



Microalgae Culture Media

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Algal nutrient solutions or culture medium are made up of a mixture of chemical salts and water. The culture medium provides the nutrient needed for the growth of algae. These nutrients solutions are formulated specifically for its use in aquatic environments and their consistency is more precise for laboratory culture. The culture medium is constituted with the addition of macronutrients, micro nutrients and vitamins. Macro nutrients include nitrate, phosphate and silicate. Micro nutrients contain various trace metals. Vitamins like thiamin (B_1), cyanocobalamin (B_{12}) and sometimes biotin (B_7) are commonly required for the growth of most of the micro algae.

i. Macronutrients

Nitrogen and phosphorus are the important macronutrients, for the growth and metabolism of algal cells, which are added to the culture medium as Nitrate (NaNO₃ /KNO₃) and phosphate (NaHPO4.H2O). Nitrogen is a key element for the formation of protein and nucleic acids accounts to 7-20% of micro algal cell dry weight. Phosphorous plays a major role in the formation of energy carrier molecule (ATP) which forms 1% dry weight of the algae. Algae require inorganic carbon source in the form of CO₂, carbonate or bicarbonate for its photosynthesis. Silicate is necessary for the cell wall development of diatoms and is added in sodium silicate form (Na₂SiO₃ 9H₂O) to the culture medium.

ii. Micronutrients

Micro nutrients are trace metals which are present in algal cells in extremely small quantities (<4 ppm), which are essential for the physiological growth of algae. Iron (Fe,) Manganese (Mn), Cobalt (Co), Zinc (Zn), Copper (Cu) and Nickel (Ni) are the most important trace metals required by algae for the various metabolic functions. Deficiencies in trace metals may lead to slow algal growth and excess concentration may inhibit growth, impair photosynthesis and finally damage the cell membrane of the algae. Typical trace metal stock solutions may consist of chloride or sulphate salts of zinc, cobalt, manganese, selenium, and nickel, and they are kept in a solution containing the chelator EDTA. Iron is an important trace metal required for the algae





for its normal growth, photosynthesis and respiration. Iron is usually kept as a separate solution, may be added as ferric chloride or ferrous sulphate. EDTA is used as chelator and is available as disodium salt (Na₂EDTA.2H₂O) that is readily soluble in water.

iii. Vitamins

Vitamins are organic micronutrients, which are essential for photosynthetic microalgae. Algal species require different combination of vitamins, mostly Vitamin B_{12} (Cyanocobalamin), Vitamin B_1 (Thiamine) and Vitamin B_7 (Biotin). The general order of vitamin requirements for algae is vitamin B_{12} > thiamine > biotin. Vitamins are normally added aseptically (through a 0.22-mm filter) after the medium has been autoclaved.

Stock Solutions preparation

In order to simplify routine medium preparation, stock solutions are prepared with accurate weighing of the chemicals in the specific culture media, dissolved in the specific volume of distilled water. There are two terms used in stock solution preparation, working stock and primary stocks. Working stocks are the small quantity (aliquot) of solution which is directly used for the preparation of final medium so that adding a small volume of liquid stock solution is easier and quicker than weighing of individual dry chemicals. Primary stocks are formed from several single substance solutions and finally combined to form the working stock. Stock solutions made from some chemicals (EDTA) may need heat treatment to dissolve completely in the water, otherwise it may lead to unnecessary precipitation of the nutrients in the medium. But, vitamin stocks should be prepared with normal distilled water and should not be exposed to any heat treatments and are advised to be kept in dark bottles.

Culture media

The selection of culture media mainly depends on the type algae species cultured. Diatoms like *Chaetoceros,Skeletonema, Thalassiosera, Tetraselmis* etc., need silicates for the formation of silicious cell wall in addition to nitrate, phosphate, trace metals and vitamins. Most of the culture media for micro algal culture are composed of chemicals, trace metals and vitamins. Most commonly used culture media used for stock culture and mass culture of micro algae in the laboratory is 'Conway' or 'Walne's medium (Walne,1974) and Guillard's F/2 media. This media is mainly used for indoor culture of *Nannochloropsis, Chlorella,* and diatoms like *Chaetoceros, Skeletonema, Thalassiosera* and *Tetraselmis.*





Composition of Conway / Walne's medium used for mic	ro algal culture		
Solution (A)			
1. Potassium Nitrate (KNO ₃)	100gm		
2. Sodium di-hydrogen orthophosphate (NaH ₂ PO ₄ .2H ₂ O)	20gm		
3. EDTA di-sodium salt (Na ₂ EDTA)	45gm		
4. Boric Acid (H ₃ BO ₃)	33.4gm		
5. Ferric Chloride (FeCl ₃)	1.3gm		
6. Manganous Chloride (MnCl ₂ .2H ₂ O)	0.36gm		
7. Distilled Water	1 lit		
Solution (B)			
1. Zinc Chloride (ZnCl ₂)	4.2gm		
2. Cobalt Chloride (CoCl ₂ .6H ₂ O)	4.0gm		
3. Copper Sulphate (CuSO ₄ .5H ₂ O)	4.0gm		
4. Ammonium molybdate ((NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O)	1.8gm		
5. Distilled Water	1 lit		
Solution (C)			
1. Vitamin B ₁ (Thiamine)	2gm		
2. Vitamin B ₁₂ (Cyanocobalamin)	100mg		
3. Distilled Water	1 lit		
Solution (D)			
1. Sodium silicate (Na ₂ SiO ₃ .5H ₂ O)	40ml		
2. Distilled water	1 lit		
Stock culture: Only autoclaved seawater should be used.			
Working Solution for mass culture: Add 1ml of Solution A, 0.5ml of B, 0.1 ml of C and 1ml of D into 1lit sea water. D: Only for Diatoms (Chaetocerossp.,			

Skeletonemasp., Thalassioserasp.)





Composition of Guil	llard's F/2 media usec	l for mi	cro algal culture
Solution (A)			
1. Sodium nitrate (No	ıNO ₃)	75g	1 L distilled water
2. Sodium phosphate	(NaH ₂ PO ₄ .H ₂ O)	5g	
P.S.Solution*			
1. Copper Sulphate (CuSO ₄ .5H ₂ O)	10g	1 L distilled water
2. Zinc Sulphate (ZnS	O ₄ .7H ₂ O)	22g	1 L distilled water
3. Cobalt Chloride(C	oCl ₂ .6H ₂ O)	10g	1 L distilled water
4. Manganous Chlori	de (MnCl ₂ .4H ₂ O)	180g	1 L distilled water
5. Sodium Molybdate	e (Na ₂ MoO ₄ .2H ₂ O)	6g	1 L distilled water
Prepare each solution	separately in 1lit bottles		
Solution (B)**			
1. EDTA di-sodium sa	lt (Nα ₂ EDTA)	4.36g	1 L distilled water
2. Ferric Chloride (Fe	Cl ₃ .6H ₂ O)	3.15g	
Add 1ml of each s mixed solution	olution (P.S solution1-5) e	each to 1	lit of EDTA & FeCl ₃
Solution (C)***			
1. Thiamin HCl		20g	1 L distilled water
2. Biotin		100mg	g 1 L distilled water
3. Cyanocobalamine	(B ₁₂)	100mg	g 1 L distilled water
Add 5ml of each solut	ion into 1lit of sea water		
Solution (D)****			
1. Sodium Silicate (N	a ₂ SiO ₃ .9H ₂ O)	35g	1 L distilled water
Stock culture: Only a	utoclaved seawater shoul	d be use	ed.
*P.S. Solution: Each s bottles	olution (1L) should be pr	epared s	separately in 5 different
**Solution B: 1L of E	DTA & FeCl ₃ mixed solution	on with P.S	S. solution (1ml of each)





*** Solution C: 1L of sea water with Thiamin, Biotin and Cyanacobalamin solution (5ml each)

**** Solution D: Only for Diatoms (Chaetoceros]

Working Solution for mass culture: Add 1ml of each Solution A, B, C and D into 1 L sea water.

D: Only for Diatoms (Chaetoceros sp., Skeletonema sp., Thalassiosera sp.)

References:

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