

WISSARD Biological Clean Access Testing: Phase II **WIS-Spot test site, Ross Ice Shelf, 17-18 December 2012**

Introduction

The WISSARD hot water drilling system includes a water treatment system designed to remove micron and sub-micron sized particles (biotic and abiotic), irradiate the drilling water with germicidal UV radiation, and pasteurize the water to reduce the viability of persisting microbial contamination. The purpose of this system is to generate and deliver a clean and reliable supply of hot water for drilling boreholes through the ice sheet and into Subglacial Lake Whillans (SLW) and the interconnected subglacial drainage system. Figure 1 provides a schematic of the drilling system components, together with the location of selected sampling ports used for collection of the water for microbiological analysis.

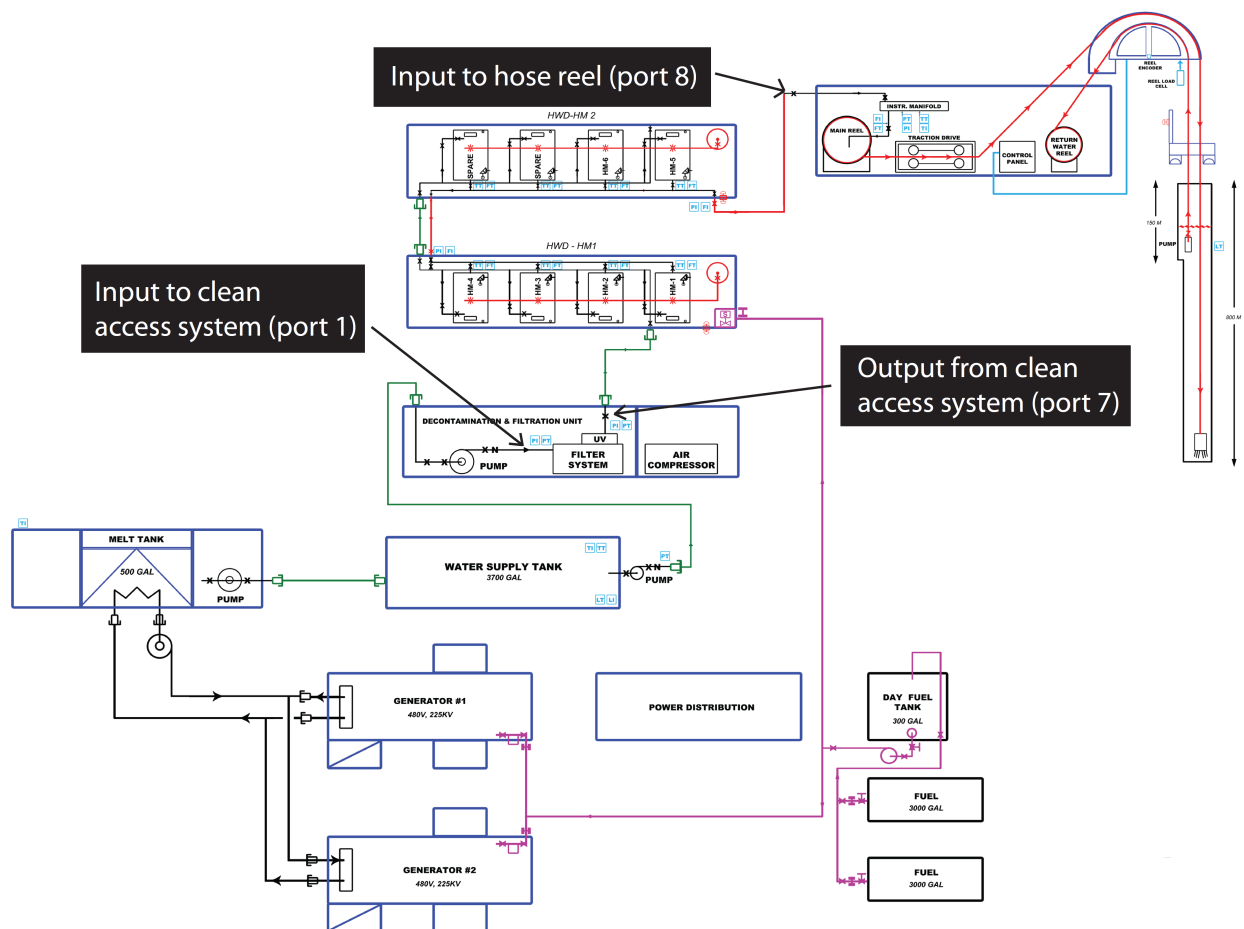


Figure 1 WISSARD hot water drill showing the location of ports used to assess the efficacy of the clean access system during testing on the McMurdo Ice Shelf during 17-18 December 2012. The recirculating pump shown was not used during the test.

Objectives

During January 2011, we conducted a test of the filtration and UV system at Montana State University (Phase I), the details of which are reported in Priscu et al. (in press). In summary, this test indicated that one passage of a volume of pond water through the filtration component of the system reduced the total number of microbial cells by more than 4-log units. Cells that remained in the water after filtration were reduced another 3.5 log units by the combined effect of UV irradiance and pasteurization.

The specific objectives of the field test (Phase II) were as follows:

1. Demonstrate the operational capabilities of the clean access system with large volumes ($\sim 115 \text{ m}^3$) of melted Antarctic snow when integrated into the drill system under field conditions.
2. Determine the effectiveness of the clean access system to remove and kill cells from the effluent.
3. Establish the effect of high loads of submicron particles on the flow rate through the filters.

Methods

The Phase II test was conducted from 17-18 December 2012 in the Windless Bight area of the McMurdo Ice Shelf, Antarctica. Water supply for the drill system was provided using a loader to recover snow from the area surrounding the drill site and place it into a snow melter. The melter was capable of supplying up to $\sim 190 \text{ L min}^{-1}$ of water during drilling operations. This water was pumped through the filtration modules and UV system, heated, and then transferred via a 3.1-cm diameter hose line 1,000 m long to discharge through the drill head nozzle (Fig. 1). During hot water drilling at SLW, water will be continuously removed from the borehole using a submersible pump positioned near the top of the borehole and returned to the filtration system, thus causing water in the borehole to circulate upward. However, during Phase II test operations on the McMurdo Ice Shelf (77.890 South, 167.008 East), the return pump was not used and water was not circulated to prevent seawater from entering the system. During the Phase II test, water temperature, flow rate, length of hose deployed, and tension on the hose were monitored continuously.



Figure 2 Water collection of from port 1 and 7 in the WFU (left) and port 8 (right).

Approximately 115 m³ of water passed through the water filtration module (WFU) during the field test until the 0.2 µm filters were clogged (i.e., output from the 0.2 µm filtration module was < 15 PSI). Prior to clogging, discrete samples were collected from ports plumbed along the water flow path (Fig. 2) and the concentration of total and viable cells determined.

Before the start of drilling on day 1 of the test, a sample was taken from port 1 (the input to the 2 µm filtration module) while the system was charged with water and circulated at 190 L min⁻¹ for 5 h. This sample served as a baseline for the initial cleanliness of the fully assembled drill system prior to any passage of water through the clean access module. On day 2, water was sampled from ports 1 and 7 at the initiation of drilling (T = 0 h), 2 h later (when the drill was at a depth of ~27 m and within the firn layer), and 4.5 h (after the drill had penetrated the 56 m ice shelf and the hose was at a depth of 62 m below the surface). At the 4.5 h time point, a sample was also collected from a port that was plumbed after the heater units, but prior to the input to the hose reel (port 8; Fig. 1). This sampling scheme is summarized in Table 1.

Table 1 Summary of samples collected during the Phase II test and rationale for each sample.

| Day | Port | Rationale |
|------------|-----------|--|
| 1 | 1 | Examine initial cell concentration associated with system after assembly. |
| 2; T=0 h | 1 & 7 | Determine cell reduction through WFU at the initiation of drilling. |
| 2; T=2 h | 1 & 7 | Determine cell reduction after passage of ~28 m ³ of water through the WFU. |
| 2; T=4.5 h | 1, 7, & 8 | Determine cell reduction after passage of ~51 m ³ of water through the WFU and cell contamination associated with passage through the heater units. |

Procedures for measuring the concentrations of cells in water from the Phase II test followed the same protocols used in the Phase I test (Priscu et al. in press). Briefly, water collected for direct cell counting was fixed with formalin (5% final concentration), 10 or 100 mL of sample was filtered onto 0.2 µm black polycarbonate filters, stained with SYBR Gold (Molecular Probes, Inc.), and enumerated by counting 60 fields using an epifluorescence microscope (Zeiss, Akioskop 50). SYBR Gold stains intracellular DNA and thus represents the number of cells containing DNA. The concentration of cellular adenosine-5'-triphosphate (ATP), a sensitive proxy for metabolically viable microbial biomass, was measured using the luciferin/luciferase assay (Lundin 2000) and 60 or 100 mL of concentrated sample from port 1 and 7, respectively. Measurements were made with a 20/20n luminometer (Turner Bio Systems) after the addition of 50 µL of the luciferase/luciferin reagent. Light emission was integrated over a 10 s period for each measurement. The ATP concentration was used to estimate the metabolically viable cell density based on a conversion factor of 2×10^{-18} mol of ATP per viable cell (Lundin 2000; Biothema Corp.).

Results and Discussion

The concentration of DNA-containing cells (i.e., stained with SYBR Gold) entering the WFU (port 1) ranged from 2.7 to 7.6 x 10³ cells mL⁻¹ and water exiting the UV modules (port 7) contained 1.2 to 3.8 x 10² cells mL⁻¹ (Fig. 3A). The metabolically viable cell concentration estimated from measurement of ATP at port 1 ranged from 2.5 to 5.6 x 10² cells mL⁻¹, whereas the concentration at port 7 was estimated to

be $< 6.5 \times 10^1$ cells mL^{-1} (Fig. 3B). Due to a technical error, no sample was available to analyze cellular ATP from port 8. Statistical analysis of the data from port 7 indicated that the cell density measurements from the 2h and 4.5 h samples (Fig. 3A) were significantly different from the blank control (Mann-Whitney test; $p < 0.008$), whereas the ATP measurements revealed that the 2 h and 4.5 h samples (Fig. 3B) were not above the level of detection under the conditions tested ($p > 0.05$). Overall our results indicate that passage of snow melt through the WFU and UV lamps resulted in a 99% reduction in the concentration of microbial cells, and of the total assemblage remaining, $< 50\%$ of these cells were inferred to be viable. Based on measurement of water from port 8 (Fig. 1), passage of water through the boilers and associated plumbing downstream of the WFU, which had not been cleaned since tests in the US, added a negligible concentration of cells (maximum increase of 4-fold observed between port 7 and 8; Fig. 3A).

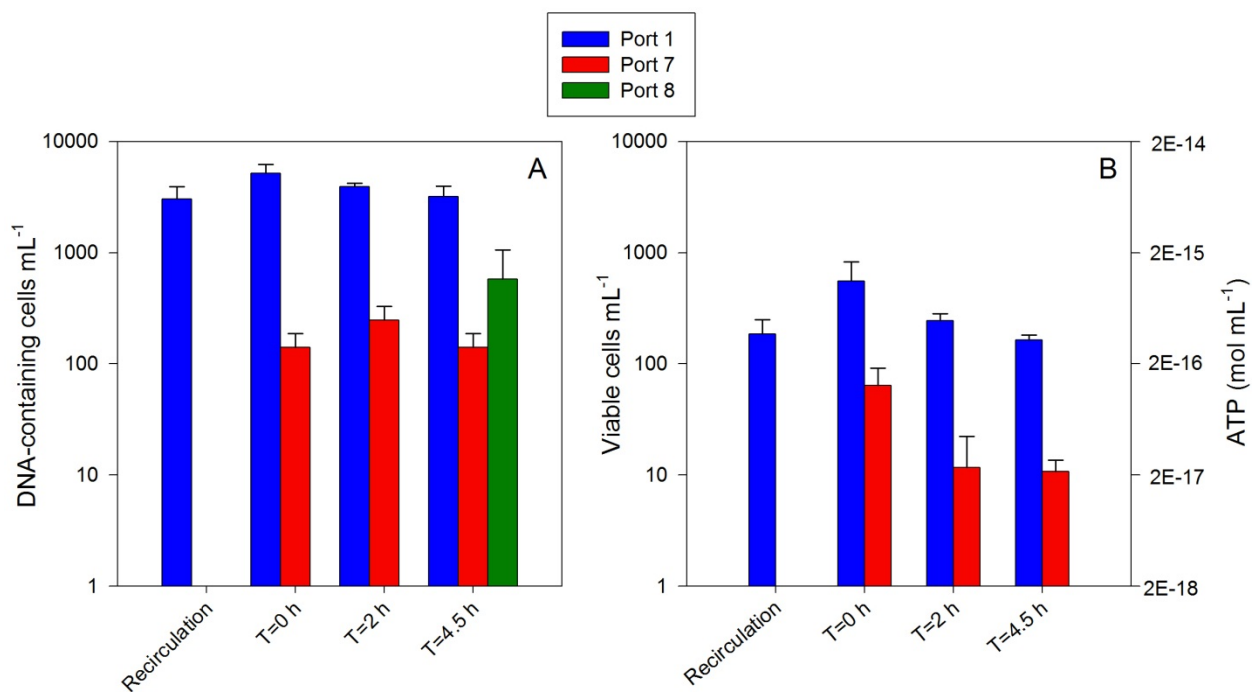


Figure 3 Concentration of directly enumerated DNA-containing cells (A) and cell density estimated using the concentration of extracted ATP (B). Error bars indicate the standard deviation from the mean ($n=60$ in panel A; $n=3$ in panel B). See Table 1 for details on sample collection and rationale.

Conclusion

Recommendation 7 of the 2007 NRC report, *Exploration of Antarctic Subglacial Aquatic Environments: Environmental and Scientific Stewardship* states that:

“Drilling in conjunction with sampling procedures will inevitably introduce microorganisms into subglacial aquatic environments. The numbers of microbial cells contained in or on the volume of any material or instruments added to or placed in these environments should not exceed that of the

basal ice being passed through. Based on research to date, a minimum of 10^2 cells mL^{-1} should not be exceeded, until more data are available.” (NRC 2007)

Based on results from an integrated test of the WISSARD drill system, in concert with those obtained during our bench tests during Phase I (Priscu et al, in press), the clean access technologies that WISSARD will employ during drilling and sampling operations at SLW meet the standards recommended by the NRC and SCAR Code of Conduct (2011). The sampling strategy used in both the Phase I and Phase II tests will be employed during drilling operations at SLW to ensure that the subglacial environment is accessed in a clean manner.

References

Lundin, A. 2000. Use of firefly luciferase in ATP-related assays of biomass, enzymes, and metabolites. *Methods Enzymol.* Part C 305: 346-370.

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