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 We use this protocol and it's working

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Tangential flow filtration (TFF) concentration of phytoplankton V.3

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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. & Vaultot, 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.](#)

GUIDELINES

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

MATERIALS

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☒ MilliQ water **Contributed by users**

☒ Filtered Seawater (0.2 μ m) **Contributed by users**

☒ 0.1 M NaOH **Contributed by users**

☒ Ethanol 10% **Contributed by users**

STEP MATERIALS

☒ MilliQ water **Contributed by users**

☒ Filtered Seawater (0.2 μ m) **Contributed by users**

☒ Distilled Water **Contributed by users**

☒ 0.1 M NaOH **Contributed by users**

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☒ MilliQ water **Contributed by users**

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Necesssary equipement

- Vivaflow Cartridge 100,000 MWCO (Regenerated Cellulose - RC) VF20C4 for viruses
- Vivaflow Cartridge 0.2 μ m (PES) VF20P7 for plankton
- Masterflex Pump 6-600 rpm (ref Bioblock F39671) - It is critical to have a 600 rpm pump, lower speed will not work. The rate of the pump must be adjustable.
- 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 (Falcon tube)
- Masterflex Tygon tubing size 16
- Plastique pipettes (1 mL) - this is used to plunge in the sample
- Clamps with screw (to control retentate speed)

Solutions

- MilliQ water : 1L
- NaOH 0.1 N : 500 mL
- EtOJ 10% : 500 mL

Rinsing cartridge

- 1 Get Vivaflow catridge out of storage
- 2 Mount Vivaflow catridge as Fig. 1 (image de C. Brussaard) in open circuit.

- 3 Remove the clamps
- 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)
- 5 Rinse the cartridge with about 250 mL of MilliQ water (longer is cartridge has been stored in ethanol)

250 mL
00:10:00

MilliQ water **Contributed by users**

Rinse cartridge

- 6 Replace MilliQ water by sample in 6 L bottle
- 7 Take sample for flow cytometry to compute concentration factor.
- 8 Rinse cartridge with about 250 mL of sample

Concentrate

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

- 10 Clamp the retentate line to increase filtrate flow so that Manometer gets up to 2.5 bars.
- 11 Concentrate sample until about 250 mL remains (6 L takes about one hour)
⌚ 01:00:00
- 12 Transfer sample to a smaller bottle (250 mL) then finally to a 50 mL tube with conical bottom
⌚ 00:10:00
- 13 Continue to concentrate very carefully, lowering the pump speed until the sample volume is reduced to 15-20 mL

Note

It is very important to lower the pump speed in order to avoid losing the sample.

Recirculation

- 14 When final volume is about 10 mL, clamp filtrate tube and recirculate slowly (no change of volume should take place)
- 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
- 16 Take sample for flow cytometry count (to compare with original sample concentration and estimate concentration factor)
- 17 Store concentrated sample for later use (e.g. flow cytometry sorting, culture etc...)

Rinsing

- 18 Go back to Fig. 1 configuration (Open circuit)
- 19 Rinse 1 min with filtered sea water
00:01:00
Filtered Seawater (0.2 µm) **Contributed by users**
- 20 Rinse 1 min with distilled water
00:01:00
Distilled Water **Contributed by users**
- 21 Rinse with 50 mL NaOH 0.1 M
50 mL
0.1 M NaOH **Contributed by users**
- 22 Put all three tubes (feed, retentate, filtrate) in bottle containing NaOH 0.1 M

0.1 M NaOH **Contributed by users**

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

00:20:00

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

250 μ L

MilliQ water **Contributed by users**

Storage

25 Stop the pump and clamp all three tubes

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

Ethanol 10% **Contributed by users**