F1-055

CALFED

I. EXECUTIVE SUMMARY

CALFED Bay Delta Water Quality Action 19 Bioremediation of Petroleum Refining Industry Selenium Discharge

PRINCIPAL INVESTIGATOR:

Terrance Leighton (Lawrence Berkeley National Laboratory & University of California Berkeley)

CO-PRINCIPAL INVESTIGATORS:

Bob B. Buchanan (LBNL & UCB)
David Jenkins (UCB)
Roger Prince (EXXON Corporate Research)

COLLABORATORS:

Greg Balmer (EXXON Research and Engineering)
Sally Benson (Earth Sciences, Lawrence Berkeley National Laboratory)
Norman Edelstein (Chemical Sciences, Lawrence Berkeley National Laboratory)
Stan Goldman (Life Sciences, Lawrence Berkeley National Laboratory)
Dick McDonald (Nuclear Sciences, Lawrence Berkeley National Laboratory)
Al Middleton (EXXON Refinery Benicia, California)
Tetsu Tokunaga (Earth Sciences, Lawrence Berkeley National Laboratory)

OBJECTIVE:

The deliverable for this project will be a demonstrated and validated aerobic activated sludge selenium removal technology for use by the Bay Area petroleum refining industry.

BACKGROUND:

The subsurface geology of central California, including the San Joaquin Valley, is highly seleniferous. The processing of San Joaquin Valley crude oil by San Francisco Bay Area oil refineries results in the wastewater discharge of approximately 2,200 kg/year of selenium into the Bay Area ecosystem. Selenium is toxic to fish, shellfish, water fowl and small mammals. There is currently no cost effective technology to treat high-volume selenium contaminated waste streams. The absence of such treatment technology threatens the environmental quality of San Francisco Bay and has lead to litigation, oil refinery discharge permit limits that are one order of magnitude lower than current effluent levels, adverse economic impacts on refinery operations, and limitations on the quantity of San Joaquin Valley crude oil that can be used as feedstock.

We have discovered a novel and cost effective aerobic bacterial selenium treatment technology. Certain soil and aquatic bacteria that inhabit high selenium environments are able to bioconcentrate and bioimmobilize toxic forms of selenium into species that can be removed with the bacterial biomass. This aerobic biovalence treatment system has been extensively characterized and optimized at UCB/LBNL. Under optimal conditions, the UCB/LBNL system can achieve a 10,000 fold concentration and removal of selenite, the toxic selenium species found in refinery effluent, from bulk liquid phase. Based on our bench-scale experience and results from limited larger-scale field trials, we anticipate that these systems will minimally reduce selenium effluent loading of San Francisco Bay by 50%, and that with proper optimization we should be able to achieve a 80 - 90 % reduction in selenium effluent loads. The focus of this proposal is to migrate the present technology to a demonstrated and validated pilot-scale selenium biological treatment technology.

APPROACH/TASKS/SCHEDULE:

1. Configure and operate a pilot-scale UCB/LBNL selenium treatment system by March 31, 1998.
2. Configure and operate a pilot-scale BIOX selenium treatment system by September 30, 1998.
3. Complete documentation, validation and optimization of the UCB/LBNL pilot-scale treatment system by March 31, 1999.
4. Complete documentation, validation and optimization of the BIOX pilot-scale treatment system by September 30, 1999.
5. Select the optimal treatment system technology by December 31, 1999.
6. Support the migration of the selected treatment technology to full-scale field implementation beginning January 1, 2000.

UCB will make available to this project pilot-scale activated sludge research reactors, contiguous laboratory space and support facilities at the Richmond Field Station. Beam time required for XAS at the SSRL and the LBNL Advanced Light Source will be made available for this project. The LBNL Center for Environmental Biotechnology (CEB) and the UCB/LBNL Bioremediation Education Science and Technology (BEST) program will provide facilities for low background gamma-ray spectroscopy, microbial physiology, microbial ecology, analytical chemistry and CLPP.

Anticipated Construction Start Date: 10/31/97

Anticipated Construction Completion Date: 10/31/98

Grand Total: **$2,100,000.00**

Estimated Operation and Maintenance Cost: Approximately $400,000 per year.

Estimated Agency Contributions: $1,000,000 over the three year project period.

II. TITLE PAGE

CALFED Bay Delta Water Quality Action 19
Bioremediation of Petroleum Refining Industry Selenium Discharge

PRINCIPAL INVESTIGATOR:

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Dick McDonald (Nuclear Sciences, Lawrence Berkeley National Laboratory)
Al Middleton (EXXON Refinery Benicia, California)
Tetsu Tokunaga (Earth Sciences, Lawrence Berkeley National Laboratory)

RFP PROJECT GROUP TYPE:

Other Services

III. PROJECT DESCRIPTION

OBJECTIVE

The deliverable for this project will be a demonstrated and validated aerobic activated sludge selenium removal technology for use by the Bay Area petroleum refining industry.

BACKGROUND

The subsurface geology of central California, including the San Joaquin Valley, is highly seleniferous. The processing of San Joaquin Valley crude oil by San Francisco Bay Area oil refineries results in the wastewater discharge of approximately 2200 kg/year of selenium into the Bay Area ecosystem. Selenium is toxic to fish, shellfish, water fowl and small mammals. There is currently no cost effective technology to treat high-volume selenium contaminated waste streams. The absence of such treatment technology threatens the environmental quality of San Francisco Bay and has lead to litigation, oil refinery discharge permit limits that are one order of magnitude lower than current effluent levels, adverse economic impacts on refinery operations, and limitations on the quantity of San Joaquin Valley crude oil that can be used as feedstock.

Over the past ten years, the Western States Petroleum Association has investigated a variety of potential selenium refinery effluent treatment technologies including: ion exchange, reverse osmosis, chemical precipitation, physical-chemical sorption, anaerobic microbial reduction, and several proprietary technologies. All of these potential treatment technologies were found to have severe limitations, such as: lack of performance in selenium removal; inability to treat high-volume waste streams (1 - 2 MGPD); generation of excessive hazardous waste; or excessive cost (See Table 1 on the following page). We have discovered a novel and cost effective aerobic bacterial selenium treatment technology. Certain soil and aquatic bacteria that inhabit high selenium environments are able to bioconcentrate and bioimmobilize toxic forms of selenium into species that can be removed with the bacterial biomass. This aerobic biovalence treatment system has been extensively characterized and optimized at UCB/LBNL. Under optimal conditions, the UCB/LBNL system can achieve a 10,000 fold concentration and removal of selenite, the toxic selenium species found in refinery effluent, from bulk liquid phase. Based on our bench-scale experience and results from limited larger-scale field trials, we anticipate that these systems will minimally reduce selenium effluent loading of San Francisco Bay by 50%, and that with proper optimization we should be able to achieve a 80 - 90 % reduction in selenium effluent loads. The focus of this proposal is to migrate the present technology to a demonstrated and validated pilot-scale selenium biological treatment technology. This microbial selenium treatment system has the advantages of low capital cost, low operating costs, robustness to variations in wastewater flow rate and chemistry, use of a reproducing biocatalyst that does not require regeneration when fully metal loaded, and nonpathogenic biomass from which high-value elemental selenium can be easily recovered.

PREVIOUS WORK AND PROOF OF CONCEPT

1. *Scientific and Technical Approach*

The advantages and disadvantages of various selenium treatment technologies are summarized in Table 1. As a result of litigation, the Bay Area Oil Refineries have until July, 1998 to implement interim selenium treatment technologies. It is likely that most refineries will select either iron precipitation or

Table 1

Treatment Technology	Construction Cost	Operating Cost	Performance in Selenite Removal	Hazardous Waste Generation
Ion Exchange	High	High	Moderate	Low
Reverse Osmosis	High	High	Low	Low
Precipitation	Moderate	Moderate	Moderate	High
Sorption	Moderate	Moderate	Moderate	High
Biological Treatment	Moderate	Low	High	Low

I-001421

iron sorption as a temporary treatment system. Precipitation and sorption treatment systems generate large quantities of hazardous waste and are not particularly effective in selenite removal. It is unlikely that either of these technologies will be capable of achieving drinking water selenium effluent standards.They are however, the only currently available technology for reducing selenite discharge. As a long-term solution to this problem, our strategy is to develop a much more efficient and environmentally acceptable biological selenium removal technology. Refineries could retain the precipitation or sorption processes as a backup system in case of a process failure in the biological treatment system.

2. *Previous Work - Biological Treatment System Development*

The project team consists of microbial ecologists, microbial physiologists, analytical chemists, and chemical engineers from UCB/LBNL and EXXON. The goal of this group has been to develop the potential of biological treatment systems for attenuating the selenium liquid waste discharge from Bay Area petroleum refineries. In the context of this project, an *in situ* treatment system refers to bioengineering modifications to the existing EXXON Benicia refinery BIOX (BIological OXidation) and preBIOX reactors, while an *ex situ* treatment system refers to a designed and engineered polishing reactor system for selenium attenuation based on UCB/LBNL technology.

EXXON has provided approximately $200K to LBNL/UCB to investigate the feasibility of a microbial selenium removal process for their Benicia refinery. The results of this feasibility study have demonstrated that a well characterized laboratory strain of the common soil Gram-positive bacterium, *Bacillus subtilis*, detoxifies selenite by aerobic reduction to an insoluble and nontoxic form, elemental selenium (Garbisu et al., 1995; Garbisu et al., 1996; Garbisu et al., 1997; see Terrance Leighton CV for references). *B. subtilis* is able to grow and detoxify selenite at concentrations up to 400 ppm. At the lowest selenite concentration studied - 100 ppb - *B. subtilis* was able to remove 96% of the selenite from the liquid phase (see Figure 1).

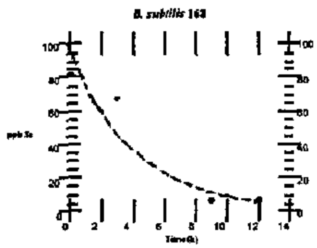


Figure 1 Selenite Removal During Growth of *B. subtilis*

This selenite concentration is typical of oil refinery liquid waste stream selenium burdens. A high energy carbon source, such as glucose, supports optimal selenite reduction. The selenite valence transformation to nontoxic elemental selenium is not affected by a ten-fold excess of nitrate or sulfate - alternate electron acceptors which block selenite reduction in anaerobic treatment systems (Garbisu et al., 1995). We conclude that selenite is not reduced via dissimilatory electron transport but rather via a novel detoxification system. These results indicate that the soil bacterium *B. subtilis* and related organisms form the basis of a very promising technology for bioremediating selenite in an *ex situ* polishing reactor configuration. These microorganisms can be easily and inexpensively cultivated in large-scale fermentation systems. The microorganisms are nontoxic and Generally Regarded As Safe (GRAS).

Data collected by Benicia EXXON plant personnel suggested that there may be a thirty percent drop in selenium burden across the BIOX activated sludge wastewater treatment system. We have begun investigations of the microbial ecology of the BIOX system to determine whether selenium detoxifying microorganisms were present and could account for the observed removal of selenite. Two hundred individual bacterial strains have been isolated from the EXXON Benicia refinery BIOX activated sludge system. A majority of these isolates are Gram-negative bacteria that were able to detoxify selenite with kinetics similar to well characterized laboratory strains of *B. subtilis*. At the lowest selenite concentration studied - 150 ppb - these EXXON BIOX isolates were able to remove >98% of soluble selenite from the liquid phase (see Figures 2 and 3).

I-001422

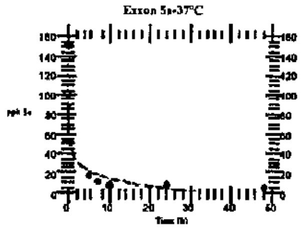


Figure 2 Selenium Removal During Growth of EXXON BIOX Strain 5a

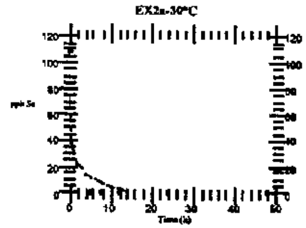


Figure 3 Selenium Removal During Growth of EXXON BIOX Strain 2a

Electron microscopic examination of *B. subtilis* and the EXXON strains grown in the presence of 1 mM selenite suggested that the electron dense elemental selenium detoxification product (identity confirmed by x-ray microprobe analysis) was accumulated in the extracellular periplasmic region of *B. subtilis*, while most of the EXXON BIOX strains accumulated dense intracellular elemental selenium granules. It is clear from the data that the microorganisms resident in the EXXON BIOX reactor are capable of detoxifying selenite, the major toxic selenium species present in the stripped sour water feed. On-going studies are directed at taxonomically identifying the selenite detoxifying members of the BIOX microbial population (principally Gram-negative groups such as pseudomonads, and Gram-positive bacilli), developing strategies for maximizing their population size and detoxification kinetics, and assessing the effects of seasonal and plant operation parameters on the BIOX microbial ecology.

3. *Previous work - Advanced Environmental Measurement Technologies for Selenium*

In collaboration with Roger Prince from EXXON Corporate Research and Ingrid Pickering at the Stanford Synchrotron Research Laboratory (SSRL) we have explored the feasibility of applying x-ray Absorption Spectroscopy (XAS) techniques to the *in situ* characterization of biological selenium wastewater attenuation systems. The goal of the project has been to speciate selenium contaminants incorporated into biological sinks for selenium valence transformation and bioimmobilization in natural environments. There is an urgent need for innovative environmental measurement technologies that are capable of *in situ* condensed phase toxic metal speciation. The XAS techniques pioneered at SSRL have the potential for *in situ* speciation of toxic metals, such as selenium, in sediments and microbial biomass at ppm concentration levels, without physical or chemical manipulation of the sample. There are no alternative analytical technologies for these environmental applications.

XAS spectra reflect the wavelength-dependent intensity reduction of the incident beam when it passes through a sample. Inner shell electrons of the absorbing atoms, excited by x-ray photons to the continuum and interacting with neighboring atoms, cause a modulation of the absorption coefficient. The fine structure characterizing the spectra, called Extended X-ray Absorption Fine Structure (EXAFS) and X-ray Absorption Near Edge Structure (XANES) can be used to determine the short range order surrounding specific species and to obtain chemical bonding information. EXAFS refers to the sinusoidal variation of the x-ray absorption coefficient as a function of x-ray photon energy. Oscillations in the post edge region arise from back-scattering of the emitted electron wave by neighboring atoms. The demonstration by Pickering and coworkers (*Environ. Sci. Technol.* 1995, *29*, 2456) of the ability of XAS to speciate toxic metals, such as selenium, *in situ* has created a powerful new tool for the analysis of undisturbed metal species in native environmental samples. Analyses at SSRL of EXXON BIOX biomass and microcosm models has established that EXAFS is capable of *in situ* speciation of inorganic and organic forms of selenium in these samples. BIOX microcosms were established and incubated for 48 hours in the presence of 0 - 50 ppm of added selenite. The biomass was harvested, frozen and analyzed by EXASF. Table 2 illustrates the effects of increasing selenite concentration on the fractional selenium species distribution in the BIOX biomass. The initial selenite concentration in the BIOX fluid was 80 ppb. As selenite concentration increased, an increasing fraction of the total biomass selenium was in the elemental form. The high fraction of organic selenium species observed at the lower selenite concentration ranges is consistent with our analysis of the microbial ecology of the BIOX system. The predominant microbial populations in the BIOX are Gram-negative bacteria that are known to incorporate selenite into seleno-amino acids. At higher and more biologically stressful levels of selenium, the majority of the selenite is detoxified to the non-toxic elemental species. Similar previous studies (Buchanan et al., 1995) have shown that all of the selenium in the UCB/LBNL *ex situ* treatment system biomass is present as elemental selenium. This result is expected as the Gram-positive microorganism used in this system, *B. subtilis*, is not known to incorporate selenite into seleno-amino acids.

Table 2

Selenite Addition (ppm)	0	0.5	1	10	50
Selenite (% of total)	10.3	7.5	5.9	2.7	0
Selenocyanate (% of total)	5.8	7.3	8.2	8.0	0
Selenomethionine (% of total)	62.3	63.4	63.4	23.1	0
Elemental Selenium (% of total)	21.6	21.8	22.5	66.2	100

In collaboration with McDonald's group at LBNL, we have performed gamma-ray spectroscopic quantitation of the total selenium concentration in the BIOX biomass. Gamma-ray spectroscopy is the only method available to measure selenium concentration in a sample without chemical extraction and manipulation. These *in situ* measurements, which have been independently confirmed by Atomic Absorption analyses, indicate that the selenium concentration in the BIOX biomass is approximately 185 ppm. Based on these data, the BIOX system is able to achieve a 2000 fold concentration and removal of selenite from the bulk liquid phase under optimal conditions. Similarly, the UCB/LBNL polishing system can achieve a 10,000 fold concentration and removal of selenite from bulk liquid phase. Based on our bench-scale models and results from limited larger-scale field trials, we anticipate that these systems will minimally reduce selenium effluent loading by 50%, and that with proper optimization we should be able to achieve a 80 - 90 % reduction in selenium effluent loads.

4. *Comparative Analysis of Biological Treatment System Options*

Each treatment strategy has merits. Metabolic engineering of the existing EXXON BIOX system to increase its efficiency of selenium removal would build on an established field-scale treatment technology. The disadvantage of this strategy is that only a portion of the total selenium is present as an easily extractable and non-toxic elemental species. The UCB/LBNL technology is attractive in that the bulk of the treatment system biomass selenium appears to be the non-toxic elemental form. The disadvantage of the UCB/LBNL system is that it has not yet been operated and validated at field-scale, although *B. subtilis* has been grown for over thirty years at large commercial scale for the production of industrial enzymes.

5. *Biological Treatment System Monitoring and Process Control Technologies*

The inability to characterize *in situ* microbial communities rapidly and economically is a severe barrier to the efficient optimization and control of biological treatment processes. Successful development of a functionally-based high-throughput instrument for the characterization of treatment system microbial communities, the community-level physiological profile (CLPP), would provide a critical enabling technology for minimizing the environmental impacts of refinery wastewaters.

We have demonstrated the power of the CLPP technology is a three year pilot study of the microbial community residing within the EXXON BIOX activated sludge waste treatment system. This project has two goals: (i) the development of monitoring tools to assess microbial community adaptation and adjustment to variations in treatment system operating parameters; and (ii) the use of CLPP as a high-throughput screening system to engineer the microbial community metabolically for optimal toxic metal bioremediation. CLPP has proven to be a very sensitive and incisive tool for assessing the integrity and functionality of the treatment system microbial community. CLPP has been used to track a treatment system upset that was caused by an alteration in the food to mass ratio of the wastewater feed stream. This perturbation resulted in significant negative impacts on treatment system nitrification and settling parameters. The three figures in the Appendix plot the CLPP signatures of the Normal BIOX Community (Figure 4), the Upset BIOX Community (Figure 5), and the Recovering BIOX Community (Figure 6). In each of these figures the respiration rate is plotted for a particular test substrate. The surprising result

I-001424

from these studies is that BIOX microbial communities have very simple and diagnostic CLPP signatures. We interpret these results to mean that BIOX communities are highly differentiated metabolic specialists, rather than metabolic generalists which would be expected to exhibit far more complex CLPP signatures than we have observed. The "normal BIOX" CLPP pattern has been stable over three years of treatment system monitoring. Based on the promise of these findings, EXXON is establishing an on-site Biolog monitoring capability at its Benicia refinery.

We will use pilot-scale activated sludge reactors to allow the intentional creation of process upsets, such as elevated carbon to nitrogen ratios, elevated levels of refining process additives that may have biotoxic effects, variations in process stream mixtures, and variations in process control set points for pH, dissolved oxygen concentration, sludge age and hydraulic retention time. The pilot reactor experiments will facilitate correlation of CLPP data from the full-scale BIOX system to carefully controlled and repeatable perturbations of the pilot-scale reactors. CLPP stability/recovery analysis of these typical process upsets will create an expert system that can predict the occurrence and nature of treatment system failures before the integrity of the microbial community and effluent permit levels are violated.

METHOD OF ACCOMPLISHMENT:

1. *Technical Objectives*

OBJECTIVES: 1. Configure and operate a pilot-scale UCB/LBNL selenium treatment system by March 31, 1998. **2.** Configure and operate a pilot-scale BIOX selenium treatment system by September 30, 1998. **3.** Complete documentation, validation and optimization of the UCB/LBNL pilot-scale treatment system by March 31, 1999. **4.** Complete documentation, validation and optimization of the BIOX pilot-scale treatment system by September 30, 1999. **5.** Select the optimal treatment system technology by December 31, 1999. **6.** Support the migration of the selected treatment technology to full-scale field implementation beginning January 1, 2000.

2. *Tasks, Project Phases and Deliverables*

The project involves the following tasks.

TASKS:

The tasks in **phase 1** of the project include: 1. Configure and instrument a 500 GPD pilot-scale aerobic granulated carbon bioreactor employing the UCB/LBNL technology. 2. Commence operation of the UCB/LBNL treatment system with BIOX effluent feed and appropriate metabolic amendments identified from previous bench-scale studies. 3. Monitor treatment system biological, chemical and physical parameters.

The tasks in **phase 2** of the project include: 1. Configure and instrument a 500 GPD pilot-scale BIOX bioreactor. 2. Commence operation of the BIOX treatment system with EXXON BIOX effluent feed and appropriate metabolic amendments identified from previous bench-scale studies. 3. Monitor treatment system biological, chemical and physical parameters. 3. Characterize the selenite detoxification end product produced by the UCB/LBNL treatment system.

The **phase 3** tasks include: 1. Optimize the UCB/LBNL treatment system feedstock control parameters for selenite bioremediation. 2. Characterize the detoxification end product produced by the treatment system. 3. Demonstrate sustained operation of the treatment system. 4. Document and validate the bioreactor design; feedstock source selection; feeding strategy selection; on-site microbial population monitoring methods; on-site microbial selenite detoxification monitoring; and the use of advanced environmental measurements technologies for selenium characterization. The latter include *in situ* selenium speciation of bioreactor sludge by x-ray absorption spectroscopy and *in situ* quantitation of selenium levels in bioreactor sludge by low background gamma-ray spectroscopy.

The **phase 4** tasks involve: 1. Optimize the BIOX treatment system feedstock control parameters for selenite bioremediation. 2. Characterize the detoxification end product(s) produced by the treatment system. 3. Demonstrate sustained operation of the treatment system. 4. Document and validate the bioreactor design; feedstock source selection; feeding strategy selection; on-site microbial population monitoring methods; on-site microbial selenite detoxification monitoring; and the use of advanced environmental measurements technologies for selenium characterization. The latter include *in situ* selenium speciation of bioreactor sludge by x-ray absorption spectroscopy and *in situ* quantitation of selenium levels in bioreactor sludge by low background gamma-ray spectroscopy.

The **phase 5** tasks involve: 1. Preparation of final reports and analyses of UCB/LBNL and BIOX treatment system performance, economics, operational requirements, environmental impacts, and regulatory compliance. 2. Preparation of recommendations for petroleum refining industry treatment system selection criteria.

The **phase 6** tasks include: 1. Support the field-scale deployment of biological selenium treatment technologies by the Bay Area refining industry. 2. Provide technology transfer and technology support for bioreactor design; feedstock source selection; feeding strategy selection; on-site microbial population monitoring methods; on-site microbial selenite detoxification monitoring; and the use of advanced environmental measurements technologies for selenium characterization. The latter include *in situ* selenium speciation of bioreactor sludge by x-ray absorption spectroscopy and *in situ* quantitation of selenium levels in bioreactor sludge by low background gamma-ray spectroscopy.

3. *Facilities and Resources*

UCB will make available to this project pilot-scale activated sludge research reactors, contiguous laboratory space and support facilities at the Richmond Field Station. Beam time required for XAS at the SSRL and the LBNL Advanced Light Source will be made available for this project. The LBNL Center for Environmental Biotechnology (CEB) and the UCB/LBNL Bioremediation Education Science and Technology (BEST) program will provide facilities for low background gamma-ray spectroscopy, microbial physiology, microbial ecology, analytical chemistry and CLPP.

Summary

This project builds on a successful partnership between UCB/LBNL and EXXON Corporation. The participants have been developing a number of innovative technologies for the bioremediation of industrial toxic metal contaminants.

Microorganisms have an unusually high surface to bulk phase volume ratio and are more efficient than other life forms in adsorbing, precipitating or complexing toxic metals within their wall matrix. In addition, microorganisms can detoxify metals by valence transformation, extracellular chemical precipitation, or volatilization. *B. subtilis* and other aerobic soil bacteria have been shown by the Principal Investigators to perform valence transformations of toxic metals to species which are much less toxic and less mobile (chromium [VI] to chromium [III]; selenite to elemental selenium). The precipitated metals, such as selenium, are retained in the sludge or settling from the aerobic zone. If the metal content of the biomass is sufficiently high, the solids from the aerobic zone can be directly extracted for metal recovery.

Properly engineered microbial waste treatment systems have a number of advantages: (1) low construction and operating costs; (2) robustness to variations in flow-rate and the chemical composition of co-contaminants and toxic metals; (3) the ability to lower toxic metal levels to below EPA standards; (4) the use of a reproducing biosorbent not requiring replacement once the sorbent is loaded with metals; and (5) the biocatalytic generation of products such as SH^-, HPO_4^{2-}, and CO_3^{2-}, which facilitate toxic metal precipitation.

Activated sludge systems are extensively employed for the treatment of high-volume municipal and industrial wastes worldwide. Despite their large-scale application, very little is known about the microorganisms present in activated sludge, or the physiological interactions which are critical to their effectiveness in wastewater treatment. The CALFED project team will create this important knowledgebase while developing a specialized toxic metal biological treatment technology for application by the Bay Area Oil Refining Industry. We anticipate that these new microbial treatment system tools will have wide applicability beyond the remediation of refinery selenium discharges. Other potential applications for these technologies include the treatment of toxic metal laden agricultural, mining, semiconductor and chemical process wastestreams that impact the Bay Delta ecosystem.

I-001426

SUMMARY OF PRINCIPAL INVESTIGATOR'S BACKGROUND:

Dr. Leighton has been a Professor of Microbiology and Biochemistry at UC Berkeley for the past twenty years. He is a Senior Staff Scientist in the LBNL Earth Sciences Division and directs the UCB/LBNL Bioremediation, Education, Science and Technology Center. Professor Leighton is an expert in *B. subtilis* postexponential phase microbial biology, the genetic regulation of bacterial sporulation, the molecular mechanisms which regulate hazardous metal detoxification and biosorption in Gram-positive bacteria, and the microbial ecophysiology of wastewater treatment systems and damaged environments.

ADMINISTRATIVE POSITIONS:

Director UCB - LBNL Bioremediation Education Science and Technology Center
East Bay Base Conversion and Reuse - Environmental Committee
UCB Biocomputing Administrator/UCB Genome Project Biocomputing Coordinator
CoDirector UCB - CalEPA Bioremediation Validation and Certification Laboratory
Director UCB Advanced Undergraduate Biotechnology Research Program
Founding UCB Member - Science Education Academy of the Bay Area (SEABA)
UC Systemwide Biotechnology and Bioengineering Grant Review Committee

RECENT RESEARCH PUBLICATIONS:

Melhorn, R. J., Buchanan, B. B. and T. Leighton. 1993. Bacterial chromate reduction and product characterization *in* "Emerging Technology for Bioremediation of Metals", Chpt. 3, p. 26. Lewis Publishers.

Yang, W. H., Yang, J. R., Yee, A., Buchanan, B. B. and T. Leighton. 1993. Microbial removal of sodium selenite from culture media by *Bacillus subtilis*. Proc. 23rd Mississippi Water Resources Conf. p.161. Water Resources Research Institute, Mississippi State University.

Buchanan, B. B., Bucher, J. J., Carlson, D. E., Edelstein, N. M., Hudson, E. A., Kaltsoyannis, N., Leighton, T., *et al.* 1995. A XANES and EXAFS Investigation of the Speciation of Selenite Following Bacterial Metabolism. Inorganic Chemistry 34: 1617.

Garbisu, C., Gonzalez, S., Yang, W.-H., Yee, B. H., Carlson, D. L., Yee, A., Smith, N. R., Otero, R., Buchanan, B. B. and T. Leighton. 1995. Physiological Mechanisms Regulating the Conversion of Selenite to Elemental Selenium by *Bacillus subtilis*. BioFactors 5: 29.

Garbisu, C., Ishii, T., Smith, N. R., Yee, B. H., Carlson, D. E., Yee, A., Buchanan, B. B. and T. Leighton. 1995. Mechanisms regulating the reduction of selenite by aerobic gram (+) and gram (-) bacteria *in* "In situ and On-site Bioreclamation," pp. 125- 131, Battelle Press, Columbus, Ohio.

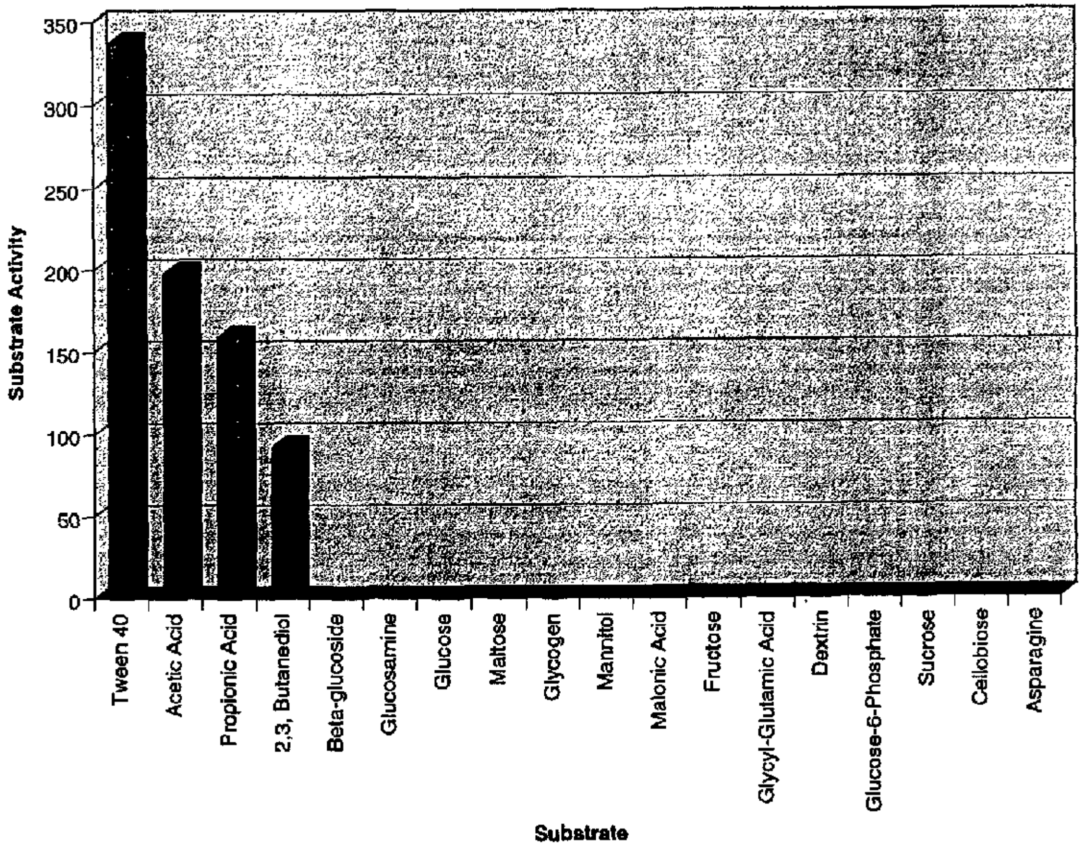
Coombs, G. F., Garbisu, C., Yee, B. H., Yee, A., Carlson, D. E., Smith, N. R., Magyarosy, A. C.. Leighton, T. and B. B. Buchanan. 1996. Bioavaliability of selenium accumulated by selenite-reducing bacteria. Biol. Trace Elem. Res. 52: 209.

Garbisu, C., Ishii, T., Leighton, T. and B. B. Buchanan. 1996. Bacterial reduction of selenite to elemental selenium. Chemical Geology, 132: 199.

Garbisu, C., Ishii, T., Smith, N. R., Yee, B. H., Carlson, D. E., Yee, A., Buchanan, B. B. and T. Leighton. 1997. Mechanisms regulating the reduction of selenite by aerobic gram (+) and gram (-) bacteria *in* "In situ and On-site Bioreclamation," Lewis Publishers, In press.

Garbisu, C., Magyarosy, A. C., Carlson, D. E., Smith, N. R., Ratliff, M., Stroh, M., Martinez, B., Sauri, D., Joyner, D., Leighton, T. and B. B. Buchanan. 1997. Laboratory assessment of the selenium bioremediation potential of microorganisms indigenous to an industrial activated sludge system. Gas, Oil and Environmental Biotechnology, Institute of Gas Technology, In press.

Appendix Figure 4
Normal BIOX Microbial Community Breathprint



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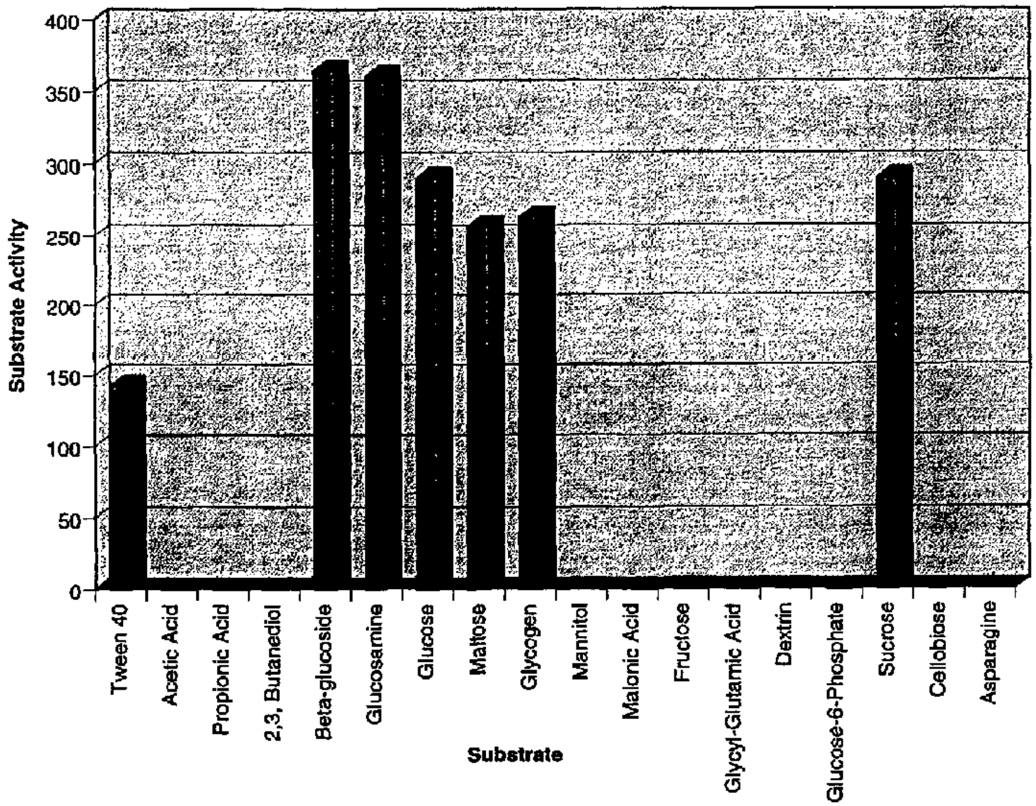
Appendix Figure 5
Abnormal BIOX Microbial Community Breathprint 6/28/95



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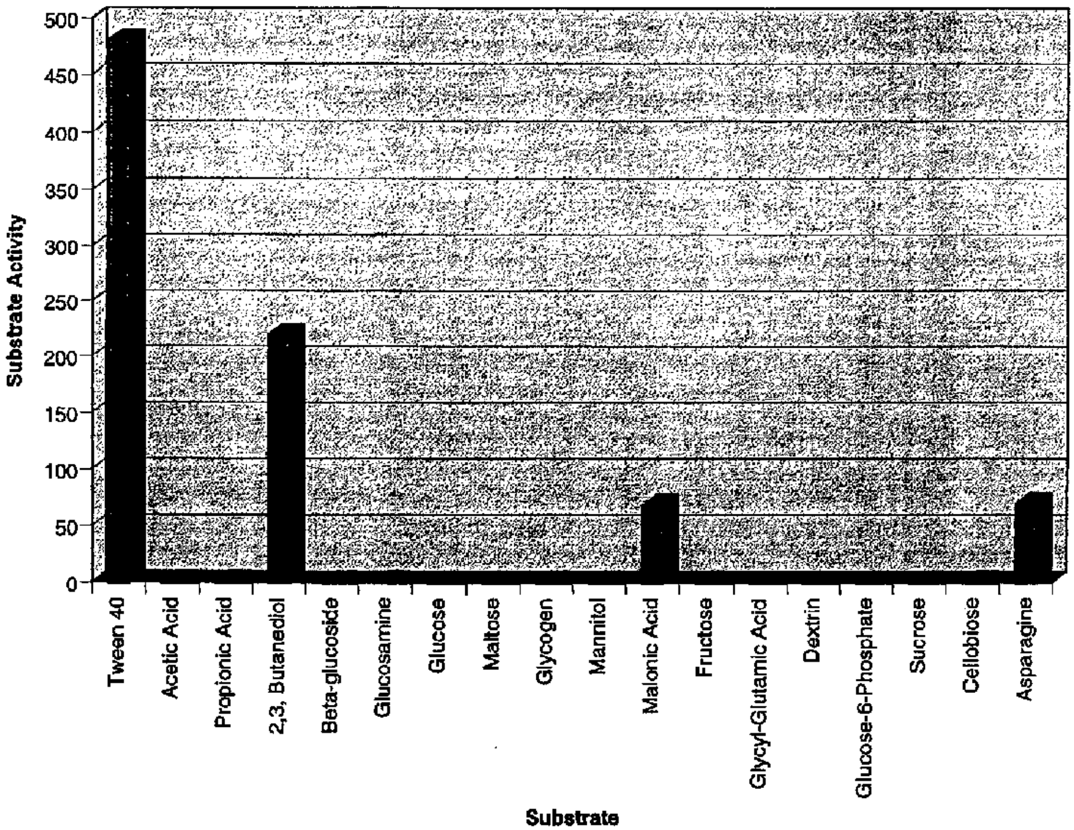
Appendix Figure 6
Recovering BIOX Microbial Community Breathprint 8/18/95



POTENTIAL WATER QUALITY PROJECTS FOR CALFED EARLY IMPLEMENTATION

Project Name: Bioremediation of Petroleum Refining Industry Selenium Discharge

Project Manager: Dr. Terrance Leighton, Center for Environmental Biotechnology, Lawrence Berkeley National Laboratory, MS 70A-3317, Berkeley, California 94720

Telephone: 510 642-1620
FAX: 510 643-5035
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Title of Project Manager: Senior Staff Scientist

Other Involved Agencies:

Department of Molecular and Cellular Biology
University of California at Berkeley

Department of Microbial Biology
University of California at Berkeley

Department of Civil Engineering
University of California at Berkeley

Department of Environmental Engineering
University of California at Berkeley

Richmond Field Station
University of California at Berkeley

Earth Sciences
Lawrence Berkeley National Laboratory

Chemical Sciences
Lawrence Berkeley National Laboratory

Nuclear Sciences
Lawrence Berkeley National Laboratory

EXXON Corporation
Benicia, California

EXXON Corporate Research
Annandale, New Jersey

IV. COST AND SCHEDULE TO IMPLEMENT PROPOSED PROJECT

	In Progress	Completed	Expected Completion	Not applicable
Study	X		1/1/98	
Design			9/30/98 - 9/30/99	
Field Testing			9/30/98 - 12/31/99	
Environmental Compliance			9/30/98 - 12/31/99	

Anticipated Construction Start Date: 10/31/97

Anticipated Construction Completion Date: 10/31/98

Table 3 Budget estimates

Project Phase and Task	Direct Salary and Benefits	Overhead Labor (G&A and Fee)	Material and Acquisition Contracts	Miscellaneous and other DirectCost Costs	Total
Phase 1	$99.6K	$81.9K	$55.0K	$15.5K	$252K
Phase 2	$111.6K	$95.7K	$92.0K	$15.7K	$315K
Phase 3	$214.8K	$168.6K	$58.0K	$20.6K	$462K
Phase 4	$230.4K	$180,9K	$62.0K	$9.7K	$483K
Phase 5	$72.0K	$57.0K	$23.0K	$16.0K	$168K
Phase 6	$217.2K	$166.8K	$30.0K	$6.0K	$420K
Grand Total:					**$2,100,000.00**

Estimated Operation and Maintenance Cost: Approximately $400,000 per year.

Estimated Agency Contributions: $1,000,000 over the three year project period.