Video Article

Large Volume (20L+) Filtration of Coastal Seawater Samples

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URL: http://www.jove.com/details.php?id=1161
DOI: 10.3791/1161

Keywords: Molecular Biology, Issue 28, microbial biomass, filtration, sterivex, GF/D, nucleic acids, seawater, fjord, hypoxic, Saanich Inlet,

Date Published: 6/18/2009

Citation: Walsh, D.A., Zaikova, E., Hallam, S.J. Large Volume (20L+) Filtration of Coastal Seawater Samples. J. Vis. Exp. (28), e1161, DOI : 10.3791/1161 (2009).

Abstract

The workflow begins with the collection of coastal marine waters for downstream microbial community, nutrient and trace gas analyses. For this method, samples were collected from the deck of the HMS John Strickland operating in Saanich Inlet. This video documents large volume (≥20 L) filtration of microbial biomass, ranging between 0.22µm and 2.7µm in diameter, from the water column. Two 20L samples can be filtered simultaneously using a single pump unit equipped with four rotating heads. Filtration is done in the field on extended trips, or immediately upon return for day trips. It is important to record the amount of water passing through each sterivex filter unit. To prevent biofilm formation between sampling trips, all filtration equipment must be rinsed with dilute HCl and deionized water and autoclaved immediately after use. This procedure will take approximately 5 hours plus an additional hour for clean up.

Video Link

The video component of this article can be found at http://www.jove.com/details.php?id=1161

Protocol

1. **Set up of filtration apparatus**
    1. To set up the filtration apparatus, use tweezers to place a grade GF/D filter into the prefilter housing unit. Loosely tighten the housing by hand. As a rule of thumb, do not over-tighten or the unit will leak once the pumping starts.
    2. Replace the carboy cap with the autoclaved cap and attached tubing by carefully inserting the internal tubing into the carboy without touching it. If necessary, use tweezers to guide the tubing into the carboy.
    3. Pass the external tubing through the peristaltic pump.
    4. Attach prefilter housing units to the end of both external tubes (i.e. 2 units per carboy). Tighten the connection with pliers.

2. **Filtration of seawater**
    1. To begin filtering the seawater, turn on the pump and set the flow speed to 1. Watch to ensure that water is being pumped in the proper direction.
    2. Allow 500 ml of water to flow freely through the prefilter unit and into a 1 L Beaker.
    3. Turn off the pump, and attach a Sterivex filter to the male luer fitting at the free end of prefilter setup. Tighten the prefilter housing snugly by hand.
    4. Watch for at the prefilter housing and at the connection between the prefilter housing and the tubing that leads to the carboy. If leaks occur, turn the pump off and loosen and retighten the prefilter housing accordingly. Turn the pump back on.
    5. Collect the filtrate in 4 L flasks. Once they are full, empty the flasks into a clean carboy. Be sure to note the volume passed through each filter. Collected filtrate can be further processed, such as filtration for viruses.
    6. Continue filtering until no water is left in the carboys. Usual filtration times range between 4-5 h / 20 L carboy; however, this depends dramatically on the amount of biomass within the water.
    7. Once filtration is complete, switch the pump off and disconnect the Sterivex filters.

3. **Storage of filters**
    1. To store the filters, first expel any remaining water with a 30-60 ml syringe.
    2. Using a pipette, add 1.8ml lysis buffer to the filter, keeping ~ 200 ul of space in the filter for later addition of reagents.
    3. Seal the bottom and top of the Sterivex filter with a small piece of Parafilm, and label the filters with the date and sample identification.
    4. Store the filters in a pre-labeled 50 ml falcon tube at -80°C
    5. Remove the filters from the housing unit and place them into labelled 50 ml Falcon tubes containing 2 ml of lysis buffer. Store these at -80°C.

4. **Cleanup**
    1. For final cleanup, place the tubing that was inside the carboy into a flask containing 0.1 M HCl
    2. Direct the tubing that lead to the 4L flask into the original carboy.
    3. Switch on the pump and run at 3, collecting the flow through run in the original carboy.
    4. Finally, rinse the tubing with autoclaved water.

Representative Results: The final result consists of two Sterivix filters each containing microbial biomass from approximately 10 L seawater in the size range of 0.22µm and 2.7µm. This is stored in lysis buffer and ready for DNA extraction. In addition, this procedure collects and

concentrates biomass greater than 2.7 µm, on the GF/D prefilters. This is also stored in lysis buffer and ready for DNA extraction. If additional processing steps are taken, the 0.22 µM flow-through can be concentrated down to collect the viral fraction as well.

Discussion

It is imperative to wipe down benches and pumps with a damp towel after use to remove any lingering traces of salt water that would otherwise corrode equipment. Although the procedure is simple, the time requirement can be extensive depending on the concentration of biomass or debris in the sample being filtered.

Acknowledgements

We would like to thank the Canadian Foundation for Innovation, the British Columbia Knowledge Development Fund and the National Sciences and Engineering Research Council (NSERC) of Canada for supporting ongoing studies on low oxygen regions of coastal and open ocean waters. D.A.W. was supported by fellowships from NSERC, Killam and the TULA foundation funded Centre for Microbial Diversity and Evolution.

References

