Opportunities for Geomicrobiological Characterization and Experimentation During DUSEL Site Selection and Development. "ProtoEarthLab"

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In addition to the geomicrobiological experiments that are proposed in the EarthLab document (www.earthlab.org) for implementation in a completed DUSEL facility, there are multiple opportunities for geomicrobiologists to contribute to and to benefit from the site selection and development of DUSEL. Several candidate DUSEL sites have been identified. While clearly not all of these will be fully developed into underground laboratories, each offers unique opportunities for geomicrobiological characterization and experimentation. Each is an invaluable target of opportunity for geomicrobiologists. Deep subsurface science is limited by the paucity of sites; none is current available in the U.S. Interdisciplinary characterization can provide geomicrobiological, geotechnical, geochemical, and geophysical data that will help to inform decision making regarding the siting and development of DUSEL. It will also begin to generate fundamental geomicrobiological knowledge of previously uncharacterized subsurface environments at modest cost and in the near term. Sites with existing mines can serve as deep platforms for sampling groundwater and rock. Deep mine adits can serve as platforms for drilling exploratory boreholes that probe the lower limits of the biosphere. Well developed tunnel systems can be exploited for preliminary reactive transport experiments. Sites without preexisting underground infrastructure offer "green fields" with largely unknown and uncharacterized microbial communities and biogeochemical processes that can be accessed via exploratory boreholes from the surface. Cores from these boreholes will provide detailed geological information, and the boreholes will also be available for geophysical characterization of proposed and developing DUSEL sites. Samples obtained at these sites will enable detailed geochemical, molecular, and physiological studies of indigenous subsurface microbial communities and the geochemical processes that they mediate.

As discussed at the Blacksburg, VA DUSEL meeting (November 12-13, 2004), a number of exploratory boreholes and other geological characterizations are planned for the candidate DUSEL sites as part of Solicitation 2 activities. These provide excellent targets of opportunity for geomicrobiological characterization. We can, in essence, initiate EarthLab activities during the coming year with a modest increase in cost, primarily the cost of using geochemical and microbiological tracers. We have outlined below 1) some examples of the types of samples that would be of greatest interest for geomicrobiology, and 2) the technical requirements for geomicrobiological sampling.

Samples of interest for geomicrobiology:

1. Core samples from deep (>2 km below land surface (kmbls)), pristine subsurface environments. Sites with steep geothermal gradients (>20°C/km) are of particular interest.
2. Core intervals that include geological interfaces. Interfaces between organic rich, fine textured sediments and coarser textured aquifer materials are of particular interest.
3. Flowing water samples from old (> 1 million years, (>1 Ma)) ground water.

4. Flowing water samples and/or core from deep sites (>1 kmbls) with mineralogy that may be conducive to abiotic, geochemical generation of H_2 (e.g., basalt, serpentinized ultramafic rock, Fe(II)-rich minerals, U-rich minerals).
5. Flowing water samples and/or core from deep sites (>1 kmbls) with evidence of biological sulfate reduction (significant H_2S in ground water) or methanogenesis (significant CH_4 in groundwater or measurable partial pressure of CH_4 in localized areas of mine atmosphere). Note: CH_4 accumulation in the mine atmosphere indicates a potential serious safety hazard.
6. Biofilm samples from tunnel walls may be of interest. These are easy to collect, and in some cases, we may be able to identify the major dominant microbial process (e.g., sulfur oxidation or iron oxidation) based on morphology. From this, we may be able to infer something about the chemistry of the groundwater source for the biofilm.

Technical requirements for geomicrobiological sampling:

1. Core diameters of $\geq$2 inches ($\geq$5 cm) are preferred in order to obtain subcores from within the cores.
2. Preferred drilling methods are highly site specific. Drilling fluids (water, mud, air, foam, etc.) should have tracers (see below) added at the surface and quantified in outer core parings and in inner subcores).
3. Ideally, an anaerobic glove bag or chamber should be available on site or at a nearby laboratory for processing samples to maintain viability of O_2-sensitive anaerobes and for maintaining lower redox geochemistries. Temporary, disposable glove bags can also be used. Alternatively, samples can be frozen as quickly as possible after collection for culture-independent analyses (e.g., nucleic acid and lipid analyses).
4. Core barrels should be steam cleaned before use. Ideally, core barrel liners should used. These are available in Lexan. Samples can be collected for geomicrobiology without using core barrel liners, but the chances of introducing O_2 are greater and the core barrel will be unavailable for sampling until after the core has been processed.
5. Tracers:
   a. Solute tracers can be as simple as bromide and/or rhodamine dye or as sophisticated as perfluorocarbon tracers. Br^- can be used as LiBr at 500 mg/L in drilling fluids and quantified by ion-specific electrodes or ion chromatography. Rhodamine can be used at 20 mg/L and quantified by fluorimetry. Inert perfluorocarbons (e.g., perfluorohexane, perfluoromethylcyclohexane) have a low aqueous solubility but can be quantified over many orders of magnitude by gas chromatography. These are added at ~1 mg/L using an HPLC pump.

   b. Fluorescent microspheres make a good fluorescent tracer of bacterial-sized particles. We've used latex, carboxylated (for negative charge) 1-µm diam microspheres from Polysciences. These are placed into Whirl-Pak bags in the shoe of the corer in such a way that the bag breaks on contact, bathing the core in a suspension of beads. These are then quantified in the fluid surrounding the core, in the parings, and in the subcore by fluorescence microscopy. Cost of beads is ~$115/10 ml. A 10-ml suspension is sufficient for one core sample.

Further information on sampling is available in Fredrickson, J.K., and T.J. Phelps. 1997. Subsurface drilling and sampling, pp. 526-540, In: Manual of Environmental Microbiology (C.J. Hurst, Ed.), ASM Press, Washington, DC. [pages 679-695 in the 2002 edition].

Please feel free to contact Tom Kieft (tkieft@nmt.edu), 505-835-5321 or Tommy Phelps (phelpstj@ornl.gov) 865-574-7290 to discuss the scientific and technical aspects of any potential geomicrobiological sampling opportunity.