Peristaltic Pump-Coupled Chambers for In Situ Microbial Cultures

Samantha C. Lewis  
Mentor: Dr. Martin Fisk  
College of Oceanic and Atmospheric Science

Background and Introduction
Observation of bio-weathering by lithotrophic bacteria is a challenging and yet extremely desirable prospect. Microbial bio-weathering may be vital to the future of astrobiology and geology, because these "bio-signatures" indicate the presence of microbes long after they are gone. Patterns similar to those created by basalt-loving lithothrophs have been observed on Mars meteorites, thus bio-weathering may even aid in the discovery of life on Mars. However, because such bacteria live in extreme environments deep inside the Earth they are best collected from wells and hot springs. Their collection in the field may be extremely difficult and time-consuming. A procedure and apparatus were designed that would allow lithothrophic bacteria to be "trapped" and then cultured and identified in the lab so that weathering may be observed. Close proximity with basalt minerals inside the chambers, low flow-rates and high temperatures were desired to aid inoculation and growth.

Materials, Methods and Procedures
Two chamber prototypes were designed in AutoCad in association with Hans Jannasch of the Monterey Bay Aquarium Research Institute and created out of high density polyethylene. The design features four core components: (1) Pre-filter for blocking large debris and organisms (Sun) (2) Growth Chamber filled with mineral sand where microbes are expected to colonize and grow (3) Post-filter series to remove bacteria from water flow (1, 45, 2, 2 um) (4) Control Chamber to observe chemical reactions between minerals and the water. Use of a control chamber was instigated to distinguish chemical effects from microbial weathering. Chambers have four growth beds each containing four different minerals: olivine, pyroxene, glass (silica) and feldspar.

The chambers were tested in the lab for water tightness, microbe retention and ease of use with water collected from the Otatile Spring in Central Oregon already known to contain lithothrophs. In the final design chambers were machined out of Teflon for additional stiffness and because of Teflon's inert qualities. All filters, membranes, tubing and fittings are autoclavable as a unit to increase sterility.

Data Collection
Lithotroph-inoculated water was run through the apparatus twice, 7 days each trial. Minerals from each growth bed and control bed were then removed and water samples were plated on LB media in a F-series dilution using standard sterile procedures. Plates were counted after 48, 96 and 144 hours of growth. No new colonies or substantial growth were observed after the 144 hour count.

Results and Analysis
Multiple procedures and materials had to be modified or replaced to reach our goal: colonization of the growth beds with minimal or no colonization of the control chamber. Results of the initial trials indicated that colonization of both chambers was equal. After inspection of our procedures and setup, we discovered that several key components, such as the interior of the growth beds and the water used to plate the chamber samples were not being completely sterilized. To counteract that, we increased autoclave times for all components and instigated a step-wise sterilization procedure that involves autoclaving the chamber beds three times, waiting two days between each session to catch any recovering microbes. To increase the development of microbes in Trial 2 we used less nutritious media: 1/10 concentration LB, whereas in the first trial we used standard LB. As can be seen in Figure 2, colonization in the Unfiltered Chamber in Trial 2 decreased as the microbes progressed through each growth bed in order, which is to be expected as microbes set out onto the minerals. Colonization of the Unfiltered Chamber in Trial 1 was misleading as all the contaminated water increased colony counts across all beds. Colonization of the Filtered, or Control Chamber was drastically decreased in Trial 2 by the rigorous sterilization procedures we employed.

Thanks
Dr. Steve Giovannoni, Dept. of Microbiology, OSU  
Dr. Rick Cowell, COAS, OSU  
Dr. Raul Pena, Dept. of Microbiology, Portland State University  
Hans Jannasch, Monterey Bay Aquarium Research Institute