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Institut des dynamiques de la surface terrestre

Determination of Chl *a* in EtOH 90 %

Equipment

- 1 vacuum pump
- 1filter unit
- Filter membrane GF/F (porosity 0.7 μm)
- 90% ethanol prepared from ethanol absolute for analysis, ACS,ISO,Reag. Ph Eur, purity ≥ 99,9% (Merck)
- 15 mL falcon tubes
- uv-vis spectrophotometer

Filtration of water

Depending on the phytoplankton load of the sample, 100 to 1000mL of water is filtered through GF/F filters with a diameter of 47mm. The filter is then placed in a 15 mL Falcon tube covered with aluminum foil and annotated as follows:

Sampling site - date - replicate no. – volume filtred

The filter is then frozen at -20°C for at least a few hours prior to extraction. Freezing/ thawing causes the cells to burst, which facilitates the extraction of Chl *a.*

Extraction

Important notes :

- As Chl *a* is photodegradable, it's essential to protect the samples from light by wrapping a sheet of aluminum foil around the tubes.
- Ethanol is a relatively toxic solvent when ingested, it's also toxic by inhalation and skin contact. It must therefore be handled with gloves and under a fume hood.

1) **After thawing the filter, add 7.5 mL of 90% ethanol.**
Close the tube tightly to prevent evaporation of the solvent.

2) **Shake the tube vigorously to destroy the filter until it forms a dough.**

3) **Place the tube for 10 min in the water bath at 70°C.**

4) **Leave to cool at room temperature for one hour before measuring with the spectrophotometer or leave to stand for about 16 hours at 4°C and in the dark.**

Absorbance measurements with spectrophotometer

1) **Centrifuge 5 min at 4000 rpm and 10°C**. Most of the filter sludge will form a pellet but some fragments will remain stuck to the walls of the tube, so the tube must be tilted to re-suspend these pieces in the ethanol 90%.

2) **Centrifuge 10 min at 4000 rpm and 10°C to** form the pellet.

3) Take 1 mL of the supernatant with a micropipette and place it in a semi-micro glass or polystyrene cuvette. **Read the absorbance at 665nm** (wavelength corresponding to the maximum absorption of *Chl a*) **and at 750 nm** (for evaluation of the turbidity of the sample).

- If the measurement at 750 nm is too high (> 0.005 for absorbance levels of 0.1-0.2), then particles form the filter were present in the supernatant. These particles have therefore distorted the absorbance measurement, so centrifugation is necessary and absorbance measurements should be repeated.

- The sample can also be too concentrated. Above 0.8-1 absorbance unit, the extract must be diluted with 90% ethanol, because it's no more in the linear part of the reading range. Conversely, you should always try to reach absorbance > 0.1, i.e. beyond the detection limits.

First make a blank with 90% ethanol, then measure three times per sample.

Estimation of the Chl a concentration (according to Talling ,1963)

If it's certain that the analyzed sample contains only *Chl a*, the concentration in the raw water in g/L is calculated as follows:

$$\mathbf{[Chl\ a] = 12.05 * (DO_{665} - DO_{750}) * (VE / VF)}$$

with **VE : extraction volume (mL)**

VF : filtered volume (L)

On the other hand, if the water sample contains other chlorophyll pigments, the method of Wintermans & de Mots (1965),still with 90% ethanol as solvent, in addition to *Chl* ***a***, an estimation of C*hl* ***b*** (en µg/L)**.**

$$\mathbf{[Chl\ a] = [13.7 * (DO_{665} - DO_{750}) - 5.76 * (DO_{649} - DO_{750})] * (VE / VF)}$$

$$\mathbf{[Chl\ b] = [25.8 * (DO_{649} - DO_{750}) - 7.6 * (DO_{665} - DO_{750})] * (VE / VF)}$$