

Biology at DUSEL

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Science (1-2 pages)

DUSEL offers a unique opportunity to explore how life persists, thrives and evolves and ultimately what limits life in Earth's deep terrestrial ecosystems. Resource availability and isolation at depth likely impose constraints on microbial activity and dispersal patterns that differ from the shallow subsurface. Processes that mediate microbial evolution and genome diversification may also vary considerably in these habitats. As has been proposed for microbial communities extant in parts of the deep seafloor, the deep terrestrial subsurface environment may serve as an analogue to primordial microbial ecosystems, to prebiotic systems and even to subsurface life on other worlds. DUSEL also offers a unique opportunity to examine the adaptation and evolution of surface organisms transported into a subsurface environment. The 125 year long mining activity is sufficient time for micro and macroorganisms that were transported deep into tunnels to adapt to elevated temperatures, distinct carbon sources and absence of light. These adaptations may have led to the development of useful metabolic capabilities that can be exploited for bioremediation and biofuel production. Documentation of the dispersal and evolution of microbial immigrants into the Homestake mine also facilitates the recognition of those microorganisms that are true inhabitants of the deep terrestrial subsurface.

Specific hypotheses to be addressed by the first suite of experiments include the following:

1. The importance of chemoautotrophs increases relative to heterotrophs as depth, temperature, degree of isolation from the surface increases.
2. The rock(metamorphic)/water interactions are important not only in generating chemical energy sources BUT also as a source of essential macronutrients (e.g. Fe, Mn, N, HPO_4^{2-}) and micronutrients (e.g. V, Co and Mo) for microorganisms.
3. Microbial growth and biomineralization reactions in fractures generate a significant feedback by altering hydrologic flow, rock strength and reactivity. Conversely, fracture formation enhances microbial growth and activity by providing living space and increasing nutrient flux.
4. Diversity declines and novelty (novel functions) increases with increasing depth or environmental extremes.
5. The subsurface environment selects for guilds of microorganisms from a diverse exogenous microbial input (extending from historic to present).
6. The ability of genetic elements (transposons, CRISP elements, phages, plasmids) to share functional capability may be infrequent and is limited by the abundance of microbial cells and life supporting nutrients in isolated fracture environments. Their activity will increase during changes in the environment.

7. From Neutral Theory and much empirical data: small genetic population sizes and/or low rates of genetic recombination impose more rapid rates of protein evolution, resulting from inability to purge mildly deleterious mutations.
8. Deep subsurface microorganisms use quorum sensing and motility as a survival strategy during periods of extreme nutrient deprivation.

Testing these hypotheses requires the successful implementation of biological investigations at DUSEL, which depends critically upon their staging during laboratory construction and upon their integration with other activities. Four high priority projects should be implemented as part of the initial suite of experiments and a research and development program in new life-detection technologies involving a synergistic collaboration between physicists and biologists should be initiated.

Initial Suite of Experiments (1-2 pages)

1. Biological Inventory & Contemporaneous Science Objectives

The overall goal is to begin immediately by leveraging opportunities that DUSEL would provide for biological sampling, survey and *in situ* experiments. This work is all envisaged as being ‘inside the shell’ of the existing mine and its boreholes. They do offer an opportunity to carry out immediate science objectives and at the same time will provide valuable baseline data for future experiments as well as an opportunity to establish laboratory procedures for ongoing biology experiments.

Objective 1. Survey and Sampling

- Water sampling during the current dewatering program at DUSEL is to determine water chemistry, isotopic composition and age, and the microbial composition. The success of this program will depend on early interaction with scientists and the mining technicians that are carrying out the dewatering.
- It is important to survey and sample microbial biofilms and sediments whose formation is to an extent a function of introduction of air/oxygen into the mine. Preliminary evidence indicates that extensive Fe-rich and to a lesser extent, S-rich microbial biofilm communities occur in the mine.
- Air sampling should be started soon and would be a continuing program throughout DUSEL. Analysis will be done on the microbial composition and the chemistry of important volatiles.

Objective 2. *In Situ* Experiments and Bioprospecting

- Identify specific substrates in boreholes or within tunnels to look at specific microbial metabolisms including metal transformations and cellulolytic activities associated with wood remains from the old mine.
- Search for antibiotic producing microbes and antibiotic resistant microbes; this latter activity is suspected to be correlated with metal resistance in bacteria.
- Study biofouling and biocorrosion processes under controlled conditions in the mine. This could involve the establishment of controlled flow regimen microcosms and mesocosms in different piping systems to simulate natural microbially induced corrosion and biofouling systems.
- Establish a depth transect of existing boreholes within the mine that could be closed off and then sampled at regular intervals for long-term studies to investigate the long-term stability of the microbial populations that were established in them.

Since some of this work is expected to begin soon, an above ground laboratory should be constructed that is well equipped to carry out modern molecular biology work and has good imaging facilities, and contains a full suite of analytical instruments for chemical analyses. A laboratory for radio isotopic analyses should also be considered, perhaps in conjunction with the development of a low level counting facility. Underground facilities for carrying out basic microbiology including anaerobic handling of samples, incubations, and a wet chemistry laboratory should also be constructed, some of which should be mobile. Since these labs are envisioned to be user facilities, a permanent staff to maintain equipment and carry out routine analyses, such as water chemistry will be required. A final consideration should be given to establishing a repository for the microorganisms and important environmental samples that are collected. This would provide a curated archive of microbes that are isolated from the site, as well as archived water, biofilm, core and DNA samples.

2. Ultra-deep biological observatory

An array of three 2,500 m (8,200 ft.) boreholes, drilled from the 2,440 m (8,000 ft.) level of the mine will probe to at least 16,200 ft. (4,940 m) below land surface; a depth at this location approaching the expected lower biosphere limit (e.g. the 120°C isotherm).

Objective 1. Explore the upper temperature limit of life as we know it.

To achieve these objectives extraordinary care will be taken to avoid contaminating these boreholes. Aseptic drilling will involve filtration of the drilling water and decontamination of the drilling rods, as well as chemical and particulate tracers will be used to minimize and document drilling-associated contamination. The interiors of cores will be examined to document changes in microbial biomass, diversity, and activity with increasing depth. If, as expected, the boreholes intercept subsurface fluids, aquatic chemistry, dissolved gases, and microbial community structure and function will be measured initially at multiple depths and tracked over time. Microbial community variables will be documented by the best available technologies enabling the reconstruction within a geological context of metagenomes and metabolomes from presumably simple and verifiably subsurface derived communities. Borehole fluids will be isotopically dated as appropriate for the expected age of the water (e.g. ^{14}C , ^{36}Cl , noble gas composition). Wire line geophysics and borehole imaging will be performed on open boreholes to map the distribution of fractures. Since these bore holes will extend beyond the zone where mining has altered the ambient geological stress field, one may be selected for rock mechanics studies and/or *in situ* stress instrumentation. Hole completion will include sterile, non-metal packer and multilevel sampling systems to isolate fracture zones and at least one will be maintained at formation pressure.

Objective 2. Explore for life as we do not know it and abiogenic organic processes.

This facility will be designed to last indefinitely and support repeated sampling over decades in response to the availability of new technologies. Long-term experiments will include real-time monitoring of multiple parameters including pressure, temperature, water and gas compositions. Fluids will be brought out of the sealed off fractures and into a mobile laboratory equipped with anaerobic glove bag and flow through cartridges to study which factors limit the growth of indigenous microorganisms or hyperthermophilic strains from other extreme environments on the earth. This laboratory

has to be capable of performing these experiments under hyperthermophilic conditions (>120°C). Facilities will also enable the long-term study of *in situ* mineral weathering or precipitation experiments and organic/inorganic interactions. Because this site will be tapping regions beyond the known limits of life, the insertion of fluids into the borehole after completion must be stringently avoided.

3. Pristine Fracture Zone Experiments

Explore, detect, characterize and instrument isolated fracture systems that should be outside the cone of depression for mine dewatering. Given that the fracture system will be at high pressure the boreholes have to have high pressure valves and pressure gauges for fluid management. Special care will be required in coring into high pressure fractures and obtaining uncontaminated fracture surfaces in cores. Accommodation for borehole viewers, cross borehole geophysical instruments and multi-level samplers will be required. Characterization will require conservative tracer experiments to determine fracture porosity and interconnectivity. We propose to examine two different rock types; 1) metasedimentary and 2) metavolcanic, as we theorize distinct microbial communities will occur in these geological strata. On-site laboratories will circulate fracture fluids drawn from the fracture zone through flow-through bioreactors located in on-site laboratories to test hypotheses regarding the interaction of the indigenous microorganisms and organic/inorganic substrates. Because of the proximity of the fracture zone to the tunnel *in situ* experiments can also be performed and monitored in the borehole array with the following objectives.

Objective #1: Microbial biodensity and activity determinations in fractured rock.

This can be determined from analyses of the rock cores and from analyses of the fracture fluids. The *in situ* pressure during fluid removal needs to be monitored to ensure that the fluid is being replaced in the fracture. This characterization provides a baseline against which future *in situ* experiments can be compared. Enrichment and isolation of microorganisms from the fracture fluid or core is also essential for the second objective.

Objective #2: Microbial response(s) to biostimulation and bioaugmentation.

The goal is to determine to what degree extant microbial communities will undergo genome diversification, evolution and adaptation by horizontal gene transfer, HGT, mechanisms, i.e., transduction, conjugation, and transformation in the presence of elevated nutrient flux. The degree to which chemotaxis and quorum sensing control the migration and aggregation of microorganisms within the fracture will also be examined. The experimental design will include the introduction of microorganisms to act as a “gene source/sink” to capture genes undergoing HGT. The microorganisms injected into the fracture represent isolates of the fracture community grown up to a higher cell density and isotopically or genomically tagged. Control experiments will be conducted with and without the introduced ‘gene capturing’ microbial community. In addition, viral population diversity and abundance will be determined as a function of microbial stimulation. These genetic transformation processes will be base-lined prior to biostimulation experiments to determine whether changing environmental conditions force adaptation via these mechanisms.

4. Coupled fracture propagation and microbial activity experiments

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Objective: To determine the effect of tectonic activity on subsurface microbial activity and biodiversity by observing the changes (i. how large; ii. temporal aspects, i.e., how quickly/slowly colonization occurs) in local geochemistry, hydrology and microbiology during the creation of the artificial fractures (i.e., endogenous H₂ production; diffusion from matrix pores). A hypothesis is that tectonic activity alleviates the limiting nutritional factors that control/cap biomass and microbial activity. These studies will be coupled and leveraged to rock mechanic research activities.

Table 1. Experiments versus hypotheses.

Hypotheses	1	2	3	4	5	6	7	8
Biological Inventory	x	x		x	x		x	
Deep biological observatory	x	x		x	x	x	x	x
Fracture Zone Experiments	x	x	x	x	x	x	x	x
Coupled Experiments	x	x	x			x		

Roadmap (rough estimate of cost and time line) (1 page)

The initial inventory establishes the levels of SUSEL and DUSEL that should be made permanently available and those that can be sealed off with the idea of reducing operating costs for DUSEL.

The leveraging of biology studies by collaboration with the rock mechanics community during coring, borehole installation and fracture propagation experiments maximizes cost savings.

1. Estimated Costs (1/2 page)

Table 2. Estimated Total Costs.

Item	Cost
Characterization, inventory and archiving of core, water and microbial samples	\$1,500,000
Surface Laboratory and Archives	\$15,000,000
Shared usage subsurface laboratories (4850' and 8000')	\$1,000,000
Four mobile underground labs – (includes instrumentation)	\$4,000,000
Drilling Costs, Mobile drilling water treatment, Logging, Installation of down hole samplers and monitoring systems.	\$7,000,000
Development of new life detection technology	\$2,500,000
Total	\$31,000,000

2. Time Line (1/2 page)

- Immediately -2011 – Construction of surface laboratory and initiation of inventory of tunnels down to 4850' of present day communities and development of E&O and mobile laboratories.
- 2011 to 2015 – inventory down to 8000', flood water and establishment of “sentinel” boreholes
- 2015 – 2017 – installation of deep boreholes and fracture zone experiments and coupled processes experiment and characterization of initial state.

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- 2017 – 2020 execution of first tier experiments.
- 2020 – 2050+ six generations of five year projects

Research and Development Needs (1/2 page)

- Development of a mobile, drill water sterilization package to enable aseptic acquisition of rock core. This will be critical to the successful drilling for the deep biology observatory.
- Development of down hole instrumentation that would improve upon existing cameras, and initiate a new line of microscopic imagers and miniaturized, fiber optic UV-visible spectrophotometers, fluorimeters, Raman and Mössbauer spectrometers that could detect biomarkers, microbial cells, organics and specific mineral types. A new generation of portable, high sensitivity, down hole neutron and magnetic resonance spectrometers would benefit petroleum and natural gas exploration and extraction. Collaborations with the DUSEL physicists and with NASA are viewed as critical to this development.
- Development of tunable laser spectrometers for real-time compositional and isotopic characterization of gases evolved from borehole. These instruments are particularly critical of the biostimulation experiments.
- Development of extremely low counting capability for low energy β particles will significantly enhance the ability to measure in situ microbial activity and could help constrain the cellular turnover time for deep subsurface microorganisms. This requires close collaboration with the physicists responsible for the low background counting facility.
- Mobile robotic drilling and sampling devices with the ability to enter intact fractures will be considered. Collaborations with NASA engineers could enable this research program.

How to arrive at realistic cost and schedules (1/2 page)

- Establish a committee (with experts including scientists and field engineers) that can evaluate the proposed estimate and timeline and recommend realistic cost accordingly based upon most recent construction time lines and dewatering schedules (see Table 3).
- Request competing bids from private companies for subcontracted work (e.g. drilling and cavity construction).
- Hire personnel to coordinate sampling, scheduling, and follow-ups.
- Organize workshops to report research activities and funding opportunities and to develop network among scientists from cross-disciplines and coordinate sampling schedules.

Education and Outreach (1/2 page)

- REU after South African model (proposal already submitted to NSF).
- Summer school for graduate students patterned after the Woods Hole Summer School.
- PIRE proposal could also bring in international microbial ecologists.

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- IPTAI guidebook and DVD integrated with Sanford Laboratory teacher workshop (Sayler mobile education lab).
- On line access to authentic research data for K-12 classroom experiments. Ultimately experimental data collected and transmitted real time.
- 300' level available for public tour and K-12 student research.

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Table 3. Costs as a function of laboratory construction (does not include coupled experiments).

	Upper Campus 4100 MWE (First Occupancy ~2011)						Lower Campus 6400 MWE (First Occupancy ~2016)					
	Cavity Size (m ²)	Support Space (m ²)	Notes	Excavation Cost (\$M)	Instrument Cost (\$M)	Operating Costs (\$M/yr) ¹	Cavity Size (m ²)	Support Space (m ²)	Notes	Excavation Cost (\$M)	Instrument Cost (\$M)	Operating Costs (\$M/yr) ¹
Biological Inventory	0	300	shared usage laboratory for GEO, ENG, BIO to process samples, sterilization facilities and storing equipment	0	0.5	0.75	0	300	small laboratory for processing samples, sterilization facilities and storing equipment	0	0.5	0.75
Mobile Underground Laboratories (MUL's)	0	0	two labs - each lab fully equipped for handling fluids/gases at high pressures, aseptically and anaerobically	0	2	0.2	0	0	two labs - each lab fully equipped for handling fluids/gases at high pressures, aseptically and anaerobically the cavity has to be 20 meters in height to provide clearance for the drilling rig and the footprint includes space for the fluid handling systems and MUL's. Excavation includes geophysical and video logging of three boreholes. Installation includes	0	2	0.2
3. Ultradeep Biological/Geophysical Observatory	0	0		0	0	0	0	200	insertion of	2.5	1.5	2.15

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straddle packers, high pressure fluid handling systems, down hole strain gauges and seismometers (if necessary).

			<p>this may be located at 4850' or at 6100'. this may require 1 km drift in order to access pristine environment. the excavation costs include this drift plus 20 boreholes ~100 meters deep into fracture zone. excavation costs also include cross borehole tomographic surveys from cased boreholes. Instrumentation cost include high pressure, multilevel samplers and 20 fraction collectors/autosamplers.</p>						
4. Pristine Fracture Zone Experiment	0	300		2.3	0.7	2.15			
Annual Costs \$M/yr						3.1			3.1
Capital Costs				2.3	3.2	15.5	2.5	4	15.5

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Total \$M

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¹ Based upon \$200k/year for each investigators, so \$2M/year would support 10 investigators. Also, 10% of instrumentation costs are included as the maintenance cost of the hardware for the experiment. This would include repair and replacement of seals, pumps, valves, etc.

Cost

Estimates:	Drilling	~\$50/ft	~\$150/m
	Drifts	~\$500/ft	~\$1500/m
	Caverns	\$500/ft ²	\$5000/m ²

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