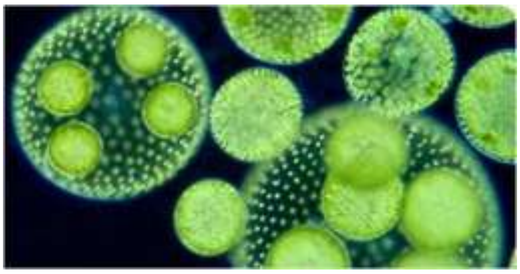
Practical ecology and physiology of algae

For 3rd year

Chemistry & Microbiology Students

Faculty of Science



By

Staff members of

Botany & Microbiology Department

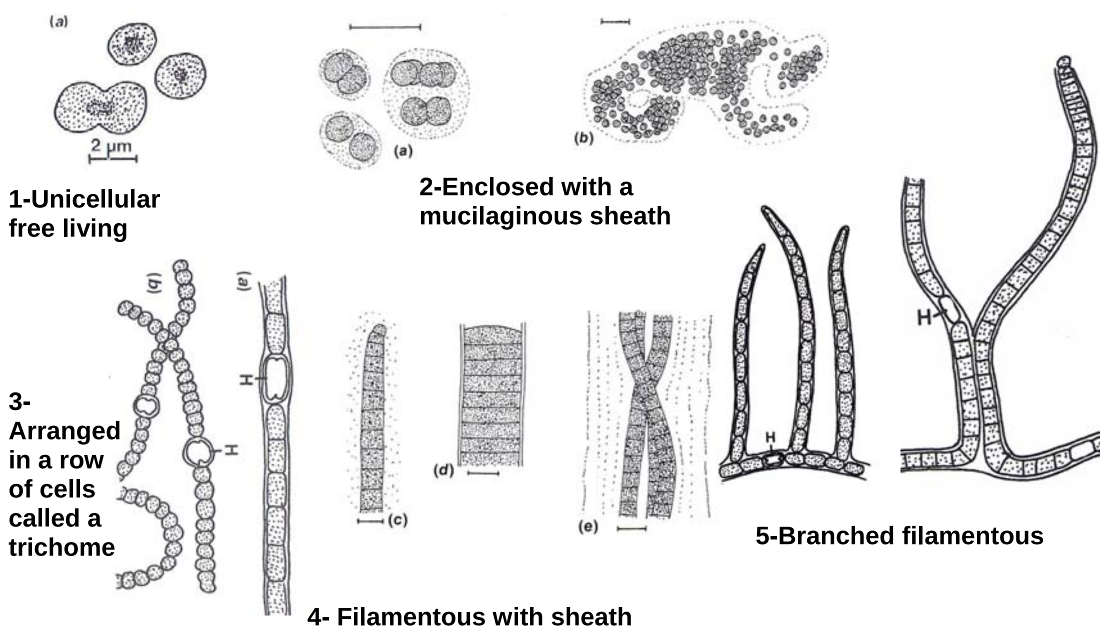
Phycology (Practical part)

Lab (1 +2)
Cyanobacteria
Kingdom: Monera
Division:Eubacteria
Class: Cyanobacteria

Prokaryotic that contains chlorophyll a, phycobiliproteins, glycogen as storage product and cell walls consisting of amino acids and amino sugars.

Morphology
1- unicellular free living
2-Enclosed with a mucilaginous envelope
3-Arranged in a row of cells called a trichome
4- Filamentous: the trichome (one or more) is surrounded by a sheath.
5-Branched filamentous which could be uniseriate (one row of cells) or multiseriate (more than one row)

Morphology of Cyanobacteria



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

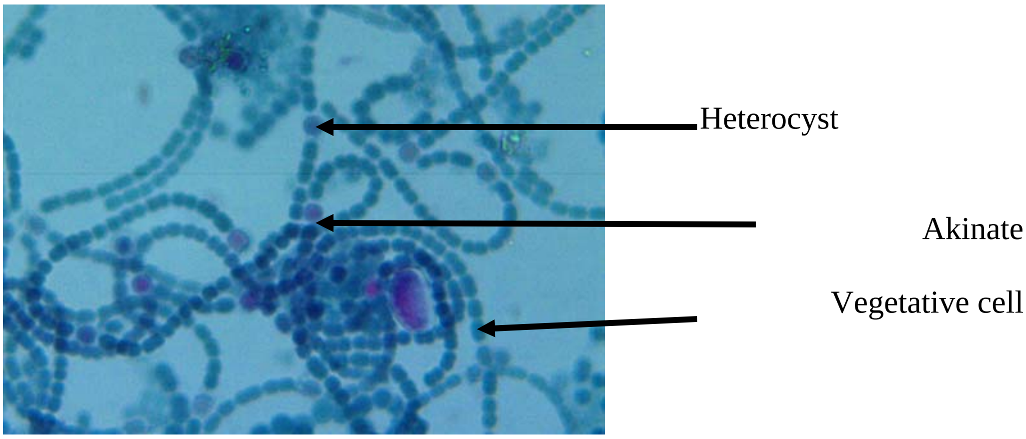
The faculty aims to excel at local level and regional throughout:

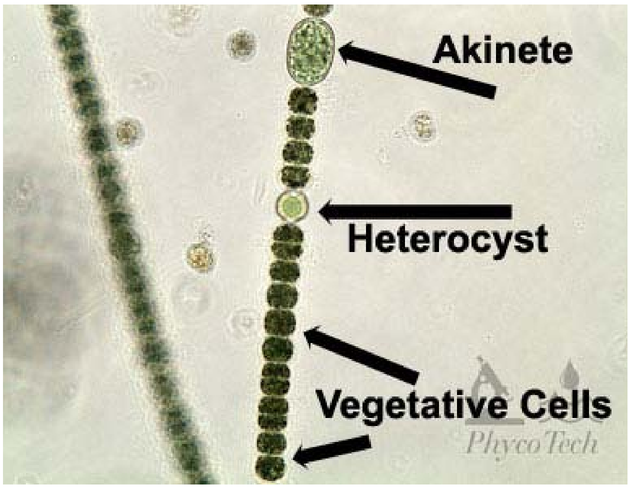
- Providing distinguished educational service to provide the market labor with graduates of high efficiency.
- Cooperating with universities and scientific institutions, regional and international.
- Academic research studies, and purposeful applied
- Providing community services and distinguished scientific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

Course Syllabus :

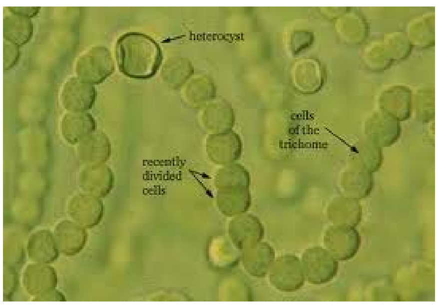
- **INTRODUCTION**
  - **ALGALPRODUCTION:**
  - **Algae cultures of limited volume (Batch culture)**
  - **Algal Growth in Continuous Culture**
  - **Microalgae Isolation Techniques**
  - **Indices of growth of algae**
  - **Inorganic Nutrients of Algae**
  - **Algal Nutrition**
- **PHOTOSYNTHESIS**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

Anabaena

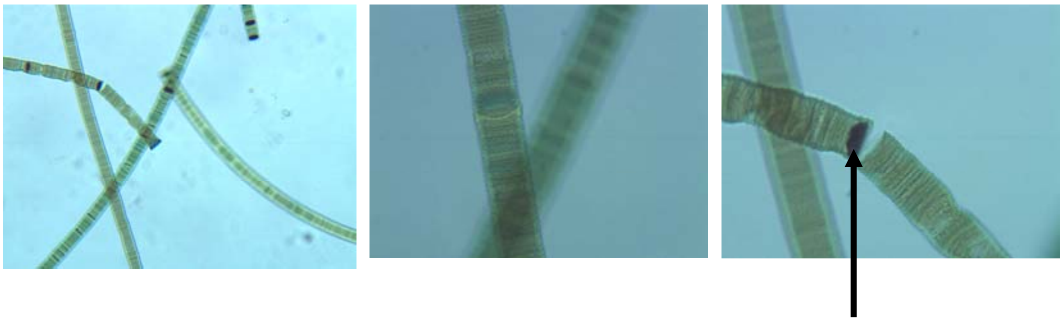




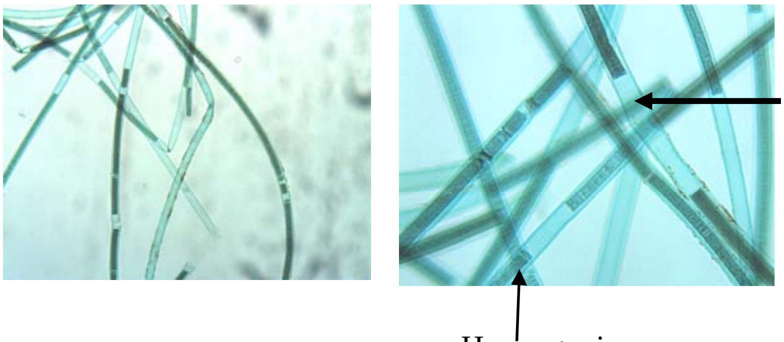
Nostoc (from specimen)



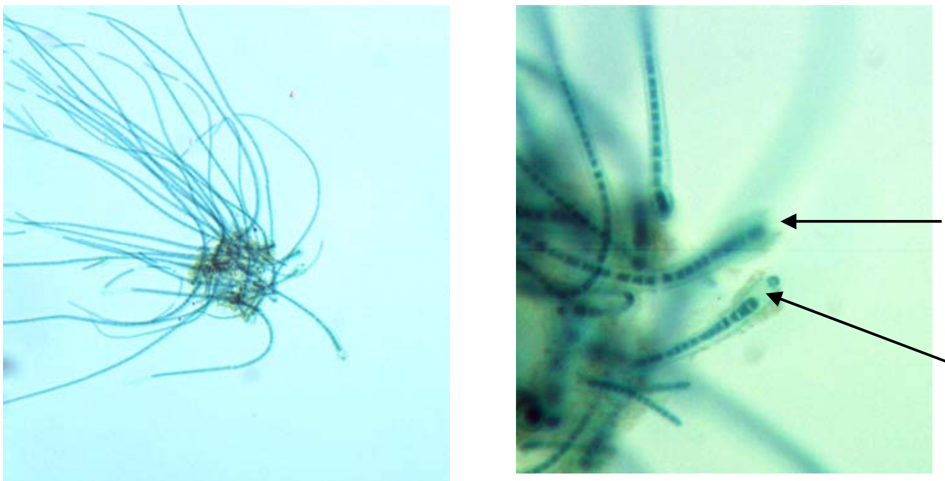
Oscillatoria



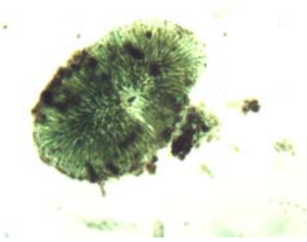
Lyngbya

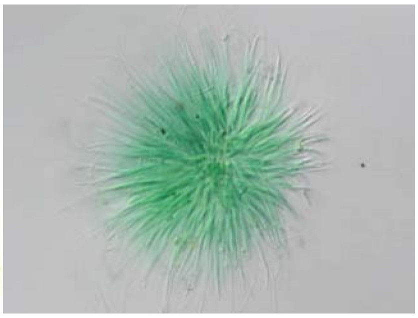


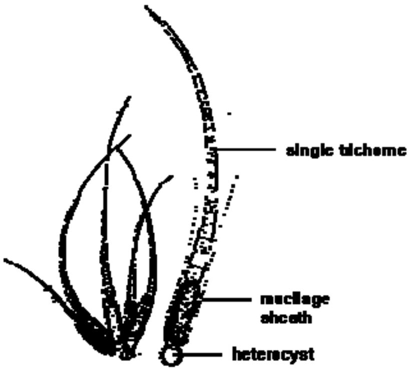
Rivularia



Gloeotrichia

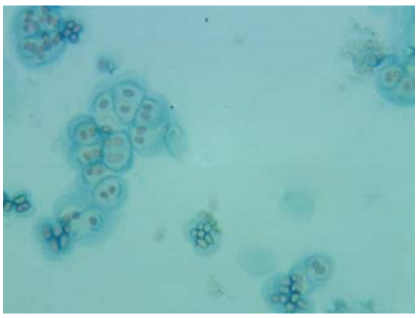


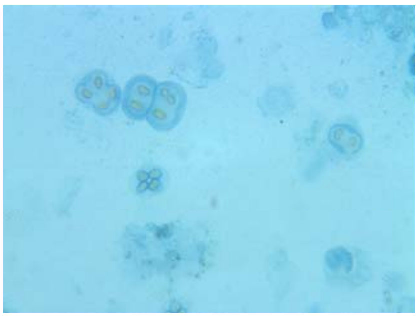


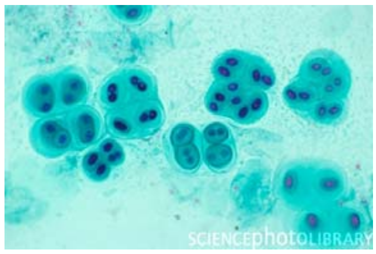


Note the polarity of filaments and the basal hetercysts and akinates

Gloeocapsa

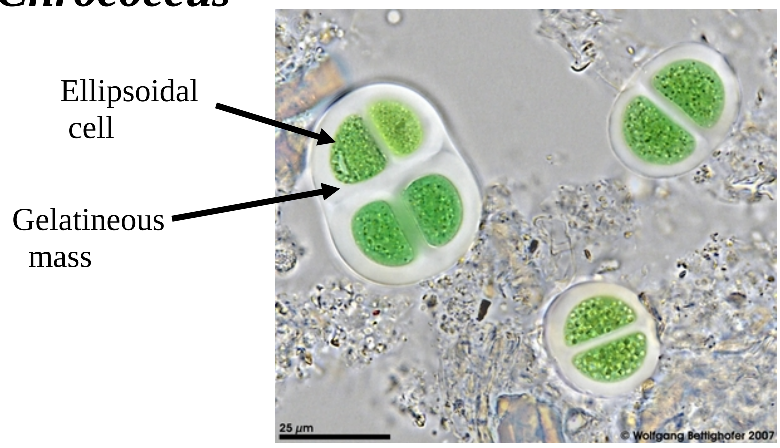




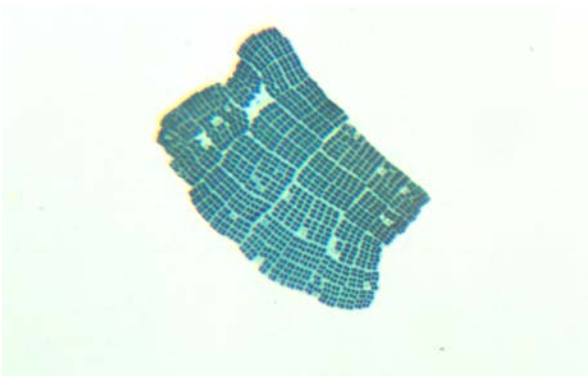


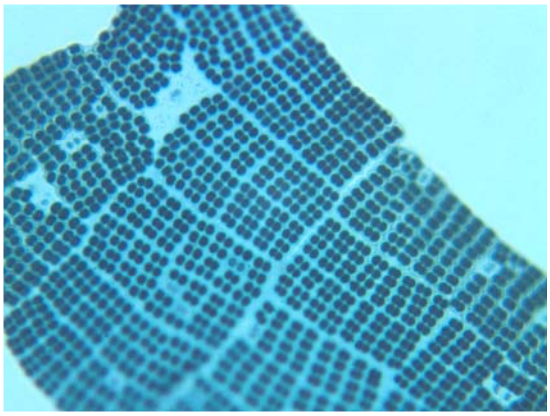
Note the mucilage sheath and the
Spherical and hemispherical cells

Chrococcus



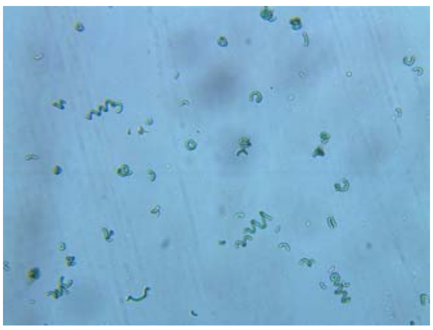
Merismopedia





- Cells are ellipsoidal.
- The arrangement is due to the limitation of cell division from two sides only.
- The division results in increase in colony size rather than in multiplication o the individual.

Spirulina

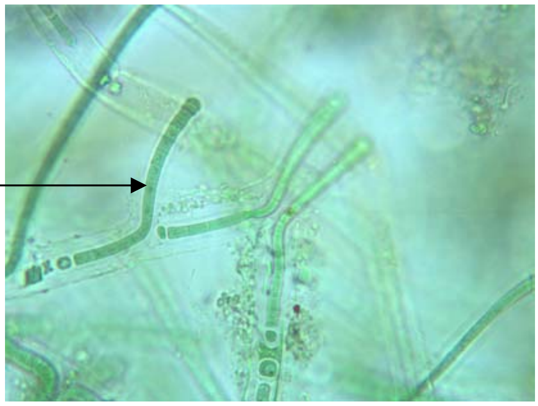


Scytonema

False branch

Heterocyst

False branch



Stigonema

As it appears in water

True branch

Multiseriate filaments

Lab (3)

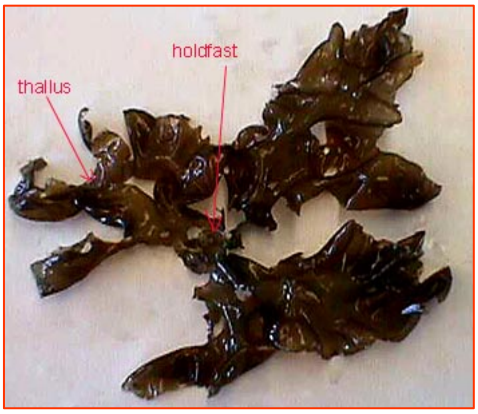
Kingdom Protista (Eukaryotes)

Algae

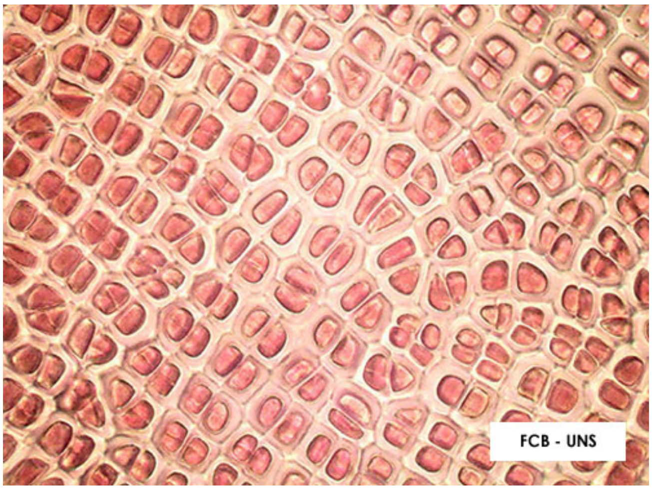
Division: **Rhodophyta**

- **Rhodophyceae** (red algae) comprise the only class in the division.
- Lack flagellated cells.
- Have chlorophyll a and d, phycobiliproteins,,
- Floridean starch granules are the storage product (outside chloroplast).
- No chloroplast ER.
- The majority of seaweeds are red algae (~400 species which is more than all other seaweeds groups).
- They live at depth as great as 200 m.
- About 200 sp. Are found in freshwater with smaller size than seaweeds.

(1) ***Porphyra***, commonly known as **nori**, is the most widely consumed seaweed in the world. This alga attaches itself to the rocks by multicellular rhizoidal attachments, usually disc-shaped. The thalli begins life as uniseriate filaments but this stage is eventually replaced by parenchymatous sheets of cells (1 to 2 cells thick).



As seen in the sea



As seen under the microscope: Note the parenchymatous appearance of uninucleated cells

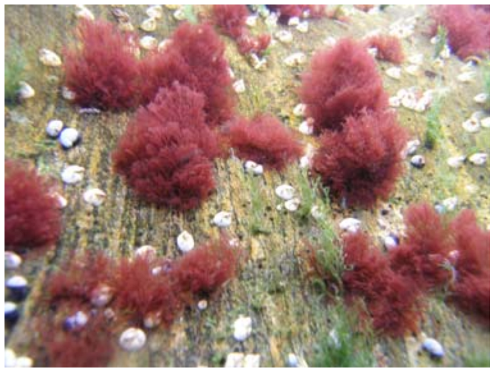
(2) ***Nemalion*** spp. This red algae grows as a slender and sometimes branched "worm" on rocks in the intertidal zone, especially where there is very active water. The algae are softly cartilaginous because of the rather firm mucilage in which the filaments are encased

Nemalion helminthoides as seen in sea water

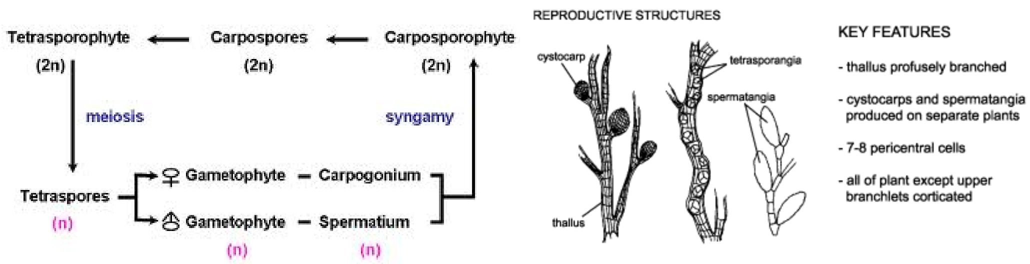
Carpogonial branches with carpospores
Note the phcoerythrin

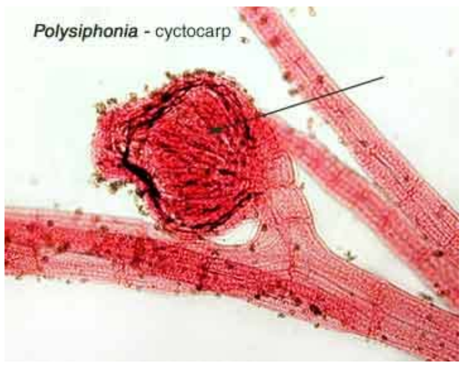
(3) *Polysiphonia*

Polysiphonia has separate male and female gametophytes that are identical in appearance. The tetrasporophytes resemble the gametophytes in size and form.

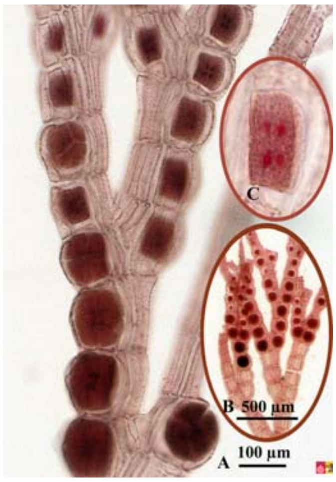


Polysiphonia elongate as seen in sea water



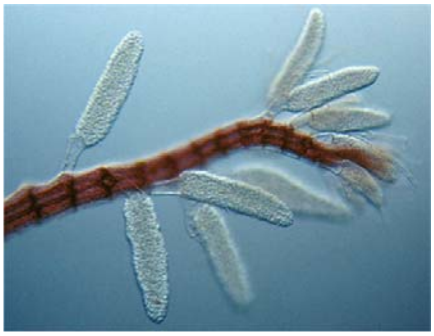


Polysiphonia Cystocarp



Carpospores with tetraspores

Multiseriate

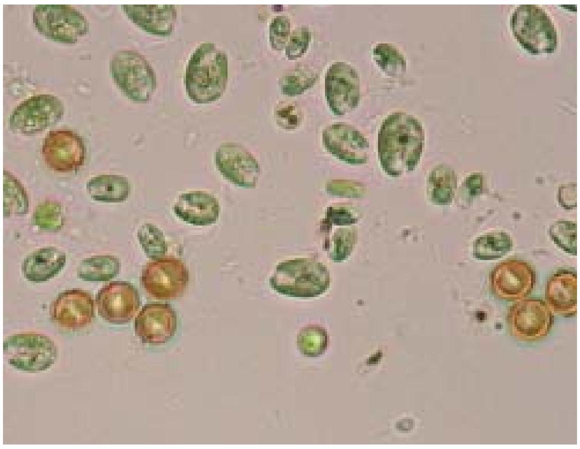
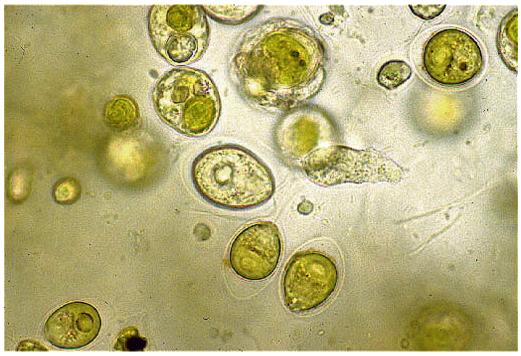


Spermatangia

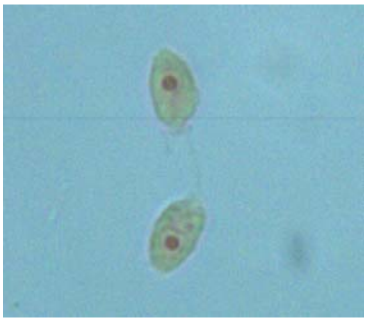
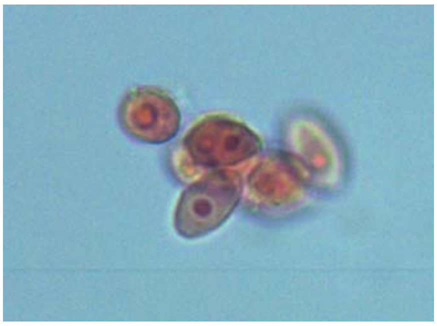
Division Chlorophyta

- have chlorophylls a and b.
- form starch with the chloroplast, usually in association with a pyrenoid. The Chlorophyta thus differ from the rest of the eukaryotic algae in forming the storage product in the chloroplast instead of in the cytoplasm.
- No chloroplast endoplasmic reticulum occurs around the chloroplasts.
- The Chlorophyta are primarily freshwater; only about 10% of the algae are marine, whereas 90% are freshwater.
- Some orders are predominantly marine, whereas others are predominantly freshwater or exclusively freshwater. The freshwater species have a cosmopolitan distribution, with few species endemic in a certain area.
- In the marine environment, the green algae in the warmer tropical and semi-tropical waters tend to be similar everywhere in the world. This is not true of the Chlorophyta in the colder marine waters; the waters of the Northern and Southern hemispheres have markedly different species.

Chlamydomonas

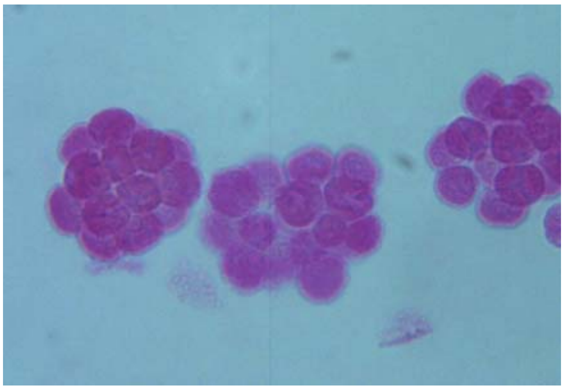



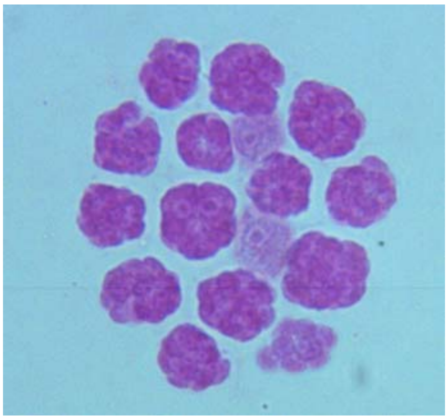
Note the flagella, the pyrenoids and the cup-shaped chloroplast


- *Chlamydomonas* is a unicellular motile alga with two flagella.
- Each cell contains single massive chloroplast
- The chloroplast may contain one or more pyrenoids.

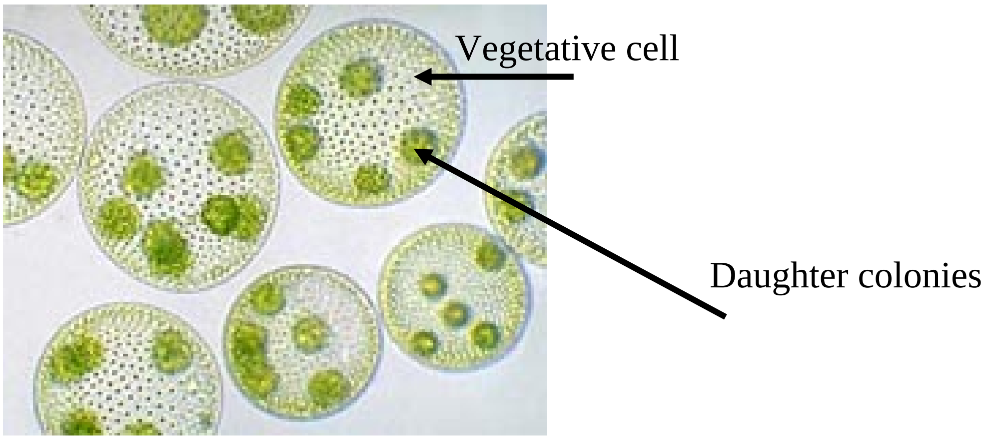
Pandorina

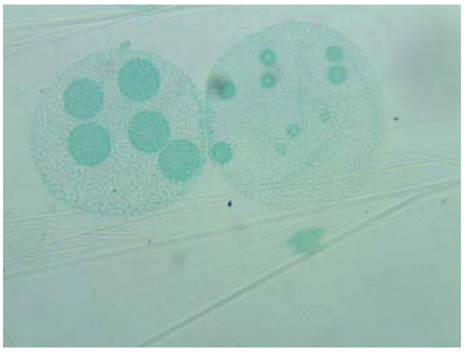


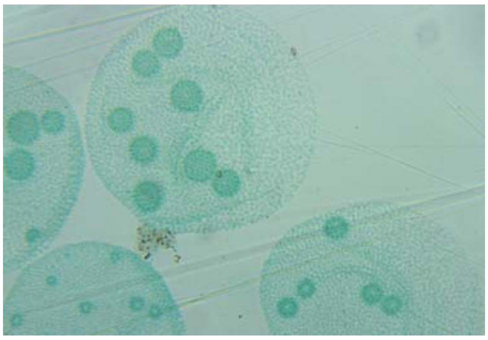


- *Pandorina* consists of 16-like *chlamydomonas* cells.
- Arranged in an almost solid, ovoidal Colony.
- Each cell is flattened at its anterior pole and narrowed posteriorly.
- The chloroplast is massive and contains a basal pyrenoid.
- The single nucleus lies in the colorless central cytoplasm.
- All of the cells in the colony are similar in size.
- After attaining the maximum size characteristic of the species, the colonies sink to the bottom of the pond and initiate autocolony formation (miniature of a parental colony).
- In autocolony formation, each of the parental cells undergoes repeated nuclear and cytoplasmic division until miniature 16-celled colonies are produced.
- The minute cells of the autocolonies then develop flagella, and the coloy begins to move slowly within the matrix of the parent colony until liberated by its dissolution.

Volvox

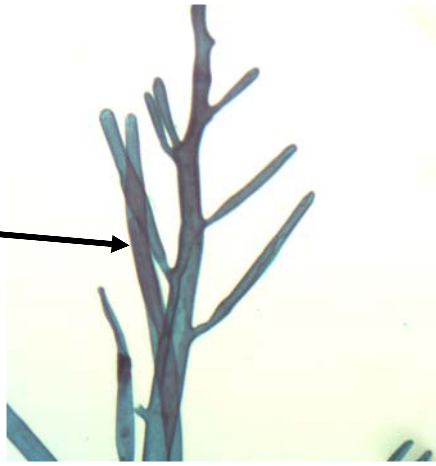






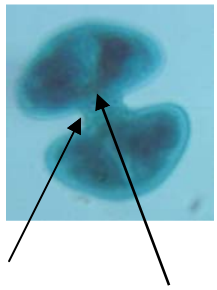
- *Volvox* consists of thousands of *chlamydomonas* like cells.
- The protoplasts of the individual cells are connected by delicate protoplasmic extensions.
- Duaghtr colonies reproduce sexually by formation of zygote, and asexually by formation of gonidia.
- The remaining cells are purely vegetative and disintegrate when the adult colony liberates its daughter colonies.

Bryopsis

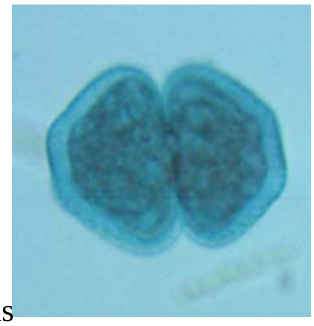


- Grows attached to rocks in shallow marine waters.
- Growth is apical.
- Coenocytic and tubular type of body organization
- At maturity, certain of the branches become segregated from the main axis by the formation of the septa and become transformed into gametangia.

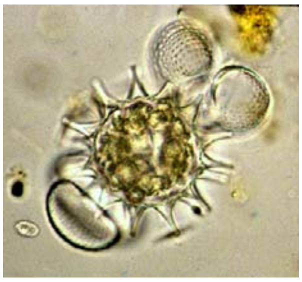
Desmids *Cosmarium*

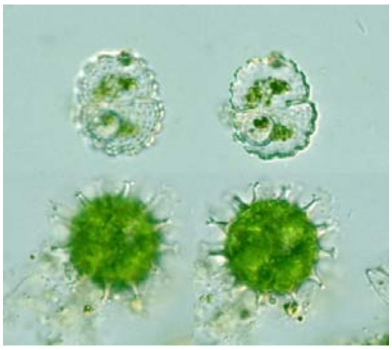


Sinus Isthmus

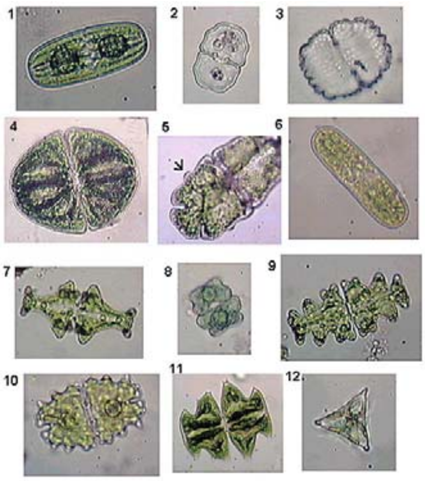


2 semi-cells

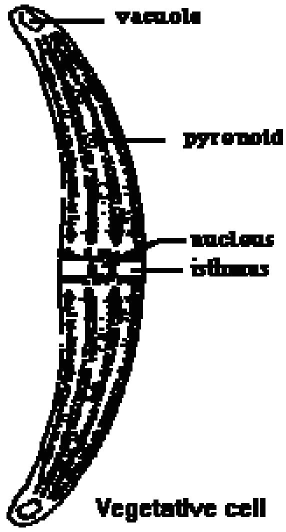


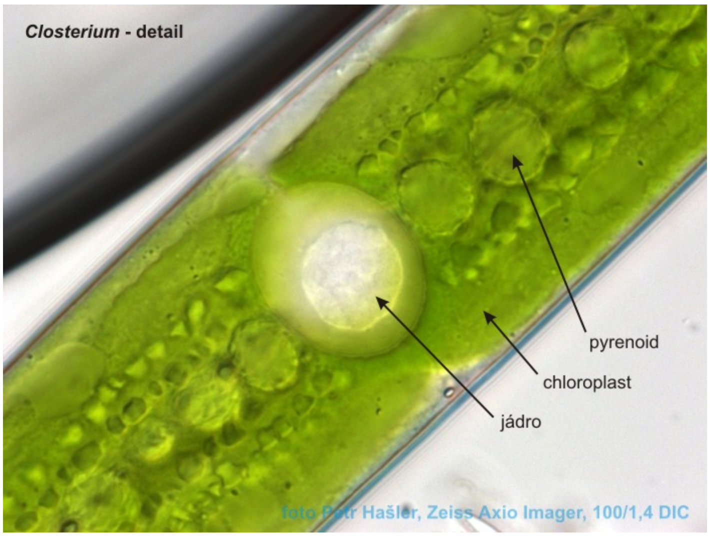


Zygote

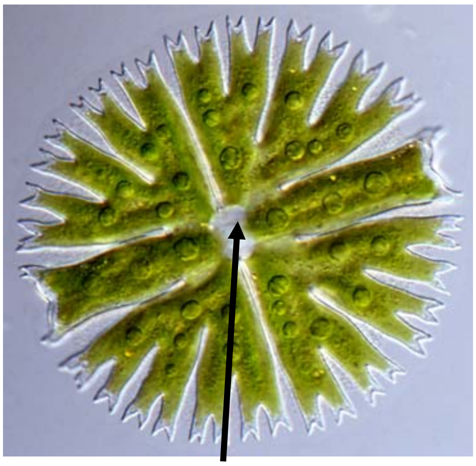
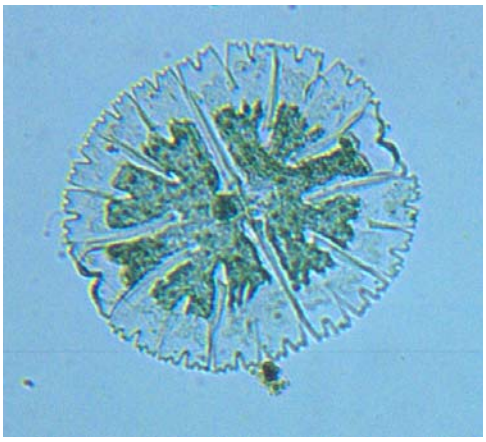


Desmids *Closterium*



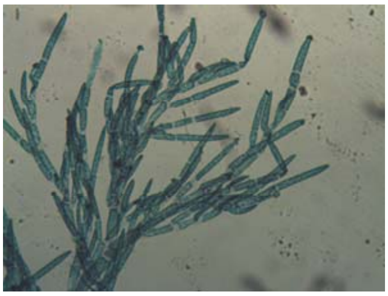
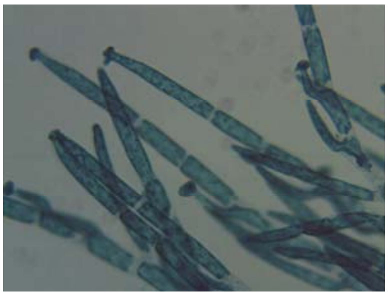


Desmids *Micrasterias*

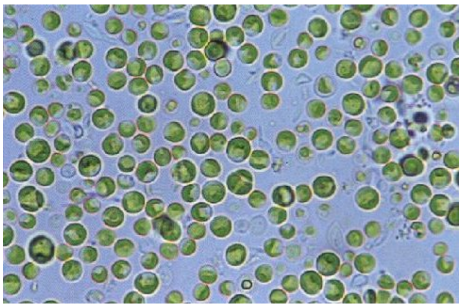
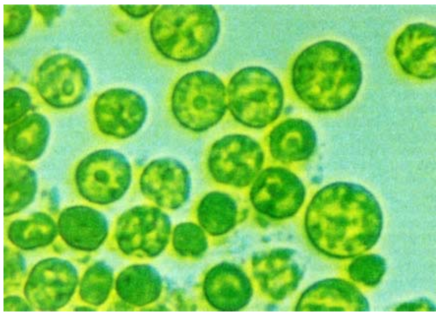
Sinus

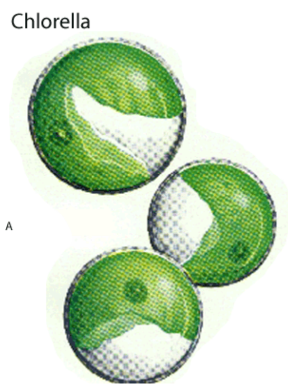
Cladophora

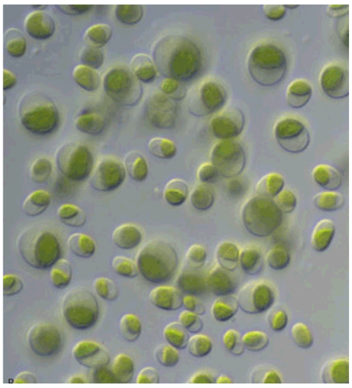
  

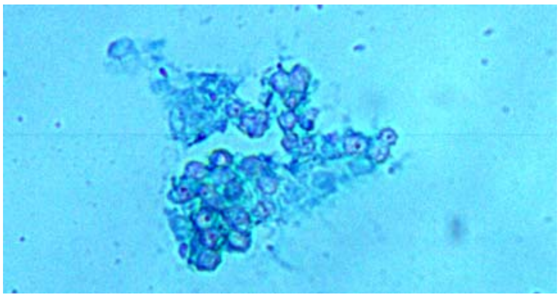
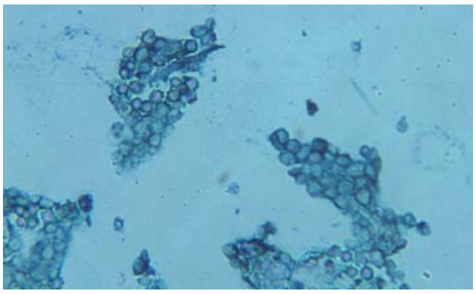
- *Cladophora* is an isomorphic with multinucleated cell.
- The gametes are biflagellated (sexual reproduction).
- The zoospores are quadriflagellated (asexual reproduction).
- The structure of the chloroplast varies with the age of the cell. In younger cells it is a continuous network, but in older ones it is largely peripheral and composed of irregular segments, in some of which pyrenoids are embedded.

Clorella

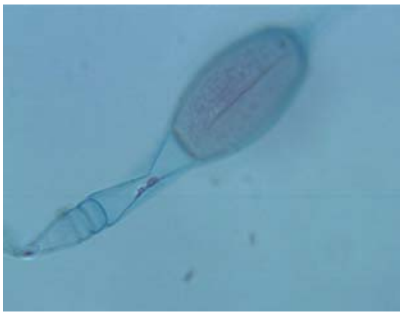
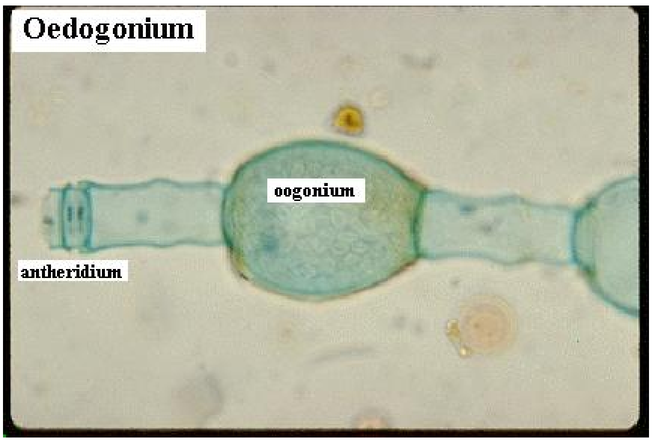




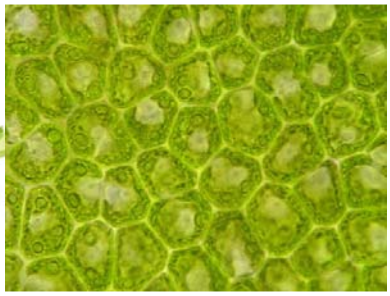



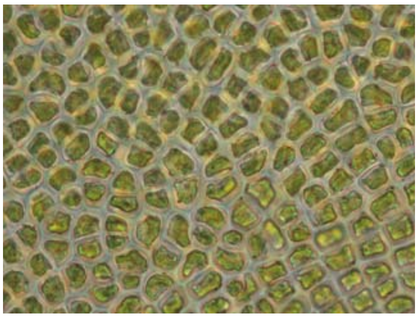
- The cells of most species are minute green spheres.
- The protoplast is composed of cuplike chloroplast, which may or may not contain a pyrenoid.
- The cytoplasm is colorless in which a minute nucleus is embedded in its center.
- A serious of bipartitions may occur, forming four or eight protoplasts endogenously.
- Delicate cell walls are then developed, and after they have begun to enlarge, they are liberated by rupture of the mother cell wall (asexual reproduction).
- Such asexual reproductive cells, which have no capacity for motility, are known as autospores.

Oedogonium

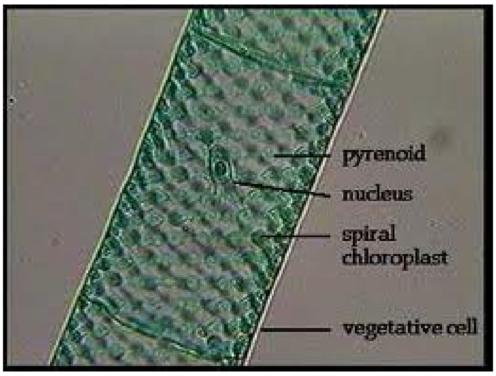
Ulva

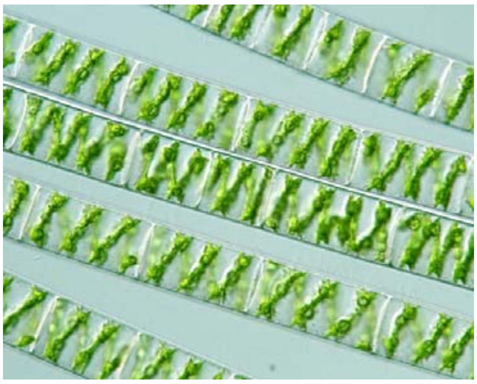


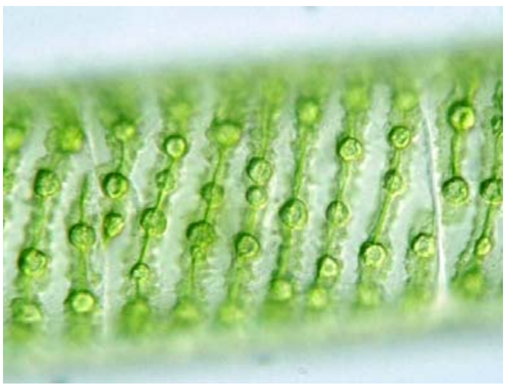


- *Ulva*, the sea lettuce is a green membranous alga.
- Grows attached to rocks, woodwork, and larger marine algae.
- The body is bladelike, often lobed and undulated, and anchored by a multicellular holdfast composed of cells with rhizoidal protuberances.
- The cell walls are thick so to withstand some desiccation when exposed at low tide.
- Each cell contains a single chloroplast with one or more pyrenoids.
- The cells of the blade are uninucleate, but those of the holdfast may have several nuclei in their rhizoidal processes.

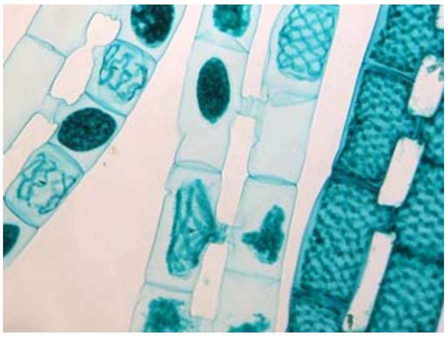
Spirogyra



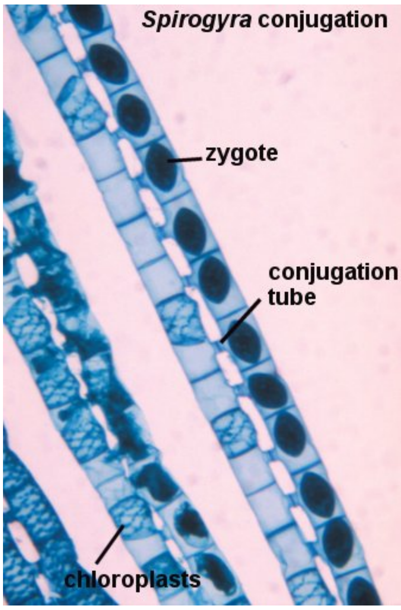


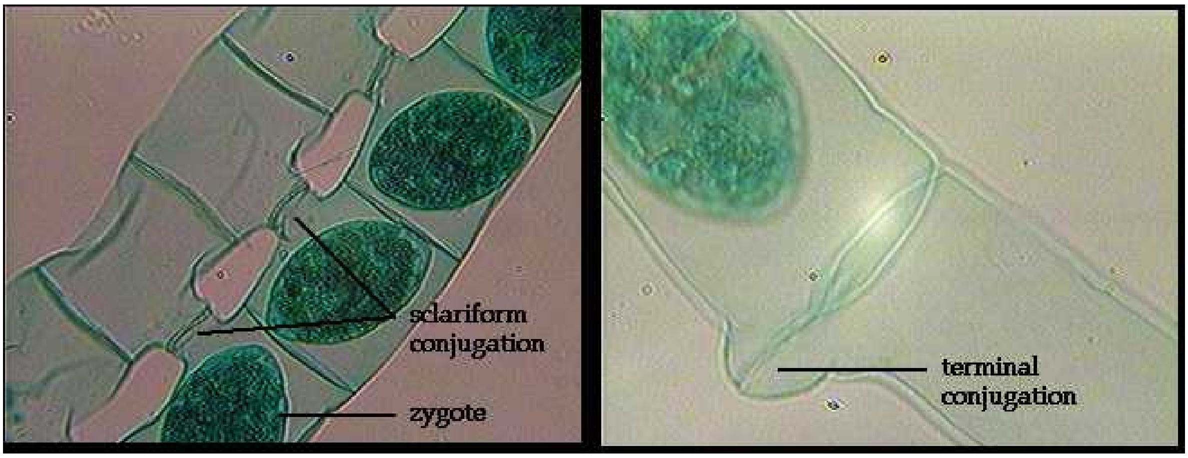


Vegetative filament



Scalariform conjugation





<u>Types of reproduction in *Spirogyra*</u>

1. Asexual reproduction (vegetative filament fragmentation).
2. Sexual conjugation

<u>A- Lateral Conjugation-</u>

1. It occurs in homothallic species.
2. Here two cells of a filament take part in gametic union.
3. Movement of gamete occurs through a passage formed either in partition wall or across the partition wall or two adjacent cells.
4. It does not look like a ladder.

<u>B- Scalariform Conjugation-</u>

1. It occurs in heterothallic species.
2. Here two cells of two different filaments take part in gametic union.
3. Movement of gamete occurs through a passage formed by the lateral walls of two filaments.
4. It looks like a ladder.

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

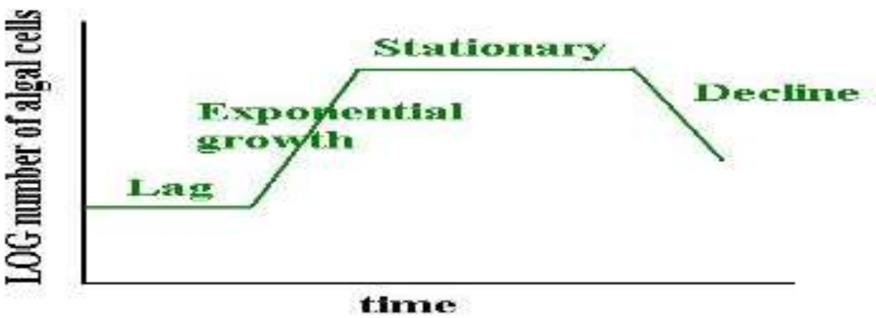
In order to grow algae in the classroom you will need to make up some growth media. In their natural habitats algae obtain all the nutrients, minerals and vitamins they require from the water in which they live. To grow them in the lab you must provide them with all of these essential the algae grow 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

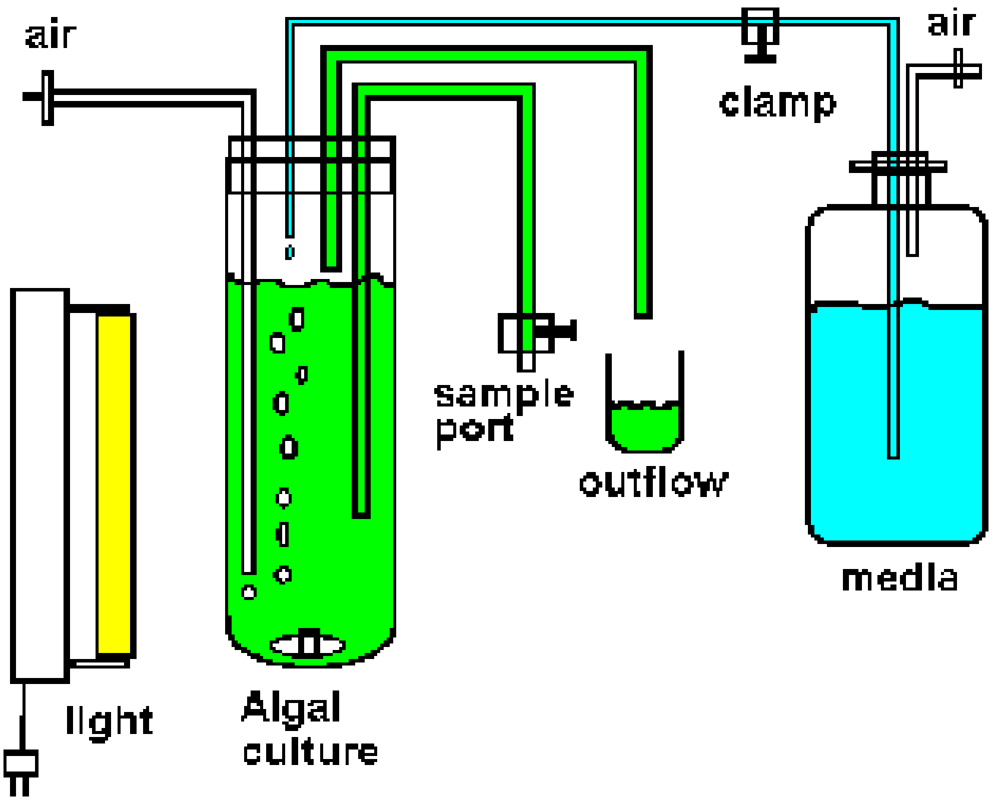
This method of culturing algae differs from the batch culture method in that fresh medium is added to the culture at a constant rate and old media (and some of the algae cells) is removed at the same rate. Two categories of continuous cultures can be distinguished:

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.

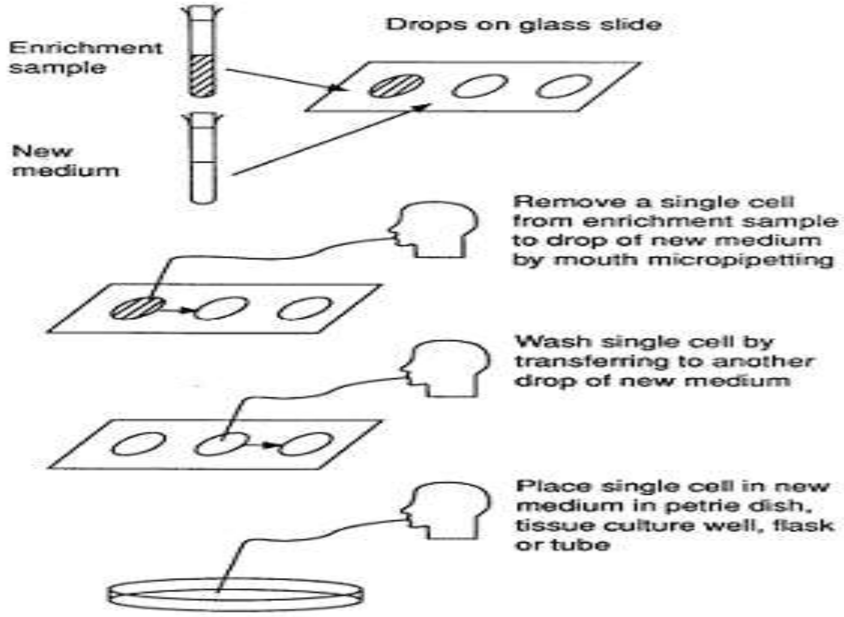


Air is also pumped into the culture vessel. This air passes down a long glass tube to the bottom of the culture and bubbles up. This serves to keep the culture well suspended as well as high in oxygen and CO2. The air flowing into the culture vessel flows out through an outflow tube. As fresh medium 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.

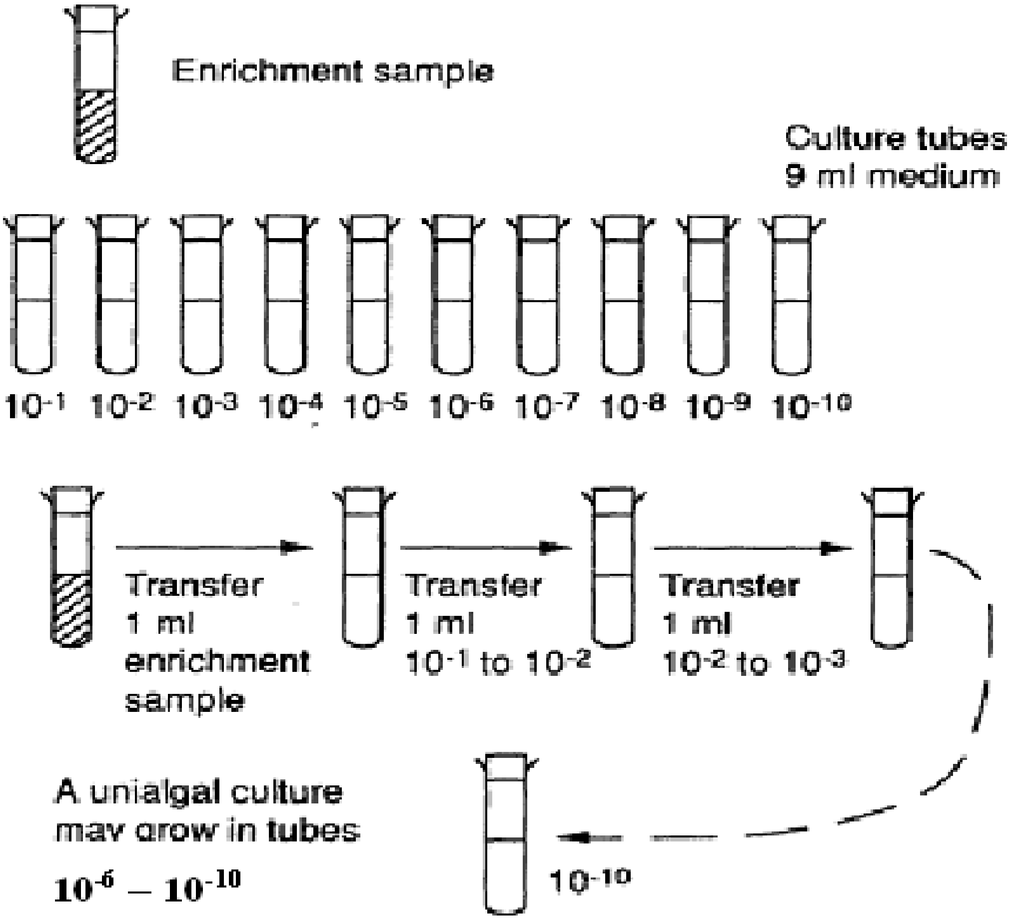
Microalgal Isolation Techniques

(a) Micromanipulation:



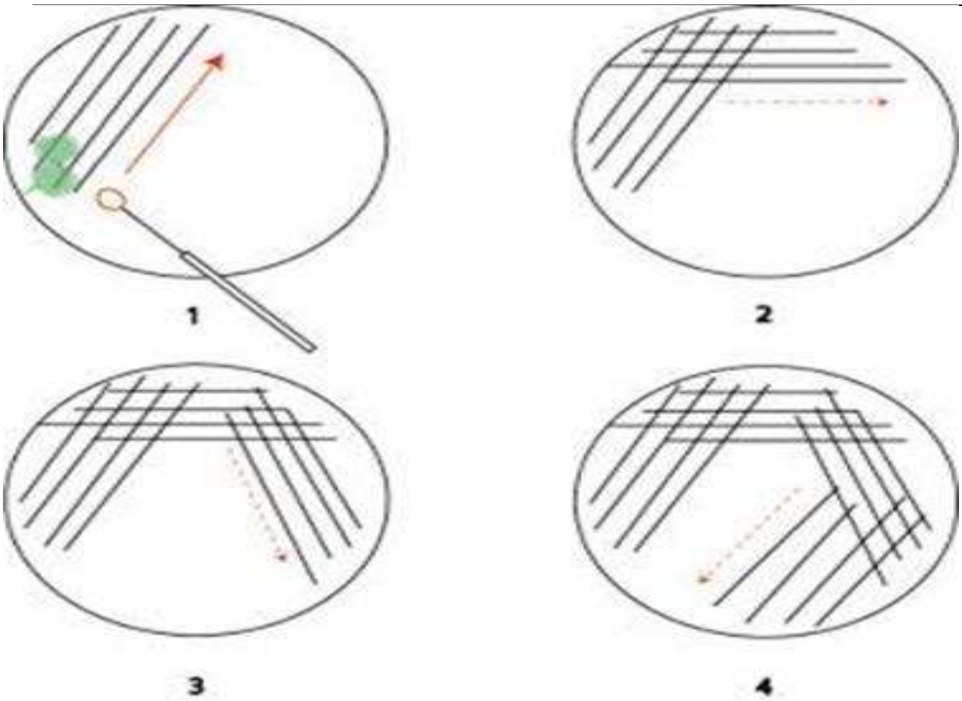
(b) Serial dilution:

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 (CO2 - carbon and oxygen) and water (H2O- 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.