water will be retained in a series of stainless steel chemostat bioreactor vessels above ground until the biodegradation has lowered the concentration of contaminants to an acceptable level. The treated water will then be aerated and mixed with fresh cultures of bacteria before being injected back into the ground upstream of the contamination areas. This continuous addition of high concentrations of enriched bacterial cultures should allow for extensive biodegradation of the hydrocarbon product remaining in the soil and at the moving soil/tide water interface. At least two, and possibly three, separate bacterial bioreactor treatment and delivery systems of this type are planned for the site.

In collaboration with Alton Geoscience, Inc., CytoCulture International will conduct further soils and groundwater investigations to maximize the effectiveness of the planned bioreclamation systems behind Buildings A and B and to determine whether a separate system will be needed in the C & D area. Eleven monitoring wells will be drilled by Alton Geosceinces in consultation with Blymyer & Sons Engineers; several of these wells will later be integrated with the bioreactor systems to continuously monitor the impact of the remediation program. CytoCulture International, Alton Geoscience and Sybron will be responsible for the design and operation of the bacterial bioreactor treatment and delivery systems. The bioreactor systems will be equipped with automated process controls for continuous delivery of bacteria to designated groundwater injection sites and occasional batch delivery to the subsurface PVC perforated pipe system beneath Buildings A, B and C. Although the Augmented Bioreclamation program is designed for nearly a year of site remediation, prior success and early closures at two similar hydrocarbon groundwater decontamination projects in California strongly suggest this site could be closed much earlier.

PROPOSED BUDGET

Task #	Task Description	Allocation
1.	Start up studies, assessment of prior data site visits by hydrogeologists and reports	
2.	Applications for required permits; presentations to local regulatory agencies	
3.	Installation of eleven groundwater monitoring wells in keeping with scope of work	
4.	Additional soil and water investigations of Pad K area, and areas east of Buildings A & B (assume 6 borings) with tests and reports	
5.	Construction of concrete pads with enclosed fence for each of three bioreactor systems	
6.	Excavation, placement and gravel bedding of french drains (assume 3) with plumbing to groundwater pumps	
	Excavation, plumbing and gravel bedding of piping for 3 infiltration trenches and manifolds for connecting to existing subsurface PVC perforated pipe (Bldgs A-C)	
7.	Design, construction and installation of steel tankage, pipes, pumps, monitors, and controls for three complete bioreactor systems	
	Installation of pumps and start up of bioreactors	
8.	Start up costs for bacteria, materials and labor, including preliminary testing for RWQC Board	
9.	Monthly operational costs over 10 months including bacteria, supplements, labor, equipment parts & service, water testing services and reports	
10.	Sample analyses: Total Hydrocarbon, phenol and polyaromatic hydrocarbons as required by RWQC Board	
11.	Final report, presentation of results to regulatory agencies, evaluations, and technical recommendations	

PROPOSED WORK SCHEDULE

TASK	FIRMS *	MONTH											
		1	2	3	4	5	6	7	8	9	10	11	12
1	ALT	X											
2	ALT	X											
3	ALT	X											
4	ALT	X											
5	ARC		X										
6	HAT		X										
7	ALT SYB	X	X										
8	SYB		X										
9	SYB ALT			X	X	X	X	X	X	X	X	X	X
10	SYB												X

* Participating subcontracting firms:

SYB = Sybron Chemicals
ALT = Alton Geoscience
ARC = Arrow Construction Co.
HAT = Hatton Construction Co.

PURPOSE AND OBJECTIVES

The purpose of the proposed site remediation project is to render the P.I.E. trucking site to levels of contamination acceptable to the State of California Department of Health Services, the San Francisco Bay Regional Water Quality Control Board, and Alameda County Department of Health. The contamination on site consists of petroleum hydrocarbons, primarily diesel fuel, from leaking underground storage tanks, above-ground storage tanks, and product delivery lines. The leaking equipment has since been removed from the site prior to the commencement of the current construction of a new shopping center.

Contamination exists as free-product on the groundwater and is adsorbed in the soil. The soil in the most heavily contaminated areas has been scraped down to six feet below the surface and is currently being treated on site by another contractor with enhanced bioreclamation techniques (spraying nutrients to enhance the growth of indigenous bacteria in the soil). The scraped soil has been replaced with engineered fill but the mud beneath the fill is still heavily contaminated in several areas. Saturation of the soil with diesel fuel at the level of the groundwater has been observed.

The primary objective of the proposed site remediation project is to design, construct and operate an Augmented Bioreclamation system using Sybron bacterial cultures to biodegrade the diesel fuel contaminating both the soil and the groundwater so as to bring the site into compliance with current regional water quality regulations. Treatment will involve both above ground bacterial bioreclamation of water extracted from the contaminated water table and subterranean bioreclamation of the contaminated soil with aerated bacterial cultures injected back into the ground with the treated water.

A second, and equally important, objective is to achieve compliance with regional water quality regulations in as short a time and as cost-effectively as possible. Therefore the project has been designed to optimize the application of bacterial Augmented Bioreclamation technology to this particular site by employing three individual bioreactor systems at the three most heavily contaminated areas on the site. If reasonably high flow rates (> 3 gal/min) of contaminated water through the system can be maintained, the site could potentially reach compliance levels of contamination in less than one year. Sybron Chemicals and Alton Geoscience are already setting precedents in California by applying to state regulatory agencies for early closures at two similar Augmented Bioreclamation sites involving polyaromatic hydrocarbon contamination of soil and groundwater.

CYTOCULTURE PROJECT LEADERSHIP

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

Dr. von Wedel is President and Director of Research for CytoCulture International, Inc., San Francisco, CA. He is a biochemist with extensive experience in project management ranging from biomedical research programs in both industrial and university laboratories to environmental biotechnology projects in the field. Besides establishing CytoCulture as a R & D and consulting firm in biotechnology, he has also set up a consulting firm, AmbienTech, in San Juan, Puerto Rico to deal directly with the application of bioaugmentation technologies to local municipal and industrial wastewater or groundwater contamination needs. Dr. von Wedel will oversee the construction, testing and operation of the bioreactor treatment and delivery systems on site. He will be coordinating the various sub-contractors participating in all phases of the project from the site evaluations through the construction of the bioreactors. He will then be responsible for submitting all reports to Blymyer & Sons Engineers, P.I.E. Nationwide and the various construction and water quality control regulatory agencies.

Project Manager: Jose F. Mosquera, M.S.

Mr. Mosquera, a Research Engineer in Sanitary/Environmental Engineering at the University of California, Berkeley, will work for CytoCulture International as the Project Manager for the proposed Augmented Bioreclamation program at the P.I.E. trucking site. He will be responsible for overseeing the day to day operations of this project at the site. He will be there to monitor, adjust and maintain the three proposed bioreactor systems described in this proposal. His extensive experience in designing and maintaining bacterial bioreclamation systems will be essential for his key role in this project.

The Curricula Vitae for Dr. von Wedel and Mr. Mosquera are enclosed in Appendix 1.

Dr. von Wedel and Mr. Mosquera have also submitted a Step I (Feasibility Study) Grant Application to the California Hazardous Waste Reduction Grant Program sponsored by the Office of Alternative Technologies, Toxic Substances Division of the State Department of Health Services. The title of the grant proposal is:

Bacterial Biodegradation of Hazardous Wastewater: Phase I Study

A copy of this proposal is available for background reading.

THE JOINT VENTURE WITH SYBRON CHEMICALS

For over a year, CytoCulture International, Inc. has been the Northern California representative and contracted distributor for the Sybron Biochemical Division of Sybron Chemicals, Inc.. involved with the production of novel bacterial cultures sold for municipal and industrial wastewater treatment. Sybron Chemicals has been a world leader in this field for nearly forty years. An arrangement has been made between the companies whereby Dr. von Wedel, as Project Director and Mr. Jose Mosquera, as the CytoCulture Project Manager, will work closely with Mr. Gary Hater, Manager of soil and groundwater treatment technology for Sybron. The Joint Venture will become a formal, binding agreement when CytoCulture is awarded the contract for this project. See the Letter of Collaboration from Mr. Hater, the C.V.'s for Mr. Hater and Dr. Goldsmith and additional technical literature from Sybron in Appendix 2.

THE COLLABORATION WITH ALTON GEOSCIENCE

Alton Geoscience, Inc. has been working on two similar Augmented Bioreclamation projects with Sybron Chemicals as their contracted representative for soil and groundwater applications in southern California. Alton Geoscience is a recognized leader and pioneer in the development of alternative remediation technologies for contaminated soil and groundwater, including air stripping of hydrocarbons, high efficiency incineration and Augmented Bioreclamation (in collaboration with Sybron). Alton, Sybron and CytoCulture are working to establish a closer business relationship for future Augmented Bioreclamation projects in Northern California. Please refer to the Letter of Collaboration from Mr. Jeff Wiegand and technical literature from Alton Geoscience in Appendix 3.

PROJECT DESCRIPTION BY TASKS

The following Task Descriptions summarize the scope of work, estimated costs and projected schedule for the implementation for the proposed Augmented Bioreclemation project at the P.I.E. trucking site in Emeryville. The proposed costs and scheduling have been summarized on pages 4 and 5 respectively.

1. Start Up Studies, Evaluations and Assessment of Prior Data

CytoCulture and Alton Geoscience will review data from previous consultants regarding prior site investigations and activities. This will involve inspections on site, assessment of the data and an evaluation of the validity if the conclusions of the previous reports. A brief report will be filed. Work would begin within 1 month of receiving a contract from P.I.E..

2. Applications for Required Permits

CytoCulture and Alton Geoscience will be submitting applications for permits pertaining to drilling, construction and treatment operations on the site. These permit applications will be filed within the first month of the contract period.

3. Installation of Eleven Groundwater Monitoring Wells

Alton Geoscience will be subcontracted to perform the drilling, installation, testing and operation of the eleven monitoring wells specified in the original scope of work. The sites for these wells will be selected in consultation with CytoCulture, Sybron and Blymyer & Sons Engineers with the intention of making as much use of these wells as possible in the normal operation of the bioreactor treatment and delivery systems. The drilling would begin within 10 days of receiving approval from the regulatory agencies and would be completed within 2 weeks.

4. Additional Soil and Groundwater Investigations

Alton Geoscience will be subcontracted to carry out additional site borings (at least six) to characterize the soil and groundwater contamination in the Pad K area and along the access road to the east of buildings A and B. This phase of the project has been advanced to first priority to accomodate construction plans on site.

5. Construction of Concrete Pads and Fencing for Bioreactors

Arrow Construction Company (Novato, CA; California General Building Contractor License Number 420628) will be sub-contracted to design and build the concrete pads (10 ft x 16 ft) which will support the bioreactor systems. Each 5 inch slab will be reinforced with # 4 reinforcing rods spaced 1 ft on centers.

Chain link fencing (6 ft) with redwood slats will be built around each pad and equipped with a 10 ft swinging gate at one end to allow complete access to the pad for installing or maintaining the equipment. Construction would begin by the end of the first month of the contract period.

6. Excavation and Installation of French Drains and Injection Trenches

Hatton Construction Company (San Bruno, CA; California Excavation Contractor's License # 319158) is one probable backhoe service which would be sub-contracted to dig trenches for the installation of the french drains, injection pipes and the subsurface PVC pipe manifolds (Buildings A, B and C).

Three french drains are planned at this time, to be located, for example, between buildings B and C, west of building A and west of the Pad K area. These locations are fairly speculative and would be determined for certain after consultations with Blymyer & Sons Engineers and Alton Geoscience, following their borings, monitoring well installations and site characterization. The french drains, from which contaminated water would be drawn for treatment in the bioreactor systems, will be bedded in pea gravel at about 12 feet below the surface, well below the high mark for the rising tidal groundwater. The piping will consist of 6 inch well casing pipe with 0.2 inch cuts. Connections to the bioreactor will be made at both ends or in the center. The trenches will be on the order of 50 feet in length, oriented parallel to the nearest building wall.

The injection trenches will be designed to maximize the surface area for returning treated water to the soil with fresh cultures of bacteria. One likely possibility, to save cost and effort, would be to dig a six foot trench parallel to the south sides of buildings B and C, and the east sides of buildings A and B, just beyond the ends of the subsurface PVC pipes protruding from the buildings (3 ft below the surface). In this way, the manifold can be connected to each pipe in the same trench where a perforated PVC pipe can be installed six feet from the surface. This greatly cuts the cost of labor and materials (pea gravel) and minimizes the disturbance to the existing site. The injection piping could be on the order of 50

to 75 feet in length, providing a large surface area for infusing fresh bacteria. The entire trench would be filled with gravel to maximize diffusion.

The PVC manifold for the subsurface PVC piping would be equipped with a manual valve to allow periodic (e.g., once a month) batch infusion of bacterial cultures under the buildings. The continuous infusion of bacteria into the soil will otherwise only occur by way of the injection trenches, the exact location and specifications for which will be determined jointly with Alton Geoscience, Sybron and Blymyer & Sons Engineers.

7. Design, Construction and Installation of Bioreactor Systems

Alton Geoscience has also been selected to contract with CytoCulture in the design, fabrication and installation of the bioreactor systems, complete with microcircuitry logic controls, groundwater pumps and process controls. As indicated before, Alton has worked closely with Sybron on similar Augmented Bioreclamation projects in California for which they also supplied the bioreactor hardware. CytoCulture will assist in the design and operation phases of the bioreactor development.

8. Start Up for Initiating Treatment

Sybron and CytoCulture would work together to test the bioreactor systems and initiate the Augmented Bioreclamation project with Sybron bacteria. This would involve at least two people from Sybron (see Appendix 2 for details) and two people from CytoCulture over a period of nearly a week. Sybron will provide bacteria, supplements, testing equipment and labor for this phase of the work. This work would begin in the second month of the contract period if there are no major delays in the installation of the drains, pumps, plumbing and bioreactors.

9. Monthly Operation of Bioreactors

CytoCulture would assume the primary responsibility for the day to day monitoring and maintenance of the three bioreactor systems once the systems were up and running. At this time, we are projecting a running period of 10 months, with an overall contract period of just one year. Sybron will make a monthly visit for mechanical and biological maintenance (see Letter from Mr. Hater in Appendix 2).

Alton Geoscience will also provide monthly mechanical maintenance. The CytoCulture Project Manager will be on site almost daily and will perform weekly chemistry tests on the cultures as well as to take weekly samples for total hydrocarbon and polyaromatic hydrocarbon analyses.

10. Routine Analysis for Monitoring Hydrocarbon Levels

CytoCulture will collect samples of treated and untreated ground water at the site for routine analysis (probably at Technical Analytical Laboratory, Hayward) according to the guidelines specified by the Regional Water Quality Control Board in authorization #2198.11 (see Blymyer's call for proposals).

11. Final Report and Presentation of Results

At the conclusion of the 10 month treatment period, a report will be submitted to P.I.E./Blymyer & Sons Engineers detailing the progress of the Augmented Bioreclamation project. The report will emphasize the communication which will be maintained with the state and regional water quality control boards as a demonstration of our "best efforts" policy to maximize the efficiency of the bioreclamation project. This report will include our evaluation of the various phases of the project and our technical recommendations how to proceed with or close down the bioreclamation project at that time. Out of that report will come a formal report to be submitted to the state and regional water qualtiy control boards and Departments of Health.

Several factors could reduce the budget total substantially, such as the decision to only install two bioreactor systems (e.g., delete the Pad K area) or reduce the projected length of the project or simplify the construction scheme presented in this proposal. These are issues which will be discussed at upcoming meetings with Blymyer & Sons Engineers.

October 6, 1987

BLYMYER & SONS ENGINEERS INC.
1829 Clement Avenue
Alameda, CA 94501-1396

RE: In Situ Site Remediation - P.I.E. Emeryville Site

CytoCulture/Sybron Remediation Project

ITEMIZED SCHEDULE OF PAYMENTS

Task	Description of Services	Estimated Billing Amt.	Estimated Billing Date
4	Soil and water investigations Pad K area and areas east of Bldgs. A & B, with reports Alton: $8,500 Cyto: $1,500	$10,000	October 15
1	Start up studies, assessment of prior data; site visits by hyrdrogeologists; reports Alton: $3,000 Cyto: $1,000	$ 4,000	October 30
2	Acquire permits for drilling, construction, excavation, surface treatment of contaminated water and plans for reintroducing treated water and bacterial cultures Alton: $4,000 Cyto: $2,000	$ 6,000	November 13
3	Drill 11 monitoring wells with sampling and analysis Survey, purge & sample wells submit final report Alton: $ 29,000 Cyto: $ 7,000	$36,000	within 1 month of NTP*

* NTP - Notice to Proceed

CytoCulture/Sybron Remediation Project: Schedule of Payments

Task	Description of Services	Estimated Billing Amt.	Estimated Billing Date
5	Construct concrete pads, fence for 3 bioreactor systems Arrow: $ 8,800 Cyto: $ 1,200	$10,000	within 1 month of NTP
6	Excavate trenches for french drains and reinjection pipe; Provide gravel bedding Remove fill from site Connect plumbing to existing subsurface PVC Hatton: $ 6,000 Cyto: $ 4,000	$10,000	within 1 month of NTP
7	Design and fabricate pumps, controls, micro-circuitry, plastic tanks (two/system) and ancillary equipment Alton: $37,000 Cyto: $ 6,000	$43,000	within 1 month of NTP
	Install and start up mechanical equipment, tests Alton: $10,000 Cyto: $ 2,000	$12,000	within 1 month of NTP
8	Start up bacterial cultures 5 day site visit by Sybron Testing of indigenous strains Nutrients, supplies Sybron: $24,500 Cyto: $ 3,500	$28,000	within 1 month of NTP

CytoCulture/Sybron Remediation Project: Schedule of Payments

Task	Description of Services	Estimated Billing Amt.	Estimated Billing Date
9	Comprehensive Maintenance: Monthly Sybron visits Monthly supply of cultures, nutrients, testing Sybron: $4,200	$ 8,000	within 5 days after each month of service
	Monthly Alton visits and equipment tune up & repair Alton: $1,800		
	Weekly/semi-weekly CytoCulture maintenance and testing with reports on system operation Cyto: $2,000		
	ASSUME 10 MONTHS OF OPERATION:	$80,000 total	
10	Test total hydrocarbon and polyaromatic hydrocarbons of discharge water:		
	Start up Phase Analysis with Reports to Regional WQCB Tech.Anal.: $3,000 Cyto: $1,000	$ 4,000	within 2 months of NTP
	Monthly analysis of weekly samples Monthly Reports to Regional WQCB (Costs subject to increase with number of samples required for Regional WQCB)	$ 1,000	within 5 days after each month
	Cyto/Tech.Anal.: $1,000/mo. ASSUME 10 MONTHS OF OPERATION:	$14,000 total	
11	Prepare and submit final reports to P.I.E. and agencies Cyto: $5,000	$ 5,000	within 5 days of completion circa 1 yr after NTP
	Total Estimated Billing:	$258,000	

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

October 5, 1987

Chris Falbo
BLYMYER & SONS ENGINEERS, INC.
1829 Clement Avenue
Alameda, CA 94501-1396

Dear Chris:

Thank you for your letter of intent of September 18, 1987 authorizing CytoCulture International, Inc. to proceed with Tasks 1, 2 and 4. As we have discussed, we are pushing up the time table on Task 4 to take boring samples from the Pad K area of the site as early as possible. Accordingly we have notified our collabortor and sub-contractor for the hydrogeology aspects of the project, Alton Geoscience Inc. They have agreed to send up a hydrogeologist to supervise the drilling and sampling of the borings now scheduled for this Wednesday, October 7. We are therefore issuing a confirming Purchase Order to Alton for this task only and will continue to submit additional purchase orders for Tasks 1 and 2 as we complete our negotiations on the final contract between Blymyer/P.I.E. and the CytoCulture/Sybron joint venture. CytoCulture has already inquired on permits and permission needed for drilling and sampling borings on the site.

In finalizing the contract for this joint venture, we have already discussed our request for a clause which specifies that the funds received by CytoCulture as payments for this project could be assigned to our bank. We would also request a clause in the contract which states that the cost of defending against any investigatory or enforcement actions by any official environmental or regulatory agency shall be borne by Blymyer & Sons Engineers and/or P.I.E. Nationwide. Of course, CytoCulture and Sybron will be available and willing to assist in responding to any official inquiries or enforcement actions.

The Statement of Insurance from Sybron Chemicals Inc. is enclosed. We await copies of the Certificates of Insurance from both Sybron Chemicals and Alton Geoscience; other Statements of Insurance for our other subcontactors will be available soon.

A draft Schedule of Payments is enclosed for your review and inclusion in the contract. Please let me know if there is any other information I can provide you to facilitate the completion of the draft contract.

Sincerely,

Randall J. von Wedel, Ph.D.

1208 Fourth Avenue San Francisco CA 94122 USA 415/564-1516

MCI Mail ID: 308-4597 Telex (via WUI): 6503084597

BLYMYER & SONS engineers, inc.

July 8, 1987
BSE Job No. 8648

CYTOCULTURE
1208 Fourth Avenue
San Francisco, CA. 94122

Attn: Dr. Randall von Wedel

SUBJECT: REQUEST FOR PROPOSALS
IN-SITU SITE REMEDIATION
5500 EASTSHORE HIGHWAY
EMERYVILLE, CALIFORNIA

Dear Dr. von Wedel:

This letter constitutes a formal request for proposal for the subject work. The purpose of the site remediation is to render the site to levels of contamination acceptable to State of California Department of Health Services, San Francisco Bay Regional Water Quality Control Board, and Alameda County Department of Health. The contamination on site consists of petroleum hydrocarbons, primarily diesel fuel, from leaking underground storage tanks, above-ground storage tanks, and product delivery lines. Contamination exists as free-product on the groundwater and is adsorbed in the soil. Both phases of contamination require treatment.

The scope of work is as follows:

1. Conduct any necessary feasibility, treatability, or start-up studies as required by the proposed treatment system or systems;

2. Conduct further soils and water investigation to determine the extent of contamination on the east side of the property, most specifically in the area of Pad K on the enclosed drawing;

3. Install no less than eleven (11) groundwater monitoring wells on site to monitor groundwater quality; locations for wells shall be chosen in consultation with Blymyer & Sons Engineers and should have a minimum depth of 20 feet, or as required by the proposed treatment system;

4. Design a remediation system, based upon the above work and work done previously at the site, to remove the hydrocarbon contamination both in the soil and on the groundwater.

5. Purchase all necessary equipment for full implementation of the designed system, including any necessary tanks, pumps, piping, bacteria, nutrients, and any accessories.

6. Obtain all necessary permits from and make any required demonstrations to San Francisco Bay Regional Water Quality Control Board or any other agencies required by Federal, state or local regulation;

7. Install the remediation system as approved by San Francisco Bay Regional Water Quality Control Board, any other requisite Federal, state, or local agency, and P.I.E. Nationwide, including all necessary components, lines, and discharges; included in this phase is all drilling, excavation, trenching and resurfacing necessary for installing the system.

8. Start-up and test the system to insure proper operation and compliance with San Francisco Bay Regional Water Quality Control Board authorization #2198.11 (enclosed).

9. Maintain and monitor the system on a monthly basis to insure proper operation and compliance, including all necessary foreseeable servicing of equipment and reports to the RWQCB.

Based upon the above scope of work please provide a cost and schedule for each work step. The scope of work may be reorganized or phased for bid purposes, as long as each work step is clearly indicated in the bid. Any work conditional on a previous work step should be indicated in the proposal with possible contingencies. If precise costs cannot be specified because of a lack of data for any work step, ranges of costs should be given with applicable assumptions stated.

All bidders should have previously received copies of the following documents for use in making bids:

1) Groundwater Technology Assessment dated September 5, 1986.
2) Peter Kaldveer and Associates Assessment dated August 15, 1986.
3) Geotechnical bore logs from Geomatrix Consultants, dated October 9, 1986.
4) Laboratory analyses of soil samples analyzed for hydrocarbons, dated April 23, 1987.
5) Laboratory analyses of soil samples, analyzed for pesticides, PCB's, and metals, dated January 13, 1987.
6) Laboratory analyses of soil samples, analyzed for total petroleum hydrocarbons as diesel, dated April 27, 1987.
7) Bore logs for Wells GT-9 through GT-13, dated October 1, 1986 through December 15, 1986.
8) Laboratory analyses of soil samples, analyzed for priority pollutants, dated October 15, 1986.

July 8, 1987

In addition, the following documents are included with this request for proposal:

1) Site plans for the development currently taking place on site, including the location of PVC perforated pipe buried under buildings A, B, and part of C, for use in any soil venting or nutrient introduction system.

2) Geotechnical soils bores done in the area of Pad K, in which hydrocarbon odors were noted, by Laver R. Loper and Associates.

P.I.E. Nationwide has purchased some equipment for groundwater remediation. To save costs, this equipment may be incorporated into any designed treatment system. However, using the equipment is not mandatory for the treatment system to be considered. The purchased equipment includes one probe scavenger, one water table depression pump, and the accessories for each.

Please send a proposal by July 29, 1987, covering all above-referenced points, addressed in the following manner:

P.I.E. NATIONWIDE
C/O BLYMYER & SONS ENGINEERS, INC.
1829 CLEMENT AVENUE
ALAMEDA, CALIFORNIA 94501

If there are any questions, please call my office at: (415) 521-3773. Also, if CYTOCULTURE declines to bid on the project, please inform Blymyer & Sons of that fact as soon as possible.

Cordially yours,

BLYMYER & SONS ENGINEERS, INC.

Chris Falbo

CF/ds

Attachments

cc: Mr. John Ster-P.I.E. NATIONWIDE, JACKSONVILLE, FL.

STATE OF CALIFORNIA
GEORGE DEUKMEJIAN, Governor

CALIFORNIA REGIONAL WATER QUALITY CONTROL BOARD
SAN FRANCISCO BAY REGION
1111 JACKSON STREET, ROOM 6040
OAKLAND 94607

Phone: Area Code 415
464-1255

November 25, 1986
File No. 2198.11

Mr. Jim Skelton
P.I.E. Nationwide
P.O. Box 2408
Jacksonville, Florida 32203

Subject: NPDES Permit for Diesel Leak Cleanup, P.I.E. Nationwide, 5500 Eastshore Highway, Emeryville

Dear Mr. Skelton,

Under State and Federal law, discharges of polluted water to surface waters require an NPDES permit. We acknowledge receipt of the EPA forms 1 and 2C NPDES applications and the application fee. I cannot at this time provide you with an estimated date for the Regional Board to consider your NPDES permit application at the required public hearing. However, I believe it is in the public interest to have the discharge of hydrocarbon contaminated groundwater proceed in this specific instance, without my recommendation to the Regional Board for enforcement action, provided the following requirements are met:

1. At no time shall the total dissolved hydrocarbon content of the discharge exceed 100 ug/l. Total phenol shall be analyzed specifically and will contribute to the hydrocarbon total. At no time shall poly-aromatic hydrocarbons detected by EPA method 610 exceed 15 ug/l. If these limits are exceeded, the discharge will cease immediately and the Regional Board shall be notified at the earliest opportunity at 415-464-1255.

2. Discharge shall not occur until at least a 24 hour pilot operation of the treatment system meets the standards listed in Item 1..

3. Sampling shall occur daily for the first three days of discharge. These samples shall be analyzed on the most rapid basis practically available.

4. Following this start-up phase, sampling shall occur weekly, and analysis can occur on a more normal basis.

5. Reports shall be submitted on a weekly basis for the first three weeks of operation, and on a monthly basis thereafter, detailing the results of effluent analysis, flow rate of effluent, and general description of the operation and maintenance of the recovery system.

6. After this four week start up phase, general reports shall be submitted quarterly, describing the overall status of the investigation and recovery operation, including precise water and product levels in

groundwater monitor wells.

A detailed technical review of the investigation and remedial action proposed for this site has not been undertaken by our staff, and due to the large number of cases of this type and limited staff availability, a review of this type is not anticipated in the near future.

We appreciate your cleanup activities. Any questions on this matter should be directed to Dale Bowyer at 415-464-0846.

Sincerely,

Roger B. James
Executive Officer

cc: Mr. Chris Falbo
Blymyer and Sons
1829 Clement Ave.
Alameda, CA 94501

Mr. T. M. Gerow
Alameda County Div. of Environmental Health
470 27th St., Rm. 324
Oakland, CA 94612

CytoCulture/Sybron Bioreclamation Proposal

Appendix 1

CytoCulture International

Personnel

and

Technical Consultants

APPENDIX 1

Resumes and Roles in Project for Key Personnel at CytoCulture

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

Dr. von Wedel will be the Project Director and Principal Scientist for the multifaceted Augmented Bioreclamation site remediation program described herein. Dr. von Wedel will coordinate the various subcontracting firms in their respective tasks and will be the principal contact between them and Blymyer & Sons Engineers, Inc./P.I.E. Nationwide, Inc.

Dr. von Wedel is a biochemist originally trainied at the University of California, San Francisco Medical Center in biomedical research. His post-doctoral research in cell culture and immunology led him to consulting work for the then embryonic biotechnology industry. After two years as Research Scientist for a Bay Area biotechnology firm (large scale mammalian cell culture facility), Dr. von Wedel established an independent consulting business in 1985 which evolved into his current biotechnology research and development company, CytoCulture International, Inc. Soon after he began consulting, he became interested in bacterial biodegradation as an alternative technology suitable for hazardous wastewater treatment, an area he had been following for years.

Dr. von Wedel became acquainted with Sybron Biochemical in late 1985 and in February 1986 he completed a training course at the Sybron facility in Virginia for technical representatives and distributors. Since then, CytoCulture has assisted in making west coast contacts in industry for Sybron and exploring new sites for treatability studies. The most recent new project involves the use of bacterial cultures for the nitrification of ammonia contaminated wastewater at a major refinery in the Bay Area. Dr. von Wedel has also been involved in Sybron wastewater treatment studies in Puerto Rico through his affiliated company there, AmbienTech. Dr. von Wedel's curriculum vitae is attached.

CURRICULUM VITAE

Randall J. von Wedel, Ph.D.

Personal Data

Date of birth: September 11, 1952 Place: New York, New York
Marital status: Single Soc. Sec. No.: 584-52-7179
Home/work address: 1208 Fourth Avenue, San Francisco, CA 94122
Home/work telephone: (415) 564-1516

Education

Dartmouth College, Hanover, N.H.; B.A., Biology/Chemistry, 6/74
University of California, San Francisco; Ph.D., Biochemistry, 9/81

Professional Record

Senior Consultant, CytoCulture International, Inc. - present
Independent Consultant in cell culture research, 10/85-10/86
Staff Research Scientist, Bio-Response, Inc., Hayward, CA 1/84-10/85
Consultant to Bio-Response, Inc., San Francisco, CA; 3/83-12/83
Postdoctoral Research Fellow, Department of Pathology,
University of California, San Diego; 10/81-8/83
Postdoctoral Research Fellow, Department of Immunopathology,
Scripps Clinic & Research Foundation, La Jolla, CA; 10/81-12/82
Research Assistant, Department of Biochemistry,
University of California, San Francisco; 9/75-9/81
Postgraduate Research Biochemist, Department of Biochemistry,
University of California, San Francisco; 9/74-9/75
Undergraduate Research Assistant, Department of Biochemistry,
Dartmouth Medical School, Hanover, N.H.; 1/73-8/74
Environmental Biologist, Environmental Quality Board,
Commonwealth of Puerto Rico; 6/72-8/72

Awards

National Research Service Training Grant Recipient, 10/81-12/82
National Research Service Award Graduate Student Stipend,10/77-10/79

Professional Organizations

Tissue Culture Association
American Society for Microbiology
N.Calif. Assoc. Professional Consultants (Co-founder, Dir. Programs)

Foreign Languages

Spanish (bilingual); German (working knowledge)

Randall J. von Wedel, Ph.D.

Postdoctoral Research Areas

Characterization of antibodies and immune effector cells directed to host antigens; experimental models of auto-immune disease;
Role of persistent virus infections in triggering auto-immune disease in the CNS; experimental models of multiple sclerosis.
In vitro models of inflammation; role of anaphylatoxins
Purification, characterization of the C5a receptor from a human monocyte cell line; biological response modifiers in culture.

Current Research Emphasis

Development of practical laboratory and pilot scale mammalian cell culture systems utilizing continuous perfusion methods for the production of vaccines, enzymes, recombinant proteins and other biologicals from high density adherent cell lines;
Automated process control, biosensors, integration of cell culture with downstream processing to achieve one continuous operation
Preparative HPLC and large scale protein purification technology
Immunoconjugation and protein derivatization methods
Diagnostic assays, vaccine development for infectious diseases
Specialized bacterial cultures for toxic wastes biodegradation

Major Responsibilities as Staff Scientist at Bio-Response:

Project Director for only research contract at Bio-Response: 2 year contract, U.S. Army: $367,000; 2-4 people under my supervision to characterize cell lines secreting acetylcholinesterase, isolate high secreting variants, mass culture the cells and purify the secreted enzyme. Developed new solid phase assay for AChE. (see annual report, poster session presentations)
Project Director for human-human hybridoma collaboration for the mass culture, purification and characterization of human monoclonal antibodies to human tumor antigens. (see poster session material, SBIR proposal)
Research Proposal Writer: two proposals (pending) as Principal Investigator, one to the U.S. Army for mass culturing 16 cell lines ($689,800 over 3 years) and the other to the NIH (SBIR; Phase I, $39,764) for human hybridomas secreting antibodies to cancer antigens (scored 182 - see enclosed NIH response and critique)
Patent Officer for Bio-Response, culminating with a personal interview in Washington with 3 patent examiners to defend 3 pending patent applications (one since issued)

BIBLIOGRAPHY

Randall J. von Wedel, Ph.D.

Papers

1. Cooke, R., M. Clarke, von Wedel, R. J. and Spudich, J. A. (1976) Supramolecular Forms of Dictyostelium Actin. In Cell Motility, Book B: Actin, Myosin and Associated Proteins, R. Goldman, T. Pollard and J. Rosenbaum, eds., Cold Spring Harbor Laboratory, New York, p. 575.

2. Kelly, R. B., von Wedel, R. J. and Strong, P. N. (1979) Phospholipase-dependent and Phospholipase-independent Inhibition of Transmitter Release by Beta-bungarotoxin. In Neurotoxins: Tools in Neurobiology, Advances in Cytopharmacology, Vol. 3, B. Ceccarelli and F. Clementi, eds., Raven Press, New York, p. 77.

3. Sanes, J. R., Carlson, S. S., von Wedel, R. J. and Kelly, R. B. (1979) Antiserum Specific for Motor Nerve Terminals in Skeletal Muscle. Nature 280: 403-404.

4. von Wedel, R. J., Carlson, S. S. and Kelly, R. B. (1981) Transfer of Synaptic Vesicle Antigens to the Presynaptic Plasma Membrane During Exocytosis. Proc. Natl. Acad. Sci. USA 78: 1014-1018.

5. von Wedel, R. J. Nerve Antibodies and Presynaptic Neurotoxins as Biochemical Probes of Transmitter Release at the Neuromuscular Junction. Ph.D. Dissertation in Biochemistry, University of California, San Francisco; September, 1981.

6. Oldstone, M. B. A., Sinha, Y. N., Blount, P., Tishon, A., Rodriguez, M., von Wedel, R. and Lampert, P.W. (1982) Virus-induced Alterations in Homeostasis: Alterations in Differentiated Functions of Infected Cells In Vivo. Science 218: 1125-1127.

7. Rodriguez, M., J. von Wedel, R. J., Lampert, P. W. and Oldstone M. B. A. (1983) Pituitary Dwarfism in Mice Persistently Infected with Lymphocytic Choriomeningitis Virus. Laboratory Investigation 49: 48-53.

8. von Wedel, R. J. and Chenoweth D. E. (1987) Induction of Specific Receptors for the C5a Anaphylatoxin in a Human Monocyte-like Cell Line. Manuscript in Preparation.

9. von Wedel, R. J. (1987) The Mass Culture of Murine and Human Monoclonal Antibodies (Chapter 9) in The Commercial Production of Monoclonal Antibodies, S. Seaver, ed.; Marcel Dekker Publishers, N.Y. In Press.

10. von Wedel, R. J. (1987) Scale-Up Techniques for the in vitro Production of Monoclonal Antibodies to Tumor Antigens in Immunological Approaches to the Diagnosis and Therapy of Breast Cancer, Dr. Roberto L. Ceriani, ed.; Plenum Publishing Corp., N.Y. Manuscript in preparation.

Abstracts

1. Strong, P. N., von Wedel, R. J. and Kelly, R. B. (1977) Evidence that Beta-bungarotoxin Binds Directly to Nerve Terminals: Separation of Binding and Enzymatic Activities. Neuroscience Abst. 3: No. 1209.

2. von Wedel, R. J., Sanes, J. R., Carlson, S. S. and Kelly, R. B. (1979) Binding of Anti-Synaptic Vesicle Antiserum to Nerve Terminals in Vertebrate Skeletal Muscle. Neuroscience Abst. 5: No. 1663.

3. Chenoweth, D. E., Soderberg, C. S., and von Wedel, R. J. (1984) Dibutyryl cAMP Induced Expression of C5a Receptors on U937 Cells. Abstracts of 10th International Reticuloendothelial Socities Congress, Ito, Japan. J. Leukocyte Biology.

4. von Wedel, R. J. Mighetto, P. I., Juarez-Salinas, H. and Brooks, T. L. (1985) The Mass Culture of Hybridomas: Production, Purification and Characterization of Mouse Monoclonal AE-1 Antibody, 4th Annual Hybridoma Congress, San Francisco, CA Hybridoma, Feb. 1985.

5. von Wedel, R. J., Dorian, R., Scott, M. H., Mighetto, P. I., Costello, M. A. and Brown, P. C. (1985) Progress on Isolating a Cell Line Secreting High Levels of Human Acetylcholinesterase, Proceedings of the 5th Annual U. S. Army Medical Research Institute of Chemical Defense Bio-Science Review, Columbia, MD, May 1985, No. 66.

6. von Wedel, R. J., Glassy, M. C., Gaffar, S. A., Mighetto, P. I. and Oakley, R. V. (1985) The Mass Culture of Human-Human Hybridomas Secreting Antibodies Directed Against Several Human Solid Tumors, 36th Annual Meeting of the Tissue Culture Association, New Orleans, LA; In Vitro, June 1985, No. 25.

7. von Wedel, R. J. (1987) Automation of Research/Pilot Scale Cell Culture Bioreactors Integrated with Biosensors and Downstream Bioprocessing Systems, Proceedings of the Fourth Annual Congress for Automation, Scale-Up and the Economics of Biological Process Engineering, March 4, 1987; San Francisco, CA; Scherago Associates and Genetic Engineering News, New York.

8. von Wedel, R. J., Peterson, J.A. and Pugh, G. (1987) A Scale-Up Technique for Producing Monoclonal Antibodies to Tumor Antigens from Bench Top Perfusion Cultures of Immobilized Hybridomas, Second International Conference on Monoclonal Antibody Immunoconjugates for Cancer, March 12-14, 1987, San Diego, CA; UCSD Cancer Center, San Diego, CA

9. O'Shannessy, D.J. and von Wedel, R.J. (1987) The Site-Directed Modification of Antibody Oligosaccharide Moieties, Second International Conference on Monoclonal Antibody Immunoconjugates for Cancer, March 12-14, 1987, UCSD Cancer Center, San Diego, CA

Trade Journal Articles

1. von Wedel, R.J. (1986) Laboratory Scale Fermenters Can Solve In-House Mammalian Cell Culture Needs, Genetic Engineering News 6(7): 16-17 (July/August), Mary Ann Liebert, Inc., N.Y.

2. von Wedel, R.J. (1986) Integrated Cell Culture Systems Appear Ready to Emerge in Marketplace, Genetic Engineering News 6(10): 52-53 (November/December), Mary Ann Liebert, Inc.

3. von Wedel, R.J. (1987) The Retrofit Modification of Microbial Fermentors for High Density, Perfusion Culture of Mammalian Cells, J. Soc. Indus. Microbiol., Manuscript in preparation.

Invited Speaker Presentations

1. "Presynaptic Probes of Neurotransmitter Release at the Frog Neuromuscular Junction" given at the Department of Neurobiology of the Salk Institute, La Jolla, CA, April, 1980.

2. "Transfer of Presynaptic Antigens to the Plasma Membrane of Nerve Terminals during Exocytosis" given for the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, CA, April, 1980.

3. "The Mass Culture Technique (MCT) at Bio-Response", Symposium on Mammalian Cell Culture Scale Up Techniques, (W. Tolbert, Chairperson), Annual Meeting of the Tissue Culture Association, Houston, TX, June 1-6, 1984.

4. "The Mass Culture of Murine and Human Hybridomas" at the following three Symposia on The Preparation and Purification of Monoclonal Antibodies, sponsored by Bio-Rad Laboratories, Inc., Richmond, CA:

   Oakland, CA (10/22/85) Cambridge, MA (10/29/85)
   Dusseldorf, West Germany (11/19/85)

5. "Scale Up Techniques for the In vitro Production of Monoclonal Antibodies to Tumor Antigens" given at the Second Annual International Workshop on Monoclonal Antibodies and Breast Cancer, San Francisco, CA (11/21/86) sponsored by the John Muir Cancer and Aging Research Foundation, Walnut Creek, CA.

6. "Automation of Research/Pilot Scale Cell Culture Bioreactors Integrated with Biosensors and Downstream Bioprocessing Systems" at the Fourth Annual Congress for Automation, Scale-Up and the Economics of Biological Process Engineering, San Francisco, CA March 4, 1987; organized by Scherago Associates and Genetic Engineering News, New York.

REFERENCES AVAILABLE ON REQUEST

CytoCulture/Sybron Bioreclamation Proposal

Project Manager: Jose F. Mosquera

Mr. Mosquera is a Master's Degree graduate student in the Division of Sanitary/Environmental Engineering in the Department of Civil Engineering at Berkeley. A native of Barcelona, Spain, Mr. Mosquera is a graduate Chemical Engineer with considerable experience in the design and construction of new industrial wastewater treatment plants. In 1986 he established his own wastewater treatment consulting company in Spain to distribute selected bacterial cultures (manufactured by TBA, Paris) for industrial wastewater biodegradation.

Mr. Mosquera was awarded a Fulbright Fellowship to attend Berkeley last August. His prior experience and academic credentials have enabled him to complete his program in one year. He received his Master's Degree in May of this year (with a grade-point average of 3.9). His direct experience with industrial wastewater treatment projects of the type described here will be invaluable for the documentation of site-specific data for this project in preparation for Step II/III grant proposal applications. His resume is attached.

CURRICULUM VITAE

Jose F. Mosquera

Personal Data

Date of birth: October 30, 1959 Place: Barcelona, Spain
Marital Status: Single
Campus Address: 759 International House
2299 Piedmont Avenue
Berkeley, CA 94720

Telephone: (415) 643-3016 or 642-9490

Education

Division of Sanitary/Environmental Engineering, Department of Civil Engineering, University of California, Berkeley: Master's Degree — 5/87

Georgetown University, Washington, D.C.
Fulbright Fellowship Grantees Orientation Course — 8/86

Instituto Quimico de Sarria (Barcelona)
Chemical Engineering Degree — 12/85

Centre de Formation et de Documentation sur l'Environment Industriel (Paris)
Course of physico-chemical treatment of wastewater — 4/85

American High School of Barcelona
Diploma — 6/78

Institut Francais de Barcelone
(Universite de Toulouse)
Certificate of French Language — 6/78

Professional Record

Research Engineer, David Jenkins' laboratory — 5/87 - present
Division of Sanitary/Environmental Engineering
Department of Civil Engineering, UC Berkeley

Consulting Engineer (bacterial applications)
Techniques et Biochimie Apliquies (Paris) — 4-7/86

Intern Chemical Engineer (water treatment)
Sociedad General de Aguas de Barcelona — 1-3/86

Quality Control and Environmental Engineer 6/84-12/85
APROCAT S.A. (Barcelona) Animal Products
Rendering Industry (designed new wastewater
treatment facility for processing cattle blood)

Technician for thermoelectric cooling systems 6-9/84
Aguafont S.A. (Barcelona)

Awards

Fulbright Fellowship (2 yrs) to attend 9/86-6/88
University of California, Berkeley

Dean's List, Department of Civil Engineering 9/86-1/87

Professional Organizations

American Society of Civil Engineers

Water Pollution Control Federation

Asociacion de Quimicos del Instituto Quimico de Sarria

Languages

Fluent in English, Spanish, French and Catalan

Publications

Mosquera, J.F. (1987) Elimination of Trihalomethanes from Drinking Water, Tecnologia del Agua, submitted.

Mosquera, J.F. (1987) Air Stripping as a Technique for the Elimination of Volatile Organics from Drinking Water, Tecnologia del Agua, submitted.

References

Prof. David Jenkins
Division of Sanitary/Environmental Engineering
Dept. Civil Engineering, UC Berkeley, 94720

Prof. R.E. Solleik
Department of Civil Engineering, UC Berkeley, 94720

Prof. Miguel Gassiott Matas
Instituto Quimico de Sarria
08017 Barcelona SPAIN

Technical Consultants

Our primary consulting resources will, of course, come from within the respective companies contributing to the Augmented Bioreclamation project. The technical qualifications of the key personnel participating in this project from CytoCulture International, Sybron Chemical and Alton Geosciences are reviewed in Appendices 1,2 and 3, respectively.

Additional technical advice will be obtained from the technical staff at Sybron Biochemical, Inc. in Salem, Virginia. Telephone and telex communication will be maintained throughout the twelve month period with the following staff scientists at Sybron as part of our current research collaboration:

Douglas Goldsmith, Ph.D. - Research Director

Lois Davis - Treatability Studies Laboratory Director

Will Stringfellow - Research Scientist

Arthur Wong - Technical Field Engineer

Mr. Wong will visit California several times during the course of this program and will be available for consultation.

In addition, through Mr. Mosquera's Masters Degree program at UCB, we expect to maintain good communication with the professors and technical staff of the Division of Sanitary/Environmental Engineering within the Department of Civil Engineering. His thesis advisor, Dr. David Jensen, will be an especially strong resource for our team.

Past Activities Related to Proposed Project

Sybron Chemical has been a major supplier of selected bacterial cultures for municipal and industrial wastewater facilities for nearly forty years. More recently, the company has embarked on a rigorous research and marketing campaign to introduce the bacterial biodegradation technologies to a much broader range of industrial applications. Groundwater clean up and soil remediation projects are now a major focus of the company under the direction of Mr. Gary Hater (see Appendix 2).

As for prior collaborations with CytoCulture, Sybron invited Dr. von Wedel to serve as an informal consultant on several bioaugmentation projects in Puerto Rico. Dr. von Wedel's affiliated company in San Juan, AmbienTech, is directly involved with the introduction of environmental biotechnologies

to the island. The company now assists Sybron representatives on the island in the biodegradation of petroleum and other industrial wastewaters, and is currently involved in establishing a wastewater laboratory technician training program for the Puerto Rico Aqueduct and Sewer Authority (to be contracted through Metcalf and Eddy, Inc.).

Additional literature, product information and case studies pertaining to Sybron bacterial cultures and their application to industrial wastewater treatment operations are available. In southern California, Sybron is already involved in gasoline contaminated groundwater treatment operations with Alton Geosciences, Inc.

Future Activities Related to the Proposed Project

CytoCulture International has been appointed the regional distributor for Sybron Biochemical and will therefore be developing both a research and marketing strategy for promoting the commercial development of specialized bacteria cultures for biodegrading toxic wastes in California. The company is building up contacts and collaborative arrangements with the University of California, Berkeley (Division of Sanitation/Environmental Engineering, Department of Civil Engineering) through Mr. Jose Mosquera, Project Manager for this proposed project. Similar contacts are being established with the Department of Chemical Engineering at Stanford to work on applications of these bacteria in trichloroethylene contaminated groundwater treatment on the Penninsula. We are also talking with Dr. Thomas Holtzer of the U.S.G.S. concerning the applications of these bacterial cultures to local contaminated groundwater treatment experiments. Lastly, we are approaching Dr. Edward Cichon, General Manager, Hazardous Waste Group, Western Division of Metcalf and Eddy, Inc. (Palo Alto) with ideas for incorporating specialized bacterial cultures into some of their new industrial wastewater treatment plants.

CytoCulture/Sybron Bioreclamation Proposal

Appendix 2

SYBRON CHEMICALS, INC.

Project Personnel

Technical Literature

Letter of Collaboration

SYBRON CHEMICALS INC.

July 28, 1987

Cyto Culture International
1208 4th Avenue
San Francisco, CA 94122

Attn: Dr. Randall Von Wedel

Re: Blymer & Sons Project in the Bay Area

Dear Randy:

Following our conversation of Monday, July 27, I am pleased to give you a short price structure for this project. As we discussed, I intend to supply two individuals, namely myself and Doug Goldsmith for the start-up with an alternate being, Russell Balderson. We intend to spend five days at the start of the project saturating the immediate system with nutrients and organisms. The project calls for in excess of 600 lbs. of organisms to start for the operation of the three systems. The first week cost will be $24,525 FOB Salem, Virginia and an applicable California sales tax of 5 1/2%. Maintenance will be performed on the system from a biological point of view once a month. The standard mechanical maintenance will be performed by your personnel. This monthly mechanical maintenance will supply chemistry organisms and nutrients for the operation of the system. Sybron's one day visit per month will make available to you bacteria counts, sole-source carbon counts, nitrogen and phosphorous values and a general system tune up. The monthly cost for this maintenance is $4,350 per month FOB Salem, Virginia and also does not include a 5 1/2% California based sales tax. Note, sales tax are based on products only not serviced and generally are about 50% of the quoted price.

Obviously, we have talked in detail about this project our intention is to supply a diesel fuel mix which is broken down into benzene, Toluene and Xylene degrading organisms with additional organisms capable of degrading PAH fractions which we think is very unique.

We trust that the enclosed information will enable you to bid the project. If you need additional assistance, please do not hesitate to call me or Doug Goldsmith, also my secretary, Margot Senior, generally can find me within an hour or so.

Most sincerely,

Gary R. Hater (Mes)

Gary R. Hater
Manager, Soil and Groundwater
Treatment Technology

cc: R. Ieva
D. Goldsmith
T. Reardon

Gary R. Hater
4286 Turf Lane
Cincinnati, Ohio 45211
(513) 574-9722

PROFESSIONAL EXPERIENCE:

February, 1984 Present

Sybron Chemicals Inc.
Biochemical Division
Birmingham Road
New Jersey 08011

Regional Manager/Program Director

Initially responsible for a North Central Sales territory. In November 1985, started soil and groundwater group. Co-inventor of the Bio-Sock and the Bacterial Contaminant Interceptor.

August, 1979 - February, 1984

Ingersoll Rand
IMPCO Division
150 Burke St.
Nashua, N.H. 03061

Area Manager/Sales Engineer

Responsible for sales of capital goods for Fortune 200 industrial accounts in Midwest; additionally co-covered pulp and paper accounts in same area.

July, 1977 - August, 1979

Western States Machine Co.
1798 Fairgrove Ave.
Hamilton, Ohio

Process Engineer

Managed test facilities, including centrifuge scale-up and field trouble-shooting.

June, 1975 - July, 1977

University of Cincinnati
Dept. of Biological Sciences
Cincinnati, Ohio 45221

Research Associate

Managed and operated an environmental research laboratory, supervising graduate and undergraduate personnel:

° Collected field data for major midwestern power companies.
° Worked on Alaskan Pipeline.

EDUCATION:

September, 1973 - June, 1975	M.S., Biology University of Cincinnati Cincinnati, Ohio
September, 1969 - June, 1973	B.A., Biology (Chemistry Minor) Thomas More College Ft. Mitchell, KY.

CHARLES DOUGLAS GOLDSMITH, JR.
640 CAMBRIA STREET
CHRISTIANSBURG, VIRGINIA 24073
Home (703) 382-2325
Office (703) 389-9361

EDUCATION

1985	Virginia Polytechnic Institute and State University	PhD	ESEN
1981	West Virginia State College - Completed Requirements	BS	CHEM
1975	Virginia Polytechnic Institute and State University	MS	F&W
1973	West Virginia State College	BS	ZOOL

EXPERIENCE

Manager of Basic and Applied Research Group, Sybron Chemicals Inc., Salem, Virginia, 1984 - present.

Analytical Chemist, Union Carbide Corporation Technical Research Center, South Charleston, West Virginia, 1979 - 1981.

Fermentations Research Technician, Union Carbide Corporation Technical Research Center, South Charleston, West Virginia, 1976 - 1979.

Instructor of Natural Science West Virginia State College, Institute, West Virginia, 1979.

Quality Control Lab Technician, Diamond Shamrock Corporation, Belle, West Virginia, May - August, 1970, 1971, 1973.

PROFESSIONAL SOCIETIES

American Water Works Association
Virginia American Water Works Association
Water Pollution Control Federation
Virginia Water Pollution Control Federation

HONORS AND AWARDS

Award for Outstanding Achievement in Biology at West Virginia State College, 1972 and 1973.

President, West Virginia State College Biology Society, 1973.

Beta Kappa Chi National Science Honorary and and delegate to the National Convention, 1972.

Magna cum laude graduate West Virginia State College, 1973.

JOB RESPONSIBILITIES

Manage a basic and applied research group consisting of one research microbiologist and three research technicians.
Coordinate industrial waste treatment and groundwater remediation university research projects funded by Sybron.
Seminars and short schools concerning principles and proper operation of waste treatment processes and bioremediation of contaminated aquifers.
Consultant to industrial and municipal treatment plant personnel nationwide.
Evaluation of field studies by Regional Technical Representatives.
Report preparation for lab and field investigations dealing with biological treatment.

RESEARCH IN PROGRESS

Biological decontamination of soil and groundwater.
Bench scale industrial wastewater treatment.
Effects of externally added bacteria on industrial waste treatment biokinetics.

PREVIOUS RESEARCH

Biodegradation of methanol and tertiary butyl alcohol in previously uncontaminated subsurface systems, 1981 - 1984.
Analytical method development for air contaminants in the workplace, 1981.
Analytical method development for alcohols, polyethylene and urethane intermediate plant processes, 1979 - 1980.
Pilot scale fermentation and supportive lab studies with chemically productive bacteria, 1976 - 1979.
Impact of lead in automotive exhausts on roadside ecosystems, 1973 - 1975.
Environmental Impact Statement data collection and preparation for the Bath County, Virginia VEPCO resevoir site, 1973 - 1974.
Physiological effects of sulfur dioxide, NSF Grant # GY6980, 1972.

PUBLICATIONS, PAPERS, ABSTRACTS

Submitted:

Goldsmith, C. D. and Balderson, R. K. 1987. Biodegradation and growth kinetics of enrichment isolates on benzene, toluene, and xylene. Microbiology of Waters and Wastewaters. International Association on Water Pollution Research and Control.

Wong, A. D. and Goldsmith, C. D. 1987. Impact of a chemostat discharge containing oil degrading bacteria on the biological kinetics of a refinery activated sludge process. Microbiology of Waters and Wastewaters. International Association on Water Pollution Research and Control.

Hater, G., Goldsmith, D., Quiros, J., and Moxley, D. 1987. Destruction of petroleum hydrocarbons from excavated and in-place soil using augmented bioreclamation. ASTM.

To be submitted:

Goldsmith, C. D. and Hogan, W. G. 1987. Biodegradation and growth kinetics of enrichment isolates on naphthalene.

Published:

Novak, J. T., K. D. White, G. T. Hickman, and C. D. Goldsmith. 1986. Organic degradation kinetics in subsurface systems. Chapman Conference-Microbial processes in the transport, fate and in-situ treatment of subsurface contaminants. Snowbird, Utah October 1-3.

White, K. D., J. T. Novak, C. D. Goldsmith and S. Bevan. 1986. Microbial degradation kinetics of alcohols in subsurface systems. NWWA Symposium - Petroleum hydrocarbons and organic chemicals in groundwater: Prevention, detection, and restoration. Houston, TX November 13-15.

Novak, J. T., R. E. Benoit, K. D. White, C. D. Goldsmith. 1985. Insitu biodegradation of alcohols. Amer. Inst. Chem. Eng. Annual Meeting. Chicago, IL November 10-15.

Goldsmith, C. D., K. D. White, J. T. Novak, and R. E. Benoit. 1985. Biodegradation of the alcohol components of gasoline in groundwater aquifers. AWWA Universities Forum, Washington, DC, June 25.

Benoit, R., J. Novak, C. Goldsmith and J. Chadduck. 1985. Alcohol biodegradation in groundwater microcosms and pure culture systems. Amer. Soc. Microbiol. 85th Annual Mtg., Las Vegas, NV March 3 - 7. (Abstr.)

Novak, J. T., C. D. Goldsmith, R. E. Benoit and J. H. O'Brien. 1985. Biodegradation of methanol and tertiary butyl alcohol in subsurface systems. Water Sci. Technol. 17:71-85.

Novak, J. T., C. D. Goldsmith, R. E. Benoit and J. H. O'Brien. 1984. Biodegradation of alcohols in subsurface systems. Seminar on Degradation, Retention and Dispersion of pollutants in groundwater. IAWPRC, Copenhagen, Denmark, Sept 12-14. (Abstr.)

Cox, D. P. and C. D. Goldsmith. 1979. Growth and napththalene metabolism by mutant strain 119 of Pseudomonas putida grown in glucose-limited culture. FEMS Microbiol. Lett. 5 (1979):277-279.

Cox, D. P. and C. D. Goldsmith. 1979. The microbial conversion of ethylbenzene to 1-phenethanol and acetophenone by Nocardia tartaricans ATCC-31190. Appl. Environ. Microbiol. 38(3.):514-520.

Goldsmith, C. D., Jr. and D. P. Cox. 1977 Growth and napththalene metabolism by a mutant of Pseudomonas putida growing continuously in glucose medium. 9th Cent. Mtg. Am. Chem. Soc. (Abstr).

Goldsmith, C. D., Jr. and P. F. Scanlon. 1977. Lead levels in small mammals and selected invertebrates associated with highways of different traffic densities. Bull. Environ. Contam. Toxicol. 17(3):311-316.

Goldsmith, C. D, Jr., P. F. Scanlon and W. R. Pirie. 1976. Lead concentrations in soil and vegetation associated with highways of different traffic densities. Bull. Environ. Contam. Toxicol. 16(1):66-70.

Goldsmith, C. D., Jr. and P. F. Scanlon. 1976. Lead levels in mammals and invertebrates associated with highways of different traffic densities. Virginia J. Sci. 27(2):44 (Abstr.)

Goldsmith, C. D., Jr., and P. F. Scanlon. 1975. Lead levels in vegetation and soils near highways of various traffic densities. Virginia J. Sci. 26:55 (Abstr.).

Goldsmith, C. D., Jr., M. N. Berkaw and P. F. Scanlon. 1974. Body weight - organ weight relationships in cottontail rabbits. Virginia J. Sci. 25:63 (Abstr.).

BIODEGRADATION AND GROWTH KINETICS OF ENRICHMENT ISOLATES ON BENZENE, TOLUENE, AND XYLENE

C. D. Goldsmith and R. K. Balderson

(Abstract)

The objective of this study was to determine the biodegradation and growth kinetics of benzene, toluene, and xylene in pure culture, sole substrate and mixed culture, mixed substrate systems. Bacterial strains capable of degrading benzene, toluene, and xylene were obtained by enrichment culturing. Several substrate concentrations were monitored for biodegradation and growth rates using 500 mL gas sampling bulbs as the batch vessel. All aromatics studied were easily biodegraded and had maximum utilization rates of 0.13 - 0.26 hours^{-1}, first order specific substrate utilization rates of 4.45×10^{-3} to 6.94×10^{-3} L/mg-hr, maximum growth rates of 0.36 to 0.90 hours^{-1} and K_s values ranging from 30 - 38 mg/L. Cell yields obtained from absorbance - biomass correlations and from the biokinetic constants μ and K were 2.72 - 3.92 based on mg/L aromatic substrate and 0.87 - 1.28 based on substrate as COD. This data can be used to estimate the time required to remediate contaminated aquifers and to properly operate above ground chemostats for the removal of aromatic contaminants.

Biodegradation and Growth Kinetics of an Enrichment Isolate on Naphthalene

C. D. Goldsmith and W. G. Hogan

(Abstract)

The objective of this study was to determine the biodegradation and growth kinetics of an enrichment isolate on naphthalene as a sole source of carbon. A bacterial strain capable of degrading naphthalene was obtained by enrichment culturing. Several substrate concentrations were monitored for biodegradation and growth rates using 500 mL gas sampling bulbs as the batch vessel. Naphthalene was easily biodegraded and had a maximum utilization rate of 0.185 hrs^{-1}, first order specific utilization rate of 0.046 L/mg-hr, and a K_s of 4 mg/L. The maximum growth rate was 0.54 hrs^{-1}, the first order specific growth rate was 0.098 L/mg-hr and the K_s was 5.5 mg/L. Cell yields were calculated to be from 2.1 to 2.9 based on mg/L of naphthalene and 0.9 based on the COD of naphthalene. This data can be used to estimate the time required to remediate PNA contaminated aquifers and to properly operate above ground chemostats in the field.

GROWTH OF MICROBES ON ANTHRACENE

BY

C.D. GOLDSMITH, JR.
MANAGER OF BASIC AND APPLIED RESEARCH
SYBRON CHEMICALS INC.
BIOCHEMICAL DIVISION
SALEM, VA 24153

W.G. HOGAN
LABORATORY RESEARCH TECHNICIAN
SYBRON CHEMICALS INC.
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SALEM, VA 24153

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are multi-aromatic ring compounds arranged in many forms. These compounds are often referred to as PNAs or polynuclear aromatics.

PNAs are found in many oils and have been studied in soils (landfarming) extensively. They present a problem in the environment due to their persistence after an accidental release. An excellent review of the literature was done by the American Petroleum Institute (1984) and is included in the Appendix.

The objective of this study was to isolate bacterial strain(s) capable of utilizing anthracene as a sole source of carbon.

METHODS AND MATERIALS

Culture Development

Anthracene degrading bacteria were isolated from soil contaminated with creosote and tar by enrichment culturing. Five grams of creosote and tar contaminated soil was placed in a 250 mL Erlenmeyer flask containing 100 mL of minimal salts media with 0.1 grams of anthracene as the sole source of carbon. The flask was placed on a Lab-Line Junior Orbit Shaker at 130 rpm and 25 C for three days. Ten milliliters of the acclimated soil flask was transferred to 100 mL minimal salts media with 0.1 grams of anthracene. This flask was transferred as before and allowed to incubate for 13 days. A fourth transfer was made and incubated for 8 days. The flask contents were streaked onto plate count agar, minimal salts agar containing anthracene, and minimal salts agar only. These plates were incubated at 35 C.

RESULTS AND DISCUSSION

Culture Development

A microscopic examination was made of the flask contents at each transfer. Good growth was observed at each transfer and consisted of motile rods. Two colony types were found on the plate count agar after 24 hours. One was slightly green and the other appeared an ivory color. The bacteria grew on the minimal salts agar with anthracene and equally as well on the minimal salts agar alone. Each colony was removed from the plate count agar and inoculated into 100 mL minimal salts broth separately to determine which bacteria was responsible for degrading anthracene. Each flask was placed on a shaker for three days and found to be capable of growing on anthracene. The colonies were streaked on

plate count slants and sent to Lewis-Gale Hospital Laboratories for identification and sensitivity testing. The organisms were identified as Pseudomonas aeruginosa and Pseudomonas maltophilia. P. maltophilia exhibited resistance to the thirteen antibiotics tested and will not be used for further work.

It was not possible to determine the biokinetic coefficients for anthracene, since anthracene is soluble in water to approximately 0.1 mg/L. The equivalent of 1000 mg/L anthracene was added to each flask for the study. The presence of crystals in solution proved to be enough substrate to stimulate growth.

CONCLUSIONS

Two bacterial strains have been identified that are capable of growing on anthracene crystals in an aerated flask.

Advantages of using Sybron Bacterial Cultures

There are several important advantages to using Sybron bacteria to establish an Augmented Bioreclamation program:

These bacteria have been carefully selected and blended in the laboratory to degrade specific toxic substrates present in contaminated ground water and soil. As a result, they are much more tolerant than "wild type" bacteria to toxic shock loads in the treatment areas.

Because of their tolerance to toxic hydrocarbon products, these bacteria can grow to high cell densities (two orders of magnitude higher than indiginous strains)and effectively degrade toxic contaminants under conditions that would have been lethal for ordinary bacteria.

Blending of bacterial cultures allows for symbiotic strains to partially breakdown hydrocarbons and complex toxic substances in such a way that the net result is total destruction of the contaminating substances.

Cyto Culture
INTERNATIONAL
INC

SYBRON
Biochemical

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

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

AUGMENTED BIORECLAMATION™

for

Soil and Groundwater Remediation

- SELECTIVELY ADAPTED BACTERIAL CULTURES
- MICRO AND MACRO NUTRIENTS
- FEASIBILITY STUDIES
- PILOT TRIALS
- CONCEPTUAL SYSTEM DESIGN
- PERMIT FILING ASSISTANCE
- PROJECT START-UP AND MAINTENANCE
- TECHNICAL AND ANALYTICAL FIELD SERVICES

ADVANTAGES

Versus alternative cleanup and disposal technologies.

Augmented Bio Reclamation™ (ABR)™ provides:

- **Cost Effective Cleanup** — most cleanup costs are half those of alternative technologies, e.g. incineration, excavation and hauling, etc
- Minimal Site Disruption — many materials can be treated at the site or in-situ
- **Ultimate Disposal Technology** — no contingent liability associated with containerized or landfilled materials which can escape into the environment
- Enhanced Kinetic Rates — selectively adapted cultures breakdown refactory compounds at accelerated rates

ABR Landfarming & Biological Surface Treatment

ABR Landfarming is used in the treatment of contaminated excavated soil. The soil is placed on a liner to a maximum depth of 18". ABR-cultures are grown on-site in portable growth chambers. After addition of an Accelerator formulation the site is augmented with bacteria from the growth chamber. Depending on the contaminant, weather and permitting agencies the landfarm may be covered with plastic.

Intermittantly the soil is mechanically turned to mix the culture with the contaminant and to provide aeration.

Biological Surface Treatment is the same as previously described but applied to shallow surface spills that are not excavated.

Deeper soil matrices can also be remediated by supplying oxygen through a piping system.

In-Situ Treatment of Soil and Groundwater

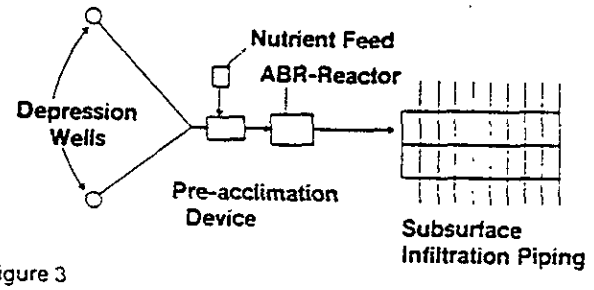


Figure 3

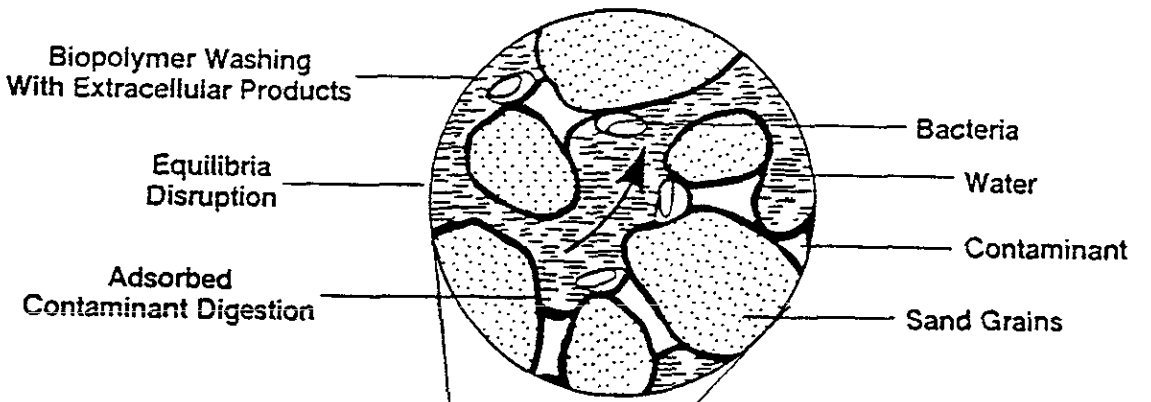
As shown in Figure 3 a cone of depression is formed and the contaminated water is brought to the surface. Contaminated water is pumped into a PreAcclimation Device (P.A.D.) where acclimated ABR-bacteria are grown sole source on the contaminant.

Final contaminant removal is completed in an above ground bioreactor. ABR organisms are seeded continuously from a BIO-SOCK* and grown in large numbers for IN-SITU injection into the saturated and unsaturated zones.

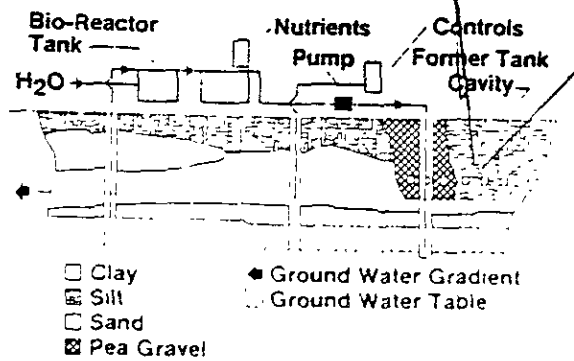
OTHER AREAS: Insitu Infiltration with Soil Ventilation, Landfill Leachate Digestion, Bio-Surtains

* Patent Pending

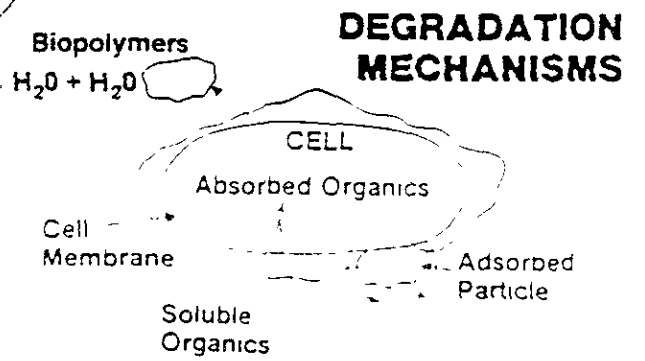
Soil Matrix/Contaminant/ Bacterial Dynamics



BIORECLAMATION SYSTEM



MICROBIAL DEGRADATION MECHANISMS



All of the elements found in nature, e.g carbon, nitrogen, etc. are available in finite and fixed quantities in nature. These elements are continuously recycled in form through what are known as the "biogeochemical cycles". When substances or compounds become waste these elements are usually released back into the environment as the material decomposes. The ultimate decomposers in all the biogeochemical cycles are bacteria and other microorganisms.

With the development of "anthropogenic or "man-made" substances it has become increasingly difficult for naturally occurring bacteria to develop the abilities to break down certain recalcitrant compounds. At Sybron we have given the natural evolutionary process a helping hand by "training" microorganisms to break down these materials through processes of selective adaptation using well known enrichment culture techniques.

Augmented Bioreclamation™ (ABR)™:

Organisms selectively adapted to rapidly biodegrade a waste or contaminant are cultured, blended, and shipped to the site for incubation. Incubation takes place in either a continuous flow or batch system depending on the application. Bacteria are dosed according to specific design criteria and site analysis resulting in a dramatic increase in the microbial population. This augmented population can be up to two orders of magnitude greater than the indigenous population. Figure 1 depicts this concept. Figure 2 compares biodegradation by selectively adapted organisms to indigenous bacteria.

Figure 1

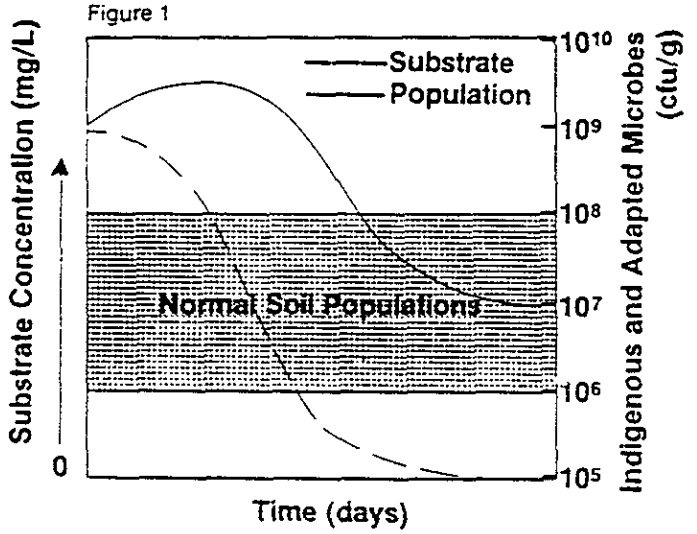
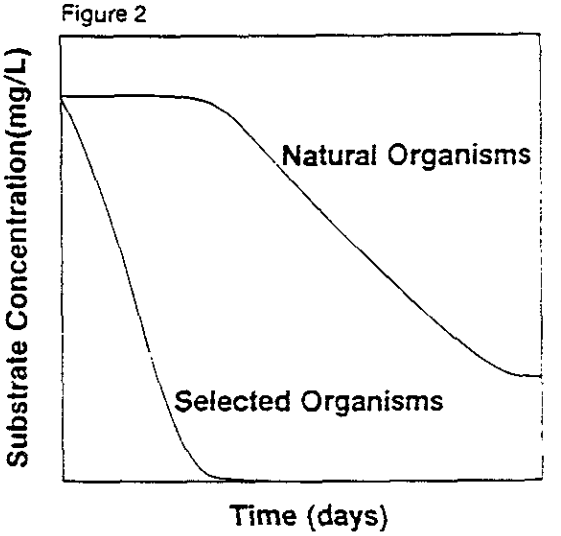


Figure 2



ABR™ Cultures:

Organisms isolated from the soil and/or groundwater by conventional enrichment techniques are documented to be safe and non-pathogenic. These cultures are grown on a sole source of carbon similar or identical to the target substrate and then transferred to bran fiber. The bacteria are mixed into a blend so that all the fractions of the contaminant are digested simultaneously. ABR cultures can comprise up to twelve different species of organisms.

ABR™ CULTURES INCLUDE:

- **ABR-GASOLINE**
- ABR-CREOSOTE
- ABR-DIESEL
- ABR-NON-CHLORINATED SOLVENTS
- ABR-CHLORINATED SOLVENTS

ABR™ NUTRIENTS INCLUDE:

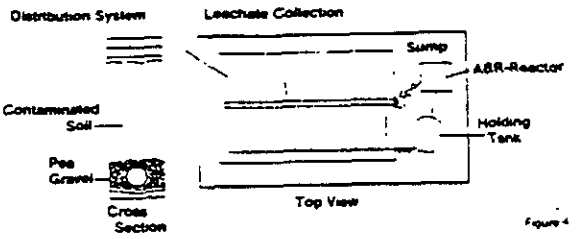
ACCELERATOR:

I. GROWTH MEDIA
II. NITROGEN+PHOSPHORUS
III. NITROGEN (no NO3)
IV PHOSPHORUS
V CHLOR-MEDIA
VI NITRATE MEDIA
VII TRACE METALS

ABR Recirculating Leachbed

Excavated soil is piled to a maximum leachable depth after a liner and drainage medium has been installed as shown in Figure 4.

Bacteria are applied to the surface of the leachbed with water and nutrients. Bacteria begin to degrade contaminants in situ while excess water leaches free product from the soil. The contaminant laden leachate is collected and biodegradation takes place in the ABR reactor. Leachate provides food for new bacteria which are redistributed using a continuous level control system.



ABR Production Pit Remediation

Oily petroleum waste from the production of crude oil and/or natural gas classically results in a spoils pond. These ponds/pits contain up to several percent oil by weight.

For treatment the pits are converted into a batch digestion system by inoculating them with ABR organisms, Accelerator and oxygen. In some cases emulsifying agents are used to enhance treatment. Use of emulsifiers to accelerate solubilization and breakdown is acceptable in most states.

Testing and Research

- SOLE SOURCE FEASIBILITY STUDIES
- MICROCOSM STUDIES
- IN LAB/ON SITE PILOT PLANTS
- ORGANISM SELECTION/ISOLATION
- AFFILIATION WITH MAJOR UNIVERSITY RESEARCH PROGRAMS
- DETAILED BACTERIA DATA ANALYSIS
- INDIGENOUS/ABR-CULTURE COMPARISONS

Areas of Expertise

WITH THE FOLLOWING CLASSES OF CHEMICAL COMPOUNDS

- PETROLEUM HYDROCARBONS (GASOLINE, DIESEL, FUEL OIL)
- CREOSOTE
- PHENOLS, ALCOHOLS, ALDEHYDES, ORGANIC ACIDS AND KETONES
- HYDROGEN SULFIDE
- CHLORINATED SOLVENTS

Corporate Offices and Sales:

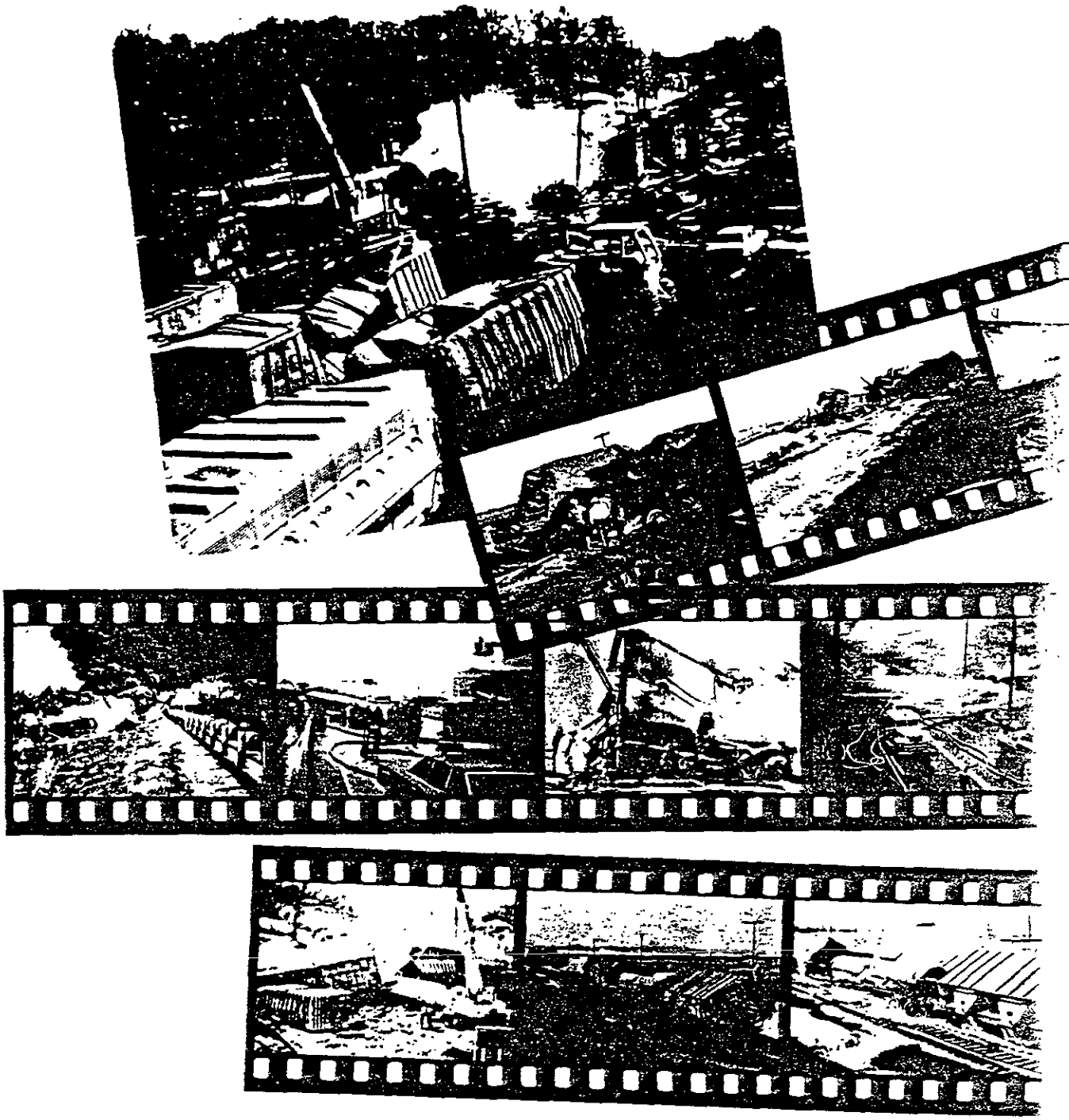
Birmingham Rd.
Birmingham, NJ 03061
800-257-9428
609-893-1100

Production, Testing, and Research:

111 Kessler Mill Rd.
Salem, VA 24153
800-654-6952
703-389-9361

Regional Offices and Affiliates in Key Areas

SYBRON CHEMICALS INC /BIOCHEMICAL DIVISION
BIRMINGHAM NEW JERSEY 08011 USA
PHONE 609-893-1100 TELEX WUI 685-1227/WUD 834446 (IONAC/SYBRON)



BI-CHEM® DETOXSOL SERIES

THE GROUND SPILL CLEANUP
PROGRAM THAT CAN SAVE YOU MONEY.

SYBRON | CHEMICAL DIVISION

DETOXSOL

can be very costly, because stringent legislation, including RECRA, now enforces strict cleanup procedures. Proper disposal may even require the collection of contaminated soil for shipment to secure landfills. The cost of such collection can be charged to the generator of the waste, even after it has left his property.

Not a pleasant prospect for your bottom line.

WE'VE GOT A COST-EFFECTIVE SOLUTION

We've developed just the solution for your toxic ground spill problems. Sybron/Biochemical Ground Spill Cleanup Cultures. Exclusively formulated from BI-CHEM mutant bacteria, Ground Spill Cleanup Cultures attack pollutant substances in-situ, utilizing safe biological processes, allowing speedy detoxification, and freeing you from the confines of stringent toxic chemical legislation.

THE BIOLOGICAL TIME MACHINE

In many instances it would take naturally occurring soil cultures years, if not decades, to adapt themselves to the complex and toxic loadings spilled on the ground.

When you have had a spill, you can't wait for nature to adapt or develop an effective bacterial population, so we decided to do something about it.

By accelerating nature's process through in-situ genetic engineering techniques, Biochemical has developed the DETOXSOL Series of organisms. These Ground Spill Cultures can also accelerate the degradation of toxic oily or recalcitrant compounds and rapidly reduce the offenders to a safe level.

MUTANT BACTERIA ALONE IS NOT ENOUGH

Therefore applying only mutant bacteria to a spill does not insure cleanup. A strange and hostile environment can force a slow and uncertain bacterial adjustment.

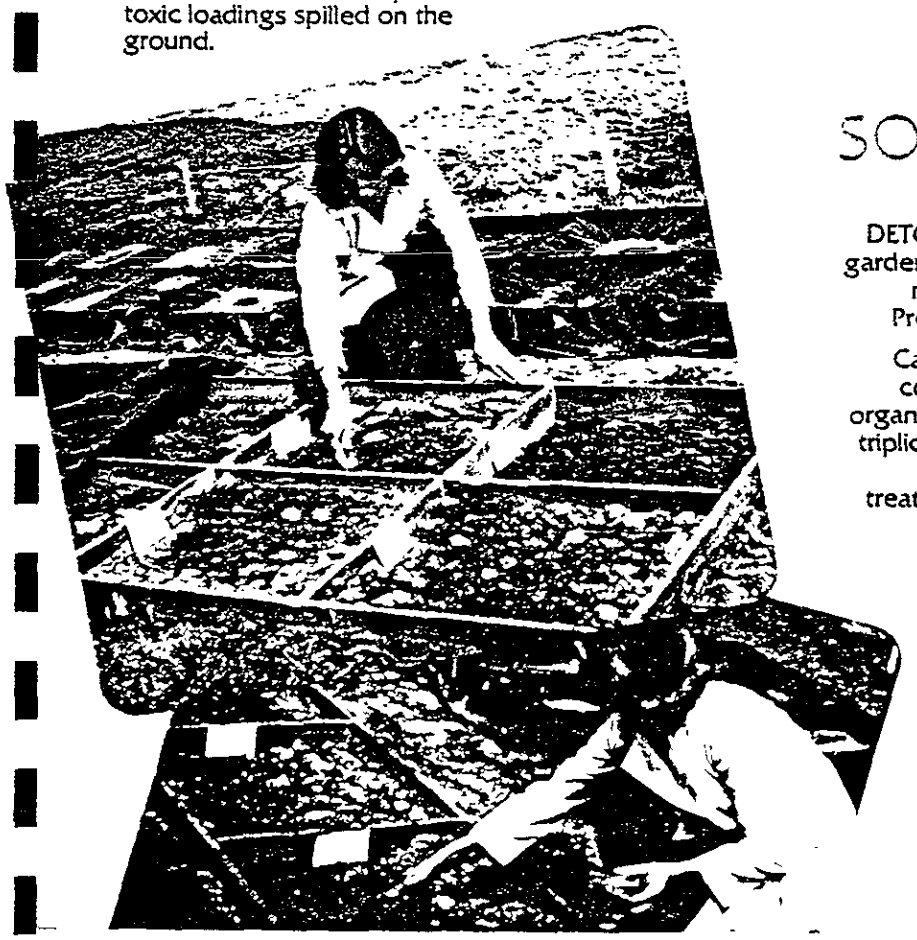
That's why BI-CHEM DETOXSOL Cultures contain an exclusive blend of buffer nutrients, growth stimulator and detoxifying agents. These substances work in conjunction with the mutant bacteria to encourage optimum biological degradation, while securing the critical establishment of the culture. BI-CHEM DETOXSOL Cultures foster the prompt, biological detoxification of intrusive chemicals with no harmful side effects.

THE PROOF IS IN THE CLEANUP

STYRENE. A major spill from a railroad car contaminated a residential area. Application of BI-CHEM Ground Spill Culture proceeded at a rate of one pound per 120 sq. ft. The culture degraded the styrene from a level of 25 to 30 parts per million to less than 1 part per million in twenty-one days.

PETROLEUM DISTILLATE. The spill covered an area of more than four acres. The initial concentration of petroleum hydrocarbons was 1200 ppm. Three weeks after application of the Ground Spill Cleanup Program, this had been reduced to one part per million.

BIPHENOL, OILS, Chlorinated Hydrocarbons, Dimethylamino Ethanol, Propylene Dichloride. BI-CHEM spill cultures are blended to take-on the toughest commercial fuel oils and petroleum by-products.



SOIL TESTING MODEL

DETOXSOL Cultures are all rigorously tested on garden soil plots. A simulated contaminant spill is made, after which the BI-CHEM DETOXSOL Product specific to the contaminant is added.

Carefully monitored, the concentration of the contaminant, moisture and the population of organisms are tested at regular intervals — using triplicate composite samples. Laboratory culture plates filled with contaminated soil are also treated and tested for confirmation of microbial degradation.

Weekly additions of BI-CHEM DETOXSOL are repeated until the contaminant is reduced to an acceptable level. Every BI-CHEM DETOXSOL Product adhers to these same strict testing procedures. Because delivering a product which helps you stop more serious problems — before they start — is as important to our business as it is to yours.

CytoCulture/Sybron Bioreclamation Proposal

Appendix 3

ALTON GEOSCIENCES, INC.

Personnel

Technical Literature

Letter of Collaboration

ALTON GEOSCIENCE

July 28, 1987

Dr. Randall von Wedel
CYTO CULTURE INTERNATIONAL INC.
1208 Fourth Avenue
San Francisco, California 94122

SUBMISSION OF A PROPOSAL TO PROVIDE
TECHNICAL SERVICES FOR THE BLYMER SITE

Dear Dr. von Wedel,

In accordance with our discussion, Alton Geoscience is pleased to submit this proposal to provide technical services to Cyto Culture International Inc for the assessment and remediation of the Blymer Site. Our capabilities for assessing and mitigating sites underlain by contaminated soil and/or ground water are documented in the accompanying Statement of Qualifications. We will gladly provide you with more business references on request.

The site in question is understood to have experienced a leak of liquid fuel onto the soil and groundwater. The depth to the ground water is believed to be approximately 8 feet. The water is tidally-influenced Bay water. Some free liquid fuel is reported to be floating on the ground water. A need exists to assess the site, to review the previous analytical efforts, and to develop and implement a cost-effective mitigation system. This system must interfere minimally with the surface activities.

Specifically, Alton Geoscience proposes to perform the following tasks:

Task 1. Review of the literature from previous work.
Cost: Not -to-exceed $ 3,000.00

Review the reports of previous consultants regarding previous site investigations and activities. Visit the site to determine the accessibility, hydrogeology, topography, utilities, and other characteristics bearing on the performance of the site assessment and mitigation. Evaluate the validity of the conclusions of the previous reports. Prepare a proposal with specific details on the necessary steps for the site assessment and site mitigation, with specific cost estimates.

Note: The cost estimate ranges for the remaining 2 tasks are typical but should not be interpreted as literal for this site. A relevant cost estimate will be submitted after the completion of Task 1.

Task 2. Site Characterization. Cost range: $ 25,000.00- $ 35,000.00

Typically includes acquiring appropriate permits. Drill and install approximately 11 monitoring wells to the ground water plus ten feet depth; install 4 inch ID PVC casing with filter pack, impermeable seals, and surface utility box. Sample and characterize the soils. Develop, survey, and sample the ground water in the wells. Where feasible, these wells may become incorporated into the recovery system. This will keep the costs of the mitigation system to a minimum. Perform chemical laboratory analysis. Analyze the hydrogeology and chemistry of the site and develop a most cost-effective mitigation system including a closed loop bioremediation system.

Task 3. Design, installation, and operation of the mitigation system. Cost range: Installation: $ 80,000.00- 100,000.00.
Operation and Maintenance: $ 4,000.00-7,000.00/month

Based on our successful completion of similar projects: design, acquire permits for, fabricate, install, and operate and maintain three bioremediation systems on the site. These systems typically include: downhole compressed air operated pumps, trenching for air supply and water return lines, above-ground tanks, controls, air compressor, and appropriate monitoring and fail-safe systems. All systems are automatic with redundancy and back-up as needed for safe and effective operation.

Bacteria will be acquired from SYBRON CHEMICALS INC., as successfully utilized in the past on similar site mitigations. The site will be visited weekly to install new bio socks, add nutrients as appropriate, and check operation of the system. Monthly analyses will be performed to determine system efficiency, and a progress report will be made each quarter, and at the end of one year.

Power and utilities will be provided by the client.

Alton Geoscience will perform this work in a manner that will represent the best interests of the client; that is, we will do all we can to seek closure of this site as quickly and economically as possible.

Please call if you have any questions.

Respectfully submitted,

Jeffery Wiegand

Jeffery Wiegand, Ph.D. CEG 331
Vice President.

ALTON GEOSCIENCE

ALTON GEOSCIENCE--STATEMENT OF QUALIFICATIONS

Alton Geoscience provides assessment and mitigation of subsurface contamination. The firm has enjoyed very rapid growth based on the ability to perform BOTH the assessment as well as the clean-up phases, and based also on its cost-effective mitigation alternatives. Alton Geoscience also provides technical investigations in the geologic disciplines. The offices of the firm are located in Irvine, California.

Specifically, the company performs the necessary functions to characterize a contamination incident, then carries out the mitigation through final regulatory closure approval. These functions include the following disciplines:

1. Hydrogeology - assess the underground contamination of soil and ground water and relate it to the hydrogeological system in order to develop the most cost-effective remedial measures.

2. Mechanical engineering - design, fabricate, install and operate customized mitigation systems including automated pumps, and effluent separation and water treatment facilities.

3. Biotechnology - perform clean up of contaminated soil and ground water utilizing specially adapted bacteria from Sybron Chemicals Incorporated. This cost-competitive technology is already operational. Alton has the capability to biodegrade contaminated soil and ground water in place as well as decontaminate soil above ground that has been excavated.

4. Field technology - perform reading of monitoring wells, pump out contaminated wells with two 600 gallon licensed trailers, and install and operate alternative mitigation systems.

5. Mobile laboratory - analyze soil and ground water samples in the field to greatly reduce the time of site clean up.

The company does NOT own its own drill rigs, nor does it haul contaminated soil. Alton does not benefit by hauling and

disposing of contaminated soil.

A typical contamination assessment and remediation project includes four phases:

Phase I: Background Review

Perform a background review of the site, including determining the local soils and hydrogeology, and nature of the incident, for a proposed site characterization plan. This plan is suitable for submission to the appropriate regulatory agency for approval. This plan is developed by acquisition and analysis of published and unpublished reports, well logs, maps, aerial imagery, and other data sources, as well as interviews with knowledgeable individuals. This phase also proposes a site investigation plan with details regarding the purpose, rationale, method and approved procedures to be followed.

Phase II: Site Characterization

This phase includes subsurface data acquisition by excavation of borings, sampling, and associated laboratory tests and analyses. Included in this phase are:

1. Perform underground utility survey as necessary to avoid encountering underground structures.

2. Acquire permits such as for drilling, as required, and drill borings and install monitoring wells. All wells are useable for extraction or recovery as well as monitoring.

3. Steam clean augers before drilling first hole and between holes. Wash sampler in trisodium poly-phosphate before taking each sample. Utilize Chain of Custody protocols.

4. The day after drilling, visit site with a truck and tank trailer and pump out four to ten volumes of water from the wells to properly develop them, then take a sample; requires a geologist on site.

5. Allow at least one day more for wells to equilibrate; visit site again and survey wells and depth to water table with a surveyor's level; tie the survey into an established bench mark. Verify all data by closing the survey. Reduce data in the office; have this part of the work confirmed and signed by a professional engineer.

6. Lockable caps and locks are installed on each monitoring well to prevent tampering.

The outcome of Phase II is a comprehensive report of findings including a preferred remedial action plan. The size of this report is kept to a minimum consistent with achieving the overall goal of regulatory closure of the incident. The report includes an index map, a site plan showing location of facilities, borings, and monitoring wells and their relation to the adjacent streets. Also included are a detailed text, boring logs and well completion schematics, cross sections, contour maps showing the extent of contamination plumes and the flow direction of the ground water, laboratory results, and Chain of Custody documents.

Phase III: Remedial Action Plan

Mitigation of the contamination is performed during Phase III. Alton has a strong commitment to utilizing alternative mitigation technologies which are most cost-effective. This commitment comes from having performed over three hundred such projects. Specifically, the alternative mitigation technologies include:

Landfarming

We have pioneered in acquiring needed permits for landfarming (aeration) of contaminated soils either on-site, or at a separate site. The latter option requires a much more lengthy permitting procedure but can be utilized for treatment of contaminated soils from several sites. Landfarming technology can be augmented or accelerated by utilizing bioreclamation at the same time. Final disposal of the soil can be at a municipal site or at an engineered landfill. The costs for this alternative will be on the order of 60-70 percent less than disposal at a Class I site.

Incineration

Alton Geoscience has performed the first test incineration of contaminated soil at an asphalt concrete batching plant. The test was successful and should soon lead to making this alternative available elsewhere. We are in the process of performing a second test incineration in the Los Angeles area. This alternative has merit when the site does not lend itself to land farming. Air quality permit acquisition is the pre-eminent issue with this alternative. The costs for this alternative will be approximately 60-70 percent less than disposal at a Class I site.

Bioreclamation

Alton Geoscience is the sole distributor in California of the augmented bioreclamation products and services of Sybron Chemicals Incorporated, a Fortune 500 firm which has been at the forefront of the biochemical waste treatment industry for over forty years. The first on-site bioreclamation project in Orange County for reclaiming contaminated soils is currently operational in Buena Park, California, by Alton Geoscience and Sybron. The process includes taking samples of indigenous bacteria, then culturing a population specific for metabolizing the contaminant. These bacteria are reactivated in a water medium with nutrients, and applied to the contaminated soils and ground water through pipes, or above ground by direct application. This alternative has immense potential for the very cost-effective mitigation of organic compounds. Bioreclamation can also be used to help clean up contaminated water for either recharge into the subsurface or discharge to the sewer.

Alton Geoscience has developed a proprietary air sparging system for volatilization of hydrocarbons in the dissolved phase from ground water. This process alone is sufficient in some cases to clean up the water to standards for discharge. There have been no problems with the permitting of this option with the air quality regulators.

We are currently performing remediation at approximately 50 sites in California; the recovery systems at several of these are unique and innovative to the industry. Several of them are automated.

Alton Geoscience has its own pump out trailers; and designs fabricates, installs and operates its own recovery/mitigation systems.

Phase IV: Monitoring and Closure

The final phase involves monitoring and regulatory approval of closure of an incident. This includes determining, with the regulatory authorities, the level of clean up that is most practical, equitable, and cost-effective.

In this regard, we believe our success rate in achieving closures is the best available in keeping with the spirit of the environmental quality regulations.

OTHER CAPABILITIES

In addition to performing assessment and mitigation of hazardous wastes, the firm performs a large number of site acquisition and site divestment studies for sellers and buyers of commercial and industrial real estate. The purpose of these studies is to establish that the pertinent property is free from contamination at the time of purchase.

Alton Geoscience also performs other earth sciences-related studies, including: engineering geology, ground water heat pump applications, geothermal exploration and development, and due diligence investigations.

MANAGEMENT AND PROFESSIONAL PERSONNEL

Trueman W. Hiller, President

Industrial engineer. Mr. Hiller is experienced in all phases of assessment and mitigation of leakage from underground storage tanks. He previously was involved in the design of service station pumping and leak control equipment.

Formerly Operations Manager for the Marley Company, Red Jacket Pumps Division, Irvine, California. Mr. Hiller was also Product Development Manager and Industrial Engineering Manager of the Red Jacket Pumps, Davenport, Iowa, Division.

Mr. Hiller is a holder of 6 patents on deep well pump and vapor control technology. Member, American Institute of Industrial Engineers (AIIE).

Jeffery W. Wiegand, Ph.D., Vice President

Engineering geologist. Formerly Vice President of Leighton & Associates, Irvine, California. Previously was Project Supervisor for D'Appolonia Consulting Engineers, Irvine, California.

Dr. Wiegand has managed the assessment and cleanup of over three hundred incidents of soil and ground water contamination by petroleum products for major oil companies. He has practiced engineering geology since 1960 in a variety of projects, including six major dams in North and South America; the national highway development program for Bolivia; harbors; military installations; and residential and commercial development. He has authored 17 published technical papers; the most recent was in May, 1987, on natural radioactivity in ground waters in Southern California.

Dr. Wiegand directs the firm's geological operations and business development program. He is a California Registered Geologist and a California Certified Engineering Geologist, and is a Registered Geologist in Arizona.

Joe M. Quiros, P.E., Vice President

Mechanical Engineer. Mr. Quiros has a comprehensive background in the design and installation of service station equipment. He is equally competent in the assessment and mitigation of leakage from underground tanks.

Formerly Engineering Manager for the Marley Company, Red Jacket Pumps Division, Irvine, California. Mr. Quiros was responsible for the West Coast engineering functions, including directing the installation of vapor control systems.

Mr. Quiros directs the design and implementation of pumping and treatment systems for underground hydrocarbon extraction and separation.

Mr. Quiros is a Registered Professional Mechanical Engineer in California and Arizona. He is also a licensed general contractor in the State of California.

He received his M.S. in Mechanical Engineering from the University of Arizona.

The following professionals are all currently working on several projects each:

1. William Hunt, Manager of Geological Services; geologist; graduated in 1985 in geology from California State University at Long Beach; has developed innovative systems for recovering free product; has performed approximately fifty investigations related to contamination from underground tanks; worked for four years as a senior technician at the Orange County Water District.

2. Michael Paules, hydrogeologist and geochemist; graduated in 1985 with an M.S. in geology from West Virginia University; worked for Murphy Petroleum for two years, and for Global Geochemistry; experienced in gas chromatography and mass spectroscopy.

3. Robert Logan, geologist; graduated in 1986 with an M.S. in geology from San Diego State University. Published technical papers on the geology of Western Arizona. Has performed approximately forty contamination investigations.

4. Erik Block, geologist, formerly with Chevron, USA, for two years; he has a Master's Degree in geology from California State University, Los Angeles.

5. Wilbert Gaston, hydrogeologist, was formerly with Gulf Oil Corporation for six years, and a petroleum engineering consulting firm. He has a Master's Degree in geology from the University of Houston (1979) and a Bachelor's in geology from Lamar University, Beaumont, Texas (1975).

6. Terrence Fox, geologist, graduated from Long Beach State University with a Master's Degree in geology in 1984, and a Bachelor's Degree in earth science from Fullerton State University in 1980. He worked for the U.S. Army Corps of Engineers for two years performing engineering geology studies.

7. Stephan Rosen, geologist, graduated from Hobart College in geology in 1982. He worked for Lamont-Doherty Geophysical institute for 4 1/2 years performing oceanologic geology studies.

8. Jeffrey Maxwell, geologist, graduated from the University of California at Santa Barbara in 1985, and did graduate work in hydrogeology at San Diego State University in 1986.

9. Eric Mears, geologist, graduated from Eastern Illinois University in 1986. Has worked for the U.S. Geological Survey for one year including performing investigations at Superfund sites for the U.S. Environmental Protection Agency.

10. Daniel Ramsay, geologist assistant, graduated from Long Beach State University in 1985 in geology. He worked for one year for a consulting geotechnical company in Orange County.

11. John Nordenstam, geologic intern, in senior year at California State University, Long Beach.

12. Marlaigne Hudnall, chemist/biologist, graduated in 1985 from the University Of California at Riverside with a B.S degree in chemistry. She was employed as an environmental chemist at Edwards S. Babcock and Sons in Riverside, California, for three years; she is experienced in gas chromatography applications and maintenance.

Project Experience

Alton Geoscience has performed the following site assessments and clean ups:

1. Major Oil Companies - 300 plus projects
2. Industrial Companies - 83 plus projects

The firm has also performed the following related work:

1. Investigation of the technical aspects of permitting a Class I disposal site; performed for Imperial County Planning Department.
2. Engineering geology investigations for private developers.
3. Geothermal energy and ground water heat pump investigations.
4. Research on a high temperature hazardous waste treatment technology.

Client List

The following are representative clients:

1. Mobil Oil Corporation
2. The Southland Corporation, 7-Eleven Division
3. General Dynamics - Convair Division
4. Cargill Corporation
5. Case Swayne
6. Warner Lambert
7. Hormel Corporation
8. Union Oil of California

BUSINESS REFERENCES

Mobil Oil Corporation
3800 West Alameda Avenue
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Los Angeles, California 91505

ENVIRONMENTAL:

Mr. Ralph Edwards
Western Regional Manager
(818) 953-2517

ENGINEERING

Mr. Harry Ericson
Los Angeles County
(818) 953-2602

Mr. Glenn Nakano
Los Angeles County
(818) 953-2608

The Southland Corporation
7-Eleven Division
1240 South State College Boulevard
Anaheim, California 92806

ENVIRONMENTAL & ENGINEERING

Mr. Larry Morris
(714) 635-7711