Peristaltic Pump-Coupled Chambers for In Situ Microbial Cultures

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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 lithotrophs 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 lithotrophic 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.

Photo-micrograph of a 30 um thin section of basalt glass. Yellow-orange smectite clay was formed as a result of microbial metabolic interactions.

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 (5um) **(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 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 Okallie Spring in Central Oregon already known to contain lithotrophs. In the final design chambers were machined out of Teflon for additional sturdiness and because of Teflon's inert qualities. All filters, membranes, tubing and fittings are autoclavable as a unit to increase sterility.

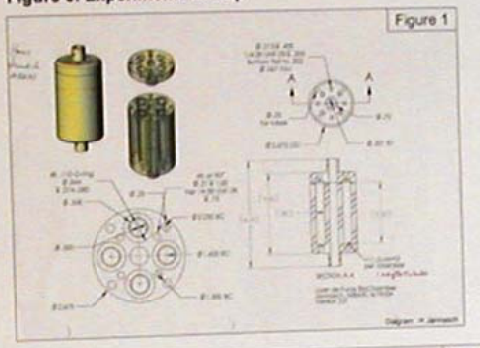
Mars meteorite containing olivine and pyroxene similar to terrestrial basalts. It is possible that subsurface organisms may have left these signatures, which are similar to those found in silicate minerals on Earth.

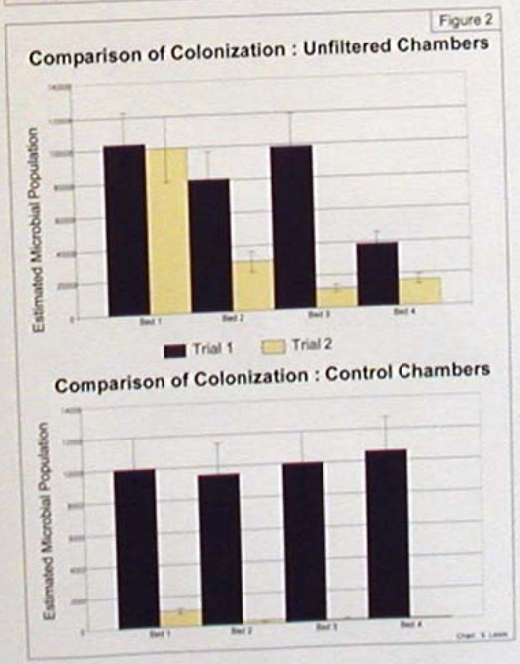
Figure 1. Chamber Design and Specifications

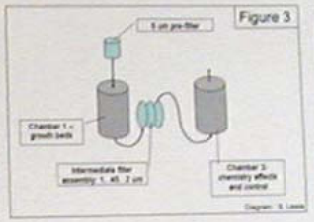
AutoCad drawing and 3D model of a chamber.

Figure 2. Comparing effectiveness of Growth and Control Chambers

Figure 3. Experimental Setup







Procedures to minimize contamination:

- Increased autoclave time for apparatus
- Increase filter membrane surface area to allow better flow-through and fewer membrane ruptures
- Used sterile procedures in a clean hood for all plating, inoculating and counting

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 7-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 trial indicated that colonization of both chambers was equal. After inspection of our procedure 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 chambers three times, waiting two days between each session to catch any recovering microbes. To increase the development time of the colonies 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 trough each growth bed in order, which is to be expected as microbes settle out onto the minerals. Colonization of the Unfiltered Chamber in Trial 1 was misleading, as 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.

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