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

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Ecology of Marine Plank... Roscoff Culture Collection

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Protocol status: Working
We use this protocol and it's working

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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.

Guidelines

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

Materials

MATERIALS

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

STEP MATERIALS

- MilliQ water
- Filtered Seawater (0.2 µm)
- Distilled Water
- 0.1 M NaOH
- 0.1 M NaOH
- MilliQ water
- Ethanol 10%
- MilliQ water
- Filtered Seawater (0.2 µm)
- Distilled Water
- 0.1 M NaOH
- 0.1 M NaOH
- MilliQ water
- Ethanol 10%

Protocol materials

- Ethanol 10%
- MilliQ water
- Distilled Water
- 0.1 M NaOH
- Filtered Seawater (0.2 µm)
- Distilled Water
- 0.1 M NaOH
- MilliQ water
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- 0.1 M NaOH
- Ethanol 10%
- Ethanol 10%
- Filtered Seawater (0.2 µm)
- MilliQ water
- Filtered Seawater (0.2 µm)
- Distilled Water
- 0.1 M NaOH
- 0.1 M NaOH
- MilliQ water
- Ethanol 10%

Troubleshooting

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) - 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.

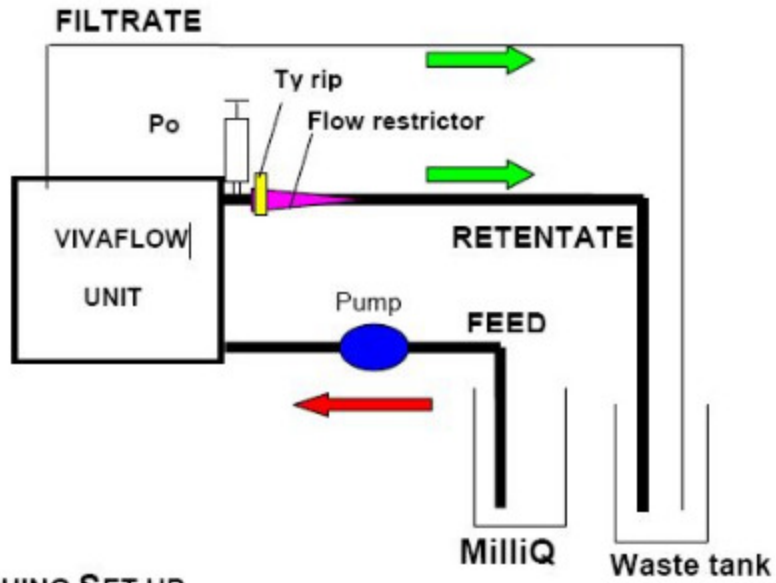

FIG 1. FLUSHING SET UP

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

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 macimum speed.

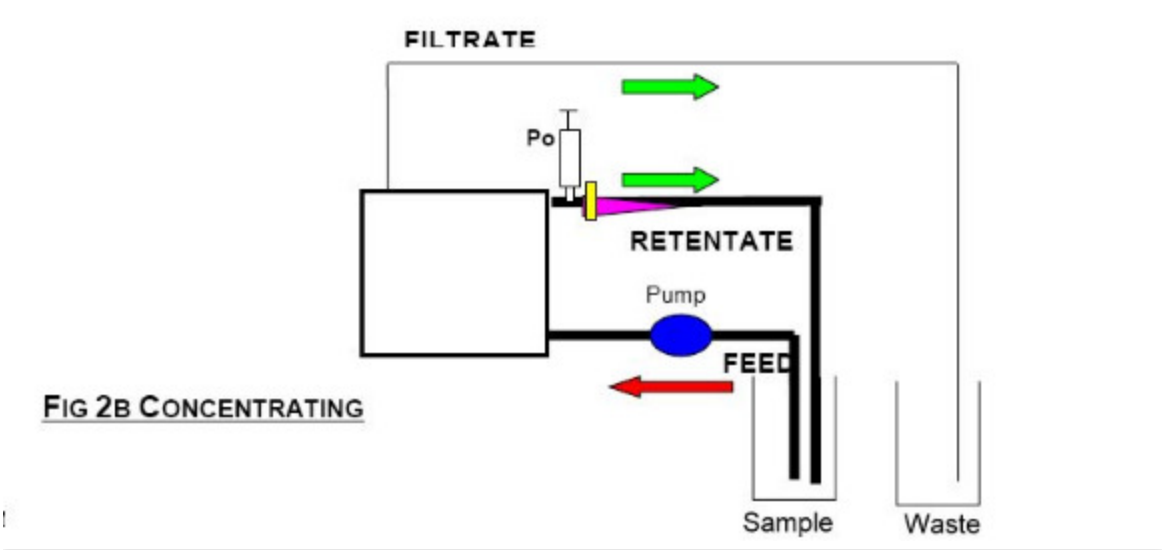

FIG 2B CONCENTRATING

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 inonder to avoid loosing 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 concnetration 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)

20 Rinse 1 min with distilled water
00:01:00
Distilled Water

21 Rinse with 50 mL NaOH 0.1 M
50 mL
0.1 M NaOH

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

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 µL

MilliQ water

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%