COVER SHEET FOR PROPOSAL TO THE NATIONAL SCIENCE FOUNDATION

PROGRAM ANNOUNCEMENT/SOLICITATION NO./CLOSING DATE/if not in response to a program announcement/solicitation enter NSF 04-2

NSF 04-2

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TITLE OF PROPOSED PROJECT **Characterizing Microbial Activity and Diversity in Hawaiian Basalt Aquifers**

REQUESTED AMOUNT	PROPOSED DURATION (1-60 MONTHS)	REQUESTED STARTING DATE	SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE
$ **287,682**	**24** months		

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☐ HUMAN SUBJECTS (GPG II.D.6)
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CERTIFICATION PAGE

Certification for Authorized Organizational Representative or Individual Applicant:

By signing and submitting this proposal, the individual applicant or the authorized official of the applicant institution is: (1) certifying that statements made herein are true and complete to the best of his/her knowledge; and (2) agreeing to accept the obligation to comply with NSF award terms and conditions if an award is made as a result of this application. Further, the applicant is hereby providing certifications regarding debarment and suspension, drug-free workplace, and lobbying activities (see below), as set forth in Grant Proposal Guide (GPG), NSF 04-2. Willful provision of false information in this application and its supporting documents or in reports required under an ensuing award is a criminal offense (U. S. Code, Title 18, Section 1001).

In addition, if the applicant institution employs more than fifty persons, the authorized official of the applicant institution is certifying that the institution has implemented a written and enforced conflict of interest policy that is consistent with the provisions of Grant Policy Manual Section 510; that to the best of his/her knowledge, all financial disclosures required by that conflict of interest policy have been made; and that all identified conflicts of interest will have been satisfactorily managed, reduced or eliminated prior to the institution's expenditure of any funds under the award, in accordance with the institution's conflict of interest policy. Conflicts which cannot be satisfactorily managed, reduced or eliminated must be disclosed to NSF.

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By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant is providing the Drug Free Work Place Certification contained in Appendix C of the Grant Proposal Guide.

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This certification is required for an award of a Federal contract, grant, or cooperative agreement exceeding $100,000 and for an award of a Federal loan or a commitment providing for the United States to insure or guarantee a loan exceeding $150,000.

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The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

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(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements and that all subrecipients shall certify and disclose accordingly.

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Project Summary

Intellectual Merit

Microorganisms are a major part of our biosphere, and recent discoveries indicate that microbes beneath the Earth's surface make up as much as half of all living material on Earth. One habitat for subsurface microorganisms is the volcanic rock that forms the ocean basins. These rocks cover at least fifty percent of the Earth's surface and are the most abundant rock types on Earth. DNA extracted from deep sea rocks and other evidence proves that the deep ocean rocks contain indigenous microbial communities. The volcanic rocks of the ocean basins are therefore the largest habitat on Earth. Major questions about the extent, diversity and activity of microbial communities in the subsurface biosphere, and how they impact the surface biosphere, are yet to be answered.

The goal of this proposal is to conduct field experiments at a site that shares many features with the volcanic rocks present in deep ocean basins in order to determine (1) the phylogenetic diversity in a basalt hosted aquifer similar to aquifers in the ocean crust, (2) the amount of microbial activity than can be stimulated in the aquifer, and (3) if and how the microbial diversity changes in response to the stimulation. These experiments are in preparation for an experiment to measure the diversity and activity of microbial communities in deep ocean volcanic rocks that has been proposed to the Integrated Ocean Drilling Program (IODP) as part of a drilling leg in the northeast Pacific Ocean. The experiment to be conducted by IODP will be a major step in our understanding of the impact of indigenous microbial communities in the subsurface biosphere on our surface biosphere, and the successful completion of the IODP experiment relies on experiments such as those in this proposal.

Broader Impacts

This project has equal components of discovery and training and learning. The work will be the primary focus of a Ph.D. studentís dissertation research, and it also will provide two years of training and research experience for undergraduate students during the academic year, and two summers of research experience for students from underrepresented groups. The students from underrepresented groups will be recruited through the Society for the Advancement of Chicanos and Native Americans and the research and training programs Haumana and MARC U*STAR of the University of Hawaii. These programs are designed to provide hands on research experience to academically qualified students whose ethnic identity is in whole or in part Hawaiian, Filipino, Samoan, and American Pacific Islander.

The work proposed here will require the development of new experimental protocols. These will be presented in widely distributed scientific publications. The new protocols will aid in the understanding of the Earth, but also they may be useful for learning how to remove nitrate from basalt aquifers which is a major environmental problem in some areas. The project is interdisciplinary in that it brings together the fields of hydrology, microbiology, and geology.

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Facilities, Equipment and Other Resources	2	
Special Information/Supplementary Documentation	0	
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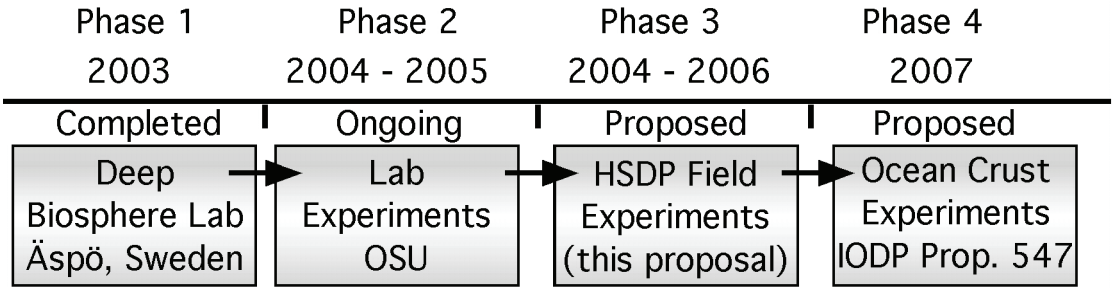
*Proposers may select any numbering mechanism for the proposal. The entire proposal however, must be paginated. Complete both columns only if the proposal is numbered consecutively.

Project Description

INTRODUCTION

Ten years ago, the microbiology of the Earthís subsurface received very little consideration from the scientific community. Today, it is an emerging field and the subsurface microbial community is thought to comprise a significant fraction of the Earthís total biology. The ocean crust is now recognized as a major microbial habitat and the "Deep Biosphere" is one initiative of the Integrated Ocean Drilling Program (IODP). In Figure 1, we depict our step-by-step approach to characterize microbial activity and diversity in basalt-hosted aquifers in the ocean crust. The culminating experiment will be conducted aboard a non-riser drilling ship and one such experiment is a part of the goals described in IODP Proposal 547 which is under consideration. The initial steps of our approach have been completed or are underway as indicated in Figure 1. This proposal describes and requests support for a field experiment in aquifers in terrestrial basalts in the Hawaii Scientific Drilling Program (HSDP) site near Hilo, Hawaii. where we will develop techniques and test the assays that have been proposed for the IODP Leg.

Figure 1. Research Plan Leading to IODP Drilling Leg.



BACKGROUND

Many investigations report evidence of a deep microbial community in the ocean crust. The evidence includes textures of the basalt glass alteration (Thorseth et al., 1995 and 2003; Furnes et al., 1999, 1996 and 2001a; Fisk et al., 1998; Furnes and Staudigel, 1999), isotopic evidence of endolithic carbon fixation and sulfate reduction (Furnes et al., 2001b; Alt et al., 2003) and molecular investigations of Bacteria and Archaea (Fisk et al., 2001; Huber et al., 2002; Cowen et al., 2003; Edwards et al., 2003, Savoie et al., 2004). Despite this evidence, confirmation of the viability, activity, and magnitude of this community is elusive due to the challenges of drilling and making *in situ* measurements. One way to investigate this biosphere is to stimulate microorganisms and measure metabolic reactions *in situ*. To accomplish this, we propose to use a technology that has been developed at Oregon State University, known as a "push-pull" test, especially suited for this type of experiment.

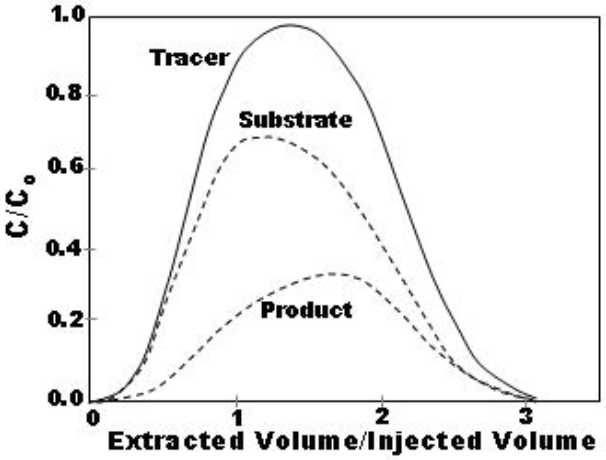
Push-Pull Tests:

Push-pull tests are used to stimulate *in situ* microbial communities. A test solution is first injected (ì pushedî) into an existing well or borehole; samples of the test solution/ground water mixture are then sampled (ì pulledî) from the same location. The test solution is prepared from ground water extracted from the aquifer to be studied and which has been amended with known concentrations of two components: a conservative tracer, such as bromide, and one or more substrates that can be metabolized by microorganisms. These may include potential electron acceptors (such as oxygen, nitrate, or sulfate) and/or potential electron donors (such as hydrogen, hydrogen sulfide, or an organic compound); the specific combination of substrates is selected to probe for specific metabolic processes. After injection, the test solution resides in the aquifer with no pumping during an incubation phase. The duration of the incubation phase is selected based on the expected rate of metabolism and the local hydrologic conditions. During the incubation phase, indigenous microorganisms consume substrates and produce metabolic products at rates directly proportional to their overall potential metabolic activity. After the incubation phase, the test solution/ground water mixture is pumped from the well and samples are

collected and analyzed for the injected tracer and substrates and for diagnostic metabolic products formed *in situ* (Istok et al., 1997). An analysis of the concentration data allows calculation of the rate of in situ substrate utilization or product formation (Figure 2).

Figure 2. Push-pull test data analysis.

C/C_o is the ratio of concentration of a component in the extracted water to the concentration in the injected water. These values start at 0.0 because the test solution is pushed into the formation with unamended ground water. The time series of concentrations of tracer, reactant, and product are plotted as the amount of water extracted from the well. This volume extracted is usually several times the injected volume. The time the reactant remains in the well can be increased if metabolic rates are low. Total reactant consumed and product produced per liter per hour can be determined from these measurements.



These tests have previously only been applied in terrestrial settings with applications to bioremediation of contaminated ground water (e.g., Istok et al., 1997; Reinhard et al., 1997; Schroth et al., 2001; Kleikemper et al., 2002; McGuire et al., 2002). However, some of these results suggest the potential application of this method in pristine environments. For example, a single push-pull test conducted in an uncontaminated control well in basalt gravel determined an *in situ* rate of nitrate reduction of 10 µM/hr (Istok et al., 1997). We propose to build on these results by conducting larger scale experiments to investigate microbial metabolism in relatively pristine fractured rock aquifers.

We have already (Fig. 1 Phase 1) applied a modified version of the push-pull method to a deep, saline, granite-hosted aquifer in *f* spˆ , Sweden and measured the microbial reduction of nitrate and the oxidation of lactate . Presently, laboratory experiments are underway in basalt sand to test the sensitivity of our measurements for detecting metabolic activity in basalt aquifers (Phase 2). Here (Phase 3) we propose to test our methods in deep aquifers in the existing HSDP site near Hilo, Hawaii. This hole intersects saline aquifers that are similar to conditions we will encounter in the ocean crust. After laboratory and field testing, we propose to conduct push-pull tests beneath the seafloor using a borehole drilled on an IODP leg to investigate the deep biosphere (Phase 4, Proposal). If successful, these tests will provide the first direct measurements of microbial metabolic activity in this environment.

HYPOTHESIS

The hypotheses we wish to test with these experiments are:

ï There is an active microbial community in basalt aquifers that can be stimulated by supplying it with exogenous electron acceptors or donors. Microbial activity can be quantified by measuring the rates of electron acceptor or electron donor utilization and the formation of metabolic products, and can be assigned to particular phylogenetic groups by correlation with changes in microbial community structure.

ï The microbial community that is stimulated is the same as the indigenous microbial community.

Based on an analysis of existing microbial community data and on the results of push-pull tests conducted in a wide range of terrestrial environments, we believe we will have success in detecting and quantifying rates of nitrate, iron, and sulfate reduction and acetate oxidation. To confirm and support our rate measurements, we will also monitor the effect of electron acceptor and electron donor additions on the structure of the microbial community, and compare communities before and after stimulation. As in any

enrichment culture, the microbes that grow are those best adapted to the culture conditions. The method chosen has the advantage of using water extracted from the aquifer which will have indigenous organisms and will be close to the in situ aquifer chemistry, and the experiments will be run at *in situ* pressure, temperature, and in the rock matrix from which the microorganisms were extracted. Duplicating these conditions in the lab experiments is extremely difficult and the experiments will be subject to questions about exact duplication of subsurface conditions.

POTENTIAL METABOLIC PROCESSES IN THE BASALT HOSTED AQUIFERS

Bach and Edwards (2003) identified likely types of redox metabolism in oceanic crust. They calculated the possible biomass production (g of carbon/yr) for each redox reaction based on a set of thermodynamic and bioenergetic assumptions (Table 1). Combined, they found that the reactions may produce 10^{12} g/yr of organic carbon. The reactions they considered are listed in order of decreasing potential production:

Table 1. Potential carbon production in the igneous ocean crust.

Process	**Reaction**	Potential Production (10^{10} g C/yr)
Nitrate Reduction	$NO_3^- + 4H_2(aq) + H^+ \rightarrow NH_4^+ + 3H_2O$	73 ± 56
Iron Reduction	$Fe^{3+} + 0.5H_2(aq) \rightarrow Fe^{2+} + H^+$	39 ± 30
Aerobic Sulfide Oxidation	$HS^- + 2O_2(aq) \rightarrow SO_4^{2-} + H^+$	20 ± 15
Aerobic Iron(II) Oxidation	$Fe^{2+} + 0.25O_2(aq) + H^+ \rightarrow Fe^{3+} + 0.5H_2O$	16 ± 11
Sulfate Reduction	$SO_4^{2-} + H^+ + 4H_2(aq) \rightarrow HS^- + 4H_2O$	9.2 ± 7.1
Anaerobic Sulfide Oxidation	$5HS^- + 8NO_3^- + 3H^+ \rightarrow SO_4^{2-} + N_2 + 4H_2O$	8.8 ± 6.5
Anaerobic Iron (II) Oxidation	$5Fe^{2+} + NO_3^- + 6H^+ \rightarrow 5Fe^{3+} + 0.5N_2(aq) + 3H_2O$	3.5 ± 2.8
Methanogenesis	$HCO_3^- + H^+ + 4H_2(aq) \rightarrow CH_4(aq) + 3H_2O$	2.9 ± 2.2

(Modified from Bach and Edwards, 2003)

The reactions presented in Table 1 are linked to oxidation of basalt and Bach and Edwards provide evidence that oxidation of basaltic ocean crust occurs mainly over the first 10 to 20 million years of crustal formation. Accordingly, the capacity of the crust to support a chemolithoautotrophic microbial community might change as a function of time on a geologic scale. Note that the calculations of potential production for nitrate reduction, iron reduction, sulfate reduction and methanogenesis are all based on hydrogen as the electron donor. In reality, the amount of hydrogen available for metabolism in the ocean crust is poorly constrained and it is unknown whether hydrogen or reduced organic carbon acts as the primary electron donor in this system (Bach and Edwards, 2003). One model of the subsurface biosphere suggests that acetogens use hydrogen to fix CO_2 and produce acetate. In turn, this acetate may serve as the energy source for a community of heterotrophic sulfate and iron reducers (Figure 3) (Pedersen, 2000).

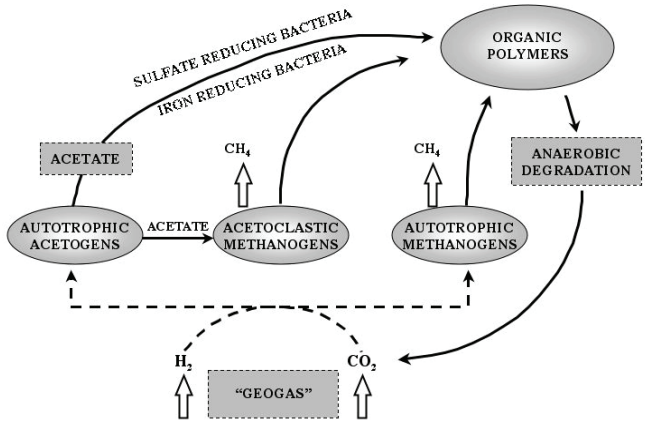


Figure 3. Culturing efforts have revealed iron- and sulfate-reducing bacteria, acetogens and methanogens to be common in aquifers hosted in igneous rocks. Hydrogen, methane and carbon dioxide have been found in μM concentrations at numerous locations. These investigations led to the suggested model of a hydrogen driven biosphere shown here. All components needed for this life cycle have been found in deep igneous rock aquifers and the expected microbial activities have been demonstrated in laboratory incubations, so the model is supported by the qualitative data obtained so far (Pedersen, 2000).

Hydrogen has been used as an electron donor in push-pull tests (Istok et al., 1997) but we propose to use acetate for our experiments. Volumes of hydrogen on the scale that we require present safety problems and there may also be problems with changes in solubility when we apply these methods to ocean settings and pump fluids into high-pressure environments. Acetate is essentially one step up the proposed microbial ecosystem (Figure 3) and may play a large role in overall subsurface activity. This makes it an appropriate choice to use as an electron donor for these experiments.

Possible Reactions to Measure:

It is logical to investigate the most energetic reactions in Table 1 which, in the absence of oxygen, are nitrate reduction, Fe(III) reduction, and sulfate reduction. Nitrate is a common ion in deep ocean water due to the remineralization of organic carbon (which releases nutrients); Fe(III) is available, making up about 1% by weight of basalt; and sulfate is the second most abundant anion in sea water. Some of these reactions have inherent challenges. For example: iron reduction could be difficult to measure using traditional push-pull methods because the insolubility of ferric iron makes it difficult to introduce into the aquifer. An alternative is to rely on the iron present in the basalt formation and testing for Fe(II) as a product. Based on these considerations, we have narrowed our investigation to test for three possible microbially-mediated reactions in the oceans: nitrate reduction, iron reduction, and sulfate reduction by measuring rates of utilization of introduced electron acceptors and the rates of production of metabolic products.

We realize that, with this approach, we may not determine the activity of the full microbial population of the ocean crust. That is, we will determine activity for only that part of the microbial population that can reduce nitrate, sulfate, or ferric iron. However, as the existence of the potentially large microbial biomass in igneous rocks has been recognized only recently, these first measurements would represent a significant advance towards understanding microbial activity of the ocean crust.

Nitrate Reduction:

After oxygen, inorganic nitrogen compounds (mostly nitrate) are some of the most commonly used terminal electron acceptors in microbial communities (Madigan et al., 2003). Nitrate reduction is a process that is capable of producing a large amount of biomass in the deep subsurface (Bach and Edwards, 2003). Nitrate can be reduced to nitrogen gas through a series of steps (denitrification) or it can be reduced to ammonia. The latter form of nitrate reduction is thought not to be globally significant (Madigan et al., 2003) but previous laboratory incubation studies suggest that it may be as important as denitrification in marine sediments (S¯renson, 1978).

Two investigations of chemical characteristics of crustal fluid appear to corroborate that nitrate reduction to ammonia occurs in the ocean crust. Cowen et al., (2003) sampled water emanating from an overpressured borehole (ODP 1026B). The concentration of ammonia in the crustal fluid was 142 times higher than in bottom seawater at the same location. This evidence, coupled with their findings of ribosomal RNA sequences related to unique nitrate reducing bacteria, suggests an active subsurface community. Wheat and Mottl (2000) present similar observations of nitrate and ammonia from a study site on the Juan de Fuca Plate. They sampled water from warm springs on a seamount (Baby Bare) and found that ammonia concentrations were ~70 µmol/kg and that the nitrate concentration was ~1 µmol/kg. Bottom seawater at a nearby seamount (Grizzly Bare) had concentrations of ammonia and nitrate of 0.3 µmol/kg and 39.2 µmol/kg, respectively. Both of these studies demonstrate the strong likelihood that nitrate reduction is an ongoing process, but existing data is insufficient to determine the rates of either nitrate utilization or ammonia production. Our experiments are specifically designed to address this issue, and link this activity to particular groups of microorganisms.

Fe(III) Reduction:

Average midocean ridge basalts (MORBs) have a composition that is 8 wt% total iron. Approximately 15 percent of this iron is Fe(III) in fresh basalt (Christie et al., 1986). The percentage of this iron that is Fe(III) changes as the fresh basalt becomes oxidized. Bach and Edwards (2003) describe a model for

basalt oxidation in which the proportion of iron that is Fe(III) rises from 15 to 45 percent over the first 10 to 20 million years of oxidation. Accordingly, when oxidized crust is buried by sediment and the circulating fluid becomes anoxic, Fe(III) may constitute ~3.6% of basalt by weight. Further, of this 3.6% ~2.4% is a product of oxidation and is likely deposited on surfaces accessible to microbes. In anaerobic systems, Fe(III) is a known electron acceptor used by microbial communities which can reduce iron using hydrogen, simple organic acids, long chain fatty acids and monoaromatic compounds as electron donors (Lovely and Chappelle, 1995 and references therein). As shown in Table 1, Bach and Edwards (2003) calculate that this type of iron reduction (by hydrogen) in the crust may be responsible for the production of 39 x 10^{10} g C/yr in ridge flanks. However, the concentrations of Fe(II) have not been reported for any ridge flank fluids, so we are not certain what to expect. We have instrumentation available that can measure Fe in water at a detection limit of approximately 100 parts per trillion so we expect to be able to measure small changes in dissolved iron. Accordingly, we propose to measure concentrations of Fe(II) in the extraction samples of our experiments. Detecting an increase in concentration of Fe(II) in response to addition of an exogenous electron donor will be strong evidence of microbial activity and we can determine a rate for this process.

Sulfate Reduction:

Sulfate is one of the principle components of sea water with concentrations on the order of 29 mM in standard ocean water (Pilson, 1998). Due to less favorable energetics, organisms using sulfate as an electron acceptor have smaller growth yields than those using oxygen or nitrate. However, due to the large availability of sulfate in marine systems, sulfate reduction may play a significant role in microbial biomass production.

Indirect evidence that sulfate reduction occurs in the ocean crust is provided by isotopic data. Biological sulfate reduction has been documented to cause fractionation of stable sulfur isotopes (Canfield, 2001). Rudnicki et al., (2001) presented evidence that a drop in sulfate concentration in crustal fluid (relative to bottom sea water) is accompanied by an upward shift in ∂^{34}S, suggesting that observed sulfate loss is due to microbial sulfate reduction. Other evidence includes isotopically heavy anhydrite observed in Ocean Drilling Program (ODP) Hole 504B (Hubberten, 1983; Alt et al., 1985), and the recovery of ribosomal RNA sequences related to a diverse array of sulfate reducing bacteria from crustal fluid (ODP 1026B), suggesting an active community of sulfate reducers (Cowen et al., 2003). It is impossible to infer rates from isotopic evidence but it is likely that the rate of sulfate reduction in the crust is very low (D'Hondt et al., 2002). As will be discussed later, the detection limit for push-pull tests depends on the length of the test and the detection limit of the analytical instrument. The length of the incubation phases and concentrations of amendments will be optimized to maximize the sensitivity of our assays.

COMPLETED, UNDERWAY, AND PLANNED WORK

As mentioned in the Introduction (Figure 1), we have sequence of experiments starting with a small scale (2-liter) system in a natural granite aquifer, then progressing to a 100-liter laboratory experiment in a model basalt aquifer, followed by a several hundred- to thousand-liter *in situ* field experiment. The small-scale experiments in the granitic aquifer in Sweden the laboratory experiments that are underway, and field experiment proposed here are described in this section.

Completed: Deep Granitic Aquifer *f* spˆ Hard Rock Laboratory (HRL):

The first experiments were completed at the *f* spˆ Hardrock Laboratory (HRL), Sweden in August and September The HRL is located on the Baltic coast, approximately 400 km south of Stockholm. The HRL consists of a 5-meter-wide tunnel into the granite bedrock of the Fennoscandian shield and is designed for a variety of research and engineering experiments related to the long-term storage of nuclear waste. An ongoing area of research is the role of microorganisms in modifying the geochemistry and redox conditions of the ground water. The ground water at this site is a mixture from marine, meteoric, and glacial inputs, and is characterized by low amounts of organic carbon (~1.4 mg/l) and a low abundance of microorganisms (~2300 cells/ml) unpublished data).

In order to facilitate the study of microorganisms *in situ*, the "MICROBE" site was established in the HRL at a depth of 450 m. Boreholes that penetrate fractures tens of meters from the sidewall of the HRL tunnel are fitted with circulation systems to allow ground water to pass through temperature-controlled, pressurized, flow cells. The flow cells contain glass slides with a total surface area of 500 cm^2 for the growth of biofilm. The flow cells and slides are installed in sterile conditions and the biofilms that form within/upon them are the result of colonization by organisms indigenous to the subsurface environment.

Experiments were conducted based on the push-pull model to measure the activity of the biofilm in the flow cells under *in situ* conditions. Nitrate reduction was measured with and without the addition of an exogenous electron donor (lactate), and lactate oxidation was measured with and without the addition of an exogenous electron acceptor (nitrate). We measured the concentration of nitrate and tracer (bromide) in samples collected during an extraction phase lasting four days. This data was used to plot the function:

$$f(x)_t = [NO_3^-{}_t]/([Br^-_t]/[Br^-_o]) \qquad (1)$$

where $[NO_3^-{}_t]$ and $[Br^-_t]$ are the concentrations of nitrate and bromide as a function of time and $[Br^-_o]$ is the initial concentration of bromide. A line fitted to this data (Figure 4) has a slope equal to the average rate of nitrate utilization in the absence of lactate, 0.08 mM/day (in prep.). This rate doubled when lactate was added. These results demonstrate that our techniques are capable of determining the *in situ* rate of potential nitrate utilization by an indigenous microbial community over a period of four days and that we can also detect the increase in activity that results from the addition of an organic substrate.

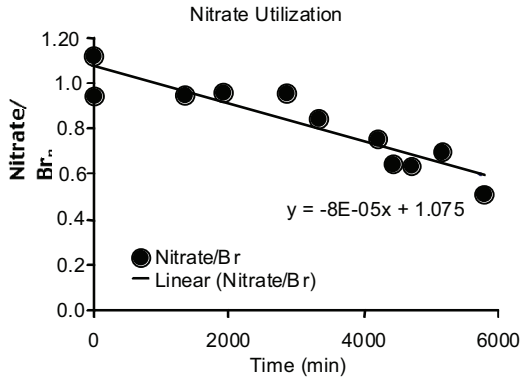


Figure 4. Nitrate consumption. Dilution-corrected nitrate concentration in the HRL pressurized flow cells (Eq. 1). Initial nitrate concentration in this experiment was 1.12 mM, and initial bromide concentration was 5.50 mM. This method of adjusting measured substrate concentrations for dilution forms the basis for the rate determinations to be made in the proposed field tests.

Underway Laboratory Experiments:

Laboratory scale experiments are underway in a physical aquifer model (PAM) constructed to approximate conditions in field tests to be conducted in a saline aquifer hosted in basalt. The PAM consists of a polypropylene reactor with a variety of injection and sampling ports (Fig. 5). The model is packed with Columbia River Basalt sand (collected from Hanford, Washington) and saturated with organic-free artificial seawater containing 40 mM of ethanol as an organic carbon source. Aerobic heterotrophic activity has consumed the oxygen in the PAM, as evidenced by the occurrence of measurable sulfide. We will conduct push-pull experiments in this anaerobic system in order to understand the chemical and physical behavior of the tracers and reactive components that we wish to test in continental and oceanic basalt aquifers. The principal goal of these experiments is to confirm that our selected tracer has the same transport and sorption characteristics as our selected reactive components, to refine our analytical techniques and the sensitivity of our method, and to obtain detailed data needed for numerical models we will use to design our field experiments. The laboratory experiments also enable us to do killed-control experiments that are not possible in the field experiments. The control experiments are helpful to distinguish between microbial and abiotic chemical processes.

Push-pull tests will be conducted using ^{15}N-labeled nitrate to study the rate of denitrification and nitrate reduction to ammonia. Nitrate can be reduced to nitrogen gas or ammonia, depending on the organisms present (S¯renson, 1978).

The use of stable isotopes enables us to trace the introduced substrate to its products in the presence of endogenous substrates and/or products. In the laboratory setting this is not critical because we can control the initial (pre-test) ammonia concentration in the PAM but will be essential in field tests where background ammonia may be present. The use of ^{15}N-labeled nitrate will allow us to determine the fraction of the observed ammonia in a sample that was produced from injected nitrate. Some of these tests will also include the use of co-injected acetylene as a specific inhibitor of the nitrous oxide reductase enzyme system. The accumulation of nitrous oxide in the presence of acetylene confirms that observed nitrate reduction is a microbial process catalyzed by denitrifying organisms. Nitrate, nitrite and ammonia concentrations will be measured using ion chromatography, nitrous oxide will be measured using gas chromatography and ^{15}N ratios will be measured by isotope ratio mass spectrometry.

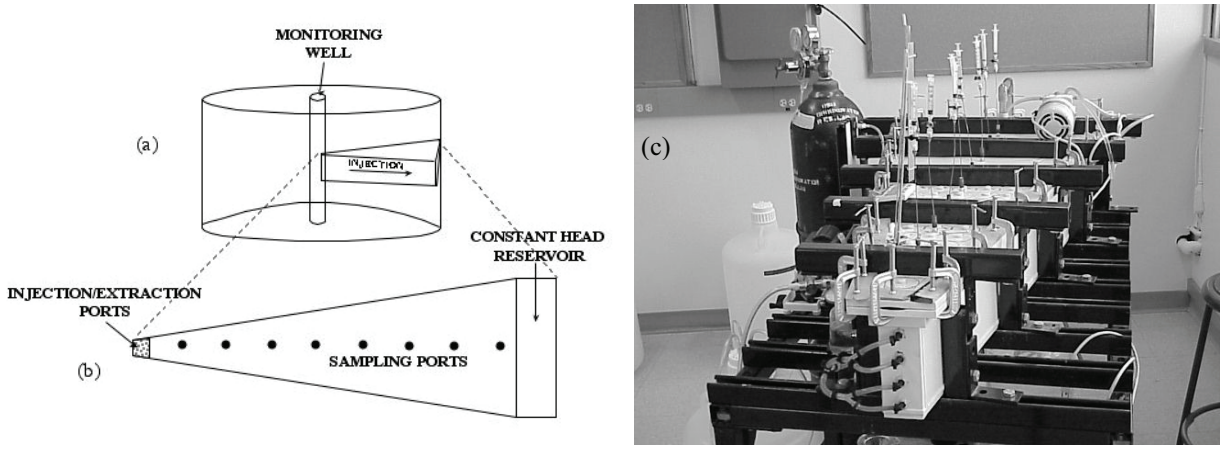


Figure 5. (a) The portion of the flow field near an injection/ extraction well represented by physical aquifer models (PAMs) used in laboratory push-pull tests. (b) Plan view of a PAM. (c) Photograph of PAM used for experiments described in the text (Istok et al., 1997)

Sulfate reduction and the sulfur isotope fractionation will also be investigated in the laboratory. Push-pull tests will be conducted in the PAM by adding acetate and then measuring the concentrations of sulfate, sulfide and the isotopic compositions of each. We will incorporate the use of stable isotopes in their natural abundance and not as a tracer in this experiment. Schroth et al., (2001) used stable isotopes in conjunction with push-pull methods to quantify rates of sulfate reduction in a petroleum hydrocarbon contaminated aquifer. The detection limit of sulfur isotope analyses is 0.15â which is much smaller than reported enrichment factors (on the order of 21â for sulfate reduction; Schroth et al., 2001). In the case of sulfur, stable isotopes will be used as a confirmation of biological activity rather than as a tracer like ^{15}N. Fractionation is qualitative evidence of biological sulfate reduction but is not quantitative because of the multiple factors that can affect fractionation such as sulfate reduction rate, temperature and carbon source (Canfield, 2001; Rudnicki et al., 2001). Some of these tests will also include the use of co-injected molybdate as a specific inhibitor of sulfate reduction to confirm that observed sulfate reduction is a microbial process.

Field Site: Hawaiian Scientific Drilling Program (HSDP) Site

The Hawaii Scientific Drilling Program (Stolper et al., 1996, 2000) drilled a three-kilometer deep hole just outside Hilo, Hawaii (Fig. 6a). The age of the sequence is a few thousand years at the surface (Lockwood and Lipman, 1987) to 500,000 to 600,000 years at 3000 m (pers. comm.). The core

collection is archived at the American Museum of Natural History in New York. Many lithologic units from this site contained alteration that appeared similar to alteration attributed to microorganisms in deep sea basalt glass (Fisk et al., 2003, Walton et al., 2003). One unit was examined in detail with multiple techniques, which confirmed the presence of microorganisms (Fisk et al., 2003). In situ organisms extracted from the rock are similar to those from the ocean crust.

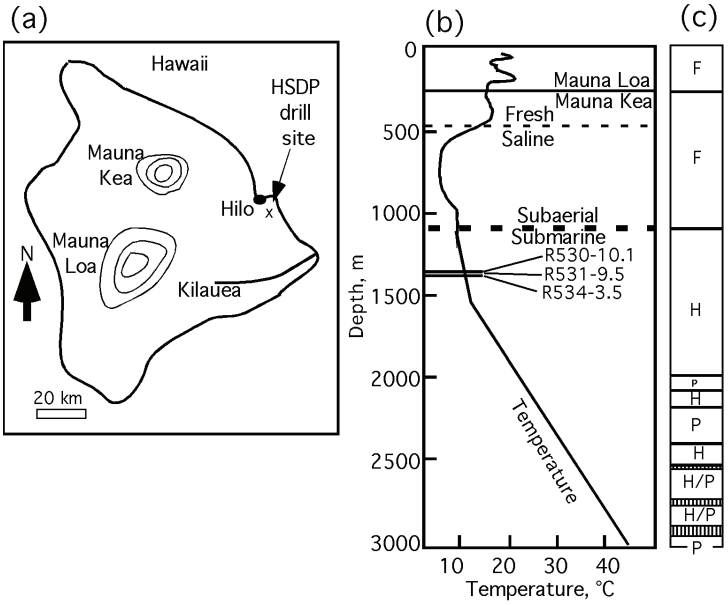


Figure 6. (a) Location of HSDP hole, indicated by an X. (b) Profile of HSDP. The solid horizontal line is the Mauna Loa and Mauna Kea boundary. The horizontal thin dashed line is the fresh - saline water boundary. The thick dashed line is the transition from subaerial to submarine eruptions. Temperatures from Thomas (2000). Sample numbers near 1350 m are in the unit in which microbes were identified (Fisk et al., 2003), however many other units had petrographic evidence for microbial presence. (c) Major lithologic units. F lava flows; H hyaloclastites; P pillow basalts; vertical ruled, intrusions. Depths are m below sea level. Modified from Hawaii Scientific Drilling Project-2 (2000).

The temperature near the surface of the HSDP site is about 16∞C and decreases to 6∞C at 600 m (Fig. 6b). This temperature profile is similar to the profile in the Pacific Ocean near Hilo (Thomas et al., 1996; Thomas, 2000). Below 600 m in depth, the temperature increases to about 15∞C at 1600 m and then increases at rate of 20∞C/km to 45∞C at 3000 m. These temperatures are hospitable for mesophilic (10 to 45∞C) or thermophilic (40 to 70∞C) microorganisms, indicating that microorganisms could survive to great depth at this location.

Abundant fresh water is present in the HSDP hole down to 470 m (Fig. 6b); below this depth the aquifers are primarily salt water. Some fresh water aquifers occur at deep levels (Thomas, 2000). These aquifers can carry inorganic and organic oxidants and reductants deep into the volcano where they could support a microbial ecosystem. The composition of two aquifers is shown in Table 2. The shallow aquifer appears to be sea water diluted with fresh water, except that it possesses sulfate levels that are higher than sea water. The deep aquifer is modified sea water, as might be expected in the ocean crust: Mg has been removed, sulfate is less than sea water and Cl is higher than sea water, possibly indicating the addition of brine. Additional aquifers have also been characterized (Thomas et al., 1996, . At the HSDP site, our experiments will be conducted in an environment that is similar to the ocean crust with respect to alteration mineralogy, water chemistry, lava age and environment, and will allow us multiple opportunities to test our protocol. In this proposal, we will examine the salt water aquifers at one or more levels in the HSDP site.

Table 2. Composition of aquifer water at two depths of the HSDP site.

Depth, m	pH	°C	Ca	Mg	Na	K	SO_4	Cl	Si	HCO_3
600	7.79	6	356	1180	9220	352	3418	17353	14	130
2079	-	23	7285	0.16	8085	91	884	22950	10	-
local SW*	7.71	10	402	1320	10400	391	3204	19923	12	140

* Near Hilo. ( ).
Concentrations in mg/l

BASIC WORK PLAN FOR HSDP

In order to apply push-pull methods to measure *in situ* microbial activity in the ocean crust we will gain expertise and refine our methods by conducting experiments in the environment described above. While every site will present unique challenges, we have developed a general work plan, summarized below, that will be used as a guide to conduct each experiment. A series of experiments will be run at each sampling event, including tests for acetate oxidation (coupled to oxygen consumption) and nitrate, iron and sulfate reduction in the presence and absence of an exogenous electron donor. All tests will include a conservative tracer such as bromide (Table 3).

Pre-Test Preparation:

Pumps and packers will be deployed in the hole with an existing drill rig available to the University of Hawaii. The water will be collected from the packed off interval of the formation after extracting 4500 liters to assure that water is from the aquifer. The chemistry and microbiology of the formation water will be determined. These data will be used to confirm the concentrations of tracer, electron acceptor and electron donors to be added and the sequence of experiments to be conducted. Microbiological analyses will also be performed to aid in field test design and interpretation and to assess the effect of test procedures on the structure of the microbial community. One thousand five hundred liters of formation water will be pumped to a sterile tank to be amended for the push-pull test.

Test Solution Preparation:

With packers deployed to seal off a section of the aquifer, the formation volume will be calculated based on the packed interval. Prior to injection, the test solution will be de-oxygenated by bubbling with 80%N_2:20%CO_2 (except for the aerobic respiration tests).

Table 3. Planned components of push-pull tests in the HSDP.

Components	$[Br^-]$	$[O_2]$	$[NO_3^-]$	$[SO_4^{2-}]$	Acetate
Aerobic Respiration	2 mM	Sat			
Denitrification	2 mM	0mM	30 µM		28 µM
Denitrification	2 mM	0mM	30 µM		28 µM
Sulfate Reduction	2 mM	0mM		30 mM	45 mM
Sulfate Reduction	2 mM	0mM		30 mM	45 mM

Concentrations are estimates and may change based on background chemistry. Experiments for denitrification and sulfate reduction will include de-oxygenating the test solution prior to injection. Concentrations of nitrate and sulfate are based on seawater values. Concentration of acetate is based on the stoichiometry required to reduce all of the added oxidant species multiplied by 1.5 (to account for endogenous oxidants).

The exact composition of the injected solution will be formulated based on the chemical composition of the aquifer being tested. For example, the amount of nitrate added will be enough to double the concentration of background nitrate in the ground water. The added nitrate will be labeled with ^{15}N at 99 atom-percent, resulting in a solution that is labeled at approximately 50 atom-percent. If there is no nitrate in the water, we will add nitrate such that concentrations are similar to those reported for bottom ocean water: on the order of 40 µM (Wheat & Mottl, 2000). Sulfate will also be added in concentrations similar to seawater values of 30 mM (Pilson, 1998). Added sulfate will not be labeled because it will not

be used as a tracer in the same manner as ^{15}N. The isotopic composition will be measured and monitored as a verification of biological transformations.

Test Solution Injection (ì Pushî):
The amount of test solution that will be pumped into the aquifer will be based on the length of the packed interval, the porosity of the formation, and the regional groundwater flow. For a 30 m packed interval and 1500 l of test solution will be sufficient to saturate an annular region 13 m in diameter. Smaller packed intervals and smaller and a porosity less than 10% (which was used in the 13m calculation) would saturate an even larger annular volume.

Incubation:
The duration of the incubation period will depend on the anticipated rate of the specific reaction and the hydrological characteristics of the aquifer. The local hydrology determines how long we can leave the solution in the aquifer and still recover a sufficient fraction and to compute rates. Rates can be determined even if only a small fraction of the original injection is recovered, as long as a measurable amount of the tracer is recovered and all analytes are above instrumental detection limits. Rates of groundwater flow in some of the aquifers of HSDP have been determined to be on the order of 2 to 14 m/yr (Thomas et al., 1996) which suggests that incubation times of a few weeks will allow us to recover some injected solution. The experiments measuring oxygen consumption (acetate injection) and nitrate reduction will likely proceed faster than the less energetically favorable sulfate reduction. Accordingly, the sulfate reduction tests will require the longest incubation period to produce a measurable signal.

Test Solution Extraction (ì Pullî):
Extraction will occur by pumping at a slow and consistent rate. Approximately 20 samples will be collected over the course of the extraction phase. Extracted volumes will be equal to 3 times the injected volume to ensure that as much of the injected fluid is recovered as possible.

Analyses:
Samples will be analyzed for both chemical and microbiological parameters. Table 4 summarizes the primary measurements that will be made. The chemical and isotope analyses will be measured at every time point as described below. The biological samples will be collected on a more limited basis (e.g. at the beginning, the end, and five time points during the course of the test).

Table 4. Summary of analyses to be measured during push pull tests at the HSDP site.

Analysis	Instrument	Facility
Inorganic Ions (Br^-, SO_4^{2-}, Cl^-, NO_2^-, NO_3^-)	Dionex Ion Chromatograph	OSU - Groundwater Research Laboratory
HS^-	Spectrophotometric Assay	
Dissolved Oxygen	Oxygen electrode	
Acetate	Dionex Ion Chromatograph	
NH_4^+	Alpkem Flow Solution	OSU ñ Central Analytical Laboratory
Fe(II)	Quadrupole ICP-MS	COAS ñ Keck Collaboratory for Plasma Spectrametry Facility
$\partial^{15}N$	Isotope Ratio Mass Spectrometer	OSU ñ Stable Isotope Research Unit
$\partial^{34}S$	Isotope Ratio Mass Spectrometer	OSU - COAS Stable Isotope Mass Spectrometer Facility
Microbiology (direct counts)	Epiflourescent Microscope	UH ñ HIMB Microbial Ecology Lab
Microbiology (community structure)	TRFLP, clone libraries	

Calculation of Rate. Zero-order reaction rates (mM/hr) for substrates are computed by dividing the quantity of substrate consumed (or product formed) by the test solution injection volume and by the mean

residence time for the test solution in the aquifer (Istok et al., 1997). First-order reaction rates are determined by fitting a regression line through the dilution-adjusted concentrations versus elapsed time (Eq. 1) (Haggerty et al., 1998).

A combination of analyses will be used to demonstrate that observed electron acceptor utilization is a biological process. These will include isotope measurements of substrates and products and biological samples. Isotopic evidence would include the incorporation of ^{15}N into ammonia (demonstrating dissimilatory nitrate reduction) or shift in the ratio of $^{34}S/^{32}S$ in remaining sulfate (Schroth et al., 2001). If a reaction is stimulated, we expect that there will be an increase in the number of active unattached cells in extraction samples; we will use total cell counts to confirm this. In addition, terminal restriction fragment length polymorphism (TRFLP) analyses supported by ribosomal RNA (rRNA) gene cloning and sequencing will be used to characterize changes in microbial community structure and link specific microbial phylotypes with functional metabolic traits.

The ribosomal RNA analyses will also be used to investigate the possibility of surface organisms contaminating the experiment. It is impractical to avoid all sources of contamination but it is highly unlikely that organisms adapted to surface conditions that are introduced into the aquifer will be able to outcompete organisms that are indigenous to the oligotrophic subsurface. The tracer data and total counts will also be used to constrain how many surface organisms were introduced during the injection using methods modeled after those presented by Pedersen et al., (1997). Finally, a comparison of rates will also be evidence of biological activity. For example, nitrate reduction experiments in *f* sp^ , Sweden yielded a rate of 0.08 mM/day in the absence of lactate but doubled in the presence of lactate (Nielsen and Pedersen, in prep.). This was interpreted as indirect evidence that observed nitrate utilization was due to microbial activity.

SENSITIVITY OF PUSH-PULL TESTS

The sensitivity of the proposed push-pull tests to detect *in situ* microbial activity depends on a variety of factors including the overall rate of potential microbial activity, the rate of dilution of injected test solutions by local ground water flow, and the detection limits of laboratory instrumentation. Since the detection limits of the instruments are known, test conditions (injection volume, rest phase duration, and extraction pumping rate) must be optimized to obtain the desired sensitivity in rates of substrate utilization. Published rates of denitrification and sulfate reduction are typically reported in units of moles of substrate utilized per unit volume of aquifer per unit time. For example, S¯renson (1978) measured rates of denitrification in marine sediments ranging from 0.10 to 0.87 $\mu m/cm^3$ per day and Canfield and Teske (1996) provide a compilation of natural sulfate reduction rates ranging from $\mu m/cm^3$day down to tens of nm/cm^3day. We expect rates in pristine basalt aquifers to be significantly lower than these reported rates, but the hydrology of the selected sites should allow for sufficient incubation times. Using these rates and estimates for the porosity of our aquifer systems we can make preliminary estimates for the length of the incubation phase in our tests. Previous push-pull tests have run as long as 10 weeks (Istok, pers. comm.) but we will determine amendment concentrations and volumes so that ours will be on the order of 10 days although longer experiments are feasible.

MICROBIAL COMMUNITY ANALYSIS

Our main goal is to determine the potential metabolic activity within aquifers, and to determine the structure of the microbial community and observe how it changes as a result of the push-pull tests. We propose a variety of techniques to investigate the microbial community including visual analysis and molecular based approaches.

Visual Analysis (Total Counts):

Visual counts of cells in the water will be made before, during and after each experiment. The data will be used to track changes in the microbial community due to the perturbations from our experiments. Water samples will be fixed with a formaldehyde solution and then filtered through a 0.2 µm black polycarbonate filter. The filters will be stained with acridine orange, which binds to RNA and DNA. The

filters will be viewed and cells counted with an epiflourescence microscope with blue excitation (450-490 nm range).

Genetic Fingerprinting of Microbial Communities:

A combination of ribosomal RNA-based fingerprinting and gene cloning and sequencing methods will be used to describe and track the microbial communities present within the basalt aquifer. While ribosomal RNA gene (rDNA) cloning and sequencing was the first method developed for describing microbial communities, several polymerase chain reaction (PCR)-based methods have been developed to generate whole community fingerprints of Small Subunit (SSU) rDNAs from microbial samples (Suzuki et al., 1998, Liu et al., 1997, Muyzer et al., 1993). Terminal restriction fragment length polymorphism (T-RFLP) analysis (Liu et al., 1997) is a rapid fingerprinting technique that yields high-resolution spatial and temporal mapping of communities, and has seen extensive use in aquatic environments (e.g. Gonzalez et al., 2000, Morris et al., submitted). This approach yields a genetic fingerprint of the entire microbial community contained within a particular sample, and has the advantage of requiring a very small amount of initial biomass. The resolution of T-RFLP far exceeds that which can be achieved by the cloning and sequencing of SSU rRNA genes alone. Briefly, SSU rDNAs are amplified from mixed genomic DNA as described above, with one or both of the oligonucleotide primers labeled with a fluorescent moiety. The fluorescently labeled amplicons are then digested with an endonuclease, and the fragments are separated via electrophoresis. Fluorescently labeled end fragments are detected on an automated DNA sequencer. The sequencer determines (a) the length of the various fluorescently labeled end fragments in the mixed template, and (b) the integrated fluorescence (≈ relative amount) of each fragment of a particular length. This approach yields a genetic fingerprint of the entire microbial community that is directly comparable through time and across sampling sites. Since this method relies on end-point quantification, it is subject to template reanealing biases (Suzuki and Giovannoni 1996, Suzuki et al., 1998), although, since very small amounts of PCR products are required for the detection of fluorescently labeled amplicons, these biases are usually minimized. Compared to other DNA fingerprinting approaches such as denaturing gradient gel electrophoresis (Muyzer et al., 1993), T-RFLP provides greater sensitivity and thus a more comprehensive measure of species richness.

Aquifer communities will be compared via T-RFLP according to the methods of (submitted), with a few modifications. Bacterial SSU rRNA genes will be amplified using primers 27F-B and 1492RY, which have been modified from Lane (1991) to include degeneracyís such that this primer set targets the widest range of bacterial taxa of any primer pair currently known (. Archaeal SSU rRNA genes will be amplified using the primers Arch20F (DeLong et al., 1999) and Arch915R (Stahl and Amann 1991). Each forward primer will be 5' end-labeled with the fluorescent molecule 5-carboxy-fluorescein (6-FAM), while the reverse primers will be labeled with 5-hexachlorofluorescein (5-HEX). PCR reactions for each DNA sample will be purified with commercially available kits and quantified by comparison to a mass ladder during agarose gel electrophoresis. Cleaned PCR products will be digested with 10U of enzyme for 6 hrs at 37∫C with each of three separate restriction enzymes: *Alu*I, *Bsu*RI (*Hae*III), and *Hin*6I (*Hha*I). Digests will be cleaned, and the entire elution for each reaction will be dried and resuspended in 1 µl sterile H_2O. Samples will be run on an Applied Biosystems (ABI) 3100 16-capillary genetic analyzer, recently purchased by the Hawaii Institute of Marine Biology with NSF-funded Hawaii EPSCoR funds, and raw data will be analyzed using GeneMapper software (ABI). When comparing fingerprint patterns, threshold values will be set such that peaks representing >1% of the total integrated peak area for each fingerprint will be analyzed. This threshold, expressed as a percentage of the total rather than absolute values for peak area, will serve to normalize the samples, which often display varying relative fluorescence intensities due to the recovery from the final cleaning step prior to analysis. Periodically, triplicate PCR amplicons will be processed in parallel from single genomic DNA samples in order to test the reproducibility of the PCR, restriction, and analysis methodology. Preliminary replicates have shown excellent reproducibility (data not shown).

In order to properly interpret the rapid TRFLP fingerprinting data described above, we will establish sequence databases from SSU rRNA gene clone libraries constructed from HSDP basalt aquifer microbial communities. Our goal is to limit the amount of cloning and sequencing to only that which is necessary for correlating TRFLP fragments with phylogenetic identity within the bacterial and archaeal domains. These libraries will be constructed and screened using TRFLP according to published protocols developed in part by the PI (, employing the bacterial and archaeal oligonucleotide primers described above. During the amplification reaction, special care will be taken to minimize in vitro artifacts by minimizing template reanealing biases (Suzuki and Giovannoni 1996) and by employing ì reconditioning PCRî to eliminate heteroduplexes in the final PCR amplicons (Thompson et al., 2002).

A database of SSU rRNA gene sequences, maintained using the ARB suite of sequence analysis programs (Ludwig et al., 2004) on a Linux-based server at the HIMB, is curated in-house by PI Rappe on a monthly basis by downloading published rRNA genes from public sequence databases such as GenBank and the Ribosomal Database Project. Sequences generated in our study will be compared to published sequences employing standard comparative sequence analysis methods (e.g. Hillis et al., 1996).

We anticipate that the rRNA-based approach we describe here will allow us to detect shifts in microbial community structure and ascribe putative functional abilities and activity levels to at least a portion of the ì uncultivatedî microbial phylogenetic groups we uncover. However, if after sampling and analysis of the molecular data collected during Year 1 we discover that we are able to detect shifts in microbial activity using the push-pull technique that are not reflected in changes in community structure, we will include a limited amount of cloning and sequencing of PCR-amplified functional genes relevant to the particular activity in question. For example, for sulfate reduction we will target the dissimilatory sulfite reductase alpha and beta subunit (*dsrAB*) genes (Wagner et al., 1998), while for denitrification we will target the nitrite reductase genes *nirK* and *nirS*, two structurally different but functionally equivalent enzymes of the dentrification process (Braker et al., 1998).

CONTAMINATION ISSUES

In all investigations of the subsurface biosphere there is a problem with surface contamination and with the fact that gaining access to make observations often perturbs the system. Advances in drilling have led to better quantification and understanding microbial contamination (Pedersen et al., 1997; Smith et al., 2000) but this does not preclude contamination from occurring. In experiments described in this proposal, the boreholes has already been drilled and although organisms will have been introduced the time since the last perturbation will be long and will allow the hole to return to ambient conditions. In addition, push-pull tests probe the aquifer at away from the borehole. We consider it unlikely that surface sea water microorganisms introduced by drilling with sea water would survive at the low nutrient setting of the subsurface. Our protocol will be to determine what organisms are present in the aquifer before and after nutrients are added to the borehole. This will allow us to determine if we the most abundant microbes in the aquifer are responding to the added nutrients or if a minor member of the community or even a likely contaminant is the most active organism when supplied with nutrients.

PUSH-PULL TESTS IN A RIDGE FLANK ENVIRONMENT.

A promising setting for push-pull tests in ocean crust is the ridge flank environment in a borehole that is sealed as a hydrologic observatory (CORK). Sites that are overpressurized can be selected based on basement topography in sediment-covered areas. Overpressured sites will favor extraction of fluid from packed off formations, and pressures are not high enough to prevent amended fluids from being pumped into the formation (over pressures are +3 kPa and +18 kPa for Holes 1025C and 1026B, respectively, Davis and Becker, 2002). The cork would be fitted with OSMO samplers at the CORK head and will have a two-year or longer sampling interval (Jannasch et al., 2004).

The borehole will be sealed long enough to geochemically equilibrate after drilling perturbation. The most promising scenario would be to revisit a CORKed hole with a submersible at least one year after

drilling. A submersible could take a pump and bladder containing the amendments we wish to introduce into the aquifer to the seafloor. The bladder will passively collect water from an overpressured hole (through a valve in the CORK) and then reinject the modified formation water into the aquifer. This has an advantage of using formation fluid for the test injection.

The injection phase would occur within the sampling window of the OsmoSamplers. Therefore, the samplers will collect fluids and microbes during the geochemical equilibration phase, the injection phase, and the incubation phase. With the Osmosamplers at the CORK head, they can be retrieved without a second submersible or ROV visit to the CORK.

BROADER IMPACTS

This project will constitute part of the Ph.D. thesis of , and as such, it is an integration of training and research. The project is part of a broad interdisciplinary program on the subsurface biosphere that is centered at Oregon State University and Portland State University. The program brings together engineers, geoscientists, microbiologists, and soil scientists to lead multidisciplinary investigations of the microbes in the subsurface. s a student in that program and is being co-advised by an engineer and a marine geologist Details of the subsurface biosphere program and a short biography of can be found at the program's web site, is now participating in IODP Leg 1 to install CORKs to investigate the hydrogeology of the Juan de Fuca Plate. This will be a major advantage to our program when we install the CORKs on our proposed IODP leg to the Juan de Fuca Ridge.

The project will also train undergraduates at both the University of Hawaii and Oregon State University. Financial support for undergraduates at the University of Hawaii is requested in the proposed budget, while support for OSU undergraduates will be requested through the NSF Research Experience for Undergraduates (REU) program (Program Solicitation NSF 03-577). Their involvement will be in research activities such as analyzing ground water, preparing for field experiments and molecular microbial ecology, as well as everyday laboratory activities and lab meetings. On both campuses, we will advertise for students of underrepresented groups to participate in the project during the academic year. At the University of Hawaii, undergraduate research positions will be specifically brought to the attention of students applying to two on-campus minority research and training programs, Haumana and MARC U*STAR, with the help of the programsí director, . These programs are designed to provide hands on research experience to academically qualified students whose ethnic identity is in whole or in part Hawaiian, Filipino, Samoan, and American Pacific Islander. Another excellent opportunity for involving students from underrepresented groups is through the Society for the Advancement of Chicanos and Native Americans (SACNAS). Each year, represents Oregon State University at the annual SACNAS conference and while there he receives numerous requests for summer internships. In will attend the SACNAS conference to recruit at least one student to participate in this project. This student will have the opportunity to run laboratory experiments and chemical analyses and participate in field measurements. To fund this student's participation, we will request an REU supplement to this grant for summer support.

This project also enhances collaborations between academic institutions in the United States. Two new collaborations will be established with the funding of this grant. has not previously worked with at the University of Hawaii. In aid of these collaborations such as this, the Subsurface Biosphere program maintains a Polycom conferencing system to hold meetings over the internet.

SUMMARY

There is a growing body of evidence that the subsurface rocks host a community of microorganisms. The goal of this proposal is to test the activity of these communities by measuring their ability to perform fundamental biochemical transformations. Successful measurements will be significant steps towards understanding the subsurface microbial communities and their role in biogeochemical cycles. Expertise

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SUMMARY PROPOSAL BUDGET YEAR 1

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION: **University of Hawaii**

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. **Assistant Research Professor**	0.50	0.00	0.00	$ 3,843	$
2.	1.00	0.00	0.00	8,693	
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (2) TOTAL SENIOR PERSONNEL (1 - 6)	1.50	0.00	0.00	12,536	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (1) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	3.00	0.00	0.00	9,016	
3. (0) GRADUATE STUDENTS				0	
4. (1) UNDERGRADUATE STUDENTS				6,000	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				27,552	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				7,472	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				35,024	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.) **Downhole Packer/Sampling System** $ 10,000					
TOTAL EQUIPMENT				10,000	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				2,200	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				7,500	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				77,130	
6. OTHER				1,000	
TOTAL OTHER DIRECT COSTS				85,630	
H. TOTAL DIRECT COSTS (A THROUGH G)				132,854	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) **MTDC (Rate: 36.3000, Base: 70724)**					
TOTAL INDIRECT COSTS (F&A)				25,673	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				158,527	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 158,527	$

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI/PD NAME

ORG. REP. NAME*

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

SUMMARY PROPOSAL BUDGET YEAR 2

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION: **University of Hawaii**

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. **Assistant Research Professor**	0.50	0.00	0.00	$ 3,920	$
2.	1.00	0.00	0.00	9,041	
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (2) TOTAL SENIOR PERSONNEL (1 - 6)	1.50	0.00	0.00	12,961	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (1) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	3.00	0.00	0.00	9,196	
3. (0) GRADUATE STUDENTS				0	
4. (1) UNDERGRADUATE STUDENTS				6,000	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				28,157	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				7,682	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				35,839	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.) **None** $ 0					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				2,200	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				7,500	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				67,085	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				74,585	
H. TOTAL DIRECT COSTS (A THROUGH G)				112,624	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) **MTDC (Rate: 36.3000, Base: 45539)**					
TOTAL INDIRECT COSTS (F&A)				16,531	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				129,155	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 129,155	$

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI/PD NAME

ORG. REP. NAME*

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

SUMMARY PROPOSAL BUDGET

Cumulative

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION: **University of Hawaii**

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR:

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. **Assistant Research Professor**	1.00	0.00	0.00	$ 7,763	$
2.	2.00	0.00	0.00	17,734	
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (**2**) TOTAL SENIOR PERSONNEL (1 - 6)	3.00	0.00	0.00	25,497	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (**0**) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (**2**) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	6.00	0.00	0.00	18,212	
3. (**0**) GRADUATE STUDENTS				0	
4. (**2**) UNDERGRADUATE STUDENTS				12,000	
5. (**0**) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (**0**) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				55,709	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				15,154	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				70,863	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.) $ 10,000					
TOTAL EQUIPMENT				10,000	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				4,400	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (**0**) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				15,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				144,215	
6. OTHER				1,000	
TOTAL OTHER DIRECT COSTS				160,215	
H. TOTAL DIRECT COSTS (A THROUGH G)				245,478	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)					
TOTAL INDIRECT COSTS (F&A)				42,204	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				287,682	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 287,682	$

M. COST SHARING PROPOSED LEVEL $ **0** AGREED LEVEL IF DIFFERENT $

PI/PD NAME

ORG. REP. NAME*

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

C ***ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET**

BUDGET JUSTIFICATION - UNIVERSITY OF HAWAII

A. Senior Personnel. One half-month of salary support is requested for each year, who has responsibility for overseeing the molecular microbial ecology and assisting with the microbiological aspects of the proposed work. Nearly all of the molecular biology-based research will be performed at the University of Hawaiiís Hawaii Institute of Marine Biology, where Rappe maintains separate molecular biology and microbial cultivation facilities. Funds are requested for one month of salary support each year for co-PI to work with the biological team in the design of specialized packer/sampling system for conducting the nutrient spiking as well as subsequent collection of samples from the deep borehole.

B. Other Personnel. PI equests funds for three months of salary per year for laboratory technician support ( ). His involvement in this project will be to (1) Assist PI train OSU graduate student] nd UH undergraduates in basic molecular biology and advanced molecular microbial ecology laboratory techniques, (2) Facilitate and expedite the microbial ecology research by preparing reagents and troubleshooting protocols in advance of stay at the HIMB, (3) Oversee the day-to-day research activities of undergraduate scientists in the laboratory, (4) Perform sequencing and other time-intensive procedures to maximize the efficient use of time at the HIMB, and (5) Complete any tasks remaining after stay at the HIMB. Funding ($6,000 per year) is requested for supporting undergraduate student research at the HIMB.

C. Fringe Benefits. Fringe benefit rates are based on University of Hawaii guidelines: Senior Personnel, 35.4%; Technician, 33.0%; undergraduate researchers, 1.0%.

D. Equipment. Support is requested for the purchase/fabrication of the wireline deployable packer and sampling system that will be required to conduct this work. The University of Hawaii currently owns a wireline winch that will be used in the deployment and retrieval of these tools.

E. Travel. In both years, a budget of $2,200 is requested to support travel by two members of the HIMB team (a combination of P technician and undergraduate scientists) to the Hawaii Scientific Drilling Hole near Hilo. This request is for two round-trip flights to Hilo with stays of 10 days per year.

G. Other direct costs. In both years, a budget of $6,500 is requested for supplies related to molecular microbial ecology and community analysis research. This includes general laboratory supplies such as microbiological media and antibiotics, general reagents and chemicals, PCR reagents and supplies, enzymes such as restriction endonucleases, disposable plasticware, etc. Qiagen 96-well format nucleic acid extraction kits (DNAesy 96 and RNAesy 96), Promega cloning kits, and Qiagen 96-well format plasmid purification kits are also included. Nominal fees for DNA sequencing and fragment analysis are also included.

Miscellaneous parts and supplies for the samplers, winch, cables and field supplies necessary for sample acquisition and preservation are estimated at $1,000 per year.

Please see below for a separate justification of the subaward to Oregon State University ($77,130 in Year 1, $67,085 in Year 2).

I. Indirect costs. The University of Hawaii requires 36.3% overhead fees on all direct costs except equipment purchases. In addition, only the first $25,000 of the subaward to OSU is included in UH

indirect cost calculations for the first year, and no UH indirect costs are calculated from the subaward to OSU in the second year.

SUMMARY PROPOSAL BUDGET YEAR 1

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION
Oregon State University

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1.	1.00	0.00	0.00	$ 6,980	$
2.					
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	1.00	0.00	0.00	6,980	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (1) GRADUATE STUDENTS				21,242	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				28,222	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				4,994	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				33,216	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				7,900	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				5,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				100	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				300	
5. SUBAWARDS				0	
6. OTHER				10,708	
TOTAL OTHER DIRECT COSTS				16,108	
H. TOTAL DIRECT COSTS (A THROUGH G)				57,224	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) **Total Direct - minus tuition ($9,258) (Rate: 41.5000, Base: 47966)**					
TOTAL INDIRECT COSTS (F&A)				19,906	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				77,130	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 77,130	$

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI/PD NAME
I

ORG. REP. NAME*

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INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

SUMMARY PROPOSAL BUDGET YEAR 2

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION
Oregon State University

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1.	0.50	0.00	0.00	$ 3,665	$
2.					
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	0.50	0.00	0.00	3,665	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (1) GRADUATE STUDENTS				21,846	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				25,511	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				3,902	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				29,413	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				5,030	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				4,200	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				100	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				150	
5. SUBAWARDS				0	
6. OTHER				11,450	
TOTAL OTHER DIRECT COSTS				15,900	
H. TOTAL DIRECT COSTS (A THROUGH G)				50,343	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) **Total Direct - minus tuition ($10,000) (Rate: 41.5000, Base: 40342)**					
TOTAL INDIRECT COSTS (F&A)				16,742	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				67,085	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 67,085	$

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI/PD NAME

ORG. REP. NAME*

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 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

SUMMARY PROPOSAL BUDGET

Cumulative

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION: **Oregon State University**

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1.	1.50	0.00	0.00	$ 10,645	$
2.					
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	1.50	0.00	0.00	10,645	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (2) GRADUATE STUDENTS				43,088	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				53,733	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				8,896	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				62,629	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				12,930	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				9,200	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				200	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				450	
5. SUBAWARDS				0	
6. OTHER				22,158	
TOTAL OTHER DIRECT COSTS				32,008	
H. TOTAL DIRECT COSTS (A THROUGH G)				107,567	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)					
TOTAL INDIRECT COSTS (F&A)				36,648	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				144,215	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 144,215	$

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI/PD NAME

ORG. REP. NAME*

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INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

C *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

Oregon State University Budget Justification (Fisk)

Senior Personnel:

A.1. is a marine geologist and has worked on the characterization of the microbial colonization of volcanic rocks for the past 10 years. He is the advisor of who will be supported by this grant. He has a 12-month appointment at Oregon State University of which 40% is supported by the university for teaching and other academic duties. The remaining 60% must be raised from other sources. One and a half months of salary support are requested for for activities described in the work plan. These include participating in the field measurements in Hawaii and the chemical and physical characterization of the igneous rocks from the field sites.

is a groundwater hydrologist who was the initial developer of the single-well, push-pull test method to be used to measure *in situ* microbial activity in this project. He has extensive field experience with the push-pull test method and has developed several related field procedures for measuring aquifer physical, chemical, and biological characteristics *in-situ*. He is the author of two textbooks on these subjects (" "). He is Co-Advisor the graduate student to be funded by this project. He will aid in the theoretical and numerical analyses required to support the design and interpretation of the push-pull tests and will directly participate in the field experiments. His participation is at no cost to the proposal except for travel to Hawaii for the push-pull test in the first year.

B.3. Graduate Student. Support for one Ph.D. student, , is requested for two years. He will develop the detailed work plan and organize and carry out the field program with the assistance of and He will determine the concentrations of constituents of the initial ground water and the extracted ground water. He will determine the level of amendments to be added to the ground water and determine the length of incubations and the volumes of water to extract. He will collect microbial samples and determine the community structure in the lab of at the Hawaii Institute of Marine Biology. He will be primarily responsible for the synthesis of the results and writing publishable reports. The amount requested is for twelve months per year at the level of an Oregon State University Graduate Research Assistantship.

B.4. Undergraduate Student. Not shown in the budget is support for one student each summer. We will request a supplement to support students from underrepresented groups through the NSF Research experience for undergraduates program (Program Solicitation NSF 03-577). Details of our recruiting efforts to fill these positions are given in the Project Summary in the Broader Impacts section.

E. 1. Domestic Travel

In the first year travel funds are requested for one experiment in the Hawaii Scientific Drilling Hole near Hilo. The experiment will require five people, three from OSU and two from Hawaii. The OSU travel is budgeted here. This request is for three round trip flights to Hilo with stays of

twelve days. The trip is for setting up and running push-pull tests as described in the work plan. Also in the first year will travel to the Hawaii Institute of Marine Biology to characterize the microbiology of the push pull experiments. Thirty-five days are requested for this trip in order to learn the techniques and make the measurements. Per diem and lodging are priced at cost in university housing.

In the second year an additional push pull test will be conducted. Funds are requested for to travel to Hawaii to conduct the push pull test (10 days) and also to return to the Hawaii Institute of Marine Biology to characterize the diversity and activity in the aquifer (25 days).

In each second year travel is requested to attend a national conference to present results from the experiments.

G.1 Materials and Supplies.
In the first year the cost of material for conducting the push pull test $1000 for a 500-gallon water tank, $2,000 for chemicals required to prepare the test solutions, $1,000 tubing and fittings required for manipulating fluids, and $1000 for shipping push pull equipment from Oregon to Hawaii and back.

In the second year, we will purchase a second set of packers $500, some tubing and fittings $700, chemicals $2000. Shipping will also be required. The 500 gallon tank from the first year will be reused. Shipping will be $1,000.

G.2. Publication costs are $100 per year for meeting abstracts fees.

G.4. Computer services are calculated at a rate of determined by the College of Oceanic and Atmospheric Sciences.

G.6. Analytical costs.
In both years, samples will be analyzed for; inorganic ions, acetate and sulfide ($700/yr). The cost of molecular biology studies are budgeted in the University of Hawaii budget. The cost of characterizing the physical and chemical properties of the basalts housing the aquifer will be $350 for SEM and $400 for electron microprobe in each year.

In years 1 and 2 graduate student tuition is also included in this category (year 1 $9,258; year 2 $10,000).

Facilities and equipment

(i) Laboratory

possesses laboratory space totaling ~1250 ft^2 on Coconut Island (Kaneohe Bay, windward Oahu), home of the Hawaii Institute of Marine Biology (HIMB), a research institute of the University of Hawaii at Manoa School of Ocean and Earth Science and Technology. A dedicated molecular biology laboratory totaling ~850 ft^2 is located in the new Pauley Laboratory complex. Available equipment in the laboratory includes a Stratagene PCR clean hood, three 96-well PCR machines with dedicated multi- and single channel pipettes (BioRad and MJ Research), three microcentrifuges, two hybridization ovens, two Precision 6M dry incubators, numerous water baths, heat blocks, vortexers, pH meters, precision balances, electrophoresis equipment, BioRad DGGE unit, and a Kodak gel imaging and documentation system. Renovation has recently been completed on a dedicated cultivation facility totaling ~400 ft^2. This laboratory has all of the equipment required for the primary isolation and cultivation of marine microorganisms, including shaking platforms, lighting systems, multiple incubators, a Class II laminar flow hood, dedicated pipettes, and other equipment used for high throughput cultivation. The laboratory is also equipped with a liquid nitrogen dewar used to store the cultures, filtration equipment, -80˚C freezer access, and an epifluorescence microscope equipped with a CCD camera served by a Macintosh computer using IPlab Spectrum image analysis software.

Most chemical analyses will be performed at the Ground Water Research Laboratory (GRL) at Oregon State University ( ). The GRL contains: two Shimadzu Model 14A Gas Chromatographs (GC) equipped with flame ionization, thermal conductivity, and electron capture detectors; a Hewlett-Packard (HP) UV visible spectrophotometer; Dionex Model 4000I and 2000I Ion Chromatographs with conductivity and spectrophotometric detectors; and a Packard Instrument Tri-Carb Liquid Scintillation Counter. The GRL contains two walk-in style constant temperature rooms and a large volume (3000 L) fluid handling/mixing/storing system used for test solution preparation and waste storage and treatment. The GRL contains a comprehensive inventory of field equipment including a laboratory-equipped cargo van, tanker trailer, variable-speed submersible pumps, water level indicators, pressure transducers, generator, field instruments for temperature, pH, electrical conductivity, specific anion concentration, and portable spectrophotometers. coadvisor to has free access to the GRL.

Elemental Analyses will be performed at the Keck Collaboratory for Plasma Spectrometry Facility in the College of Oceanic and Atmospheric Sciences at Oregon State University (http://wmkeck-icpms.coas.oregonstate.edu/). The laboratory operates a quadrupole ICP-MS, high-resolution ICP-MS, multi-collector ICP-MS, and optical emission ICP. These instruments are available for iron determinations of water collected from the aquifers.

Electron microprobe: At Oregon State University, we are now reviewing bids to replacement for our 1988-model Cameca SX-50. The new microprobe will be suited for mapping of light elements, chemical mapping of weathering textures needed to determine rates of formation of secondary minerals and rates of dissolution of primary minerals. Petrographic microscopes and facilities for preparing samples are available.

(ii) Clinical

No clinical lab space is dedicated or needed for this subproject.

(iii) Animal

No animal space is dedicated or needed for this project.

(iv) Computer

has four PCs and one Macintosh to support this project, including one laptop computer for traveling. In addition, the Laboratory Library maintains 12 PCs and one Macintosh for graduate student and visiting scholar use, all possessing high speed internet connections. Co-PI also maintains a dedicated Linux-based server for bioinformatics applications (ARB, GDE, Phylip, GenDB, etc).

(v) Office

has ~100 sq. ft. of office space, and graduate student office space of equal size, in the complex located on Coconut Island.

(vi) Other

The Hawaii Institute of Marine Biology houses a shared use molecular biology facility in the Pauley Laboratory complex, currently servicing six faculty members. Dedicated equipment includes two PCR machines, ultra pure water purification system, BioRad quantitative PCR machine, BioRad gel imaging and documentation system, BioRad Microarrayer and Microarray Scanner, two automated DNA sequencers (Beckman CEQ 8000XL, ABI 3100), benchtop refrigerated microcentrifuge, SpeedVac, two hybridization ovens, UV/VIS spectrophotometer, muffle furnace, drying oven, centrifuge with microtiter plate capability, autoclave, UV-crosslinker, and refrigerators and freezers.

Additionally, The Center for Gene Research and Biotechnology at Oregon State University operates analytical instruments for molecular genetics research, including gene sequencers, in its Central Services Laboratory (website address: www.cgrb.orst.edu). The Center has recently upgraded its gene sequencing capabilities with a capillary array electrophoresis (CAE) instrument. The Center also offers support in analysis of genetic data obtained from the sequencers.

(vii) Major Equipment (*List the most important items available for this project and, as appropriate identifying the location and pertinent capabilities of the items.)*

(viii) Other Resources *(Provide any information describing the other resources available to the project. Identify support services such as consultant, secretarial, machine shop, and electronics shop, and the extent to which they will be available for the project. Include an explanation of any consortium/contractual arrangements with other organizations).*