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BIOMASS, COMMUNITY STRUCTURE AND NUTRITIONAL STATUS ATTRIBUTES OF THE DEEP SUBSURFACE MICROBIOTA--AT IDAHO AND HANFORD SITES

Renewal Application

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ABSTRACT:

The signature lipid biomarker technique based on phospholipid ester-linked fatty acid pattern analysis (PLFA) provides data on the total viable or potentially viable communities without the necessity of: 1) Quantitative recovery from the sediments or 2) The ability to culture the organisms. Analysis of PLFA provides evidence for the nutritional status (starvation and/or unbalanced growth) in situ. PLFA analysis of SSP samples from the INEL and PNL sites vadose zones showed higher biomass at the surface with prominent Actinomyces biomarkers with lower biomasses of stressed microbiota at progressively greater depth. The biomass and community diversity increased at the water table at both sites. Both these Western sites showed lower viable microbial biomasses than the WSRS samples. Cluster analysis of the total patterns from various sedimentary horizons showed three major consortia of microbes, with surface microbiota related at both sites, low viable biomass sites closely related at both sites, with anaerobic desaturase pathway being predominant at INEL and consortia utilizing predominantly branched saturated and the aerobic desaturase pathway at both sites. Preliminary examination of the consortia recovered from NTS show a clear relationship to water level. Ester-linked fatty acid analysis of isolates from the NTS tunnels show two major groups of organisms the saturated branched PLFA groups of Arthrobacter and Micrococcus and the anaerobic desaturase patterns of Pseudomonas and Acinetobacter. The major classification of isolated microbes from the SSP by PLFA was exactly parallel from a limited analysis of the SSP culture collection from WSRS and exactly paralleled the phylogenetic relationships between these strains based on 16S RNA. SLBT analysis of samples of opportunity from contaminated subsurface sites show distinct differences from uncontaminated subsurface sites. Correlations between SLBT patterns and geochemical parameters are currently a major focus. Development of new microtechniques for extraction to eliminate as far as possible chemical noise from contaminated solvents allowed increased sensitivity in this analysis to increase the signal to noise ratio sufficiently so that organisms could be detected in the dry vadose zones of the Western sites. The finding that specific gram organisms could be detected at very high sensitivities by analysis of the lipopolysaccharide lipid A hydroxy fatty acids (LPS-OHFA).

Research proposed will be to analyze ever smaller samples for quantitative measures of microbial distribution (of both community structure and nutritional status) in new samples such as GEMHEX, in specific microcosms set up by colleagues in the SSP and to continue the analysis of the data already collected in relationship to the geohydrochemical data that is concurrently being developed on the sample sites. New research in developing

new techniques for increasing the sensitivity of the PLFA analysis and in particular the analysis of LPS-OHFA.

INTRODUCTION

The extant microbial community in a subsurface sediment is the primary resource for manipulation to provide bioremediation of organic contaminants or bioimmobilization of inorganic contaminants. The ability to define the total microbial community without requiring quantitative recovery or induction of growth is a major asset. The definition of viability or potential viability by the recovery of polar lipids which are labile and not found in fossil microbes adds another dimension to the importance of these assays. The ability to determine nutritional status and to some extent the degree of stress based on signature biomarkers provides an <u>in situ</u> picture of the critical metabolic activity of the subsurface microbiota.

The PLFA analysis has been applied to isolates and shown clear differences between the composition of surface and subsurface communities. This will be an excellent screening technique to be applied to subsurface collections to define specific isolates that can be examined for nucleic acid sequence differences indicating differential mutation rates, differential survival technologies in repair of nucleic acids, in starvation responses, or in the enzymology of storage lipid (PHA) metabolism. Direct examination of isotope fractionation in these components can in some cases help identify the nutrient sources for the microbes.

Proposed research involves increasing the sensitivity and specificity of the SLBT. Increased sensitivity will allow smaller sample sizes. This will increase the resolution in examination of the distribution of microbes along gradients and in transition zones. Increasing the specificity involves examination of the lipopolysaccharide Lipid A hydroxy fatty acids (LPS OHFA) of gram-negative bacteria. This has been shown to increase the detection of particularly important physiological groups of organisms such as facultative hetrotrophic iron reducers, organisms capable of anaerobic dehalogneation, and others.

OBJECTIVES

Objectives of this research program derive from the objectives of the transition program plan and will be relevant to future studies examining the origins of the subsurface micobiota:

a. Characterization of the subsurface microbiota by PLFA analysis for viable and potentially viable (contain polar lipids) cells and collaboration to viable and direct counts. Correlate microbial fatty acid (MIDI) analysis of isolates recovered from

these samples with the in situ measurements and nucleic acid analyses and classifications.

b. Correlate the PLFA analysis with physical and chemical characterization of the samples.

c. Correlate the PLFA analyses with geohydrochemical controls.

d. Correlate with results from different sites.

e. Correlate with GEMHEX and other transition sites.

f. Use the SLBT/PLFA analyses and MIDI (microbial identification system--based on fatty acids) on isolates and in situ samples to correlate with other properties of the sedimentary horizons to define relationships with geochemical gradients and to give insight into community responses.

g. Increase the sensitivity and resolution of the SLBT by using micro-supercritical fluid extraction (SFE) techniques and derivatizations with electron-withdrawing agents for chemical ionization mass spectrometry with negative ion detection. To increase the resolution by using the SFE and derivatization techniques to analyze PHA and LPS OHFA. These increases will be utilized to better estimate distribution of subsurface microbiota.

SIGNIFICANCE

This program of in situ analysis of the viable and potentially viable microbiota, which does not require quantitative recovery or growth of the microbiota, relates directly to the goals of the Subsurface Science Deep Microbiology Program. This provides the information base on spatial and vertical presence, abundance, diversity, and metabolic traits of the subsurface microorganisms. The analyses provide independent confirmation of the lack of significant microbial contamination in the refinements of drilling and sampling procedures for uncompromised sampling. They also provide a means for comparing the different sites based on totally unbiased analysis of the viable microbiota at each site. Comparisons of in situ analyses from contaminated sites directly relate to the development of sediment/groundwater/microbial interactions in bioremediation potential predictions. This program has already identified some of the nutritional properties that characterize the consortia from the subsurface that are active in fortuitous metabolism of halogenated hydrocarbons.

EXPERIMENTAL AND TECHNICAL PROGRESS

The initial research program showed that the sedimentary horizons containing significant clay contents that were impermeable contained so few bacteria (10^2 to 10^4 bacteria/g dry weight) that totally new methods were necessary to detect them. The initial steps involved increasing the signal over the chemical noise from contaminated reagents. This involved a

micromethod in which the extractions were performed using 100 times smaller amounts of solvents (Tunlid et al. 1989).

The next technical innovations involved the inverse serial extractions on the microscale in which the same solvent is utilized with small portions of the lyophilized sediments in laminar flow hoods. This technology when combined with the cluster analysis allowed us to define the Savannah river samples and show the correlations between the permeability and the biomass and community structure of the in situ microbial community. This analysis also clearly documented the clear differences between the biomass and community structure of the surface microbiota and the extreme differences between the microbial community structure of the subsurface and surface sediments and samples of the drilling muds (White et al. 1991). SLBT technology was used to test the correspondence between the phylogenetic relationships in methane oxidizing bacteria and the phenotypic expression of their total fatty acid patterns. Previous work had shown that unique PLFA signatures could be defined for type I and II methylotrophs (Nichols et al. 1986) and that these microbes could be induced to greatly expand their biomass in subsurface soil columns exposed to methane (and propane) (Nichols et al. 1987, Ringelberg et al. 1988). These studies also showed these new communities were able to degrade trichloroethylene and numerous other halogenated hydrocarbons (Nichols et al. 1989). In this study we were able to show essentially complete concordance between the relationship as defined by the 16S RNA and that from the patterns of the PLFA for 17 distinct species from all over the world. This was despite the fact that the cultures were grown to maximum yield for RNA extraction. With the PLFA analysis it was possible to show that detailed knowledge of position of unsaturation and conformation of the monoenoic PLFA as well as the position of the hydroxyl, methyl branches, and cyclopropane rings was critical to the concordance (Guckert, J. B. et al., 1991).

Participation in the DOE/SSP Transition program allowed analysis of samples from the three Western sites. Results of preliminary analyses from the current Deep Subsurface Science program with the samples from INEL showed the presence of Actinomycetes at the surface (18% of the profile as 10 Me16:0, 10 Me18:0). At just above the water table the viable biomass increased with a community enriched in organisms with the anaerobic desaturase and aerobic desaturase. These sediments showed microorganisms with pink and blue pigments in their lipids. The horizons with pigments showed indicators of metabolic or nutritional stress in the high trans/cis monoenoic PLFA and cyclopropane/monoenoic PLFA. The deep samples, 560 and 592, showed high PLFA and were extremely wet compared to the other samples. These samples contained high proportions of aerobic and anaerobic desaturase type microbes and the metabolic/nutritional stress biomarkers. The 560 site was a

place of high activity with ^{14}C mineralization from acetate and glucose with recovered samples. The viable biomass from the total PLFA corresponded to the INEL water table diagrams (see Figure 1). Preliminary analyses of samples from PNL are illustrated in Figure 2. The surface patterns showed a higher biomass than at INEL and again a high content of Actinomycetes (8% of the total). Subsurface samples from 155 to 176 ft also showed the presence of the 10 methyl branched saturated PLFA that are characteristic of Actinomycetes. This is the first instance of detecting these obligate aerobes in the subsurface, in our experience. The water table level again showed increases in biomass with a community with high levels of the anaerobic desaturase PLFA and in the trans/cis monoenoic PLFA ratios (indicating toxicity). The sterilized sample showed PLFA with extremely high cyclopropane/monoenoic ratios indicating that the killing may have been incomplete and the organisms that survived starved for a long period of time. There could have been incomplete killing and regrowth. It could also be that the internal and external phospholipases, which we have shown rapidly degrade the polar lipids with cell death and loss of recoverability after irradiation, chemical sterilization, and autoclaved wet sediments were somehow rendered ineffective in autoclaving these desiccated samples. Samples and isolates from the Nevada Test site (NTS) were provided P. Amy. In general there was an excellent correlation between the extant biomass and the water content. In the wettest tunnel (G) the community showed a high ratio of cyclopropane/monoenoic PLFA (Figure 3). The wetter sample of the G tunnel showed the highest ratios of the anaerobic desaturase PLFA. The other tunnels showed very low biomass. Looking at the isolates from each site (Figure 4) those from the G tunnels are Pseudomonas by the PLFA and total ester-inked fatty acids (MIDI) which are characterized by high proportions of anaerobic desaturase PLFA. Tunnel P yielded a pink isolate characterized by the MIDI system as Micrococcus roseus which shows very little branched PLFA characteristic of these tough gram positive bacteria but high levels of aerobic desaturase fatty acids (18:1w9 cis and 18:2w6 and polyunsaturated PLFA) which are characteristic of protozoa.

Initial comparisons between the extant viable microbiota at the INEL and PNL sites have been compared by cluster analysis of the PLFA (Figure 5). Preliminary analysis shows that there are three major consortia of organisms. The surface organisms from both sites clearly group together as do the samples of extremely low biomass. The rest of the system group with the biochemical pathways of fatty acid synthesis with a cluster of bacteria with high utilization of the anaerobic desaturase pathway predominantly from INEL; a group of organisms forming primarily the branched saturated PLFA from both sites; and a group utilizing the aerobic desaturase pathway again from both sites.

Cluster analysis of the PLFA patterns of isolates from the NTS site provided by P. Amy show the great majority of the organisms cluster into two main groups: Arthrobacter and Pseudomonas (Figure 6). There are subsets in the gram-positive Arthrobacter group of some of the micrococci and in the broader group of gram-negative Pseudomonas there are Acinetobacters. With this relationship established the correlations between phenotypic relationships of the isolates and localization in specific horizons or positions in the blocks such as the N-cube are being determined.

Preliminary analysis of organisms from the deep subsurface culture collection (and one from an ORNL well) grouped into the same two major groups by their 16S RNA sequences. PLFA analysis using the detailed analysis structural analysis indicated by Guckert et al. 1991, shows essentially identical relationships (Figure 7).

In preliminary experiments to increase the sensitivity of the SLBT analysis electron withdrawing derivative were formed to generate molecular mass information after resolution by capillary gas chromatography with chemical ionization mass spectrometry of negative ions. In Figure 8 the transesterification by the traditional mild alkaline methanolysis which forms methyl esters and, is the gold standard of lipidology, is compared to the formation of the electron withdrawing pentafluorobenzyl derivatives. In this sample authentic phosphatidyl ethanolamine is esterified in the presence of mild alkaline methanol (PE-MAM), mild alkaline pentafluorobenzyl alcohol (PE-PFB), and strong acid pentafluorobenzoyl alcohol (PE-PFBol). The initial experiments show that the mild alkali with PFB is nearly as good as the MAM whereas the strong acid is clearly inferior. There is no differential effects between the different PLFA. It is just a matter of adjusting conditions before the mild alkaline PFB formation by transesterification can be put in practice. With single ion monitoring with CI/NI/MS this can increase the detection by at least 10^3. This could increase the detection limit to sub-femtomolar quantities or approximately 10^2 cells/sample.

Preliminary analysis of samples from contaminated subsurface sediments show distinct differences. Analyses of these samples shows the distinct presence of the endogenous lipid storage polymer poly beta hydroxy alkanoate (PHA) in sites with carbon rich contamination in moist environments. This has been seen previously in microcosms and field samples (Nichols et al. 1989). None of the pristine Western sites showed this polymer on preliminary analysis. In table 1 we show that analysis of the microbiota from a non-DOE site in California show an excellent correlation between the ability of the microbiota to degrade TCE and a high ratio between the PHA and the total PLFA for well MB1.

The data is less obvious for well MB2. These studies are continuing.

RESULTS OF THE SSP PROJECT REVIEW MAY 22, 1991

Ratings of this project were: Scientific Merit, 9.1; Importance of Project, 9.1; Quality of Team, 9.3; Scientific Approach, 9.1; Productivity, 8.9, Probability of Success, 9.2; Overall, 9.2.

HYPOTHESES

1. The viable biomass, community structure, and nutritional status of the subsurface microbiota recovered from the subsurface in the SSP will show changes that reflect relationships between the hydrogeochemistry and hydrogeophysics of specific sites as measured by PLFA.

2. The viable biomass, community structure, and nutritional status attributes of a specific subsurface community reflect the availablity and rates of delivery of nutrients, carbon sources, and electron donors/acceptors of the bulk phase groundwater in the specific sites.

3. Heterogeneities in the substratum sediment and in the availability of the bulk phase solution will be reflected in the viable biomass, community structure, and nutritional status of the microbiota determined by the PLFA.

4. Community composition reflected in the fatty acid patterns of isolates from subsurface sites will be reflected in the in situ analysis for viable biomass, community structure, and nutritional status by PLFA.

5. The insight and more sensitive methods developed in this program will contribute to understanding heterogeneities in microbial distribution and in helping to define the origins of the subsurface microbiota in future research programs.

GENERAL OBJECTIVE

To apply state-of-the-art non-selective methods for determining the microbial viable biomass, community structure, and nutritional status developed with SSP support to determine in greater detail the relationships between the subsurface microbiota and the hydrogeochemical and geophysical properties of the sediments and the chemistry and flow rates of the groundwater. To use the insight gained to contribute to future studies to help determine the origin of the microbes, and manipulations of consortia and isolates to show responses important in the potential for site cleanup and nuclide immobilization.

PROPOSED TASKS FOR THE THIRD YEAR 1992-1993

Objective a. Characterization of the subsurface microbial community by PLFA. With increased sensitivity and resolution (objective g) developed samples of opportunity from microcosms and experiments from other collaborating SSP investigators (Kleft, Amy, Brockman) can be examined to continue the correlation between the microbiota and the hydrogeochemical and geophysical properties of the specific sediments. The opportunity to analyze the new GEMHEX samples from PNL will be a major resource for these analyses and subsequent correlations. If the microbiota along gradients through which the groundwater supply comes show consistency the likelihood that the organisms arrived in the subsurface is higher than if they are highly diverse. The cluster analysis of patterns of PLFA from isolates with the MIDI system and by the PLFA will be correlated to nucleic acid phylogenetic and functional analyses to better define the metabolic potential and community response to the local environmental niche. The appropriateness of the responses could become an important consideration in future definition of the origin of the microbes.

Objectives b, c, d, and e. Research as in a.

Objective f. We will continue the analysis of the isolates as to localization in the sedimentary horizon and into the relationships between each other (as in Figure 5). We will continue to correlate the ester-linked fatty acid patterns with the 16S RNA and other nucleic acid patterns (as in Figure 7 and the Guckert et al. 1991 paper on the methylotrophic bacteria).

Objective g. With the increased sensitivity and resolution provided by research in objective g it will be possible to examine smaller samples. With smaller samples it will be possible to define in much more quantitative terms the effects of structural heterogeneity on the distribution of the microbiota. This will be particularly useful with the GEMHEX samples from PNL and the frozen archived subsurface samples.

Objective g. Research will be continued in increasing the sensitivity and resolution of the PLFA technology. We will continue to develop a satisfactory supercritical fluid extraction technology. We have shown SFE (with prior derivatization) can be a rapid and quantitative extraction method for bacteria, cyanophytes, algae, and yeast (White et al., 1991). Continued development is necessary to fractionate the lipids into neutral, glyco- and polar lipids prior to derivatization. The rational to develop SFE is that it is faster, semiautomatable, generates essentially no toxic waste, and most important has much less potential for contamination and the chemical noise that has been the primary problem in increasing the sensitivity of PLFA.

Objective g. Research will continue in the generation electron-withdrawing derivatives so that the higher sensitivity of chemical ionization mass spectrometry with negative ion detection can be utilized. Transesterification will be the method of choice as there is less chance of derivatizing contaminating free fatty acids with the gentle conditions utilized. This will be particularly useful with the hydroxy fatty acids with two functional groups to derivatize. The liberation of the hydroxy fatty acids from PHA will be a very important measure as it allows monitoring of the unbalanced growth. Presently our measures of PHA have detection limits three orders of magnitude above those of the PLFA and this has seriously hampered analysis of this critical molecule. Increasing sensitivity by electron withdrawing group derivatization and GC/CI/NI/MS analysis will allow us to revisit some of the archived lyophilized SSP samples with very sparse microbiota. The presence of hydroxy fatty acids in the lipopolysaccharide of gram-negative bacteria provides a special bonus as we have recently been able to capitalize on two features of the LPS molecule. LPS contains the Lipid A moiety that is covalently linked to the carbohydrate chain through the unusual eight carbon carbohydrate keto deoxyoctulonic acid (KDO). KDO is very sensitive to mild acetic acid hydrolysis. If the lipid-extracted residue is incubated in 0.1 M acetic acid at 100 C for several hours the KDO is ruptured and the Lipid A becomes extractable with organic solvents. If this extract is then subjected to strong acid alkolysis in an electron withdrawing alcohol such as pentafluorobenzyl alcohol, an electron active ester is formed. Since the fatty acids of the LPS are almost exclusively 2 and 3 hydroxylated this offers an additional site for derivatization by electron withdrawing agents and thus a much greater proportion of negative ions detected in the MS. This will greatly increase the sensitivity of the analysis without the terrible problems of subjecting tannin containing extracted soils to strong acid methanolysis with the resulting contamination with a bewildering array of alcohols. The same advantages of two derivatization sites on the molecule also subtends with the monomer of the PHA molecule that we have shown is one of the critical components in the biodegradation of mixed organic waste which includes halogenated volatile hydrocarbons (Nichols et al., 1987; Ringelberg et al., 1989).

RELATIONSHIP TO OTHER PROJECTS

Our primary collaborations have been with SSP investigators in Biodegradation/Microbial Physiology and Deep Microbiology subprograms. Balkwill, Kleft, Fredrickson, Colwell, Phelps, Palumbo, Ghiorse, and Amy have provided isolates and in some cases special samples for analysis. We are awaiting the GEMEX samples and some other samples from gradients and interfaces from Long and Murphy as well as geohydrochemical data on specific samples so we can continue the correlative statistical analysis.

We will be anxious to examine anaerobic isolates and consortia provided by Phelps, Stevens and Balkwill as well as the genetic analyses of Balkwill and Nierzwicki-Bauer for correlations similar to those of Figure 7. With samples from bioreactors and other treatment facilities we expect to continue some efforts on analysis of shifts in microbial community structure and nutritional status that correlate with effectiveness in subsurface bioremediation of mixed organic wastes.

In a related collaborative program with ESD/ORNL we will utilize some of the techniques developed in this program to compare SLBT analysis periphyton from contaminated and pristine sites in surface streams. We will catalog the SLBT patterns from the in situ subsurface analyses for the eventual use in quantitative subsurface toxicity assessment. Presently we are collaborating on a project utilizing patterns from classes of lipids recovered in situ from periphyton which accumulates on artificial unglazed stones incubated in pristine and contaminated surface streams in which the contamination is monitored. The toxicity is monitored by standard toxicity tests in these systems (Pimphales minnow, Cerodaphnia, etc.) and these changes are correlated with shifts in the community structure and nutritional status of the periphyton. The changes are then modeled in the artificial streams. We feel that the pattern analysis of the PLFA of the microbiota in the subsurface can be utilized in the same way to provide toxicity assessments as well as the effectiveness of transformations of the toxicants.

NEW SCIENCE

The new science will result insights gained from the application of the PLFA with increased sensitivity to new samples such as those from GEMHEX. The development of more sensitive detection and better resolution by more effective application of PHA and LPS OHFA will give better resolution to the community structure and nutritional status determinations. Coordination between the in situ PLFA analysis and postulated origins of the subsurface microbiota will provide strong supporting evidence.

COORDINATION

With the exception of the research involved with improving the sensitivity and resolution of the PLFA our program depends on samples from collaborating investigators in the SSP. We have been successful in the past and will continue to develop and continue active collaborations.

BUDGET JUSTIFICATION

Personnel: The Co-PI's D. C. White and D. B. Ringelberg, have coordinated analysis of PLFA of the microbiota in Phase I and the earlier parts of the transition phase of the SSP.

Materials and Supplies: The ultrapure solvents, SFE gases, reagents, capillary columns are expensive. It is anticipated that 20% of the operating time and maintenance contract for the VG Trio-3 MS/MS will be supported by this research ($5,000).

FACILITIES

The Center operates from 15,000 sq ft. of laboratory space in the Pellissippi Parkway laboratories of the University of Tennessee at Knoxville. Walk-in cold rooms, laminar flow hoods, autoclaves, a dark room, refrigerated centrifuges, liquid scintillation counters, ^{14}C- combustion analyzer, lyophilizers, flash evaporators, freezers are available. HPLC gradient apparatus with fluorescence detector, UV-Vis (diode array), electrometric, and conductivity (for ion chromatography), and radiochemical detectors are available. Supercritical fluid (SFC) extraction devices and capillary SFC chromatograph are available. The laboratory contains 12 capillary gas chromatographs, 4 dedicated to on-line reactor monitoring. Analytical GC's are equipped with autosamplers, P.I.D. and Hall detectors for chlorinated hydrocarbons as well as a dual oven dual column capillary gas chromatograph, and 5 packed column gas chromatographs and 3 with purge and trap accessories. The data from these systems is currently fed to a Nelson analytical computer system utilizing an IBM compatible-AT computer network as a laboratory data system. A Hewlett Packard bench-top GC/MS and the VG Trio-3 triple quadrupole mass spectrometer, mass range of 1 to 3000 amu, with inlets for GC, thermospray/plasmaspray LC, FAB and dynamic FAB LC, desorption chemical ionization probe, and supercritical fluid chromatography inlets are available. A Nicolet 60SX Fourier Transforming infrared spectrometer (FT/IR) and a custom designed Laser-Precision ATR-FT/IR with an Analect IR Microscope for DRIFT are available. A surface enhanced solid-phase Raman spectrometer (SERS) constructed by T. L. Ferrell and R. J. Warmack of Oak Ridge National Laboratories is in the laboratory and allows non-destructive ultrasensitive analysis of Raman-active analytes. The center contains excellent facilities for the culture of organisms with laminar flow hoods, airlift, CSTR, and differential volume bioreactors some fitted with fiberoptic light sensors. These have automated temperature, Eh, and Ph control as well as computer generated input lines for substrate-response experimentation. Continuous culture apparatus for flow controlled coupon exposure are in operation. A 40l fermenter with harvesting centrifuges, walk in incubators at various temperatures, Fowler cell adhesion measurement modules, an DC potentiostats, two electrochemical impedance spectrometer systems, quartz crystal microbalance non-destructive biofouling detector, and electrochemical noise detectors with spectral analysis with Hewlett Packard and IBM-PC type computer control together with other electrochemcal sensing systems are utilized in on-line, non-destructive monitoring of microbial biofilm activities. A Perkin-Elmer-Cetus PCR, Oriel light sensors, BTA

electroproator, bioimaging analyzer for gels, pulsed field electrophoresis, radioscan analyzer for TLC plates, and various ultracentrifuges are available.

PERSONNEL

CO-PI'S:

David Cleaveland White

[redacted]

Education: Dartmouth College, A.B., Magna cum laude, Phi Beta Kappa, 1951.
Tufts University School of Medicine, M.D., 1955
Rockefeller University, Ph.D., 1962.
Internship Hospital University of Pennsylvania, 1955-1956.

Military Service: Lt. MC USNR., NAS Johnsville, Pennsylvania, 1956-1958, Aviation Medical Acceleration Laboratory, Naval Air Development Center, research in aviation medicine.

Academic Service: Assistant Professor to Professor of Biochemistry, University of Kentucky Medical Center, 1962-1972.
Professor, Department of Biological Sciences, Florida State University, 1973-1985; Associate Director Program in Medical Sciences, FSU, 1973-1984.
Founder and Director, Institute for Applied Microbiology, University of Tennessee/Oak Ridge National Laboratory, University of Tennessee, Knoxville/Oak Ridge National Laboratory Distinguished Scientist 1986-,
Professor of Microbiology/Ecology, University of Tennessee, Knoxville, 1986-,
Research Scientist, Oak Ridge National Laboratory, Environmental Science Division, 1986-
Principal Investigator, ORNL Environmental Biotechnology Program, 1990-.

Honors: P. R. Edwards Award, Outstanding Microbiologist South-Eastern Branch, American Society for Microbiology, 1981; Wellcome Visiting Professor-American Society for Microbiology and the Burroughs Wellcome Fund for 1984-1985, to the University of Oklahoma; Fellow American Academy Microbiology, 1978-1984; Foundation Speaker, American Society for Microbiology, 1971, 1978, 1979, 1985; Advisory Committee, Center for Theological Inquiry, Princeton University 1984-; Antarctic Service Medal, 1984, Scientific and Technological Achievement Award USEPA 1987; Scientific Advisory Panel, Michigan State University Center for Microbial Ecology, 1989-; Certificate of Appreciation, Deep Microbiology Program, Department of Energy, 1986-1989.
Reviewed Publications: Of 300 as of October 1991:

David B. Ringelberg
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EDUCATION

B.S. (Biology, Chemistry) Florida State University, 1984

WORK EXPERIENCE

Lab Technician - Olin Corporation 2/85-4/85
Research Assistant - Florida State University 4/85-2/86
Biological Scientist I - Florida State University 2/86-9/86
Research Associate - The University of Tennessee Knoxville, Institute for Applied Microbiology 1986- Management of operations analytical chemistry, Operatiion and maintenance of HP 5995 and VG Trio-3 mass spectrometers. Preparation of final reports describing analyses and results. Supervision and training of personnel.

AWARDS

Antarctic Service Medal for Service in the Antar[illegible]ic - 1989

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CURRENT AND PENDING SUPPORT

Current Support:

1. Electric Power Research Institute. Genetic Ecology of Biofilms and Microbially Induced Corrosion. 06/01/90 - 12/31/94, annual budget to UTK laboratories $205,000. This proposal between three laboratories will focus genetic probes and spectroelectrochemical analysis on biofilm formation and stability as it relates to MIC.

2. Office of Naval Research. Monitoring Biofilm Ecology with Bioluminescent Bacteria 04/01/91-03/31/94 $231,000/year for a total grant $691,000 for 3 years. Grant is to develop and utilize bioluminescent bacteria to test non-polluting antifouling paints in marine systems for both microbial colonization effectiveness and sub-lethal toxicity with genetically engineered bacteria. The ecology of the naturally bioluminescent bacteria will also be studied using methods derived from the EPRI System.

3. Department of Energy. On-line monitoring of aerobic bioremediation with bioluminescent probes.
three years $575,000, initiated 07/30/91. This grant supports the development of bioluminescent sensors for key parameters in bioremediation of mixed types of organic wastes. The biosensors will be consortia of bacteria including genetically engineered bacteria that will be sensed with fiber optic conduits in systems isolated from the community and confined to prevent escape.

4. Office of Naval Research. Joint Program on the Molecular Biology of Marine Organisms. Office of Naval Research and Defense Advance Research Products Agency N00014-87-K00012. The UTK portion is $125,000, 10/01/90-03/31/92. This project supported the development of the non-destructive, on-line monitoring of biofilm development and succession.

5. Department of Energy. Biomass, Community Structure, Nutritional Status and Specific Metabolic Attributes of the Deep Subsurface Microbiota. Department of Energy DE-FG05-90ER60988. $180,000 ($90,000 yearly) 06/01/90-05/31/93. This research program involves <u>in situ</u> analysis of fatty acids from deep subsurface samples, isolates from the deep subsurface, and communities from mixed organic toxicant bioreactors with the signature lipid biomarker techniques developed in this laboratory. Proposed for renewal in this application.

7. NASA. Rapid Monitoring of Microbial Contamination in Water-Reclamation Systems of the Space Station NASA. $120,000 09/24/90-10/24/92. This is a project to develop continue development and implementation of on-line biofouling monitors for ultra pure water systems.

PENDING SUPPORT

Pending research proposals:

1. National Science Foundation, proposal DCB-9118636 "Archaebacterial membrane ether lipids: metabolic turnover and environmental adaptations", 01/01/92-12/31/95, $150,000, ($50,000 first year). This grant would support graduate studies to utilize the techniques developed for ether lipids to examine the physiology of ether lipid metabolism in various Archaebacteria.

2. Office of Naval Research. Supplemental Proposal-Graduate Students "Supercritical fluid extraction-mass spectral analysis for rapid determination of biofouling community density and structure" 06/01/92-05/31/95 $107,627 ($47,041 first year). This is a proposal to support D. B. Hedrick to use develop supercritical fluid extraction/mass spectral analysis for samples from biofouling community studies.

3. Department of Energy. Biomass, Community Structure, Nutritional Status--relation to geochemical gradients and community responses. 06/01/92-05/31/95, $276,000 ($92,000 first year), continuation of the research supported in # 6 above with emphasis on in situ analysis along gradients and appropriateness of communities to the extant environment. The continuation of the application of this technology to SSP problems.

4. Savannah River Research Institute, through Oak Ridge National Laboratory. Research relative to the OTD Integrated Demonstration in in situ Methane Induced Biodegradation of Trichloroethylene. 12/15/91-06/30/92. This is a grant to study the effects of air and methane + air injection on the subsurface microbiota in the integrated demonstration grant at WSRS and to test resilience of microcosms made with consortia of subsurface microbes degrading trichloroethylene and other organic wastes.

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