Tangential flow filtration (TFF) concentration of phytoplankton Version 2

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Abstract

Concentrate phytoplankton samples about 100-fold typically from 5L down to 20 mL. Takes about 1 hour per sample. Samples can be used for flow cytometry sorting or for cultures. Enrichment by TFF usually keep growing for a longer time than unconcentrate samples.

Reference

Marie, D., Shi, X.L., Rigaut-Jalabert, F. & Vaulot, D. (2010). Use of flow cytometric sorting to better assess the diversity of small photosynthetic eukaryotes in the English Channel. *FEMS Microbiology Ecology*. 72. p.pp. 165–178.

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Guidelines

In order to estimate actual concentration efficiency measure Synechoccus, pico, and nanoeularyote concentration before and after TFF.

Before start

Necesssary equipement

- Vivaflow Cartridge 100,000 MWCO (Regenerated Cellulose - RC) VF20C4 for viruses
- Vivaflow Cartridge 0.2 µm (PES) VF20P7 for plankton
- Masterflex Pump 6-600 rpm (ref Bioblock F39671)
- Rotor 3 “galets” (ref F39110)
- 1 Head high throughput (ref F40103) (Can be replaced with quick load head)
- Replace tube provided by stronger tube with two connectors (see picture)

- Bottle 6 L
- Bottle 1 L
- Conical tube 50 mL
- Masterflex Tygon tubing size 16
- Plastique pipettes (1 mL)
- Clamps with screw (to control retentate speed)
- Clamps

Materials

- MilliQ water by Contributed by users
- Filtered Seawater (0.2 µm) by Contributed by users
- 0.1 M NaOH by Contributed by users
- Ethanol 10% by Contributed by users

Protocol

Rinsing cartridge

Step 1.

Get Vivaflow catridge out of storage

Rinsing cartridge

Step 2.

Mount Vivaflow catridge as Fig. 1 (image de C. Brussaard) in open circuit.

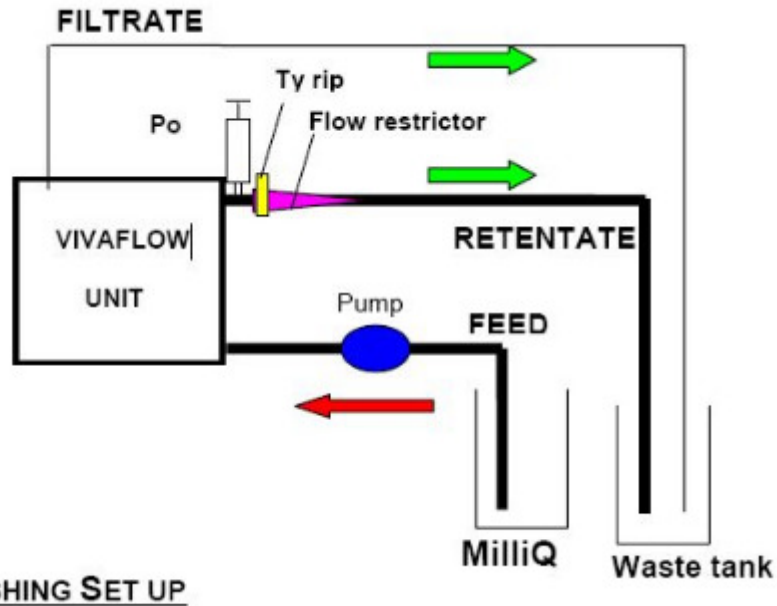

FIG 1. FLUSHING SET UP

Rinsing cartridge

Step 3.

Remove the clamps

Rinsing cartridge

Step 4.

Set the pump to maximum speed. Manometer should be at about 2.5 bars (with a new cassette

sometimes the manometer get stuck, if the value is too low there is a leak in the system)

Rinsing cartridge

Step 5.

Rinse the cartridge with about 250 mL of MilliQ water (longer is cartridge has been stored in ethanol)

AMOUNT

250 ml Additional info:

REAGENTS

MilliQ water by Contributed by users

DURATION

00:10:00

Rinse cartridge

Step 6.

Replace MilliQ water by sample in 6 L bottle

Rinse cartridge

Step 7.

Take sample for flow cytometry to compute concentration factor.

Rinse cartridge

Step 8.

Rinse cartridge with about 250 mL of sample

Concentrate

Step 9.

Put the retentate line into the sample bottle (Fig. 2B - Recirculation mode). Record sample volume and start pump increasing to macimum speed.

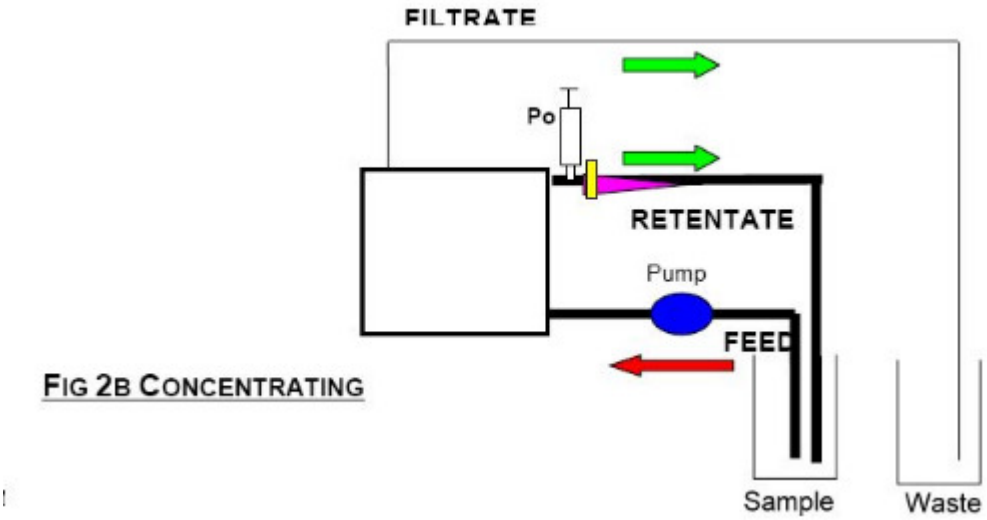

FIG 2B CONCENTRATING

Concentrate

Step 10.

Clamp the retentate line to increase filtrate flow so that Manometer gets up to 2.5 bars.

Concentrate

Step 11.

Concentrate sample until about 250 mL remains (6 L takes about one hour)

DURATION

01:00:00

Concentrate

Step 12.

Transfer sample to a smaller bottle (250 mL) then finally to a 50 mL tube with conical bottom

DURATION

00:10:00

Concentrate

Step 13.

Continue to concentrate very carefully, lowering the pump speed until the sample volume is reduced to 15-20 mL

NOTES

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It is very important to lower the pump speed inonder to avoid loosing the sample.

Recirculation

Step 14.

When final volume is about 10 mL, clamp filtrate tube and recirculate slowly (no change of volume should take place)

Recirculation

Step 15.

Leaving the filtrate tube clamped, get the feed line out of the sample in order to get back the total volume of concentrated sample

Recirculation

Step 16.

Take sample for flow cytometry count (to compare with original sample concnetration and estimate concentration factor)

Recirculation

Step 17.

Store concentrated sample for later use (e.g. flow cytometry sorting, culture etc...)

Rinsing

Step 18.

Go back to Fig. 1 configuration (Open circuit)

Rinsing

Step 19.

Rinse 1 min with filtered sea water

REAGENTS

- Filtered Seawater (0.2 µm) by Contributed by users

DURATION

00:01:00

Rinsing

Step 20.

Rinse 1 min with distilled water

REAGENTS

- Distilled Water by Contributed by users

DURATION

00:01:00

Rinsing

Step 21.
Rinse with 50 mL NaOH 0.1 M

AMOUNT
50 ml Additional info:

REAGENTS
- 0.1 M NaOH by Contributed by users

Rinsing

Step 22.
Put all three tubes (feed, retentate, filtrate) in bottle containing NaOH 0.1 M

REAGENTS
- 0.1 M NaOH by Contributed by users

Rinsing

Step 23.
Recirculate for 20 min (to get rid of everything on the cartridge filter)

DURATION
00:20:00

Rinsing

Step 24.
Rinse with 250 mL of MilliQ water (Fig. 1)

AMOUNT
250 µl Additional info:

REAGENTS
- MilliQ water by Contributed by users

Storage

Step 25.
Stop the pump and clamp all three tubes

Storage

Step 26.
Store at 4°C. For a storage beyond 1 day, store with Ethanol 10%

REAGENTS
- Ethanol 10% by Contributed by users