Cah. Biol. Mar. (2006) 47 : 443-447

A deep sea hydrothermal vent bio-sampler for large volume *in-situ* filtration of hydrothermal vent fluids

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Abstract: The Hydrothermal Vent BioSampler (HVB) currently being developed at the Jet Propulsion Laboratory is designed to collect large-volume hydrothermal vent samples, operating with fluid temperatures reaching 400°C and at vent depths of up to 6,500 metres. The primary goal of the project is to collect 'pristine' samples untainted by the surrounding waters. Analysis of the collected samples can reveal the existence of thermophillic organisms within the vent fluid, extending the upper limits of life with respect to thermo-tolerance. Any biology found at such environments can contribute to research in astrobiology, while the technology developed for the system can contribute to bio-containment techniques useful for Arctic and planetary exploration. The HVB performs *in-situ* filtering of hydrothermal vent fluids to concentrate a large sample volume to a smaller volume more suitable for transport. The HVB system is currently in the development phase. This paper provides a physical description of the current system, as well as a summary of the preliminary tests conducted in 2005 and 2006: pressure chamber tests and a two dive operations at a hydrothermal vent off the northern coast of Iceland.

Keywords: Hydrothermal vents • Water sampler • *In situ* filtration • High temperature • High pressure • Pristine samples

Introduction

Marine hydrothermal systems and the unique biota associated with them represent some of the most interesting ecosystems on the planet. These hostile environments are often composed of vents spewing super-heated fluid containing a variety of reduced compounds, many of which can be used as substrates for growth by microorganisms. While evidence of microbial growth in hydrothermal associated habitats abounds, the evidence of microbial life within the hydrothermal plume has been elusive and difficult to validate. To prove life exists in these extreme environments, '*pristine*' samples (i.e. fluids untainted by microbes entrained from the surrounding waters) must be collected.

To account for the presumed low biomass in the hydrothermal vent fluid, a sampler should be capable of collecting samples of significant volume. Most samplers being developed today have relatively small capacities ranging from tens to hundreds of millilitres (Naganuma et al., 1998; Di Meo et al., 1999; Malahoff et al., 2002; Phillips et al., 2003) to the one litre filtered by the Hydrothermal Fluid and Particle Sampler (HFPS; Johnson et al., 2003).

A novel hydrothermal vent bio-sampler (HVB) was recently developed by the JPL Robotic Vehicles Group with input from the Biotechnology and Planetary Protection Group and experts from the Monterey Bay Aquatic Research Institute (MBARI), Scripps Institute of Oceanography (SIO) and Woods Hole Oceanographic Institute (WHOI). The HVB has been designed to withstand extreme temperatures (~ 400°C) and has been pressure tested to a simulated depth of 6,500 m. In-situ sensing devices have been positioned throughout the system to monitor real-time temperature and flow rates during sampling, ensuring that samples are collected from specific areas of interest (i.e. they are '*pristine*'). The position of the nozzle within the hydrothermal plume will be directed by real-time temperature sensors ensuring that the HVB samples as pure as possible. To account for low biomass samples, the HVB employs a series of pre-filters and a large surface area collection filter which effectively concentrates the particulate matter from 20 L of fluid into a final volume of 500 mL (filtered material is collected off the filter by back flushing with 0.5 L of sterile water).

The HVB is designed to be deployed by a manned or unmanned submersible, although it can be deployed using divers if the vents are accessible. The vehicle will descend with the sampler to hydrothermal vent, where a robotic manipulator on the submersible will grasp the intake nozzle (connected to the HVB through a flexible hose) and insert it into the vent. A cable connecting the HVB to a control station provides the system with power and data signals. This allows an operator to command and monitor the HVB throughout the sample collection process.

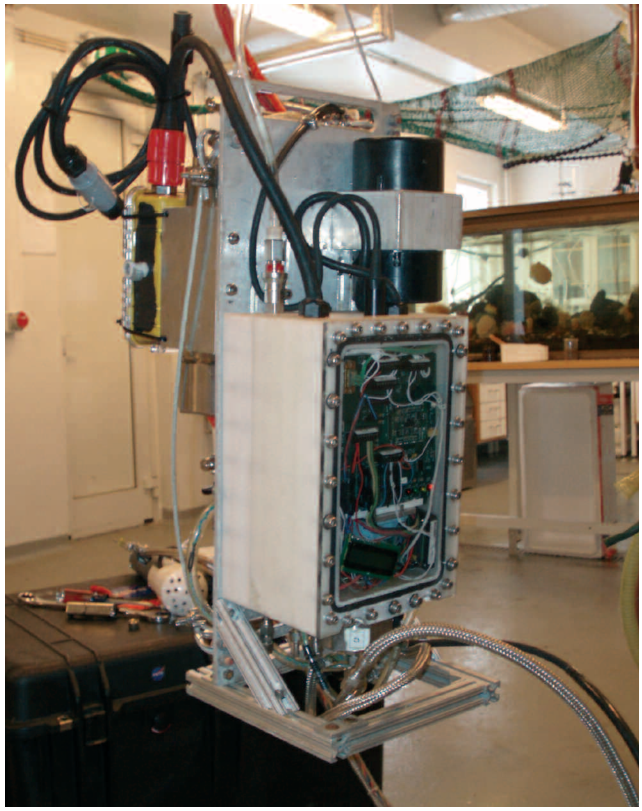

Figure 1. Hydrothermal Vent Sampler (front view). The Delrin® equipment box is mounted on the front of the HVB chassis and contains the systems electronics. The flexible tube extending from the bottom of the unit extends to the nozzle (not pictured).

Figure 1. Echantillonneur de source hydrothermale (vue de face). La boîte Delrin® est montée devant le châssis HVB et contient les systèmes électroniques. Le flexible sortant du bas de l'unité s'étend jusqu'au tube de prélèvement (non montré).

Materials and Methods

The HVB has a main aluminum chassis onto which most of the components are mounted. Affixed to the front of the chassis (Fig. 1) is an equipment box made from Delrin®, a high-strength thermoresistant polymer, which houses and protects the HVB's electrical components. The box is filled with Fluorinert™, a nonconductive and non-compressible fluid used to withstand the high pressure environment in the deep ocean. A flexible membrane filled with additional Fluorinert™ acts as a pressure compensator to fill in any residual air gaps and adjust for increasing external pressure. The equipment box also houses a pump, a servomotor to actuate a four-way valve, and a flow meter. These mechanical components are placed inside the equipment box to provide easier connection with the electronics, while the other less delicate mechanical components are placed outside the equipment box.

On the back side of the chassis are the remaining mechanical components: a series of pipes and filters constructed of 316 marine stainless steel. Three commercial in-line filters (Swagelok TF-series) with decreasing porosity (90, 60 and 7 µm) are used to prefilter the incoming fluid, and a custom-made 0.2 µm filter (Mott Corp.) with a large cross-sectional area is used to trap most of the biology in the hydrothermal vent fluid.

Currently the HVB in development has one set of such filters. In the final version, the system will have three identical sets of filter assemblies arranged in parallel, allowing for multiple sampling opportunities. Hydrothermal vent fluid enters the system through an intake nozzle located at the front end of the system. The intake nozzle (Fig. 2) is opened and closed by a servomotor and is connected to the main HVB system through a flexible hose. A stainless steel four-way valve (Swagelok, SS-45ZF8-ND) actuated by a servomotor directs the incoming fluid from the nozzle to either the bypass pipe or one of three filter assemblies (Fig. 3). The bypass pipe is used to flush the system to eliminate cross-contamination between samples. Pumping fluid through the bypass pipe prior to sample collection clears material

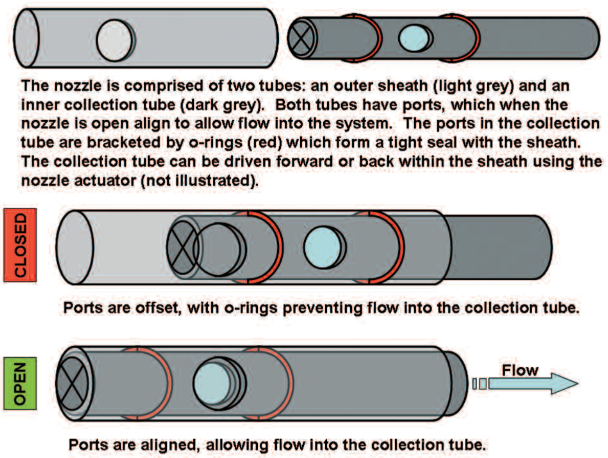

Figure 2. Diagram of the actuated HVB nozzle. Note that flow into the system can only occur when the nozzle is open. The nozzle has four ports located along its side 4 cm from its tip. The position of the ports should prevent accidental clogging during insertion into a hydrothermal vent.

Figure 2. Diagramme du tube de prélèvement HVB en action de marche. Noter que l'écoulement dans le systeme ne peut se produire que lorsque le tube est ouvert. Le tube possède quatre ports situés sur le côté à 4 cm de l'extrémité. La position des ports doit prévenir d'une obstruction accidentelle lors de l'insertion dans la source hydrothermale.

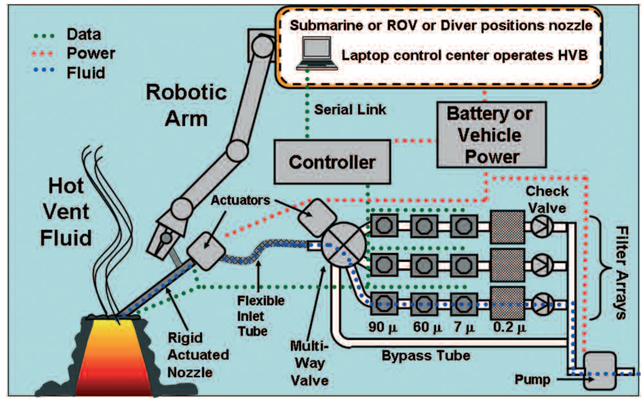

Figure 3. Cartoon illustrating the major components of the HVB. The unique nozzle design and the position of the valves (multi-way in the front and check valves at the rear) prevent contamination from bulk water.

Figure 3. Schéma montrant les composants principaux du HVB. La conception spécifique du tube et la position des vannes (vanne à sorties multiples à l'avant et vannes de contrôle à l'arrière) permet d'éviter la contamination de l'eau prélevée.

collected during previous samplings and flushes the HVB from the intake nozzle through the four-way valve. To begin sample collection, the four-way valve is rotated to one of the three filter assemblies opening the sample pathway.

The design of the HVB incorporates a bio-containment mechanism to protect samples from contamination after their collection. Each filter assembly is bracketed by the four-way valve at the front and a one-way SS Poppet check valve (Swagelok, SS-CHS4-KZ-1/3) at the rear. The check valves are opened only when the four-way valve is aligned to its filter assembly and the pump is turned on.

A peristaltic pump (Micropump GB-P35) driven by a brushless DC motor is located within the electronics box at the back of the piping system. This is done to protect the pump from high fluid temperatures at the front end of the system and eliminate the need to fully sterilize it. All components from the intake nozzle through the filters are sterilized and filled with sterile water before deployment to ensure the authenticity of the collected samples.

While the fluid pathway components (filters, pump, and flow meter) are connected together with stainless steel tubing (0.25" inner diameter), the data and electrical connections are contained within flexible hoses filled with Fluorinert™. These hoses act as additional pressure compensators; under pressure, these hoses would compress, accounting for bubbles trapped inside the electrical system.

A suite of sensors is used to monitor environmental conditions during sample collection. Several high-temperature K-thermocouples are located at the front end of the system and digital thermometers are located at each of the filters to monitor the conditions during sample collection. Two flow turbine-type flow meters (Omega Engineering, FTB-9501) monitor fluid movement through the system while a pressure sensor gives an indication of water depth.

Electronics

The electronics are placed inside the equipment box to protect them from the underwater environment. Power is supplied by a single 24 V DC supply and is fed in parallel to the DC pump motor and a power converter that provides the main circuit board with a 12 V DC supply. The idle system draws ~ 0.2 amps of current while, when active the pump draws ~ 2 amps, and each of the servomotors draws a maximum of 3 amps.

The main component of the circuit board is the OWL2pe Data Logger (EME Systems), which is built around a BASIC Stamp microcontroller alongside a real-time clock and an analog-to-digital converter. 512K of onboard Flash memory allows measurement data from the sensors to be logged.

The microcontroller communicates with most of the sensors through a serial SPI interface. The DC motor driving the pump is throttled using PWM, while the two servomotors are actuated using a Futaba-compatible control pulse. In order for the servomotors to operate in the high pressure environment, their onboard electrolytic capacitors have been replaced with solid capacitors.

Software System

The heart of the digital logic is the BASIC Stamp 2pe40. The software, written in PBASIC, has several well-defined tasks. After system initialization, the HVB polls its various sensors for measurement data. The data is logged onto the onboard memory and sent to the control station for display. It then awaits commands from the operator and performs the appropriate controls such as operating the pump or actuating the servomotors. The program's first iteration of the loop is now completed, and the system continually repeats the aforementioned tasks.

The total time needed to poll all the sensors is approximately 2 seconds, while the interval between measurement samples is configurable from 1 to 10 seconds.

Depending on the configured sampling interval, the onboard 512K of Flash memory allows for at least 2 hours of operations to be logged. The entire data log can be streamed to the control station after each sampling operation is completed.

Control Station

The control station, a graphical-user-interface (GUI) programmed in LabVIEW, can be run on a typical notebook computer. It communicates with the HVB system through a serial RS-232 interface. The control station displays measurement data from the HVB in virtually real-time. Controls are provided to actuate the pump and four-way valve servomotors. A timer function allows the operator to monitor the total amount of filtered fluid.

Results & Discussion

Testing in the lab has shown a satisfactory flow rate of around 0.6-0.7 $L.min^{-1}$ through the filtration system. At this rate, it would take the system < 20 minutes to filter 10 L of fluid. The system was run successfully in the lab for more than 60 minutes with negligible reduction in flow rate. Recent testing on hydrothermal vents in Iceland (March 2006) demonstrated the HVB capable of sampling for up to 90 minutes.

A series of tests were conducted in the field to subject the system to the environment similar to the ones encountered during deployment in the deep ocean.

The HVB was tested twice in a pressure chamber at the Scripps Institution of Oceanography (SIO) between June and August, 2005. The chamber was pressurized to 68.9 megapascal, analogous to a water depth of 6,500 metres. As of the second pressure test, all of the components were verified to be operational with the exception of a power converter. This converted as been replaced with a more robust model tested individually at 68.9 megapascal. Another full system pressure test at SIO is scheduled for the end of April, 2006.

In September 2005 and March 2006, the HVB was deployed at a hydrothermal vent off the northern coast of Iceland, near the city of Akureyri. In conjunction with the University of Akureyri, the HVB was tested to a depth of approximately 20 m, successfully filtering vent fluid reaching 77°C. Vent fluid was sampled for periods of ~ 10 minutes, 20 minutes, and approximate 90 minutes (3 different dives) before the HVB was brought to the surface and the samples recovered. The HVB maintained a constant flow of 0.6-0.7 $L.min^{-1}$ throughout all of the trials. Temperature data collected by the HVB indicated a constant 75-77°C at the nozzle, 25°C at the four-way valve, and 3-4°C at the filters. Samples from the 2006 dives are currently being processed at the University of Akureyri and JPL. Initial evaluation of the salinity and pH of the samples indicated high purity (the pH and salinity of water from these vents is known; Marteinsson et al., 2001).

The HVB and all of the tools needed for in-field servicing were brought to Iceland in the normal luggage allowance for three people on-board a commercial aircraft. This portability is a unique feature to the HVB, as other samplers (such as the HFPS) weigh in excess of 100 kg and cannot be easily transported. Because the only required interfaces for the HVB are a 24 V DC power line and a single serial link, it has proven to be easy to integrate and adapt to fit various means of deployment (boats, pressure chambers, ROVs, etc.) This was verified by easy setup and low impact that the HVB had on the University of Akureyri's boat.

Future Work

The HVB system is still in the development phase. The current model has only one filter assembly, so work remains to be done to incorporate three filter assemblies in parallel to allow collection of multiple samples. The tests conducted so far are only functional tests to verify the operation of the system. Procedures need to be established for sterilizing the unit and handling the filters to achieve proper bio-containment. Current work indicates that using a system of sterilized tubes and external pump to back flush the 0.2 μm filter with sterile water maintains sample integrity, although continued testing needs to be done to assess the effectiveness of 'in-field' sterilization.

After the system is fully developed, the HVB system is envisioned to travel onboard a research vessel, where it can be deployed by a submersible at a hydrothermal vent in the deep ocean.

Addendum

As of November 2006, a fully operational HVB with three filter pathways has undergone successful field trials along the Izu-Bonin Arc (Japan) at the Myojin Knoll and Suiyo Seamount sites. Integrated with the ROV HYPER-DOLPHIN (courtesy of JAMSTEC), the HVB successfully collected samples >10L from vents at depths of 1.2-1.4 km with water temperatures greater than 300°C.

Acknowledgements

The work described in this paper was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration. The task is funded through NASA's Astrobiology Science and Technology Instrument Development Program. The authors would like to thank the scientists and engineers at the Scripps Institution of Oceanography (Charles Coughran, Dave Agleitti), Woods Hole Oceanographic Institute (Tim Shank, Chris German, Dana Yoerger), Monterey Bay Aquarium Research Institute (Hans Thomas), the School of Ocean and Earth Science and Technology-University of Hawaii at Manoa (Alexander Malahoff), and the University of Akureyri, Iceland (Hreidar Þor Valtýsson, Hjörleifur Einarsson) for their outstanding advice and use of their facilities.

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