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Periphyton growing and sampling in situ or in mesocosm for metabolomic analysis V.2

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We use this protocol and it's working

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Abstract

This document describes the various steps involved in recovering biofilm exposed in mesocosm (on glass slides) or in the natural environment (on glass slides or natural substrates) for metabolite analysis.
The aim is to describe the procedures as exhaustively as possible so that all samples are treated in the same way. The sample fixation (quenching) step is a key step that must be performed as quickly as possible to ensure the accuracy of the results. Depending on the feasibility of quenching in the fieldor in the laboratory, a choice must be made between liquid nitrogen and dry ice.

Materials

- Glass slides of variable size (e.g. 210 × 75 × 4 mm) or microscope slides or natural substrates like pebbles
- Ice block
- Nitrogen tank with liquid nitrogen for transport or storage, or dry ice in a polystyrene cooler.
- Freeze dryer
- Freezer -80°C
- Razor blade to scrape the slides or brush (like a toothbrush) to scrape the stones
- Aluminum foil burned 8 hours in the oven at 450°C
- Solvent (ethanol or methanol (U)HPLC grade) for cleaning glassware

Safety warnings

Sample storage and transport in ice
safety rules for using liquid nitrogen and dry ice

Before start

Draw up a sampling plan and set constraints for quenching, particularly in the case of sampling in the natural environment.

Slides periphyton colonization and sampling

1d 16h

1 Slides periphyton colonization in environnemental system

8h

- Slides must be burned in oven in advance at 450 °C for 08:00:00 or washed with Methanol or Ethanol (grade (H)UPLC).
- Slides must be exposed on the field with an adapted system (e.g cages, transparent plastic box) minimum 3 weeks to have enough biomass (depending on the season)

Glasses must be in water few centimeters from the surface.

Slides on inox support in cage

2 Slides recovery

- The slides are drained and wrapped in aluminum foil.
- Label each slide on the aluminum foil with a suitable marker.
- Keep it cool in a cooler with ice packs or crushed ice.
- Immerse the slides or support in liquid nitrogen as quickly as possible using a suitable canister.

- Store it in -80°C

Equipment

Freezer	NAME
-80°C	TYPE
Thermo Scientific	BRAND
ultra-low freezers (ULT)	SKU
https://www.thermofisher.com/us/en/home/life-science/lab-equipment/cold-storage/lab-freezers/ultra-low-temperature-freezers-minus-80.html	LINK

Since it is not always possible to use liquid nitrogen in the field, dry ice can be used to quench samples. The chosen method must be applied to all samples in the project.

Quenching samples in liquid nitrogen

3 Biofilm Recovery 1d 8h

- Slides are lyophilized for 24:00:00 . *See lyophilizer quality documentation.*
- Following lyophilization, scrape each slide onto pre-calcined aluminum foil (450 °C ; 08:00:00) and an aluminum weighing dish.
- Weigh the amount of biofilm recovered in the aluminum foil.

- Ensure each sample is clearly identified.
- Store in the freezer at -80 °C .

Recovery of lyophilized biofilm on a slide

Environnemental periphyton colonization and sampling

1d 16h

4 Special case: biofilm collection from substrate

- In the natural environment, collect biofilm from a substrate such as pebbles.
- Using a brush, recover the biofilm with some surrounding water.
- Transfer into pre-labeled cryotubes.
- Quench quickly in liquid nitrogen or dry ice.
- It is important to record the sampled surface area, either by using a template to always sample the same area, or by wrapping the sampled surface with aluminum foil and measuring it later in the laboratory.
- If necessary, store at -80 °C .
- Freeze-dry the samples.

Growing periphyton for inoculum and colonize periphyton in 9 mm glass discs

5 **Colonize periphyton in Gazinet-Cestas pond**

Install glass slides (25 cm* 8 cm) in the cage, colonize at the Gazinet-Cestas pond, collect the glass slides at least after one month. If possible, avoid *in situ* colonization starting in December to February since periphyton might grow very slow at this season.

6 **Colonize periphyton in glass discs**

- Use of 6 times diluted DAUTA (Dauta 1982) as colonization solution. Adjust the pH into 7 before use.
- Alternative: use pond water with nutrients. Pond water sieved at 200 µm, add nutrients K2HPO4 and KNO_3 (final concentration: 0.23 mg/L PO_4; 2.4 mg/L NO_3). If prepare 2000 mL solution, add K_2HPO_4 (6.25 g/L) 135 µl and KNO_3 (50 g/L) 156 µl.
- Using 0.5 L beaker (110 mm) as colonization container

(1) Prepare inoculum: scratch 2 recto-verso slides (from part 5) with 100 mL 6x diluted DAUTA in a 0.5 L beaker. Sieve through a 200 µm mesh.
(2) Add 200 mL 6x diluted DAUTA in another 0.5 L beaker as colonization container.
(3) Place ~80 discs (48-well size, 9 mm) at the bottom of container

(4) Gently add 100 mL prepared inoculum to the container. The total volume is 300 mL.

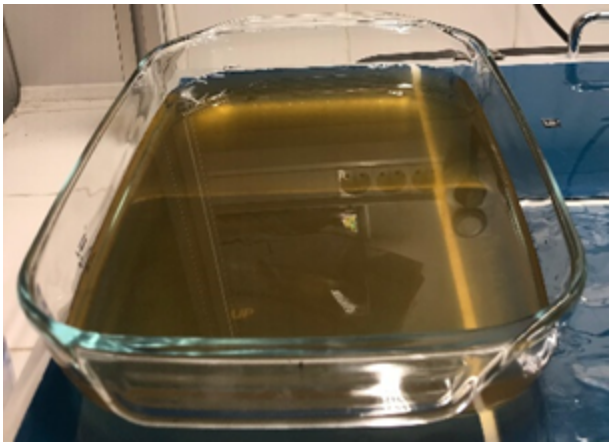
- Adaptation to one lasagna plate as colonization container

(1) Prepare inoculum: scratch 5-6 recto-verso slides with 300 mL 6x diluted DAUTA in a 0.5 L beaker. Sieve through a 200 µm mesh.

(2) Add 1.2 L 6x diluted DAUTA solution to the lasagna plate

(3) Place maximally 450 discs (48-well size, 9 mm) at the bottom of plate, leave some empty space for the ease of changing solution etc.

(4) Using pipette to gently add 300 mL inoculum to the container. The total volume is 1.5 L .

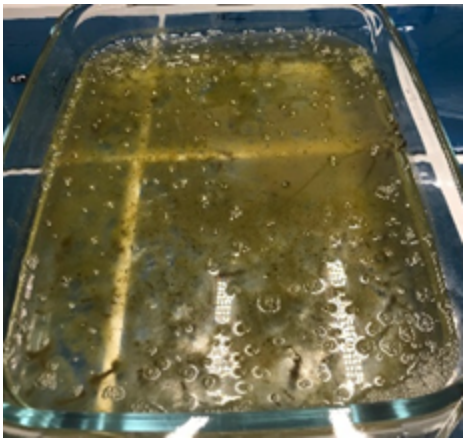


Lasagna plate as colonization container which contain 450 glass discs at bottom

- Check the inoculum biomass: Take 1 mL of homogenized inoculum into a pre-weighed 2 mL tube (4 replicates). Dry the tubes using a Genevac, then measure the dry weight. Multiply by the total volume of inoculum to calculate the total biomass. Standardize the biomass used for inoculation (e.g., 500 mg for one lasagna plate). Adjust either the number of scratched slides or the inoculum volume to achieve this fixed biomass across all experiments.
- Colonization environment: 21 °C in the mesocosom room, agitation 30 rpm, light intensity 65 µmol, photoperiod light/dark cycle 16 h/8 h.
- Media replenishment during colonization: every 2 days fill up the volume with demineralized water (to avoid increase content of minerals). Change media every week.
- The existence of worms induce substantial heterogeneity among samples, since the biomass of worm is much higher than the microbiomes in one disc. Therefore, we need to remove worms during colonization, or don't use the discs contain worms. The worms normally shown as dark cluster/tube shape.

Example of worms on the discs

- Parameters to be followed every week before change the media: conductivity, temperature, pH, oxygen in water // nutrients
- Duration of colonization: 2 or 3 weeks - "expert call". Before continue doing experiment, using 3 discs to measure Total C, community composition by PhytoPAM.



Three weeks colonization

- The colonized glass discs can be transfered into 48-well plate to perform chemical exposure experiment.

Precautions

7 Use and transport of liquid nitrogen
Refer to the use of liquid nitrogen (internal document)
- Use appropriate PPE (gloves, goggles, tongs)
- Liquid nitrogen must be transported in containers approved for "nitrogen transport."
- The nitrogen tank must be stored in a ventilated room.
The risks are:
- Severe burns
- Asphyxiation due to lack of oxygen

- Explosion

Use and transport of dry ice
Dry ice must be stored and transported in a polystyrene cooler
It can be stored -80 °C
Le transport de carbo glace nécessite une aération régulière du véhicule.

- Using the freezer -80 °C
- Use the appropriate PPE (gloves).

Handling sharp objects
- Be careful when handling and storing them.