BG-11 medium preparation Protocol and Growth Conditions

Introduction

To grow *Synechococcus elongatus sp. UTEX 2973*, BG-11 was used. This protocol originates from The University of Texas (2009). BG-11 Medium Recipe [on line]. Available at https://utex.org/products/bg-11-medium (15/05/2019)

Materials

- Stock 1 (1L)
  - Na_2MG EDTA 0.1g/liter
  - Ferric ammonium citrate 0.6g
  - Citric acid.$1H_2O$ 0.6g
  - $CaCl_2$.2H2O 3.6g
- Stock 2 (1L)
  - $MgSO_4.H_2O$ 7.5g
- Stock 3 (1L)
  - K_2HPO_4 3.05g
- Stock 4:Trace metal solution (1L)
  - H_3BO_3 2.86g
  - $MnCl_2.4H_2O$ 1.81g
  - $ZnSO_4 . 7H_2O$ 0.222g
  - $CuSO_4 . 5H_2O$ 0.079g
  - $COCl_2 . 6H_2O$ 0.050g
  - $Na_2MoO_4 . 2H_2O$ 0.391g
  - or MoO_4 (85%) 0.018g

Procedure

BG-11 Medium

1. Prepare stock 1-4: in 900 ml dH2O add components in the order specified on a magnetic stirrer. Bring the total volume to 1L with dH2O. Autoclave at 15 psi for 30 minutes at 121°C.

2. Combine the following solutions and adjust pH to 7,5 (Use 1.0M HCl or NaOH).

Stock	Per liter of medium
Stock 1	10 ml
Stock 2	10 ml
Stock 3	10 ml
Na2CO3	0,02 g
Stock 4	1,0 ml

NaNO3 1.5 g (For 100x BG-11 solution don't add NaNO3)
For **solid media:** add 1.5% agar to the liquid BG11 media.

3. Aliquot into flasks (50 ml/125 ml flask) with cotton stoppers on top and autoclave at 15 psi for 30 minutes at 121°C.

4. After autoclaving and cooling, the pH might have changed, so check and adjust.

Growth Conditions

5. The day of arrival, the culture was first put under light (50 µmol/m².s) for 20 hours, and further incubated in dark for 24 hours to avoid bleaching. Afterwards, cultures were inoculated in 30 mL of BG-11 medium and grown in conditions as described below.

6. *S. elongatus sp. UTEX 2973* was grown in filtertop erlenmeyers (0.22 µm) in 28°C at 100 rpm and a constant irradiation of 50 µmol/m².s.