Working with Algae and Cyanobacteria

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Basic Types of Algae

Diatoms

Bacillariophytes, which occur in fresh water, salt water, and terrestrially, date back to the Cretaceous Period. They are single-celled algae with shells constructed of two overlapping valves composed of pectin and impregnated with silica; these shells can be quite ornate. Although the diatoms are single-celled organisms, they can form colonies and filaments. The group comprises two main types: centric and pennate. Centric diatoms are radially symmetrical and contain numerous plasmids, while pennate diatoms are bilaterally symmetrical and contain fewer plasmids. Many diatoms have conspicuous oil droplets within the cell, which is the photosynthetic food reserve, chrysolaminarin. The plastids of diatoms contain the pigments chlorophyll a and b, alpha and beta carotene, and several xanthophylls.

Dinoflagellates

Dinoflagellates are mostly marine organisms and they compose nearly all marine plankton. They occur as free-living flagellates, sessile unicells, colonies, and filamentous forms. The fossil record of the dinoflagellates can be dated back to the Cambrian period, with some evidence suggesting they existed even earlier. The term dinoflagellate actually refers to the twirling motion exhibited by the pair of whip-like undulipodia (flagella). These flagella originate in the sulcus, or groove, of the organism. Some dinoflagellates have thecal plates embedded in their cytoplasmic membrane and are called armored, others lack these plates and are called naked. Food is stored in the form of true starch and oils. Dinoflagellates contain the pigments chlorophyll a and c, beta carotene, and several xanthophylls that often give these organisms a brownish color. Some dinoflagellates produce powerful toxins with potentially dangerous results. When "blooms" occur, the water can take on a pinkish or red hue known as a red tide. This often causes massive fish kills and can be dangerous to humans as well. Some, such as Noctiluca, are bioluminescent, and can cause ocean waves to glow at night. This is the only example of bioluminescence in the Algae kingdom.

Euglenoids

Typically green and unicellular, euglenoid flagellates live in fresh water. They have characteristics of both plants and animals yet are distinct in many ways. Most are photosynthetic, but many, lacking chloroplasts, are heterotrophs. Most do not reproduce sexually. Euglenoids lack a cellulose cell wall; instead, they have a proteinaceous pellicle just inside the plasmalemma. The plastids contain chlorophyll a and b, beta carotene, and xanthophylls. If placed in the dark over the course of several divisions, the chloroplasts of Euglena gracilis will become colorless. When returned to the light, the plastid structure is reformed and the green color returns.

Brown Algae

Multicellular and structurally complex, with no colonies or simple, unbranched filaments, the Phaeophytes, or brown seaweed, are primarily marine algae; less than one percent occur in fresh water. They are most abundant, and reach their maximum development in the colder water of the oceans. While some species of Sargassum are found floating in enormous numbers in the Atlantic, the algae are usually firmly attached to a substrate by means of elaborate holdfast structures. Food is stored as soluble carbohydrates such as lamarin, fats and the alcohol mannitol. The plastids of the brown algae contain pigments chlorophyll a and c, c-carotene, and xanthins; an accessory pigment, fucoxanthin, gives the algae their characteristic dark brown or olive green color. The Phaeophytes are an economically important resource, used for alginic acid, fertilizer, and food.

Golden Algae

Chrysophytes are a large and complex group characterized by plastids containing distinctive golden yellow pigments. The group is diverse in form, yet all feature this yellow color, permitting easy identification. Chrysophytes are usually found in cold freshwater lakes and ponds, although some marine forms are common. Synura, existing in colonies in fresh water, can cause a fishy odor in reservoirs even in low concentrations, but is not harmful.

Green Algae

Chlorophytes are a diverse group and are common in fresh water, salt water, and soil. They are very similar to plants, and most botanists agree the ancestor of higher plants can be found somewhere within this group. Chlorophyte reproduction varies greatly, from asexual division to isogamy and heterogamy to oogamy. Cell walls are constructed of cellulose and pectin. The food storage product is true starch, the same as plants. This can be demonstrated by staining with KI, which turns the starch in the algae blue-black. Green algae possess true chloroplasts, which contain the same pigments found in higher plants: chlorophyll a and b, alpha and beta carotene, and many xanthophylls.

Red Algae

Although Rhodophytes, the most abundant type of seaweed, are widely distributed in the oceans, most occur in tropical and subtropical littoral zones. Of the 4,000 species, the vast majority are marine. Rhodophytes are not mobile — they possess no flagellated or ciliated cells at any stage of their life cycle — yet all reproduce sexually. Many red algae, such as Corallina, are calcified and encrusted appearing much like coral. This calcification has made it possible to trace the Rhodophytes to the Paleozoic Period. Single-celled forms such as Porphyridium are a rarity. Rhodophytes are characterized by reddish plastids, called rhodoplasts, which contain the pigments chlorophyll a and d, alpha and beta carotene, some xanthophylls and phycobiliproteins.

Yellow-Green Algae

Xanthophytes are highly successful in fresh water and terrestrial environments, although some marine forms also exist. The yellow-green algae have pectin-rich cellulose walls. Starch is absent and food is stored in the form of oils. Xanthophytes are characterized by yellow-green plastids (xanthoplasts) which contain pigments chlorophyll a and c, several xanthins, and beta carotene. Vaucheria is a large macroscopic, filamentous form that was classified a chlorophyte until pigment analysis showed the absence of chlorophyll b and true starch. Tribonema is a typical freshwater, unbranched, filamentous form which clearly demonstrates overlapping walls.

Blue-Green Algae (Cyanobacteria)

The cyanophytes are the only prokaryotic algae. They are found in virtually every type of environment including terrestrial, freshwater, and marine habitats. Since cyanobacteria are prokaryotes, they lack membrane bound organelles. However, the external structure can range from unicellular or colonial to branched or unbranched and filamentous. Like the rhodophytes, the cyanophytes possess no flagellated or cilliated cells at any stage of their lifecycle, although, simple movements such as bending and swaying are made possible by internal pressure changes exerted on the cell wall. They are heavily pigmented with chlorophyll a, beta carotene, and several xanthophylls. The presence of several phycobiliproteins gives the cyanophyta their unique blue-green coloration. Food is stored in the form of glycogen.

Maintaining Algal Cultures

Handling Cultures

Because algae are photosynthetic, carbon dioxide and a light source are the requirements for growth. Immediately upon receipt, loosen the jar cover or test tube cap to allow gas exchange, and store in a cool area (15 – 20˚C) with dim light. A window with northern exposure is ideal. Avoid storing the algae in direct light, since it will raise the temperature in the jar or tube, creating a miniature "greenhouse effect", damaging the culture. Avoid storing the culture at temperatures over 30˚C because this temperature will damage the cells, reducing the quality of the culture. Stored in ideal conditions, the culture can retain its high quality for several days. If the culture is not going to be used within four to five days, it should be subcultured (transfered to a fresh source of nutrients).

Illumination

Generally, cultures should be grown in a 16-hour light period alternating with an 8-hour dark period. Ideally, the cultures should be illuminated by 40-watt cool-white fluorescent tubes on a timer. A 40-watt fluorescent tube at a distance of about 15 cm will provide roughly 500 foot candles of illumination. At a distance of approximately 50 cm, the illumination will fall to approximately 200 foot candles. An inexpensive light meter calibrated in foot candles can provide a simple and accurate way to regulate the light intensity in your lab.

Freshwater algal cultures should be grown under a light intensity of 400 to 500 foot candles. At this light intensity, cultures will reach optimum growth in 7 – 14 days, depending on the species and condition of the initial algal inoculum. After this period, reduce the light to 50 – 100 foot candles.

Marine algae grow best in slightly lower intensities than those required by freshwater algae — 200 – 300 foot candles. The light cycle and growth period are the same as those recommended for freshwater cultures.

Transfer Periods

Algal cultures should be transferred at different intervals, depending on the species. After the initial growth period, transfer the cultures to an area with low illumination, where they can be stored for one to six months before subculturing is required again. Some cultures, such as Volvox and Spirogyra, should not be stored and should remain under full illumination.

Make new subcultures from the most recently stored cultures. Flagellates generally require subculturing every one to three months. Filamentous algae and unicellular nonmotile algae should be subcultured every three to six months. The Volvocales should be subcultured every 7 – 10 days. Marine algae should be subcultured every 1 – 3 months.

Special Culture Techniques

General Hints

- Sterilize all transfer pipets before beginning culture transfers.
- Wash all glassware thoroughly, rinse several times, and soak in hot water for a final rinse.
- Examine each culture under a microscope before transferring. Check the culture's condition and look for possible contamination.

Use only the best cultures for subculturing.

Sterilize all algal media before using. Algal media can be sterilized by autoclaving at 15 psi for 15 – 20 minutes. Some marine media cannot be autoclaved due to high salt concentrations. Pasteurize these media before using by heating the medium to 73˚C for 15 minutes. Repeat this procedure for three consecutive days.

Subculturing

Flasks, tubes, bottles, or Petri dishes can be used as culture vessels. If using a 250 mL Erlenmeyer flask, fill the flask to approximately 150 mL with freshly prepared media and sterilize the media.

Add a small amount (5 – 10 mL) of inoculum from a stock culture, handling the inoculum according to the instructions for the particular species that is being subcultured.

Always prepare more than one subculture in case one of the new cultures becomes contaminated.

To culture on agar, sterilize the media and, using sterile technique, transfer the algae from stock culture to a tube containing fresh media with a sterile cotton swab. Be sure to cover the entire surface of the agar and replace the cap loosely to allow for gas exchange.

Some algae can be grown on agar and kept viable for up to a year. However, cultures maintained on agar may begin to exhibit abnormal morphology over the course of time and may need to be transferred to liquid before normal morphology can once again be observed. To do this , remove some of the cells from the surface of the agar with a sterile cotton swab and place them in fresh liquid medium. If pressed for time, add sterile distilled water to the culture tube to allow some of the cells to regain their normal morphology.

Demonstrations of Sexual Reproduction in Algae

Various forms of algae can be used to demonstrate sexual reproduction. These are only guidelines, so you should expect some variation in reaction time due to differences in light intensity, temperature, and media. Because the sexual process in most algae is associated with light, it is not possible to supply cultures which will be immediately sexually active. Therefore, be sure to obtain cultures well enough in advance so that they may be cultured in or acclimated to a new environment.

Chlamydomonas, Plus strain and Minus strain

1. Grow the heterothallic strains separately, on soil extract agar or Bristol's agar, for about one week under 350 foot candles of illumination.
2. The day before the demonstration, wash the cells of each strain from the agar using approximately 5 mL of distilled water. Place the suspensions of the strains in separate flasks.
3. Illuminate the flasks for several hours and then place in a dark area.
4. Two hours before the demonstration, illuminate the flasks again. After this period, mix a drop of each culture together on a clean glass slide.

Sexual reproduction is evidenced by the immediate clumping of the flagellated gametes. Pairs can be observed within a few minutes after mixing. The pairs swim about, joined at the anterior end. Fusion (plasmogamy) occurs approximately six to eight hours later. Use the usual 16-hour light period alternating with an 8-hour dark period.

Culture Media

1. Bristol's Solution (as modified by H.C. Bold, Bull, Torrey Bot. Club 76: 101-108, 1949)
Six stock solutions, 400 mL in volume, are employed. *Each contains one for the following salts in the amounts listed:*

a) $NaNO_3$	10.0 g	d) K_2HPO_4	3.0 g
b) $CaCl_2$	1.0 g	e) KH_2PO_4	7.0 g
c) $MgSO_4$	3.0 g	f) NaCl	1.0 g

10 mL of each stock solution are added to 940 mL of glass-distilled water. To this is added a drop of 1.0% $FeCl_3$ solution. Two mL of minor elements solution (Trelease and Trelease, American Jour. Bot. 22: 520-542, 1935) may also be added. Solidify with 15 g agar per 1000 mL if desired.

2) Cyanophycean Agar (Culture Collection, Cambridge University)
For each 1000 mL of medium required:

KNO_3	5.0 g
K_2HPO_4	0.1 g
$MgSO_4 \cdot 7H_2O$	0.05 g
Fe Ammonium Citrate	10 drops of 1% solution

The above should be added to 1000 mL of Pyrex-distilled water. Solidify with 15g agar.

3) Desmid Agar (E.G. Pringsheim, Culture Collection, Göttingen)
To 1000 mL of Pyrex-distilled water add 10 mL of each of the following solutions:

0.1% solution of $MgSO_4 \cdot 7H_2O$
0.1% solution of K_2HPO_4
1.0% solution of KNO_3

Solidify with 7.5 agar. It has been found that some desmid strains grow better with the addition of 50 mL/1000 mL of the supernatant from soil-water medium.

4) Erdschreiber Solution (Mary Parke, Plymouth Marine Station, England)
1000 mL filtered sea water
50 mL soil-water supernatant
0.2 g $NaNO_3$
0.03 g $Na_2HPO_4 \cdot 12H_2O$
1st day: Filter seawater through No. 1 filter paper and then heat to 73°C.
2nd day:
A) Again heat saltwater to 73°C.
B) Autoclave salt solutions (made up in distilled water so that 1 mL of each solution gives required amount for 1000 mL of culture solution).
3rd day: Add cold salt solutions to cold soil supernatant, and then add soil supernatant to cold seawater. Dispense in sterile tubes and flasks as desired.

5) Euglena Medium (Culture Collection, Cambridge)
To 1000 mL of Pyrex-distilled water add:

Sodium acetate	1.0 g
Beef extract	1.0 g
Tryptone	2.0 g
Yeast extract	2.0 g
Calcium chloride	0.01g

If desired, the above medium may be solidified by adding 15 g of agar.

6) Ochromonas Medium (W. Koch, Göttingen)
To 960 mL of Pyrex-distilled water add:

Glucose	1.0 g
Tryptone	1.0 g
Yeast extract	1.0 g
Liver extract (infusion)	40 mL

7) Polytomella Medium (E.G. Pringsheim, personal communication)
For each 1000 mL of medium required:

Pyrex-distilled water	1000.0 mL
Sodium acetate	2.0 g
Yeast extract	1.0 g
Tryptone	1.0 g

8) Porphyridium Agar (E.G. Pringsheim, personal communication)
For each 500 mL of medium required:

Pyrex-distilled water	200.0 mL
Natural sea water	250.0 mL
Soil-water supernatant	50.0 mL
Yeast extract	0.5 g
Tryptone	0.5 g
Agar	7.5 g

9) Proteose Agar
For each 1000 mL of medium required:

Bristol's solution ([2], above)	1000.0 mL
Proteose peptone	1.0 g
Agar	15.0 g

10) Soil Extract Agar
For each 1000 mL of medium required:

Bristol's solution ([2], above)	960.0 mL
Soil-water supernatant	40.0 mL
Agar	15.0 g

11) Soil-water Medium (E.G. Pringsheim, Jour. Ecology 33: 193-204, 1946)

Variations of this medium are for non-sterile culture, especially for isolation purposes and for growing algae in order to secure "normal" growth forms. Success with soil-water media depends on the selection of a suitable garden soil. This soil should be of medium, but not too great humus content and should not have been recently fertilized with commercial fertilizers. Soils with a high clay content are usually not the most suitable for most organisms.

A variety of soil-water media can be made suing a basic formula to which are added certain additional materials. The basic soil-water medium is made by placing approximately 5 mg soil per mL (5g per liter) Pyrex-distilled water. The tube is then plugged with cotton and autoclaved for 1 hour. A number of algae such as Spirogyra grow well in this basic medium. For most persumptively phototrophic algae which thrive in an alkaline medium, a small pinch of powdered $CaCO_3$ is placed in the bottom of the test tube before the soil and water are added.

Some algae like Euglena, Polytoma, Astasia, and others require additional complex nitrogenous or carbon com-pounds not present in the basic formula.

In the case of Euglena, the best results have been obtained by adding ¼ of a garden pea cotyledon to the basic medium (including $CaCO_3$) before autoclaving. For the colorless forms, the addition of a barley grain before autoclaving supplied the necessary carbon source.

12) Trebouxia Agar (V. Ahmadjian, personal communication)

For each 1000 mL of medium required:

Bristol's solution ([2], above)	850.0 mL
Soil-water supernatant	140.0 mL
Proteose peptone	10.0 g
Glucose	20.0 g
Agar	15.0 g

13) Volvocacean Agar (E. G. Pringsheim, personal communication)

For each 1000 mL of medium required:

Waris solution (do not adjust pH)	800.0 mL
Euglena medium	200.0 mL
Agar	10.0 g

14) Waris Solution (H. Waris, Physiol. Plant. 6: 538-543, 1953)

To 1000 mL of Pyrex-distilled water add 1 mL of the following solutions:

10% KNO_3
2% $MgSO_4 \cdot 7\ H_2O$
2% $(NH_4)_2\ HPO_4$
5% $CaSO_4$
Iron Sequestrene solution.
Adjust pH to 6.0 using 0.01 N HCl and 0.01 N KOH.

Iron sequestrine solution is composed of the following:

Sequestrine AA	2.61 g
$FeSO_4 \cdot 7\ H_2O$	2.49 g
1 N KOH	27.0 mL
Pyrex-distilled water	500.0 mL

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