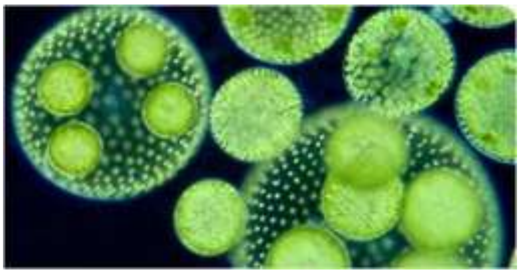
Practical Ecology of Algae

For 4th year

Chemistry & botany Students

Faculty of Science



By

Dr. Eman A. Alwaleed

Vision and Mission of the faculty

Vision

The faculty of science seeks to achieve academic community and student dominated by science, realization, culture and challenge, where all aspects are in continuing dialogue, graduating alumni equipped with information that qualifies them to be productive and creative.

Mission

The faculty aims to excel at local level and regional throughout:

- Providing distinguished educational service to provide the market labor with graduates of high efficiency.
- Cooperating with universities and scientific institutions, regional and international.
- Academic research studies, and purposeful applied
- Providing community services and distinguished scientific consulting for South Valley community
- Training and continuous improvement in the academic field to keep pace with scientific progress

Course Syllabus :

- **INTRODUCTION**
  - **ALGALPRODUCTION:**
  - Algae cultures of limited volume (Batch culture)
  - Algal Growth in Continuous Culture
  - Microalgae Isolation Techniques
  - Indices of growth of algae
  - Inorganic Nutrients of Algae
  - Algal Nutrition
- **PHOTOSYNTHESIS**
- **PLASTID STRUCTURE IN ALGAE**
- **CARBON DIOXIDE FIXATION BY ALGAE**
- **NITROGEN FIXATION IN ALGAE**

Course Goals

Dear student, by the end of this course you should be able to:

- Be aware with cultivation of algae in laboratory.
- Become acquainted with different kinds of algae cultures.
- Gain knowledge about methods of media preparation.
- Familiarize with different steps of batch culture.
- Know different kinds of continuous cultures.
- Gain knowledge about Principles of photosynthesis of algae.
- Become acquainted with different kinds of pigments involved in photosynthesis.
- Gain knowledge about structure of different pigments.
- Familiarize methods of extraction of pigments.
- Familiarize methods of estimation of pigments.
- Be aware with morphological structures of plastids in algae.
- Become acquainted with arrangement of thylakoids in algae.
- Know difference between plastid structure in algae divisions.
- Be aware with principles of carbon dioxide fixation in algae.
- Know difference between acetate and sugar algae.
- Know difference between autotrophic and heterotrophic carbon dioxide fixation by algae.
- Familiarize with different sources of inorganic carbon available for algae.
- Be aware with principles of nitrogen fixation in algae.
- Become acquainted with mechanism of nitrogen fixation.
- Familiarize with factor influencing algal nitrogen fixation.

INTRODUCTION

Plants and animals are separated by about 1.5 billion years of evolutionary history. First, plants get their energy from sunlight, not by ingesting other organisms. This dictates a body plan different from that of animals. Second, their cells are encased in semi rigid cell walls and cemented together, preventing them from moving as animal cells do. This dictates a different set of mechanisms for shaping the body and different developmental processes to cope with a changeable environment.

Physiology, as a term, was derived from the Greek words physis, meaning nature and logos, meaning discourse. Algal physiology is then, "the discourse about the nature of algae". From the physiological perspective, plants are viewed as machines that take inorganic molecules and energy from their surrounding environment and use them to assemble complex chemical structures.

In principle, algal physiology describes how algae work and focus on how algae use the energy of the sun to assimilate carbon, and how they convert that carbon to the stuff of which they are made. It also deals with several processes such as nutrient uptake and distribution, growth and development, algae response to their environment, reaction to stress and finally, the mode of algae reproduction.

This course was designed to provide skills to cultivate algae in laboratory. The importance to know the nutrients required for algal growth in order to cultivate algae and to exploit these algae in production of mass culture to be utilized in different aspects of life such as pharmaceuticals and biofertilizers and biodiesel. The need to know the indices to measure algae growth in order to monitor the different algae cultures such as batch cultures and continuous cultures. This course includes six units. Batch culture and Continuous culture, Growth media, Growth of Alga Pigments involved in Photosynthesis, Plastid structure in Algae divisions, Source of inorganic carbon, Factors influencing algal nitrogen fixation by cyanophyta and Vitamin requirements by algae.

Table of Contents

Understanding the algal groups

Understanding the common algal groups is the first step to identifying algae. Algae are classified into four major groups (Palmer 1962): Blue-greens, Greens, Diatoms and Flagellates.

By knowing the major group of your algae, verification and identification to the genus and species level with the use of a microscope and classification key becomes easier. Many traditional classification keys can be complex and daunting if you do not have an idea where to start. It is our hope that this lab reference manual will provide a good starting point.

Below are characteristics that each Algal Group will typically exhibit, however be advised that exceptions for some species do exist.

Blue Greens (Known as cyanobacteria)

- Includes species that fall under the Division Cyanophyta, Kingdom Eubacteria.
- They are referred to as algae but are actually photosynthetic bacteria that appear like true algae.
- IThey are mostly single-celled organisms or aggregations of single-celled organisms (planktonic). Planktonic algae generally do not adhere together in a mass, in that you could not easily grab a handful or mass of these organisms. Some species, such as Nostoc, are gelatinous masses that can be picked up.
- Anabaena, Aphanizomenon, Oscillatoria and Mycrocystis often dominate plankton communities and are notorious for producing potentially toxic blooms in fresh waters (note: only certain species produce toxins). These toxins can cause death and illness in livestock; and gastroenteritis, liver damage and skin and eye irritations in humans.
- High numbers of blue-greens often indicate high nutrient levels in the water, although Oscillatoria can form dense accumulations in less fertile waters.
- *Cell colour is uniform throughout (i.e. no membrane-bound coloured structures called chloroplasts) and may appear blue-green to violet, but sometimes red or green.
- *They have very simple internal structure, no definite nucleus or intracellular membrane-bound organelles.

Greens

- Includes species from the Division Chlorophyta (Green Algae).
- They are morphologically diverse (micro and macroscopically). Microscopically, they can be uni- or multi-cellular, colonial, have branched or unbranched filaments and vary in size.
- Macroscopically, they can be planktonic, filamentous or found attached to objects (epiphytic).

- Filamentous forms of algae grow as long multi-cellular threadlike filaments or masses (i.e. you can pick it up with your fingers). Early in the season these algae may develop clumps scattered on the water surface but as the season progresses they may cover the entire surface of a water body.
- *Some have a whip-like tails called a flagella. Swimming cells have 2,4, or 8 flagellae of equal length at the anterior end.
- *All species have internal membrane-bound structures (chloroplasts) that are often bright green. Other structures within the cell may also be seen at times, especially starch granules which stain dark with a preservative know as Lugols iodine solution.

* Characteristics only visible through a microscope.

Diatoms

- Includes species from the Class Bacillariophyta, of the Division Chrysophyta.
- They are often too small to see with the naked eye.
- Many are single celled, and less often colonial or filamentous. They may be planktonic, although many grow on other submerged objects.
- *They are easily identified by the cell wall (composed of silica) and shape, which are often complex and ornately marked.
- *They have shades of green, yellow, or brownish coloured chloroplasts.

Flagellates

- Includes species from various Divisions (including Chrysophyta, Euglenophyta, and Pyrrhophyta) that are motile, i.e. they have whip-like tails called flagellae.
- *They are often microscopic uni-cellular (solitary) planktonic algae.
- *They have shades of golden brown to yellow or green in the chloroplasts, or can even be colourless. Colourlessness may indicate that the species is totally dependant on het erotrophic nutrition. Some have a hard external covering (lorica) which makes them appear brown or black.

* Characteristics only visible through a microscope.

Images in this manual are microscopic images, unless otherwise indicated.
For some images, magnification or scale was not available.

Taxonomic List*

of algal genera referenced in this manual

Phylum (Division) Chlorophyta (Domain Eukaryota; Kingdom Protista)

Phylum (Division) Euglenophyta (Domain Eukaryota; Kingdom Protista)

*Adapted arrangement from G.W. Prescott (1978).

Phylum Chrysophyta (Domain Eukaryota; Kingdom Protista)

Phylum Cyanophyta (Domain Eubacteria, Kingdom Monera)

BG Blue Greens

▸ MICROCYSTIS

Microcystis and *Anabaena* (pg15) blooms appear very similar. Heavy blooms of these species create the memorable pea-soup conditions associated with cyanobacteria. Colours range from green to blue-green. Although large colonies are readily visible to the naked eye, individual cells are not visible to the naked eye and identification to genus or species requires a compound microscope. The most common species is M. aeruginosa; M. flos-aquae and M. wesenbergi can also co-occur with M. aeruginosa.

Microcystis is found throughout the water column because like other blue-greens, they possess gas vesicles (small air-filled structures) that allow the colonies to regulate buoyancy. This adaptation allows *Microcystis* to remain at optimal light levels to attain maximum photosynthetic rate.

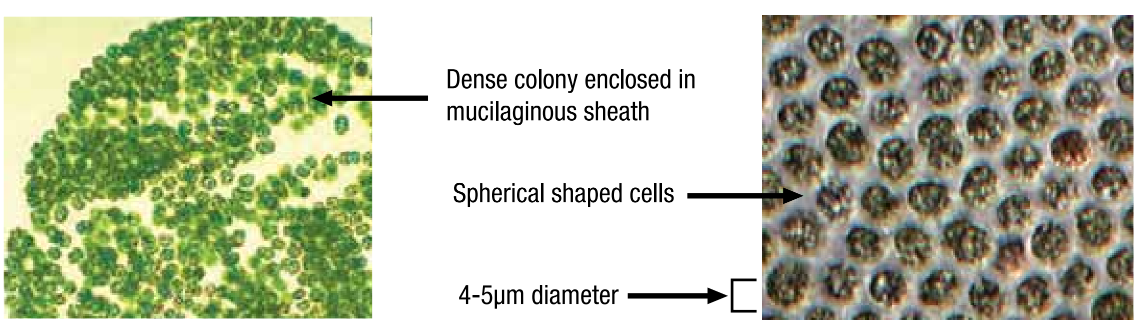
Blooms occur after periods of hot, calm weather and can develop rapidly over a few hours. This is because during periods of wind induced mixing of the water column, *Microcystis* have to produce more gas vesicles to counter the downward drag of the current. But when the wind ceases, the cells are unable to reduce the number of gas vesicles and as a result, they will migrate en masse to the surface over a few hours.

Most large blooms occur from mid to late-summer, and can through to fall.

Microcystis species are capable of producing microsystin, a hepatotoxin (liver toxin) that is lethal to wildlife, pets, livestock and humans that consume untreated or improperly treated water. Heavy blooms may cause skin irritation for recreational water users (dermatitis), as compounds on the surface of *Microcystis* colonies are skin irritants.

Under the microscope: *Microcystis* colonies are better defined and more densely clustered relative to an *Anabaena* colony. Cells are spherical shaped (~4-5μm in diameter), enclosed in a mucilaginous sheath and usually joined with other individuals within the sheath to form small detached colonies; one can find hundreds of cells in one sheath. Each cell has gas vesicles that often appear blackish blue. Cells are non-filamentous, plantonic and not attached to objects.

N.B. *Mycrocystis* is often used synonamously with Anacystis in some literature. A new naming approach, introduced by Francis Drouet (1968), has resulted in the disuse of the name Anacystis.

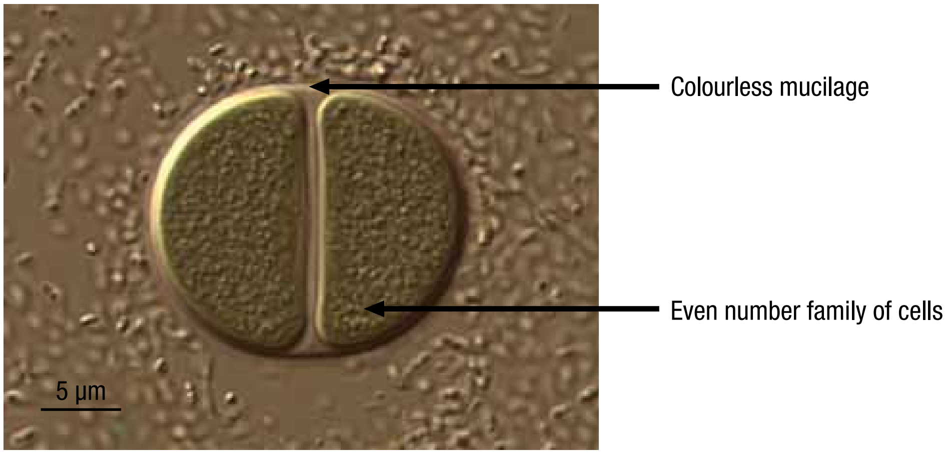


D. Krogmann and M. Schneegurt (Cyanosite 2006)

D. Patterson (micro*scope 2006)

▸ CHROOCOCCUS

Similar to *Gloeocapsa* (pg12), *Chroococcus* can be found attached to rocks or soil in or near water but have a colourless mucilaganous sheath. Under the microscope, cells are non-filmentous, plantonic and solitary or grouped in small families of 2, 4 or 8.



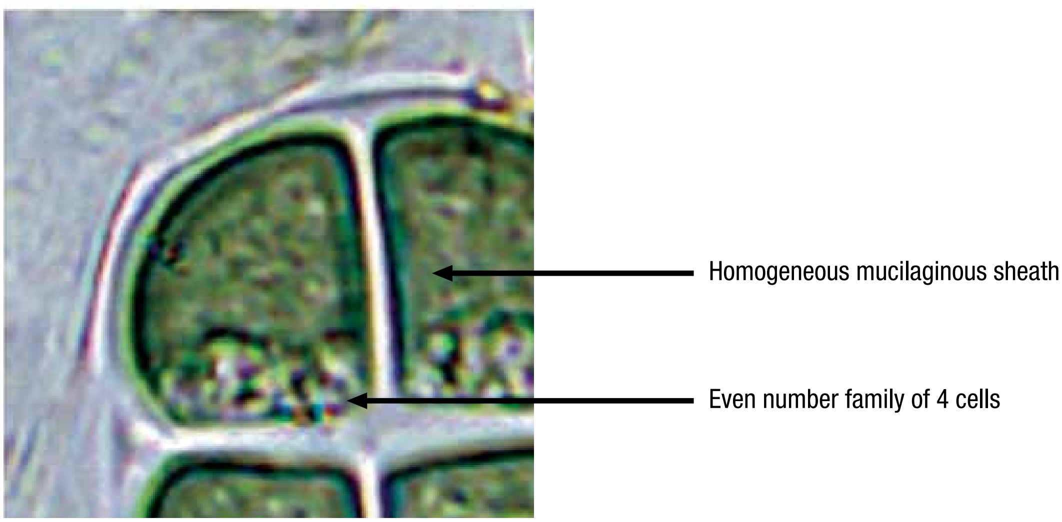
D. Patterson (micro*scope 2006)

BG Blue Greens

▸ GLOEOCAPSA

This subaerial genus often forms red, brown or orange gelatinous globs on substrates in or near water. The jelly-like colonies can be quite large and occur commonly on damp soil and rocks, but more typically on moist soil.

Under the microscope: When colonies are composed of only a few cells, *Gloeocapsa* can be confused microscopically with *Chroococcus* (pg11). These spherical non-flagellated, non-filamentous cells can be solitary or grouped in 2, 4, or 8 individuals that remain aggregated in gelatinous masses. Each individual cell is enclosed in a thick, homogenous or lamellate, mucilaginous sheath. The sheath may be yellow, brown or red, and even blue to violet.



M. Vis (Ohio University 2002)

▸ OSCILLATORIA

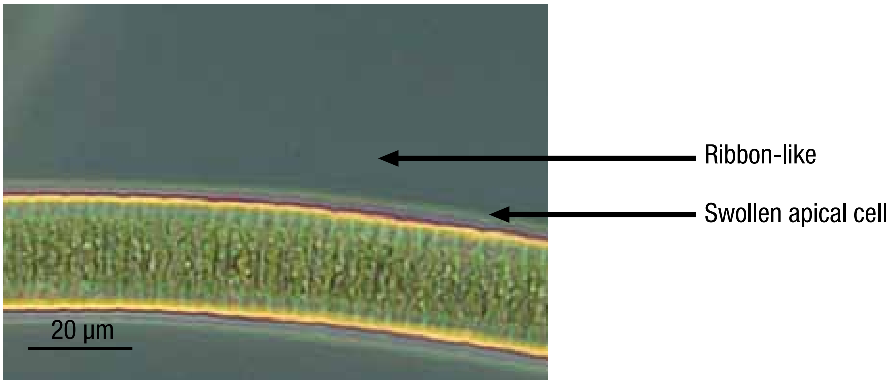
This filamentous blue-green can produce microcystin (hepatotoxin) and anatoxin-a (neurotoxin). *Oscillatoria* do not bloom in highly fertile waters, but blooms can occur in less productive (oligotrophic) lakes and reservoirs.

Oscillatoria species often range in colour such as green to blue, blue-green, purple and red. Individuals can occur singly, in aggregates with other algal communities, free-floating or attached to an object. *Oscillatoria* colonies occurring on rocks are one of the reasons fishermen wear felt-soled boots.

Oscillatoria blooms tend to occur at depths **in the water column** other than at the water's surface. Thus, water may appear clear on the surface but there may be a bloom forming three metres down. This can be important for people who have buried water intake lines in a surface water source. Blooms can be mat-forming as well. If it is left in a shallow dish in the lab, it will creep up the sides or spread itself over the bottom.

Oscillatoria rubescens is known to produce blooms under ice, turning the water brilliant red to dark purple. These blooms usually occur during late winter but may persist several weeks following ice melt in spring and contribute a red colour to the open water.

Under the microscope: *Oscillatoria* trichomes (thread of cells) often appear as dense, opaque ribbons which oscillate under the microscope. Hence, their name comes from their characteristic wavy 'oscillating' motions. The cells are short cylinders, usually wider than long. Trichomes are straight or irregularly twisted, with a swollen apical cell.



B. Anderson and D. Patterson (micro*scope 2006)

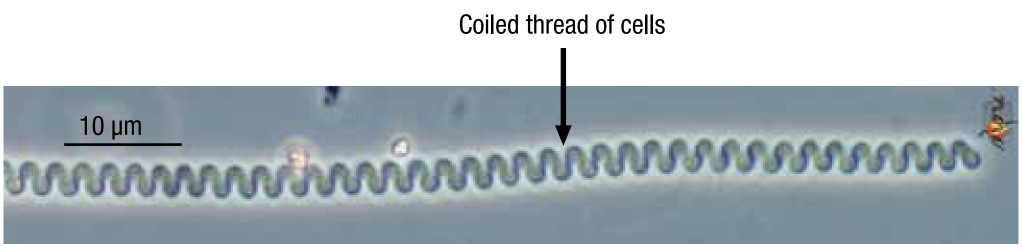
BG Blue Greens

▸ SPIRULINA

This genus does not form the intense blooms often associated with cyanobacteria nor does it produce toxins, but is of particular interest to agricultural producers because of its market value. It is most commonly marketed by health food producers as a general curative.

In the field, *Spirulina* colonies are commonly found as somewhat amorphous, light green masses either by themselves or associated with *Oscillatoria* (pg13). They are evident in standing water bodies from late spring through to fall.

Under the microscope: *Spirulina* appears as a coiled thread of cells (trichomes) and are unicelluar. Individual trichomes are essentially comprised of single cells that spiral down its entire length. Species are differentiated in part based on the tightness of the spiral. Like many blue-greens, *Spirulina* species extrude mucilage which flows along the length of the thread. This mucilage creates movement which can be viewed microscopically.



D. Patterson, L.A. Zettler and V. Edgecomb (micro*scope 2006)

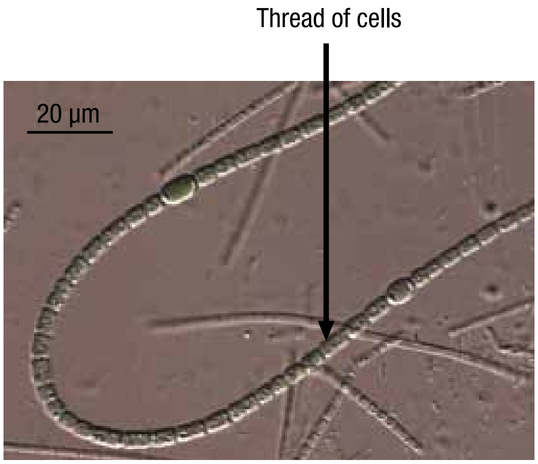
▸ ANABAENA

This genus is filamentous but does not form large colonies, and thus colonies are still distinguished in the field as planktonic with the 'open finger test'. Some *Anabaena* species are capable of producing anatoxin, or microcystin, or both. Although less common than *Microcystis*, periodic blooms of *Anabaena* can cause deaths of pets, livestock and wildlife. Heavy blooms may cause skin irritation for recreational water users.

Anabaena species are prevalent in phosphorus rich water and can cause pea-soup blooms in standing water after periods of warm, calm weather from late spring through summer. Anabeana can be especially prevalent if water is nutrient rich. There are often noxious taste and odour concerns associated with *Anabaena* blooms, especially once the bloom begins to decay. You may see tufts or flocs with aquatic plants or films on sediment.

Under the microscope: *Anabaena* is filamentous or threadlike, and characterized by the trichomes (thread of cells without its sheath) that are often coiled/spiralled. *Anabaena* threads often clump into loosely associated colonies held together by a soft mucilage. Trichomes are not parallel to each other, are multi-cellular, beadlike and not bundle forming. Each cell is spherical, beadlike, and non-flagellate.

M. Graham and S. Murrell (400X magnification)



Australian Biological Resources Study (micro*scope 2006)

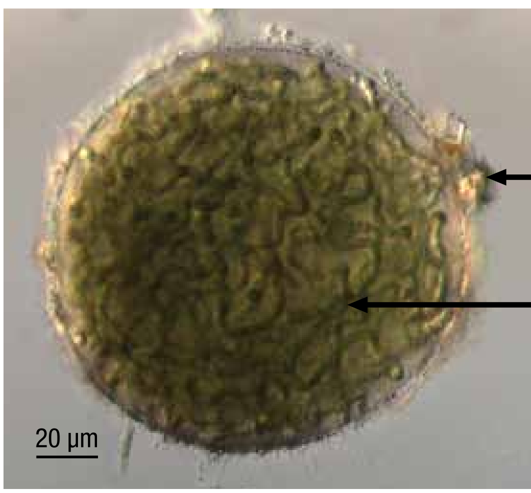
BG Blue Greens

▸ NOSTOC

Nostoc is probably one of the most readily identified cyanobacteria in the field. The colonies are found in a characteristic jelly-like ball which is actually a thick mucilage surrounding heavily coiled, bead-like threads. The rubbery colonies are often found floating in standing water bodies from mid-summer through to fall. Care should be taken with young children swimming in surface waters in summer. *Nostoc* colonies have sometimes been called 'freshwater grapes', and children have been know to eat them. Since the colonies are capable of producing cyanotoxins, this is not a recommended practice.

Colonies rarely occur in great numbers. They can be macroscopic in size, growing up to a few centimetres in diameter (between a dime to quarter in size). Colour of the mucilage ranges from yellow-clear to somewhat blueish to olive green.

Under the microscope: A colony contains a tangled mass of trichomes (thread of cells) that are enclosed in a thick mucilaginous matrix. Cells of the trichome are bead-like or barrel-shaped similar to *Anabaena* (pg15), but the trichomes are randomly tangled in a common jelly-ball sheath.



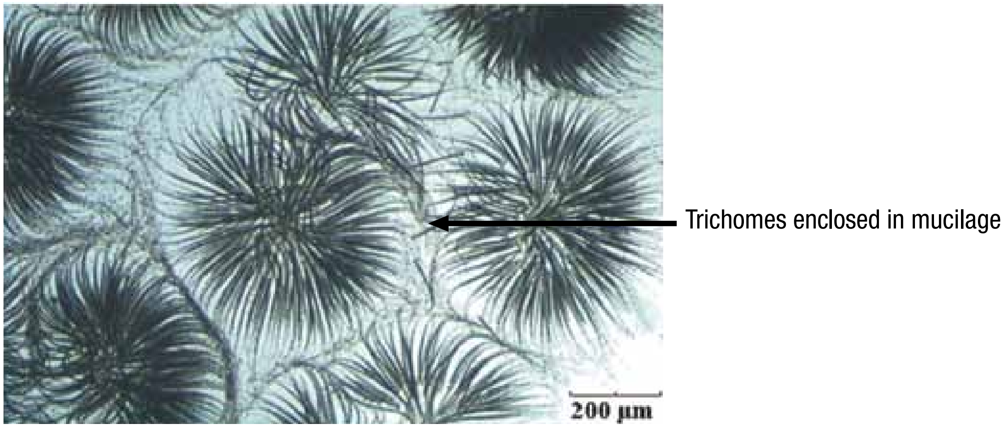
M. Bahr and D. Patterson (Micro*scope 2006)

▸ GLOEOTRICHIA

Gloeotrichia is generally recognized as non-toxic, but there is some question as to whether they are capable of producing toxins. Heavy *Gloeotrichia* blooms can however cause serious skin irritations for recreational swimmers. When mature, colonies are visible to the naked eye upon close inspection and look like numerous, tiny fish eyes, tapioca beads or pom poms. A heavy bloom will make the water appear buff or tan in colour. Some species of this generus can be green or blue. Gloeotrichica occurs primarily in lakes, ponds and other standing water, and blooms can occur from mid-summer to early fall.

Some *Gloeotrichia* species are found attached to submerged aquatic plants. Colonies are of a similar shape, but the mucilage tends to be much firmer in these epiphytic species. Colour ranges from green to black in these forms, and colony size is from 1 to 2 millimeters in diameter. Planktonic species are more soft. Common species found is *G.echinulata* and can occur in abundance in the plankton of hard water lakes.

Under the microscope: Individual threads of cells (trichomes) are enclosed in a soft mucilage resulting in small, globular colonies. Trichomes are straight or irregularly twisted, and tapered from base to apex.



P. York (York et al. 2002)

BG Blue Greens

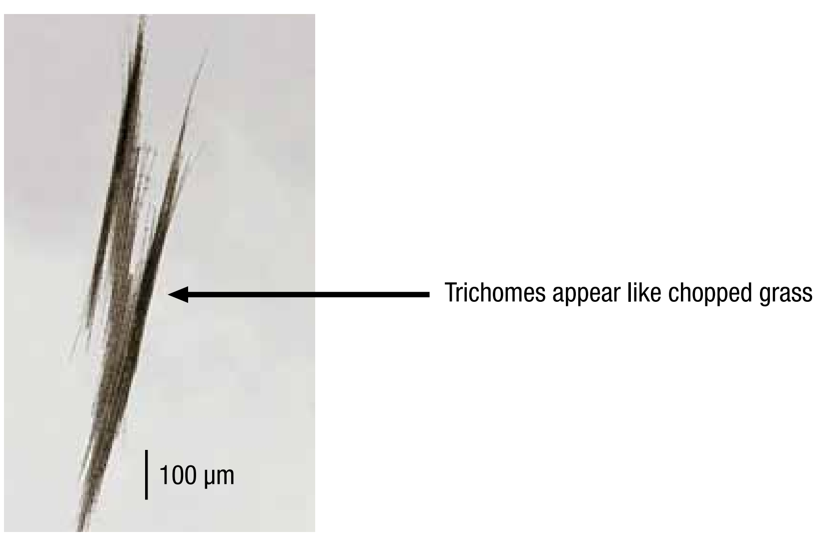
▸ APHANIZOMENON

Aphanizomenon is one of the most easily recognized genus of the blue-greens. Individuals form threads that tend to parallel one another and join into colonies to form a distinctive shape that resembles tiny grass or green fingernail clippings. In natural settings, the colonies are sickle-shaped clusters roughly 0.5 to 1.0 centimeters long. If cultured in a lab, colonies will not readily form this shape. The most common species is *A. flos-aquae*.

Heavy *Aphanizomenon* blooms also create pea-soup conditions in the water column and form after extended periods of warm, calm weather from late spring through summer. When in bloom, they tend to join into loosely formed clumps that superficially resemble some green algal colonies. However, scooped handfuls will easily break apart and pass through the open fingers of a cupped hand.

Some species are capable of releasing a neurotoxin called saxitoxin, similar to the paralytic shellfish poisin that occur in marine envrionments. In North American freshwater environments, *Aphanizomenon* appears to be primarily non-toxic though it can cause skin irritation to recreational water users. Decaying blooms also can release high concentrations of ammonia, potentially leading to summer fish kills.

Under the microscope: This genus is filamentous or threadlike, where the thread of cells (trichomes) are straight and parallel, forming a free-floating flake-like bundle. Each trichome contains 1-3 heterocysts. Heterocysts are large cells that look different in appearance- thick walled, weakly pigmented or clear compared to the other cells. Each trichome has tapered ends which appear like chopped grass. A distinctive characteristic of this genus is the akinete cell, which is cylindrical, darker in colour compared to the heterocysts and other cells, and typically found adjacent to the heterocysts. Akinete cells can help identify this genus to the species level

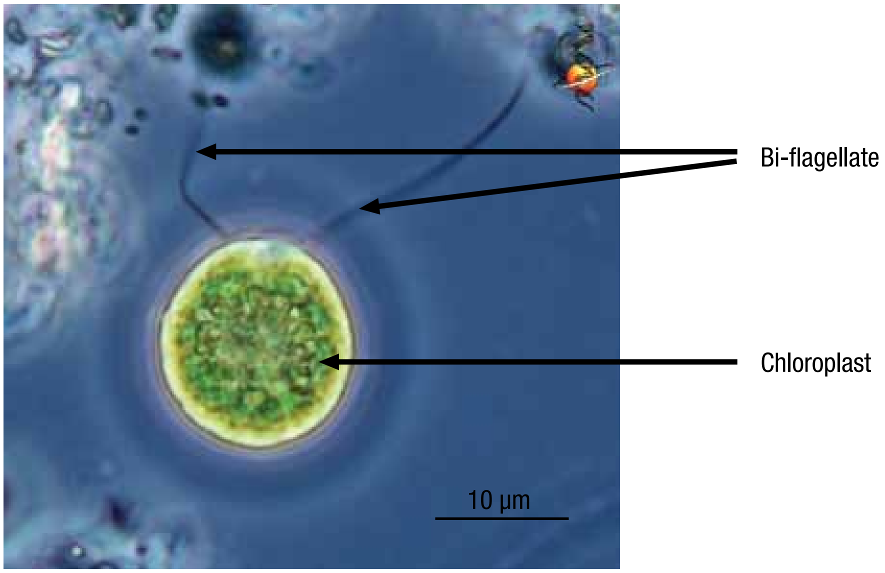


W. Bourland (micro*scope 2006)

▸ CHLAMYDOMONAS

Species of this genus are found in a wide variety of habitats, even in snow fields in the high alpine. They are common bloomers that exhibit a fishy smell in high numbers.

Under the microscope: They are solitary, small, elliptical-oval cells. They are also biflagellate (i.e. they have two whip-like tails). A tiny red **eyespot** at the base of the flagella is often evident. There is also a single large cup-shaped **chloroplast** that occupies most of the volume of the cell. They are often easily confused with other biflagellated algae or with the flagellated reproductive stages of other genera.



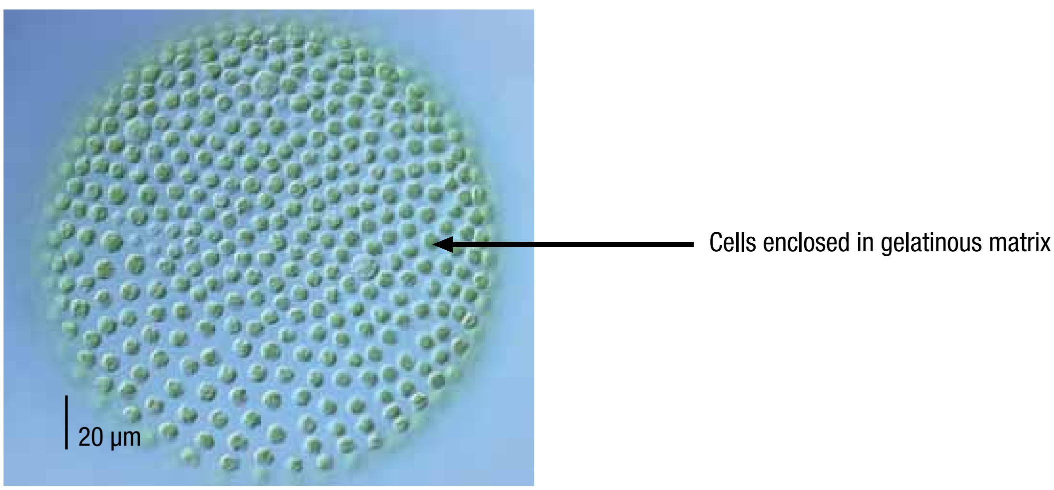
D. Patterson and A. Laderman (micro*scope 2006)

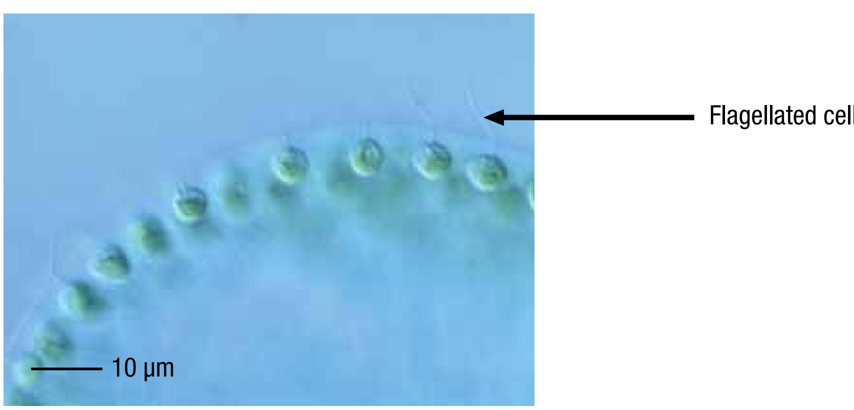
GR Green Algae

▸ VOLVOX

Volvox is often found within the general plankton of standing water systems. Blooms are infrequent but will occur over short periods during warm months, especially in waters contaminated with nitrogenous wastes or organically enriched water. Individuals of this genus join to form complex, spherical colonies that at their largest are visible to the naked eye (about 1 millimeter in diameter) and are usually yellowish-brown to light green.

Under the microscope: It is a large hollow sphere of a colony of algal cells, usually 500 or more, surrounded by a gelatinous matrix. Individuals comprising the sphere are spherical or ellipsoid, and biflagellate, i.e. they have two whip-like tails of equal length. The synchronized beating of all flagellae propels the colony through the water column. The cells are in a single layer on the periphery of the colony. Most cells are vegetative. Each cell also contains green cup-shaped internal structures called chloroplasts and large internal structures that store starch, which would stain dark with iodine.

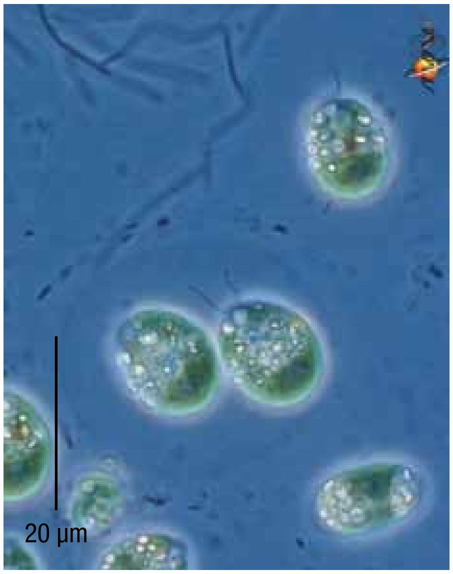




D. Patterson and M. Farmer (micro*scope 2006)

▸ TETRASPORA

Tetraspora species have colonies of minute, rounded cells in a macroscopic soft, fragile, mucilaginous common tube or sac. They are one of the first to develop in early spring. They are non-motile, non-filamentous and epiphytic (i.e. found attached to aquatic plants). They are typically associated with submerged substrata or can be found floating as colonial clumps and massive sheet-like skins in ditches, ponds, bogs and lakes.



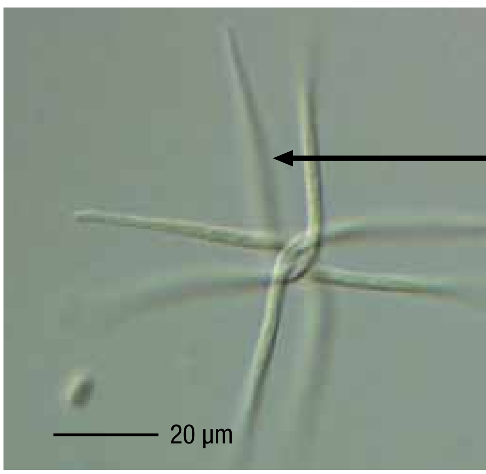
D. Patterson and M. Farmer (micro*scope 2006)

GR Green Algae

▸ ANKISTRODESMUS

These species are common within the open water phytoplankton. They will occasionally form pale green blooms **within the water column** during hotter months, but can bloom with regularity in personal aquaria or artificial pools. They are actually indicators of cleaner water since they tend to disappear from the algal community in very polluted systems.

Under the microscope: *Ankistrodesmus* exists as single cells which may form loose associations with each other (bundles, entangled with others), but not true colonies. Cells often elongate to cylindrical, straight, curved or spirally twisted shapes.



M. Bahr and D. Patterson (micro*scope 2006)

▸ CHLORELLA

Chlorella are green single-celled algae widely distributed in various fresh and marine water, soil and subaerial habitats. They are often consumed by freshwater invertebrate animals such as sponges. It has a grass-like smell because of the high amounts of chlorophyll contained within it, the highest concentration of any plant in the world. Certain species of *Chlorella* are harvested as a health food for humans and lower grades of *Chlorella* are fed to livestock. They are known to contain more than 20 vitamins and minerals including B complex, beta-carotene, vitamins C and E, iron, calcium, up to 70% protein, and 19 of the 22 amino acids.

Under the microscope: *Chlorella* is a small spherical green cell, often solitary and is non-flagellated. Its chloroplasts are thin and cup-shaped.

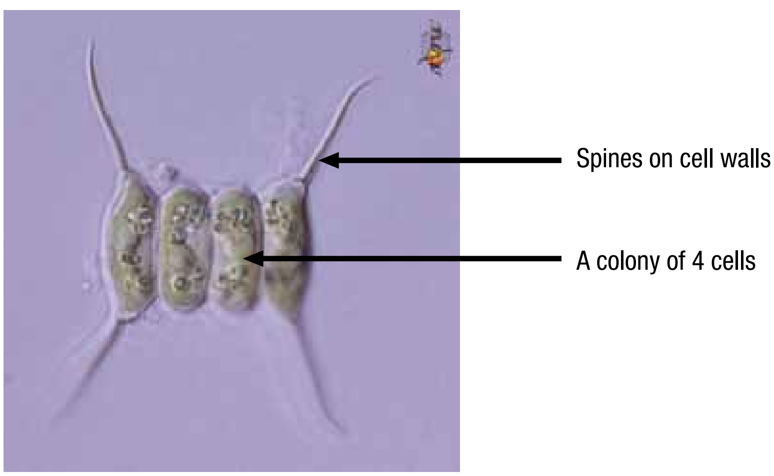
W. Bourland (micro*scope 2006)

GR Green Algae

▸ SCENEDESMUS

Colonies are too small to be visible to the naked eye. Blooms can occur in natural systems during warmer months, colouring the water a lovely shade of green. It is frequently abundant in nutrient-rich waters. This genus will typically develop along with others belonging to the Order *Chlorococcales* such as *Ankistrodesmus*, *Chlorella*, *Hydrodictyon* and *Pediastrum*. *Scenedesmus* is also a common uninvited guest of many freshwater aquaria. *Scenedesmus* is widely distributed in standing water systems. It occurs as a typical member of the plankton of open and near-shore water. The most common species is *S. quadricauda*.

Under the microscope: Colonies in this genus occur in a wide variety of forms but are comprised of 4-12 cells adjoined along the entire length of 1 cell wall. They form a coenobium, a colony that always has the same number of cells once it is formed. Cell arrangement and shape is used to distinguish one species from another, except in laboratory cultures where development is often atypical. Colonies contain chloroplasts, and are non-motile and non-filamentous. Cell walls are smooth, or with 1 or 2 curved spines or teeth. Note that not all species of this genus have the typical spines on the cell walls.



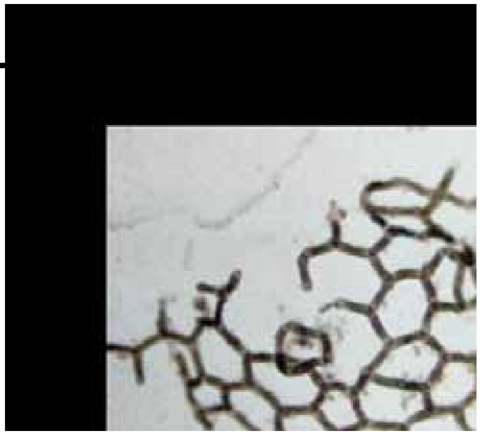
L.A. Zettler and D. Patterson (micro*scope 2006)

▸ HYDRODICTYON

Hydrodictyon is a filamentous alga which forms net-like shaped colonies. The common name for this genus is 'water net'. This is owing to its habit of growing so profusely that it can become a very bothersome, weed-like intruder. It is common in summer in standing or very slow-moving water, including irrigation ditches. It does not produce toxins but it will bind boat props and clog intake lines. Scooping up a handful of the alga will reveal the net-like appearance of the colony. This genus is found growing in hard water lakes and ponds. Dense mats are unsightly in appearance and produce unpleasant odours particularly as the organism begins to decompose. This genus is also very susceptible to copper treatment.

Under the microscope: Cells can be seen grouped into cyclical or sheet-like colonies to form definite patterns, and a network of cells. Reproduction in each cell produces not just another cell, but a replication of the entire original structure. In this manner, *Hydrodictyon* grows very rapidly and mat sizes quickly become macroscopic. They are non-motile, non-filamentous and colonial.

A network of cells



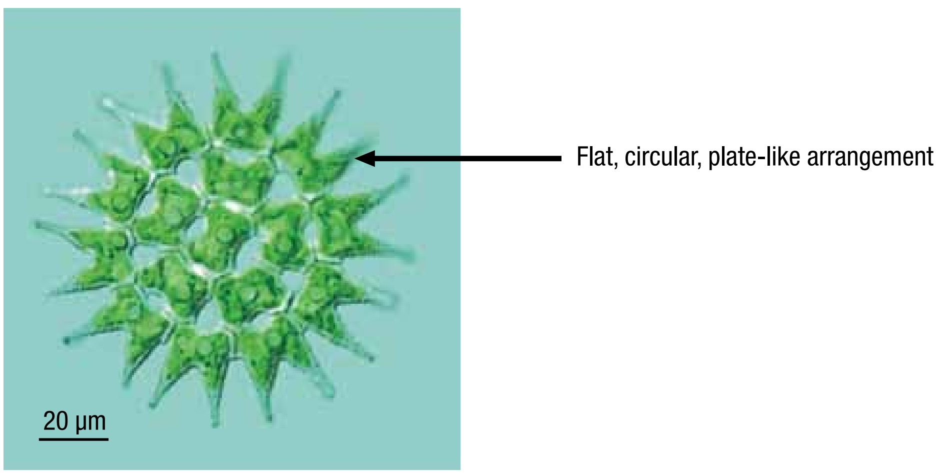
M. Vis (Ohio University 2002)

GR Green Algae

▸ PEDIASTRUM

On occasion, *Pediastrum* species will form deep green blooms during hot summer months. Blooms are usually **relegated to the water column** and do not result in the surface scums associated with cyanobacteria (do not produce film on substrate). Colonies commonly occur in ponds, small lakes and ditches intermingled with other members of the plankton community. The cell walls of *Pediastrum* are so extraordinarily rigid and resistant to decay that specimens have been found in fossil records. Blooms are often common in moderately nutrient-enriched water bodies.

Under the microscope: *Pediastrum* is colonial with a very distinctive, unmistakable star shape. Colonies are flat, circular and have a multi-cellular plate-like arrangement. Cells within the arrangement differ in shape from those outside. Outer cells form spine-like extensions of the colony. Cells are also non-motile (no flagella).

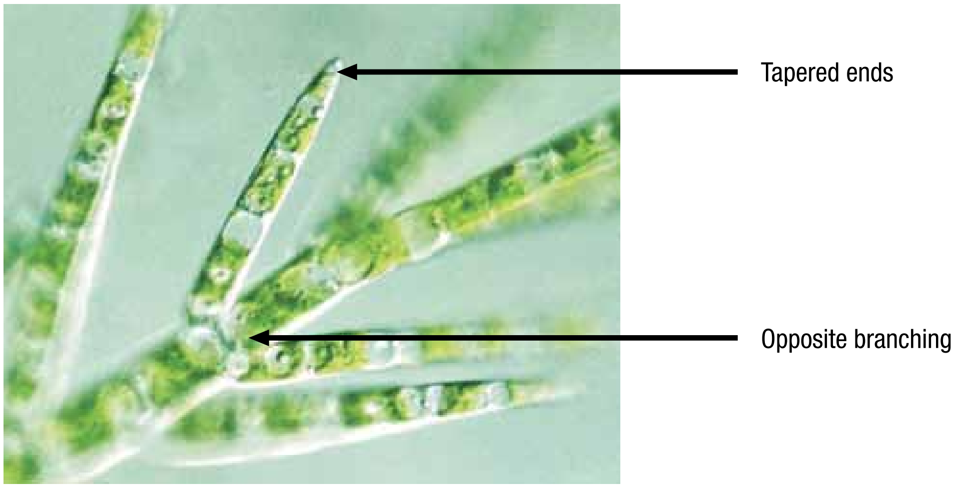


D. Patterson and M. Farmer (micro*scope 2006)

▸ STIGEOCLONIUM

A poster-genus for green algae, *Stigeoclonium* is a distinctly bright green alga often attached to rocks in fast-flowing water. Although it forms mats or streamers, it is never as large or as slimy as those of *Spirogyra* (pg30). Colonies will definitely not pass through the open fingers of a cupped hand.

Under the microscope: Identification to species are based on branching patterns of the colonies and by the overall shape of the formed tufts. Filaments grow as erect branched tufts or plumes, and the branching can either be alternate or opposite. The branches are thorn-like, short or long, and tapering to fine points.



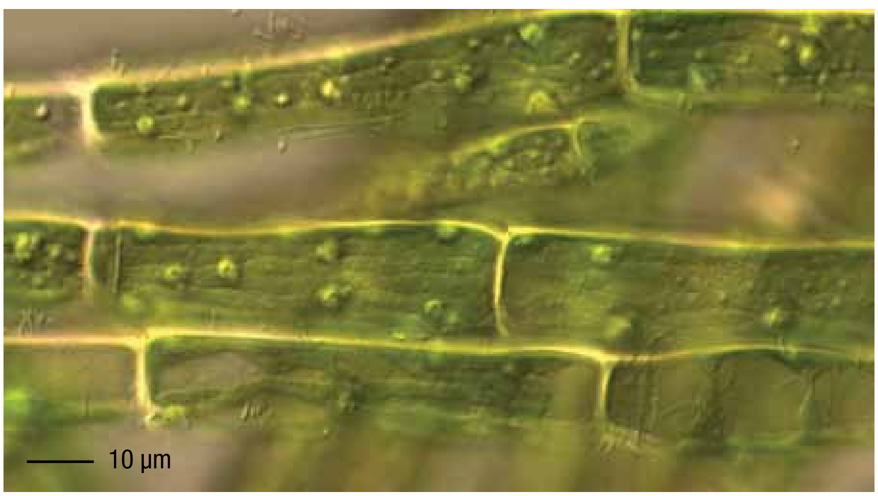
P.V. York (York et al. 2002) (22 µm cell width)

GR Green Algae

▸ OEDOGONIUM

Scooped up from the water, it adheres to one's hands rather than slipping through one's fingers like *Spirogyra* (pg30). *Oedogonium* frequently begins life as small filaments attached underwater to near-shore vegetation. Some species remain attached, and excessive growth can impede water flow in drainage canals. Some detach to form free-floating, fluffy colonies found just beneath the water's surface. Colonies are usually light green in youth, fading to a pale green or tan colour with age. They often occur in such dense associations near shore that evaporating water leaves behind dried masses of 'algal paper'. *Oedogonium* is an unbranched filament that is common in quiet fresh waters. This genus is also very susceptible to copper treatment.

Under the microscope: *Oedogonium* cannot be identified well to species if samples are collected before reproductive structures have developed. Also, many species may grow together in close association, making identification to species a challenge. Filaments of cells are not quite cylindrical but are slightly larger at the anterior end.



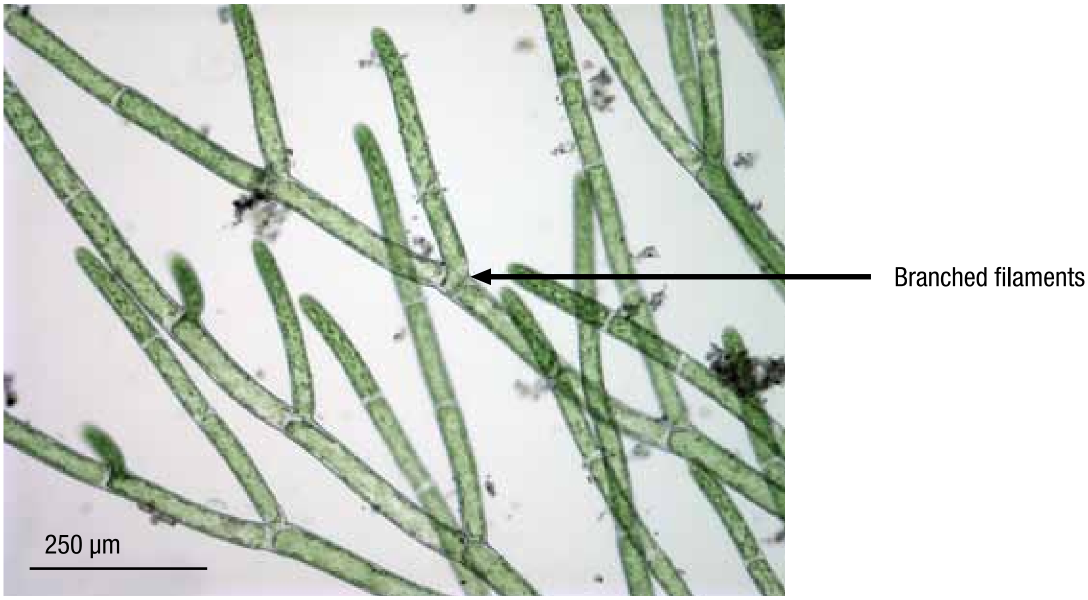
D. Patterson (micro*scope 2006)

▸ CLADOPHORA

There are a few freshwater species of this genus. Freshwater *Cladophora* species are commonly found as colonies attached to rocks, plants and almost any other stationary aquatic substrate, but can be detached by wave action. And although they are epiphytic (attaches itself to other substrate or organisms), they produce no mucilage and hence are heavily fouled by epiphytes. They occur in both flowing and standing water, although they are most often associated with streams, rivers and waterfalls.

Cladophora growth results in long, filamentous mats. The form of the filaments within the mat can vary between habitats and seasons. Mats that overwinter or constantly wave-washed will have a sturdier form than those found in slower-moving or standing water. *Cladophora* can be fairly abundant during warmer periods of the year, and ranges in color from green to yellow-brown to orange. Septic odour is present when they are abundant, which can be attributed to the decomposition of the algal mat.

Under the microscope: Filaments are branching, not tuft, and composed of multinucleate cells. Cells walls are thick, and chloroplasts can be reticulate (net-like) or divided into numerous small discs.



W. Bourland (micro*scope 2006)

GR Green Algae

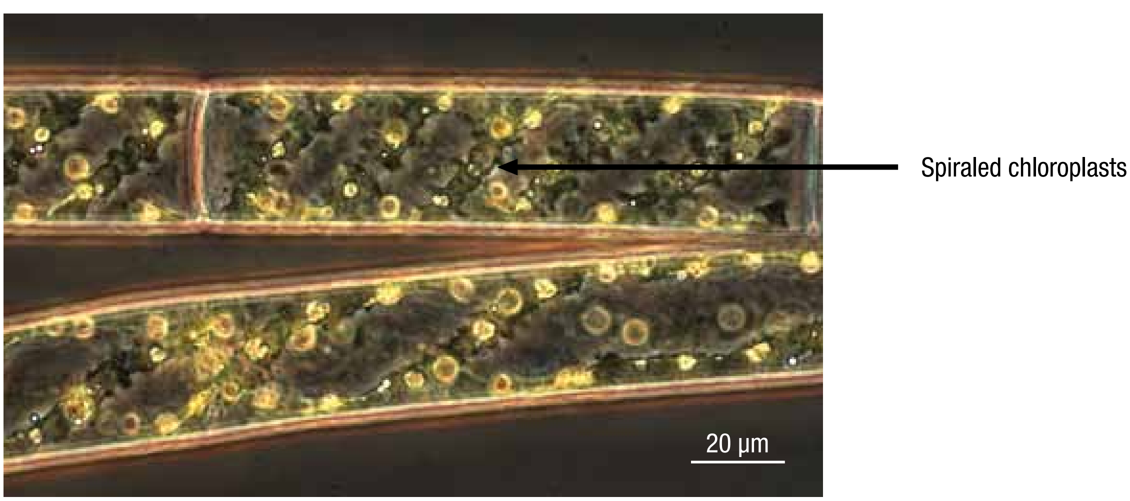
▸ SPIROGYRA

Spirogyra or 'water silk' is a common green alga found in shallow warm water where it can form extensive floating mats. It is the most frequently encountered member of the 'frog spit' Order Zygnematales. One can detect the silky quality of the filaments by trying to lift the alga from the water. This genus is very common in both standing and flowing waters. Species can be either attached or free-floating.

The floating varieties can form large mats capable of blocking stream channels and are visible as cloudy filaments just below the water's surface. Colour is usually a vibrant green while the alga is in its spring growth stage. Once it reaches a reproductive stage however, the colour can shift to a yellowish-brown. The filament mats feel very slimy to the touch, noticeably so when swimming or wading through them. Species of *Spirogyra* can be found from mid-summer to fall. Abundant growth in spring can result in clogged filters, seepage areas, drainage canals, and coves. Dense mats can deplete oxygen, interfere with fishing, or provide breeding sites for mosquitoes.

Under the microscope: The reasoning for the name *Spirogyra* is evident. While the filaments themselves do not spiral, the chloroplasts within the filament cells certainly do. The chloroplast is a bright grass-green helical peripheral ribbon. Identification to species is not possible without mature specimens. Try not to confuse the name *Spirogyra* with *Spirulina* (a cyanobacteria, pg14). They each spiral in their own special way.

Under the microscope: Filaments are branching, not tuft, and composed of multinucleate cells. Cells walls are thick, and chloroplasts can be reticulate(net-like) or divided into numerous small discs.

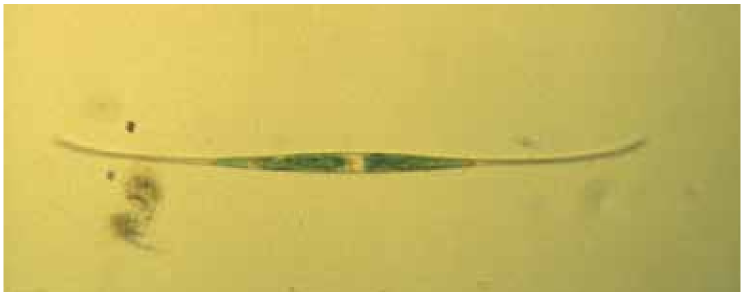


Australian Biological Resources Study (micro*scope 2006)

▸ CLOSTERIUM

Closterium is generally found free-floating in standing water, and usually mixed in with other species of green algae, especially filamentous forms. It is common in acidic (ph<7) waters, while a few species are found in nutrient-rich hard waters.

Under the microscope: Species in this genus are often single-celled (solitary) and may have a variety of shapes but are commonly elongated and crescent-shaped (bowed). They follow a pattern of being slightly widened in the middle and pointed on the ends.



M. Graham and S. Murrell (400X magnification)

D. Patterson and A. Laderman (micro*scope 2006)

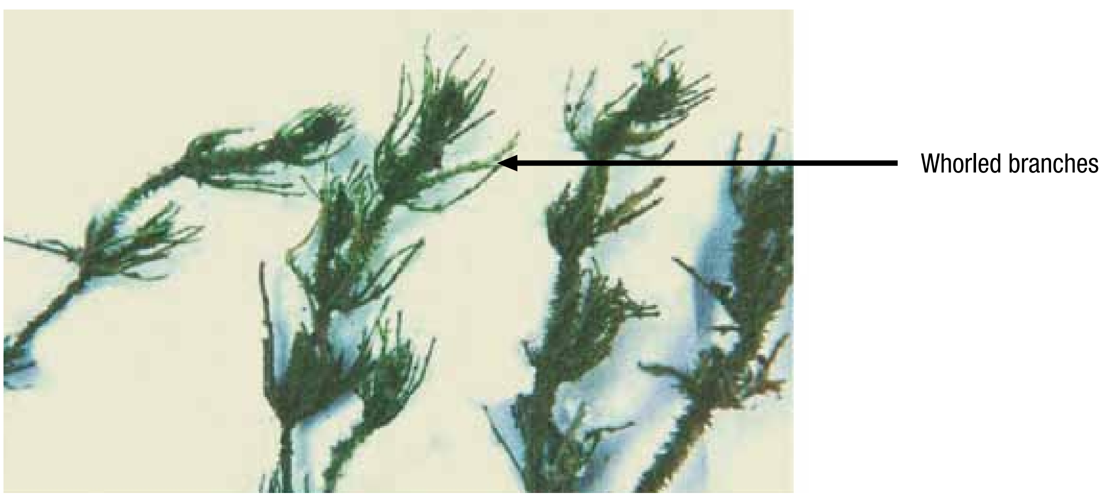
GR Green Algae

▸ CHARA

Chara, or stonewort, is a green alga that is plant-like in appearance and can grow several decimetres tall. Most grow in still, clear freshwater where they form extensive underwater meadows, anchored into the sediment by their rhizoids. They are easily recognized by their whorled branches and musky or garlicky odour.

They are particularly abundant in hard, basic waters (pH>7) where calcium is abundant. Calcareous deposits are found on the surface of the algae, making them feel rough to the touch, which gives rise to its name 'stonewort'. Problems with the species occur when dense growths impede water flow and interfere with recreational activities.

It is often confused with *Nitella* (not referenced in this manual), as both look very similar. Branches arise from each node: with *Nitella*, the branches themselves also branch; in *Chara*, the branches do not branch. Also, *Nitella* is not ill-smelling and found in soft water or acid lakes and hence, is not encrusted with lime like *Chara*.

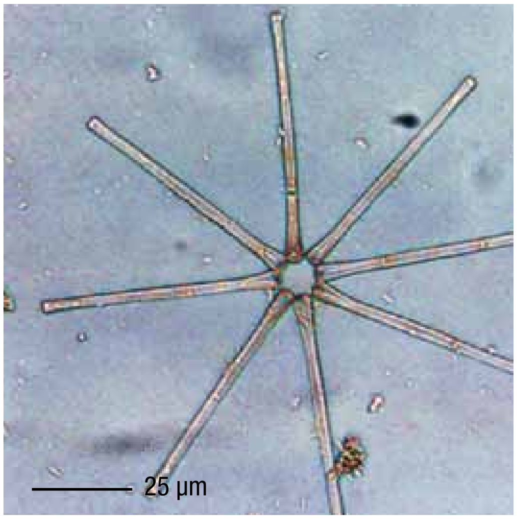


N.F. Stewart (York et al. 2002) (In situ)

▸ ASTERIONELLA

Colonies are too small to be visible to the naked eye, but they are planktonic and often very abundant in hard water lakes. They are well known to clog filters because of their rigid walls. An easy way to identify their presence is by smell- if low to moderate numbers exist, a geranium odour will likely be present. However, the odour changes to fishy when high numbers are present.

Under the microscope: Frustules (unique cell wall of silica) in girdle view are elongated and rectangular, and form a circular colony (usually 8 cells) in which the cells radiate from a common center like spokes of a wheel.



M. Vis (Ohio University 2002)

DM Diatoms

▸ SYNEDRA

Synedra, in moderate to high abundance, has a distinctive earthy to musty odour. It is also well known to be a typical filter-clogging alga because of its rigid walls. *Synedra* is common in the plankton community and in scums on substrate. Colonies are too small to be visible to the naked eye.

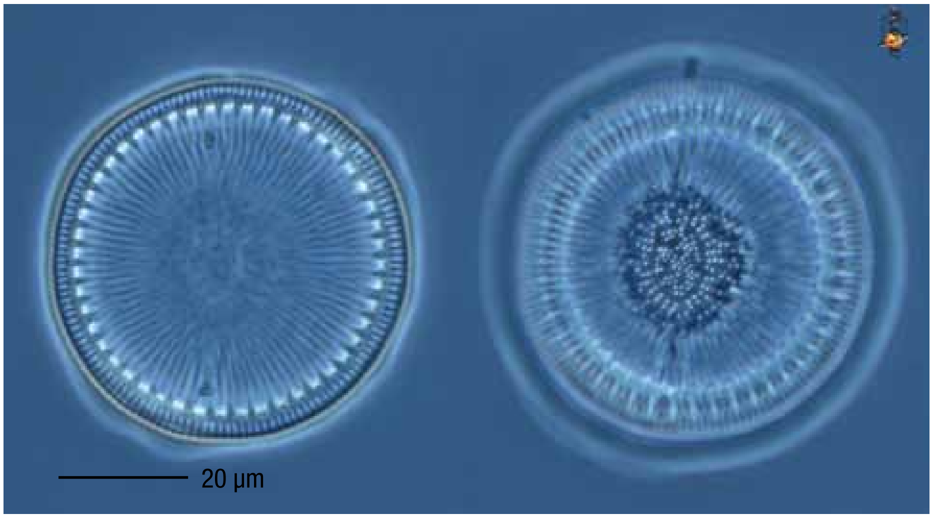
Under the microscope: *Synedra* species are large, elongated needle-shaped diatoms that are often bilaterally symmetrical. They are much longer than wide and are usually straight, although some species are curved, in which case they are not bilaterally symmetrical. Frustules (unique cell walls of silica) are either solitary or in radiating colonies attached to substrate at one end.

M. Vis (Ohio University 2002)

▸ CYCLOTELLA

Besides *Synedra* (pg34), *Cyclotella* is a common bloomer of the diatoms. It is a well-known filter-clogging alga and often found in 'clean' water. That is to say, its abundance often decreases in nutrient-rich water. Colonies are too small to be visible to the naked eye but the species are planktonic.

Under the microscope: The valve view of *Cyclotella* will show circular cells with a smooth central region and a grooved or ridged outer edge. The girdle view is narrowly rectangular.



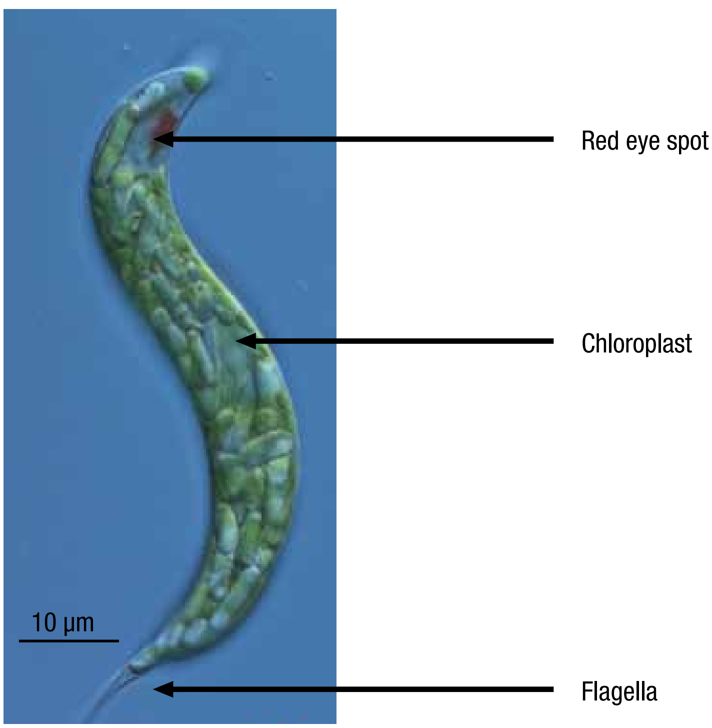
Valve view: M. Bahr and D. Patterson (micro*scope 2006)

FL Flagellates

▸ EUGLENA

There are more than 800 species in this genus. They are mostly found in freshwater habitats that are polluted by organic waste or decaying organic matter which can give rise to very dense populations of bloom proportion. *Euglena* blooms can be red.

Under the microscope: Euglenids are motile (have flagella), typically spindle-shaped and often uni-cellular. Many have disc-like chloroplasts (pigmented structures within the cell) that are able to supplement photosynthesis by taking up organic compounds. Colourless species do exist however, and are totally dependant on heterotrophic nutrition. Cells lack a distinct cell wall, do not stain (negative test for iodine) and are characterized by their red eye spot.

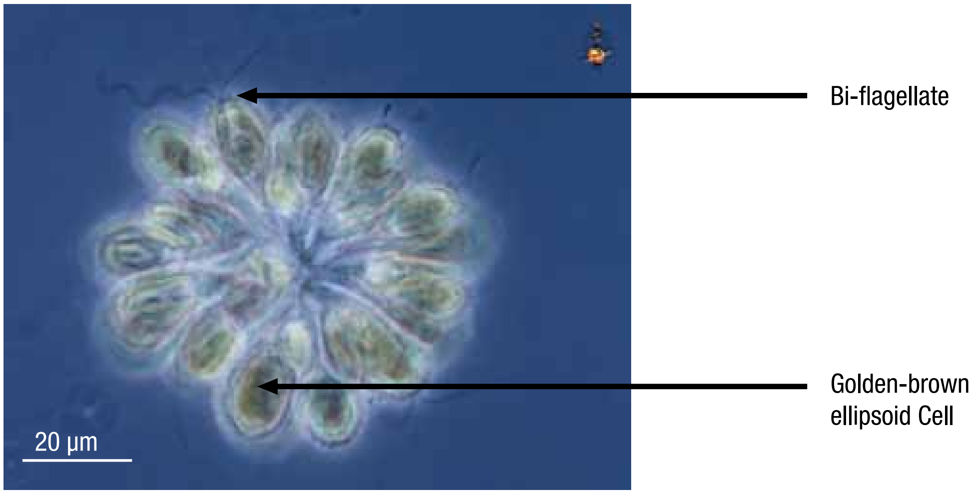


D. Patterson and M. Farmer (micro*scope 2006)

▸ SYNURA (GOLDEN ALGAE)

Synura species are common in hard water lakes. When present in high abundance, they can cause odour and taste problems. *Synura* exhibits a ripe cucumber/musk melon odour in low to moderate numbers, and a fishy smell in high abundance with other algae. When tasted, it is bitter and leaves a dry sensation on the tongue (tasting algae is not recommended however).

Under the microscope: Cells are elongate-ellipsoid and compactly arranged to form a spherical colony that is NOT in a mucilaginous sheath. Species which produce siliceous covering on the surface of the cell may have bristles or scales. Cells are biflagellate, having flagellae of equal length. Chloroplasts are golden brown, a common characteristic of Chrysophytes (golden algae).



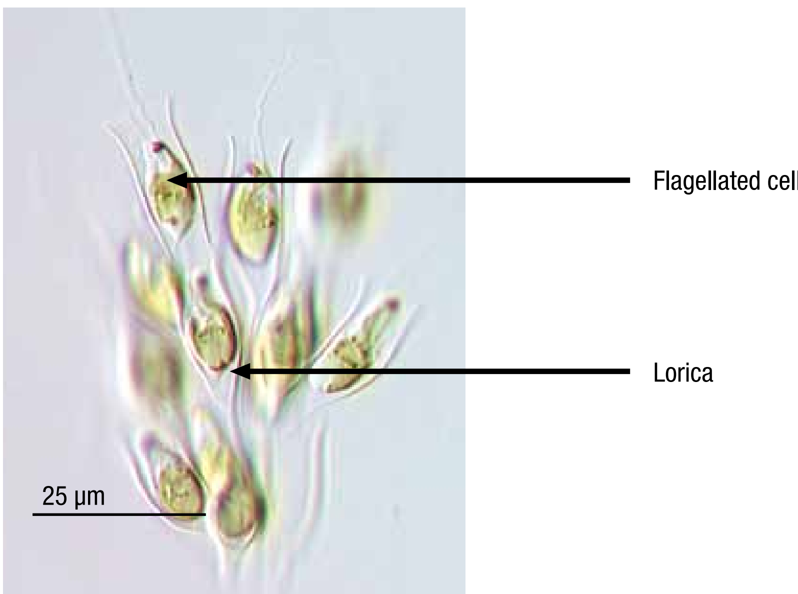
D. Patterson and A. Laderman (micro*scope 2006)

FL Flagellates

▸ DINOBRYON (GOLDEN ALGAE)

In summer, *Dinobryon* species are common bloomers of Golden Algae and are typically found in hard water and eutrophic water bodies. It is an alga of concern because it can cause taste and odour problems, especially in combination with other algae. They are also known to clog filters.

Under the microscope: *Dinobryon* species of this genus are characterized by having motile cells enclosed within a colourless envelope. They are colonial and typically have vase-shaped cones (loricas) that are stacked on top of each other which result in a branching chain. Each individual cell has 2 unequal flagellae but the cells are often absent from the lorica. Chloroplasts are golden brown, a common characteristic of Chrysophytes (golden algae).



W. Bourland (micro*scope 2006)

▸ DUCKWEED

Duckweed is often referred to as algae but it is in fact a tiny colonial, aquatic perennial plant. Individuals are small, disc-like and free-floating. Individuals are typically 2-5 centimeters long. By taking a closer look at the underside of an individual, short hair-like roots will usually be found. Common genera of duckweed include *Lemna*, *Wolffia*, or *Spirodela*.

N. Serediak (in situ)

ALGAL PRODUCTION

The most important parameters regulating algal growth are nutrient quantity and quality, light, pH, salinity and temperature

Culture medium/nutrients: Concentrations of cells in phytoplankton cultures are generally higher than those found in nature. Algal cultures must therefore be enriched with nutrients Macronutrients include nitrate, phosphate (in an approximate ratio of 6:1), and silicate. Silicate is specifically used for the growth of diatoms which utilize this compound for production of an external shell. Micronutrients consist of various trace metals and the vitamins. Two enrichment media that have been used extensively and are suitable for the growth of most algae are the Walne medium and the Guillard's F/2 medium.

Light: As with all plants, micro-algae photosynthesize, i.e. they assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this reaction and in this regard intensity. Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture 5,000-10,000 is required for larger volumes). Light may be natural or supplied by fluorescent tubes. The duration of artificial illumination should be minimum 18 h of light per day, although cultivated phytoplankton develop normally under constant illumination.

pH: The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2-8.7.

Aeration/mixing: Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients and to improve gas exchange between the culture medium and the air

Temperature: The optimal temperature for growth between 20 and 24°C

Salinity : Marine phytoplankton are extremely tolerant to changes in salinity.

Algal Culture Media

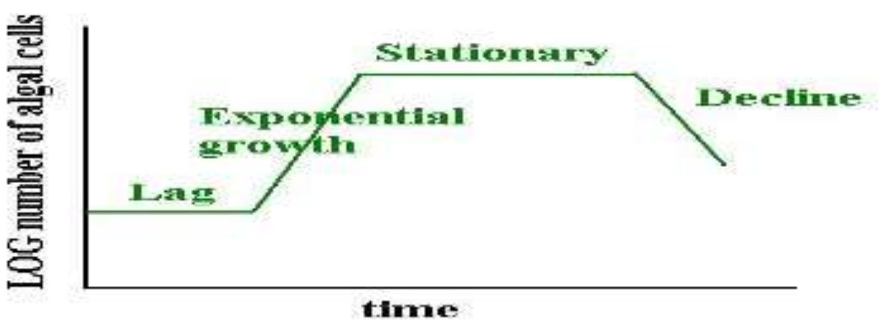
In order to grow algae in the classroom you will need to make up some growth media. In their natural habitats algae obtain all the nutrients, minerals and vitamins they require from the water in which they live. To grow them in the lab you must provide them with all of these essential the algae grow in theory culture condition should resemble the alga's natural environment as far as possible.

Algae cultures of limited volume (Batch culture)

In this method algal cells are allowed to grow and reproduce in a closed container. The batch culture consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density. In practice, algae are transferred to larger culture volumes prior to reaching the stationary phase. They have a finite amount of nutrient, and when that is exhausted, their growth stops and eventually they die. These types of cultures typically last for about one week. The most common culture system is the batch culture, due to its simplicity and low cost. This is a closed system in which there is no input or output of materials.

The photo below shows a typical batch culture set-up.

Limited volume of medium containing the necessary nutrient when inoculated with algae cells and then exposed to suitable conditions of light, temperature and aeration. Increase in cell number follows a characteristic course as:



Phases in the growth curve illustrated a typical algal batch culture

There are five phases of algal growth, lag phase, exponential growth phase, Declining growth ,stationary phases and death phase.

The Lag (induction) phase is the time where the alga is not reproduction, this lasts for about 4-6 days. This phase, during which little increase in cell density occurs.

After a while, the algae multiplies super-fast in a short period of time. This is called the **Exponential growth phase** during the second phase, the cell density increases.

Later, the algae reach a point where there is not enough space for growth and there are no more nutrients in the water so the algae stop reproducing and the growth rate are balanced, which results in a relatively constant cell density. This is called **the Stationary phase**. In the middle of this phase is the optimal time to harvest the algae.

Phase of **Declining growth** rate; cell division slows down when nutrients, light, pH, carbon dioxide light intensity, auto inhibition or other physical and chemical factors begin to limit growth.

If the algae are not harvested in the stationary phase, they will move to **the Death phase.** There is no more space and nutrients to grow so cell density decreases rapidly and the culture eventually collapses.

In practice, culture crashes can be caused by a variety of reasons, including the depletion of a nutrient, oxygen deficiency, overheating, pH disturbance, or contamination. The key to the success of algal production is maintaining all cultures in the exponential phase of growth

Continuous Culture

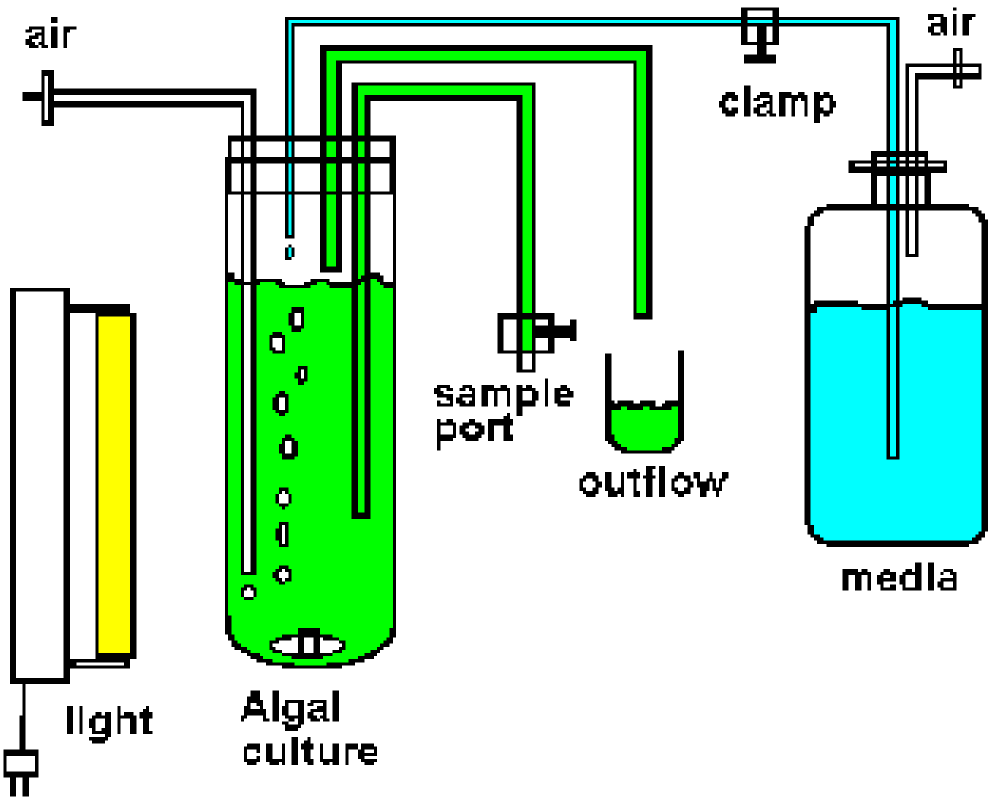
This method of culturing algae differs from the batch culture method in that fresh medium is added to the culture at a constant rate and old media (and some of the algae cells) is removed at the same rate. Two categories of continuous cultures can be distinguished:

Turbidostat culture, in which the algal concentration is kept at a preset level by diluting the culture with fresh medium by means of an automatic system.

Chemostat culture, in which a flow of fresh medium is introduced into the culture at a steady, predetermined rate. The latter adds a limiting vital nutrient (e.g. nitrate) at a fixed rate and in this way the growth

The diagram and photographs below show the parts of a continuous culture system.

First, fresh growth medium is stored in the large vessel. Air is pumped into the airspace in this medium vessel. This air pressure will push the medium through a tube which is connected to the culture vessel. By opening and closing the clamp on this medium line one can add medium to the culture vessel.

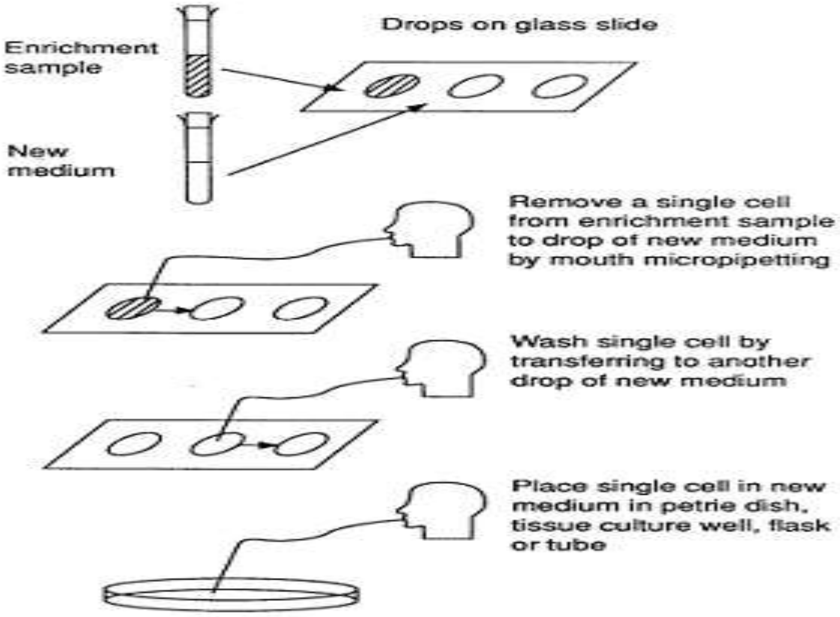


Air is also pumped into the culture vessel. This air passes down a long glass tube to the bottom of the culture and bubbles up. This serves to keep the culture well suspended as well as high in oxygen and CO2. The air flowing into the culture vessel flows out through an outflow tube. As fresh medium is added to the culture vessel the level of the liquid in the culture vessel rises. When that level reached the bottom of the outflow tube old medium and cells flow out of the culture vessel into a waste flask. There is one other glass tube in the culture vessel, the sample port. When you need a sample of cells from the culture vessel you open up the clamp on the sample port and medium and cells flow out. When you have enough you reclamp the sample port.

When choosing a culture medium the nature habitat of the species should be considered in order to determine its environmental requirements. Algae media refers to the solution or culture in which algae grow, and there are two major types of algae media, enrichment and artificial media. An enrichment medium is generally made by adding soil extracts to distilled or natural water or by simply adding chemical nutrients to seawater or chemicals and doesn't include additions of soil extracts or natural lake or sea water. This artificial medium is mostly used under laboratory conditions to exacting standards, although unknown impurities can still be present in even the most carefully prepared artificial medium.

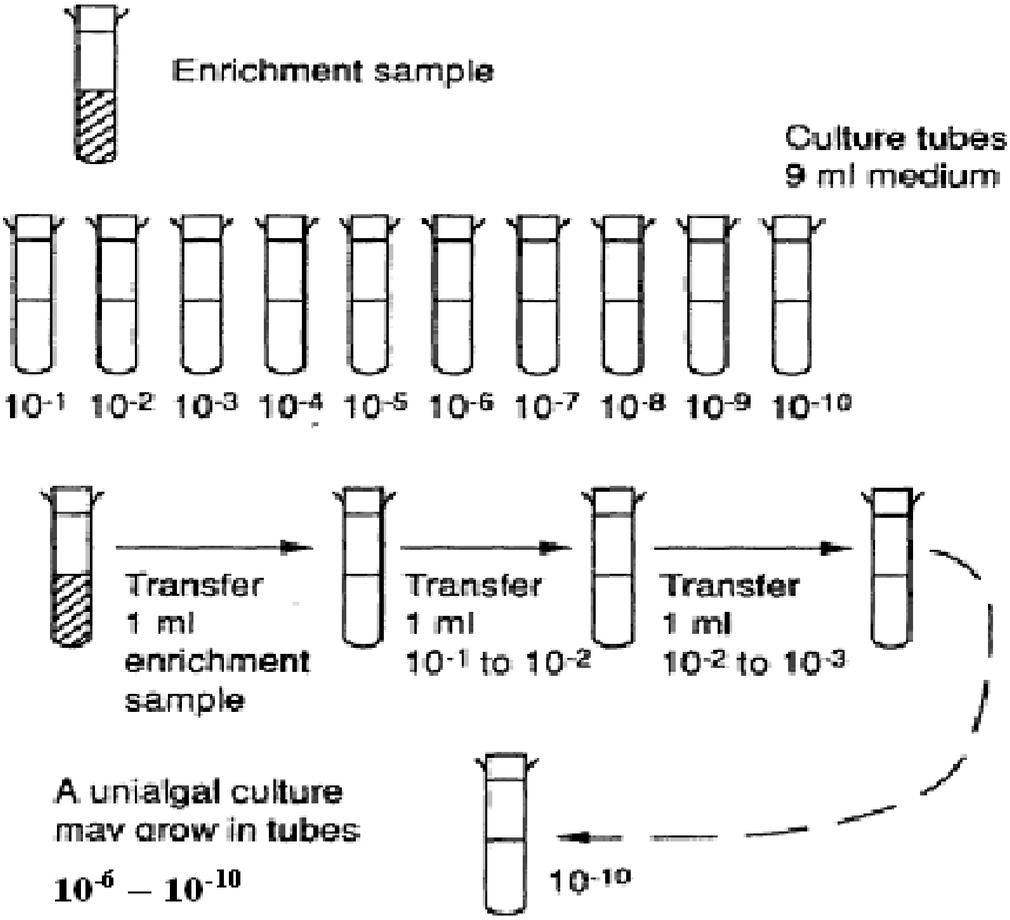
Microalgal Isolation Techniques

(a) Micromanipulation:



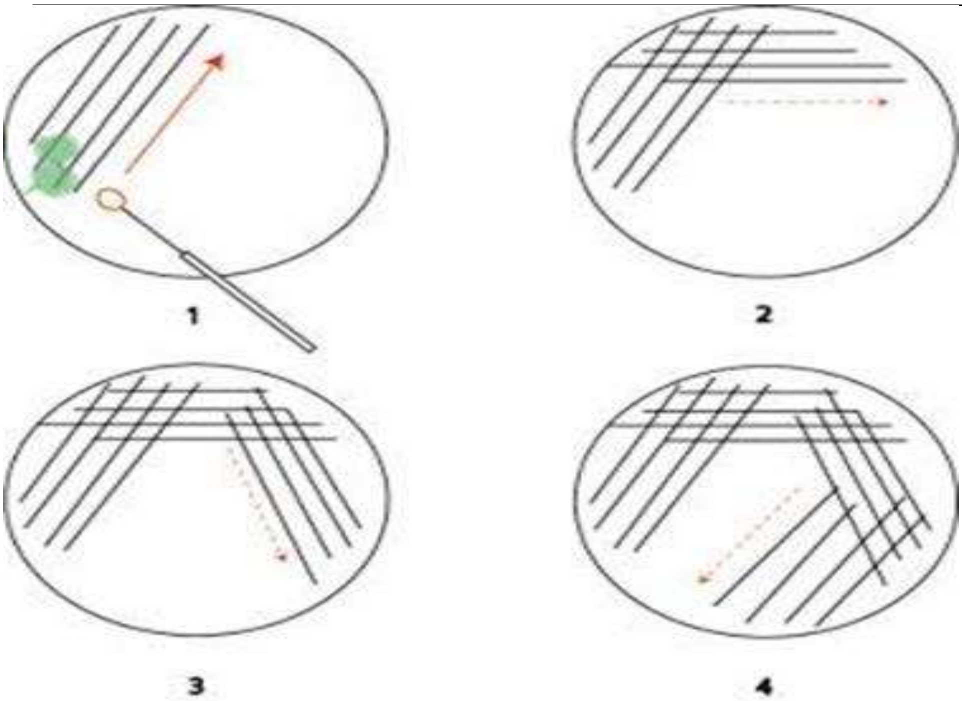
(b) Serial dilution:

dispense 9 ml of media into each of ten test tubes with sterile automatic dispenser. Label tubes 10^{-1} to 10^{-10} indicating dilution factor .Aseptically add 1 ml of enrichment sample to the first tube (10-1) and mix gently.Take 1 ml of this dilution and add to the next tube (10-2), mix gently. Incubate test-tubes under controlled temperature and light conditions



(c) Streak plating:

Prepare petri dishes containing growth medium solidified with 1-1.5% agar medium.Place 1—2 drops of mixed phytoplankton sample near the periphery of the agar. use the sterile loop to make parallel streaks of the suspension on the agar ·Remove a sample using a sterilized wire loop and place in a drop of sterile culture medium on a glass slide. Check microscopically that the desired species has been isolated and is unialgal . Repeat the streaking procedure. This second streaking reduces the possibility of bacterial contamination and of colonies containing more than one algal species.Transfer selected colonies to liquid or agar medium.



(d) **Density centrifugation and Antibiotics and specific cell inhibitors**

Indices of growth of algae:

in growing algae culture yield, dry weight, optical density of a suspension of algal cells and increase in cell number are used as a characteristic of increase of growth . Other indices of growth, such as accumulation of carbon, nitrogen, protein, or some products of cell metabolism (starch, acids) are used in growth measurement.

- **Yield as a growth indicator:** yield as an expression of organic production, is usually given in terms of fresh or dry weight of the organic mass produce over the period of the time per unite of volume or unit of area occupied by organism.

Determination of yield: **Y=X1-X0/A(or V)**

Where X1& X0 are quantitative expressions of the mass of cells at the beginning and at the end of the growth period and A (or V) the area or the volume occupied by population of microbial growth.

Algal Nutrition

(1) **PHOTOTROPHIC:** USING LIGHT TO PRODUCE CARBOHYDRATE . FROM H_2O and Co_2

(2) **CHEMTROPHIC:** employing inorganic substance

(3) HETEROTROPHIC: employing organic substance

(4) MIXOTROPHIC: autotrophic and heterotrophic

(5) Phagotrophic: which ingest organic and inorganic substance.

(6) Auxotrophic: is the inability of an organism to synthesize a particular organic compound required for its growth

Algal Nutrients: Sixteen chemical elements are known to be important to alga's growth and survival. The sixteen chemical elements are divided into two main groups: non-mineral and mineral.

- **Non-Mineral Nutrients:** The Non-Mineral Nutrients are hydrogen (H), oxygen (O), & carbon (C).These nutrients are found in the air and water .Algae use energy from the sun to change carbon dioxide (CO_2 - carbon and oxygen) and water (H_2O- hydrogen and oxygen) into starches and sugars. These starches and sugars are the alga's food.
- **The mineral nutrients**: are divided into two groups : macronutrients and micronutrients.Macronutrients can be broken into two more groups :

(1) The primary nutrients are nitrogen (N), phosphorus (P), and potassium (K). These major nutrients usually are lacking because algae use large amounts for their growth and survival .

(2) The secondary nutrients are calcium (Ca), magnesium (Mg), and sulfur (S).

(A) Macronutrients element:

(1) Phosphorus: is an essential part of the process of photosynthesis .Helps with the transformation of solar energy into chemical energy; proper plant maturation; withstanding stress.Effects rapid growth

(2) Potassium: algae require potassium ion as activator of enzymes helps in the building of protein, photosynthesis.

(3) Nitrogen: Nitrogen is a major component of proteins and amino acids.

(3) Calcium: required by most of algae for growth an essential part of plant cell wall structure

(4) Magnesium: is part of the chlorophyll in all green plants and essential for photosynthesis. It also helps activate many plant enzymes needed for growth

(5) Sulfur: Essential plant food for production of protein.

(B) Micronutrients element: Micronutrients are those elements essential for plant growth which are needed in only very small (micro) quantities.

The micronutrients are boron (B), copper (Cu), iron (Fe), chloride (Cl), manganese (Mn), molybdenum (Mo) and zinc (Zn). Providing micronutrients (as well as macronutrients) to growing plants.

Micronutrient element consider essential to all algae: An essential nutrient is a nutrient that the cell cannot synthesize on its own -- or not to an adequate amount

(1) Iron (Fe): iron required in biological oxidation and reduction reaction Essential for formation of chlorophyll.

(2) Manganese (Mn): Functions with enzyme systems involved in breakdown of carbohydrates, and nitrogen metabolism.

(3) Chloride (Cl): Aids plant metabolism.

(4) Molybdenum (Mo) Helps in the use of nitrogen

(5) Zinc (Zn) Essential for the transformation of carbohydrates.

(6) Boron (B): Helps in the use of nutrients and regulates other nutrients . Aids production of sugar and carbohydrates .

(7) Copper (Cu): Important for reproductive growth.