Sample Preservation Prior to Cyanobacteria/Microalgae Cultivation

D. Vaulot,R. Edern,C. Romero

Abstract

It is not always possible to isolate microalgae immediately after sample collection, for example on long cruises where no proper conditions are available (lack of culture chamber, sterile hoods) or on trips to remote locations. Therefore it is desirable to be able to preserve samples in such a way that microalgae can be isolated once the samples are brought back to the laboratory. One possible strategy is to use cryopreservation that is currently used to preserve live cultures of microalgae.

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Materials

Dimethyl Sulfoxide (DMSO) View by Contributed by users

Protocol

Sample pre-processing

Step 1.

Prior to preservation, samples can be pre-processed to increase chances of getting target organisms. Two possibilities have been tested:

- Pre-filtration through 2 or 3 µm. This allows removing large cells such as diatoms that may become dominant in the unfrozen samples

- Tangential flow filtration. This will concentrate the phytoplankton community about 100-fold and will, therefore, result in more cells being preserved and therefore higher recovery rates (see ***add*** link ***for sample concentration by tangential flow filtration protocol***).

PROTOCOL

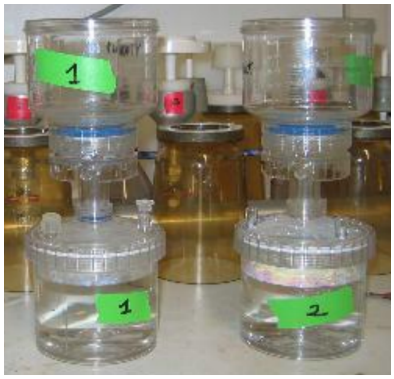
. Pre-filtration for Sample Preservation

CONTACT: Nicolas Schmelling

Installation

Step 1.1.

Install two filters on top of each other in the filtration tower to ensure maximum filtration efficiency.



Filtration

Step 1.2.

Filter 200 ml of sample by gravity without exercising any depression.

AMOUNT

200 ml Additional info: sample

Collection

Step 1.3.

Collect the filtered sample.

Cleaning

Step 1.4.

Rince very thoroughly filtration tower before proceeding to the next sample.

Sample freezing

Step 2.

The samples are preserved in 2 mL Cryotube with DMSO added (5% final concentration).

Then they are frozen according to one of three different protocols (choice most appropriate):

- Freezing into liquid nitrogen or dry ice. This is often the only method available in the field.

- Using Mr Frosty box (http://www.thermofisher.com/order/catalog/product/5100-0001) in a -80°C freezer. This allows progressive freezing but the rate of freezing is not precisely controlled.

CONCENTRATION
5 Volume Percent Additional info:

REAGENTS
Dimethyl Sulfoxide (DMSO) View by Contributed by users

Sample recovery

Step 3.

Samples are quickly unfrozen in a water bath at 25°C and then diluted into algal growth media in 50 mL flasks. The flasks are covered with aluminum for 24°C to avoid light shock and put in a culture cabinet using a temperature close to the one recorded at the sampling site (e.g. 4°C for polar waters, 15°C for temperate waters, 22°C for tropical waters). Several media can be used: K media for eukaryotes and PCRS11 media for cyanobacteria (media composition is available from http://roscoff-culture-collection.org/protocols/media-recipes). Samples can be incubated either in full concentration medium or in diluted medium.

PROTOCOL

. Preparation PCRS11-Red Sea Medium

CONTACT: Nicolas Schmelling

Preparation of Hepes-NaOH 1M Stock Solution (skip if already available)

Step 3.1.

To 250mL of H_20, add gradually 119.15g of Hepes. Adjust pH at 7.5 and complete the volume at 500mL. Store in refrigerator.

AMOUNT
500 ml Additional info: Water
AMOUNT
119.15 g Additional info: Hepes

Preparation of Na2-EDTA/FeCl3 Stock Solution (skip if already available)

Step 3.2.

- To 40mL of HCl 0.1N, add gradually 1.080g of $FeCl_3$.

- To 40mL of NaOH 0.1N, add gradually 1.488g of Na_2-EDTA.

Now mix both solutions and fill up to the final volume of 2L with sterile water.Store in refrigerator

AMOUNT
1.08 g Additional info: FeCl3
AMOUNT
40 ml Additional info: HCl 0.1N
AMOUNT
40 ml Additional info: NaOH 0.1N
AMOUNT

1.448 g Additional info: Na2-EDTA

Preparation of Sodium Phosphate Stock Solution (skip if already available)

Step 3.3.

Prepare two solutions:

- Monosodium dihydrogen phosphate (NaH_2PO_4) at 50mM (6g in 1L)

- Disodium hydrogen phosphate (Na_2HPO_4) at 50mM (3.55g in 500mL)

Make an equimolar mixture of this two solutions and adjust the pH at 7.5.

CONCENTRATION
0.05 Molarity (M) Additional info: NaH2PO4
CONCENTRATION
0.05 Molarity (M) Additional info: Na2HPO4

Preparation of Trace metals “Gaffron+Se” Stock Solution (skip if already available)

Step 3.4.

To 500mL of H_20, add gradually the following nutrients

Quantity (mg/L)	Compound Final	Concentration in media (nM)
186	Boric acid (H_3BO_3)	150
101	Manganese (II) Sulfate Monohydrate ($MnSO_4.H_2O$)	30
1.98	Sodium Tungstate dihydrate ($Na_2WO_4.2H_20$)	0.3
5.16	Ammonium molybdate tetrahydrate ($(NH_4)_6MO_7O_{24}.4H_2O$)	1.45
7.14	Potassium bromide (KBr)	3
4.98	Potassium iodide (KI)	1.5
17.25	Zinc sulfate heptahydrate ($ZnSO_4.7H_2O$)	3
9.25	Cadium Nitrate ($Cd(NO_3)_2.4H_2O$)	1.5
8.76	Cobalt (II) Nitrate ($Co(NO_3)_2.6H_2O$)	1.5
7.5	Copper (II) Sulfate ($CuSO_4.5H_2O$)	1.5
7.1	Nickel Chloride ($NiCl_2.6H_2O$)	1.5
2.4	Chromium (III) Nitrate ($Cr(NO_3)_3.9H_2O$)	0.3
1.5	Vanadyl Sulfate Pentahydrate ($VOSO_4.5H_2O$)	0.3
28.4	Aluminium Potassium Sulfate ($KAl(SO_4)_2.12H_2O$)	3
3.3	Selenium (IV) Oxyde (SeO_2)	1.5

Complete the volume at 1L and store in refrigerator.

Preparation PCRS11-Red Sea medium

Step 3.5.

To 1L of H2O, add 33,33g of Red Sea Salt. Dissolve by shake (20min on agitator)

Preparation PCRS11-Red Sea medium

Step 3.6.

Heat seawater during 20min at 100°C.

TEMPERATURE

100 °C Additional info:

Preparation PCRS11-Red Sea medium

Step 3.7.

Under the hood, to water, add these nutrients beforehand autoclaved (except vitamin):

Quantity	Compound	Final Concentration
1.0 mL	Hepes-NaOH 1M (pH 7.5) (See receipe below)	1mM
1.0 mL	Na_2-EDTA/$FeCl_3$ (See recipe above)	8µM
1.0 mL	Sodium Phosphate ($NaPO_4$) 50mM (pH 7,5) (See recipe above)	50 µM
1.0 mL	Ammonium Sulfate 400mM (NH4)2-SO4	400µM
0,1 mL	Trace metals “Gaffron+Se” (See recipe above)	
0.1 mL	Cyanocobalamin 10mg/L (Vit. B12)	1µg/L

Filter the medium on 0.2 microns.