Culture media used at BCCM/DCG

GREAT AT SMALL THINGS

f/2 + Si medium (Guillard, 1975) is the culture medium used to grow all marine species of diatoms at BCCM/DCG. **WC** medium (Guillard & Lorenzen, 1972) is applied for freshwater diatoms from habitats with pH ≥ 7, whereas **PM** medium (based on GG medium (von Stosch & Fecher, 1979)) is the standard for culturing diatoms from acidic environments. BCCM/DCG uses **3N-BBM+V** medium (Bischoff & Bold, 1963) and modified according to Starr & Zeikus (1993) to grow freshwater microalgae different from diatoms. In the table below the concentrations of the macro and micro nutrients, and the vitamins are given for each culture medium.

		marine	fresh water		
		f/2 + Si	**WC**	**PM**	**3N-BBM+V**
macro nutrients	$CaCl_2 \cdot 2H_2O$		36.76	0.37	25.00
	K_2HPO_4		8.71	2.90	75.00
	KH_2PO_4				175.00
	$MgSO_4 \cdot 7H_2O$		36.97	3.70	75.00
	$Na_2SiO_3 \cdot 9H_2O$	30.00	28.42	14.21	
	NaCl				25.00
	$NaH_2PO_4 \cdot H_2O$	5.00			
	$NaHCO_3$		12.60	3.15	
	$NaNO_3$	75.00	85.01	56.70	750.00
micro nutrients	$Na_2 \cdot EDTA$	4.360	4.360	2.180	4.500
	$FeCl_3 \cdot 6H_2O$	3.150	3.150	1.580	0.582
	$CuSO_4 \cdot 5H_2O$	0.010	0.010	0.005	
	$MnCl_2 \cdot 4H_2O$	0.180	0.180	0.090	0.246
	$ZnSO_4 \cdot 7H_2O$	0.022	0.022	0.011	
	$ZnCl_2$				0.030
	$CoCl_2 \cdot 6H_2O$	0.010	0.010	0.005	0.012
	$Na_2MoO_4 \cdot 2H_2O$	0.006	0.006	0.003	0.024
	H_3BO_3		1.000	0.500	
vitamins	B_1	1.0×10^{-1}	1.0×10^{-1}	1.0×10^{-1}	1.0×10^{-2}
	H	5.0×10^{-4}	5.0×10^{-4}	5.0×10^{-4}	2.5×10^{-6}
	B_{12}	5.0×10^{-4}	5.0×10^{-4}	5.0×10^{-4}	1.5×10^{-6}

all concentrations in mg/L

Practical considerations

Concentrated stock solutions of the different nutrients can be made. Prepare these stock solutions for each macro nutrient in a separate recipient. All micro nutrients can be mixed in a single recipient. However, to avoid precipitation and to facilitate dissolving these chemicals it is important to follow the sequence (starting with Na_2.EDTA) given in the table above. Macro and micro nutrient concentrated solutions should preferably be stored at c. 4°C. Vitamin stock solutions need to be kept sepperate and stored at -20°C. All concentrated stock solutions must be used within 1 year.

Example: prepare 200 mL of 1000 times concentrated stock solutions in standard **polystyrene** 250 mL cell culture flasks with a standard screw cap (e.g., from Greiner Bio-One). Concentrated vitamin stocks can be prepared in smaller volumes and stored in reaction vials, cryovials, polycarbonate tubes, etc.

1 liter of culture medium can be prepared as follows:

- take a glass bottle (e.g., DURAN® original Laboratory Bottle , 1000 mL)
- add at least 800 mL distilled water (fresh water microalgae) / filtered natural or artificial seawater (marine diatoms)
- add 1 mL of each stock solution (1000 times concentrated)
- adjust the volume to 1 L using distilled water (fresh water microalgae) / filtered natural or atrificial seawater (marine diatoms)
- measure pH, if you need to adjust the pH of the culture medium, use 1 N NaOH to raise the pH or 1 N HCl to lower the pH. Typically used pH values:
  - f/2 + Si, pH = 7.8 – 8.2
  - WC, pH = 6.8 – 7.2
  - **PM, pH = 4.0 – 4.5**
  - 3N-BBM+V, pH = 6.8 – 7.2
- autoclave 1 L flasks containing medium at 15 psi for 15 minutes
- add filter sterilized vitamin solution into cold autoclaved medium
- store the autoclaved bottles with culture medium at c. 4°C

Additional information

In case artificial sea water is preferred, one might consider to use commercially available Tropic Marin Pro-Reef Salt at a concentration of 35 g/L dissolved in distilled water. From our experience, this salt does not negatively affect the health and growth of diatoms.

Guillard's (f/2 + Si) Marine Water Enrichment Solution is available from Sigma Aldrich. This is a frozen 50x concentrated and sterile-filtered stock solution containing **all** nutrients and vitamins. Based on detailed testing we did not find any visible difference between diatoms growing in medium made from our stock solutions and microalgae growing in the culture medium based on the concentrated stock solution from Sigma Aldrich.

Not adding vitamins to the culture medium does not seem to affect the health and growth of diatoms.

PM medium is **original**, it is based on the GG medium (von Stosch & Fecher, 1979), but using the identical stock solutions that are also utilized in the WC medium.

Important notes from 'Algal-culturing techniques' by Anderson (2005):

It is recommended to use standard polystyrene cell culture flasks from e.g., Greiner Bio-One because:

- Phosphate stock solutions should never be stored in the polyethylene bottles since phosphate ions are strongly adsorbed onto polyethylene (Hassenteufel et al., 1963).
- Silicate stock solutions should be stored in nonvitreous material because of dissolution of silicic acid from glass vessels.

References

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