Physiology and Cultivation of Algae and Cyanobacteria

5.

Overview

- Collection of the samples
- Isolation & purification of algal culture
- Culturing
  - Methods
  - Equipments & material
  - Conditions
  - Culturing media

Collection of the samples

- purpose specific
- sample specifics (nature & environment)
- time
- concentration
- type of vessel/container
- removal of the unwanted organisms (filter)
- transfer conditions & storing

Isolation & purification of algal culture

- Equipments & suplies
  - Microscopes
  - Filters & sieves
  - Glassware, Plasticware, Utensils
- Methods
  - Sterile manipulation
  - Isolation techniques

Equipments & suplies

- Microscopes
  - dissecting (80x,..)
  - inverted
  - lighting
    - dark-field
    - fiber optic light source
    - fluorescent lamp
    - epifluorescence
- Filters & sieves
  - woven screens
  - nylon netting
  - membrane filters (material)
  - differential filtration
- Flow-box

Glassware, Plasticware, Utensils

- borosicate glassware
- plasticware (ready-to-use, culture-grade)
- sterilization technique & sterility
- dust-proof cabinet / clean containers
- caps & splips
- sterile filtration apparatus
- sterile spatula
- pens, labels
- parafilm

- growth chambers
  - test tubes, flask, culture flask, Erlenmeyer flask, Petri dish, plugs, two-steps screw
  - cultivator, tank, bioreactor

Sterilization & sterile manipulation

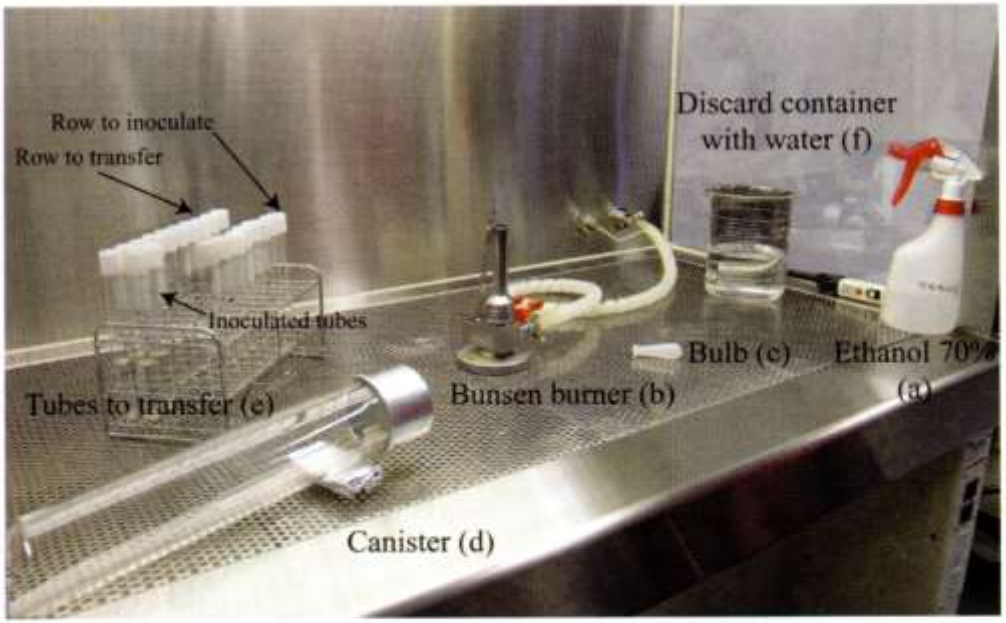
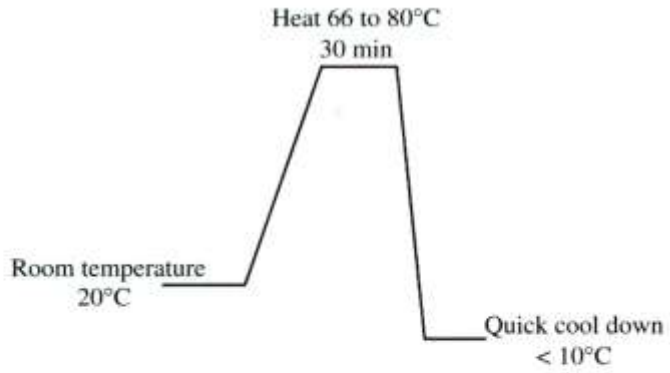


FIGURE 5.10. A laminar flow hood with Bunsen burner and supplies organized for use by a right-handed person. Placement and use order of the materials is indicated with the letters (a) to (f). Note the stopper for the cylindrical canister. Test tube arrangement is to facilitate handling and to avoid mistakes during manipulations.

TABLE 5.1. Summary of sterilization types, including applicability and limits

Category	*Sterilization method*	*Effective method*	*Application*	*Limitation*
Heat	Flame	Direct heat with fire (Bunsen burner)	Surface sterilization (test tube openings, transfer loops, glass pipettes)	Non–heat-resistant materials (e.g., most plastics)
Heat	Autoclaving	2 atm (steam pressure), 121°C; time varies (10, 20 min for small liquid vol; 1 h for large vol)	For general use: liquids and agar, glass and metal vessels, equipment	Non–heat-resistant materials; pH change; metal contamination
Heat	Dry heat	250°C, 3 to 5 h; current protocol at 150°C for 3 to 4 h	Dry goods: glass and metal vessels and equipment	Non–heat-resistant materials; liquids
Heat	Pasteurization	66–80°C for at least 30 min, followed by quick cooling (4–10°C)	Liquids with heat-labile components	Not complete sterilization (originally for killing food germs)
Heat	Tyndallization	60–80°C, 30 min, followed by quick cooling; cycle repeated 3 times in 3 d	Liquid with heat-labile components	Requires time
Filtration	Filtration	≤0.2 µm pore size filter	Liquid with heat-labile components	Small volumes, high-viscosity liquids, viruses not eliminated
Electromagnetic waves	Microwave	10 min at 700 W; 5 min with intervals at 600 W. For dry goods: 20 min at 600 W with water, 45 min without water	Liquids: small volume of media; dry goods: glassware, vessels	Small liquid volumes; dry goods with water require elimination of water
Electromagnetic waves	Ultraviolet radiation	260 nm, 5–10 min	Surface of materials, working area	Ultraviolet-sensitive plastics
Chemical	Bleach (sodium hypochlorite)	1–5 mL for 1 L water, several hours	Large volume of water for aquaculture	Cysts may survive; neutralization required (e.g., sodium thiosulfate, 250 $g \cdot L^{-1}$ stock solution; 1 mL for 4 mL of bleach)
Chemical	Ethanol	50–70% solution	Popular, general disinfection	Some resistant microorganisms
Chemical	Ethylene oxide	Airtight room or pressure cabin	Plastic and rubber products, non–heat-resistant products	Explosive; chemical residue is problematic or toxic
Chemical	Corrosive sublimate, $HgCl_2$	0.1%; add same amount of NaCl and dissolve with distilled water	Antiseptic and disinfectant	Poison; not for materials contacting live cells
Chemical	Phenol (carbolic acid)	3% solution	Antiseptic and disinfectant	Poison; not for materials contacting live cells
Chemical	Saponated cresol solution	3–5% solution	Antiseptic and disinfectant	Poison; not for materials contacting live cells
Chemical	Formaldehyde (formalin)	2–5% solution	Antiseptic and disinfectant	Poison; not for materials contacting live cells

Pasteurization



Tyndallization

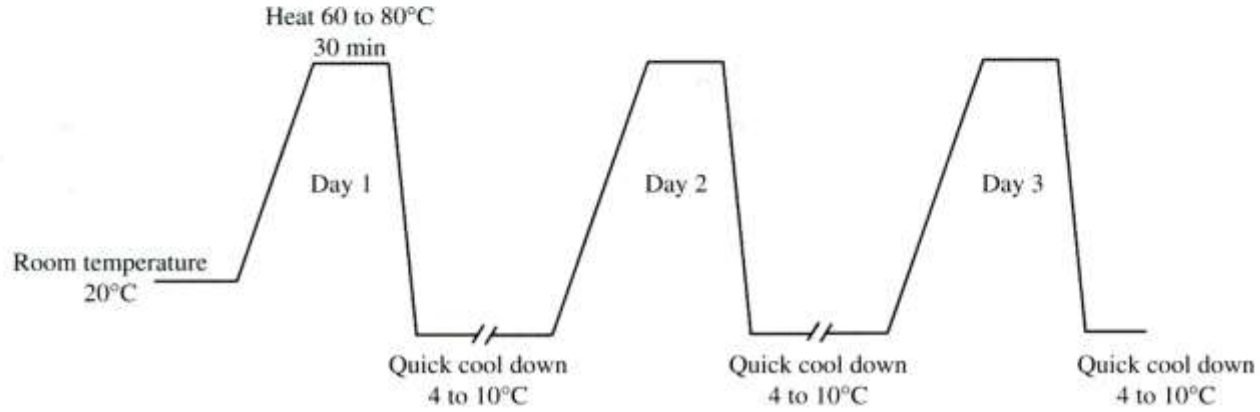


FIGURE 5.5. Schematic representation of the temperature cycles for pasteurization and tyndallization.

Methods of isolation & purification

- Enrichment culture
- Single-cell isolation
- Size separation >> filtering
- Density separation >> centrifugation
- Dilution
- Isolation with use of agar
  - streaking
  - spray
- Isolation with use of phototaxis
- Automatization (flowcytometer)

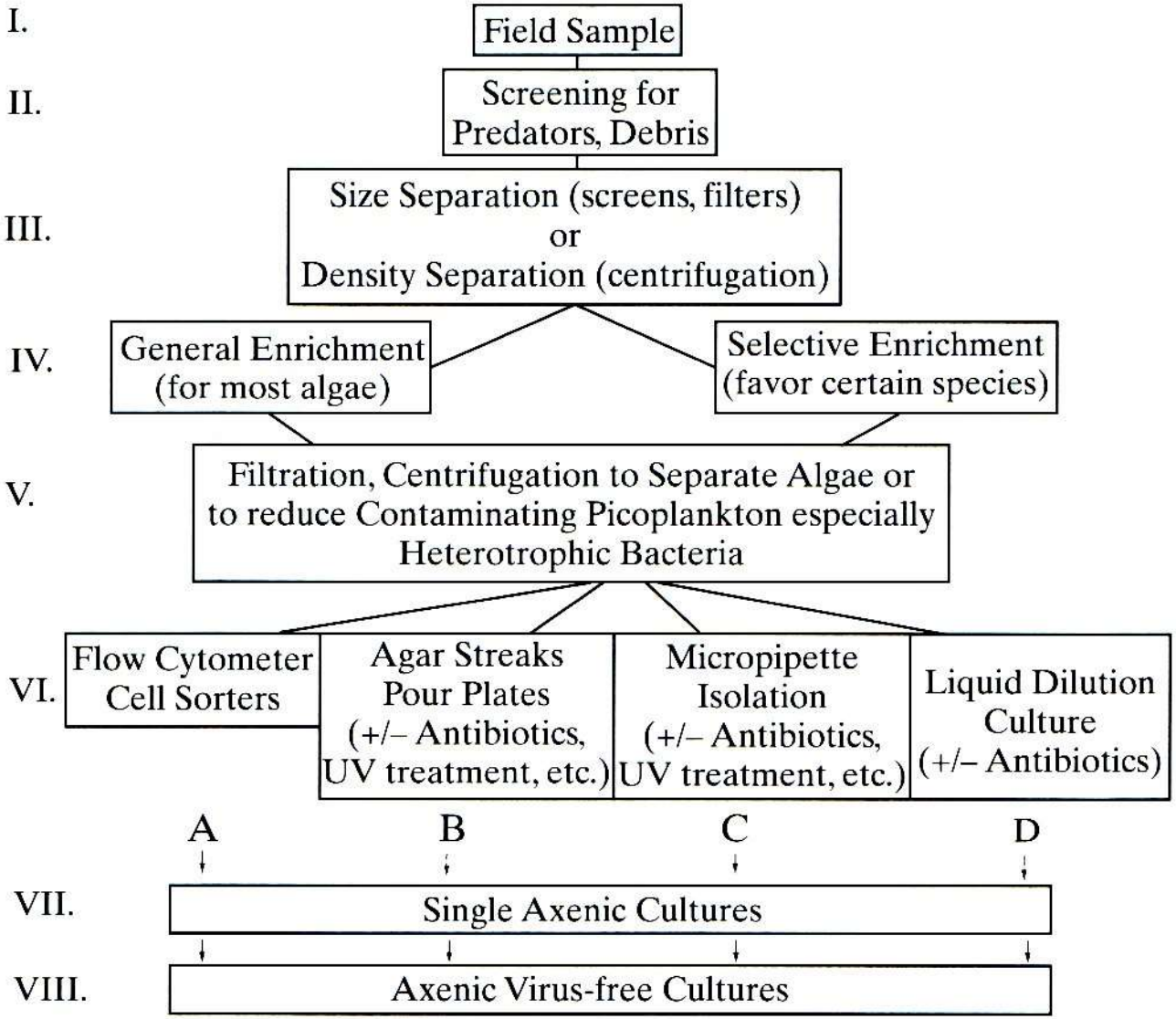


FIGURE 8.1. Flow diagram of purification steps of single-cell algae (after Guillard and Morton 2003).

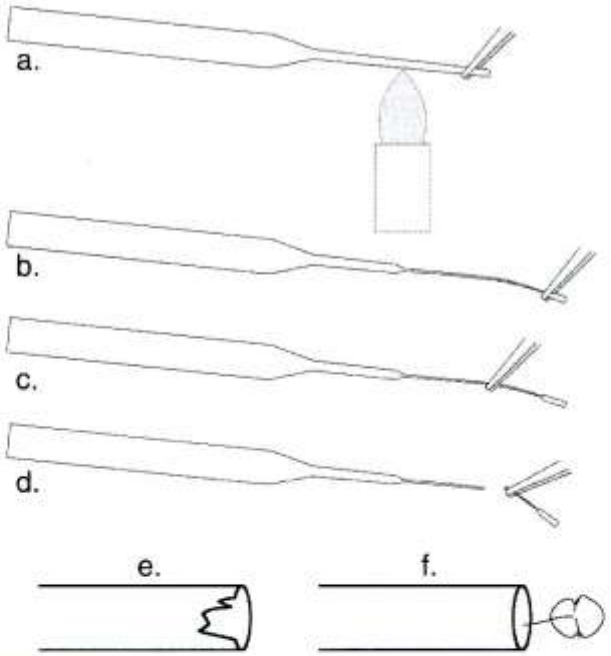


FIGURE 6.3. Preparation of a micropipette from a Pasteur pipette. **(a)** The Pasteur pipette is held in the hottest region of the flame, supported on the left by a hand and on the right by forceps. The pipette should be rotated as the glass is heated to a soft, pliable condition. **(b)** When the glass is soft, the pipette is quickly removed from the flame with a gentle pull to produce a thin tube. **(c)** The forceps is then relocated to the appropriate region of the thin tube. **(d)** The forceps is used to gently bend the thin area so that it breaks, forming a micropipette. **(e)** An enlarged tip of a micropipette, showing a jagged break; this tip is not suitable for use. **(f)** An enlarged tip with a very smooth break; this tip is suitable for use. Note that the diameter of the tip is larger than the flagellate cell (bearing microscopic scales), thus reducing the probability of shearing as the cell enters the micropipette during isolation.

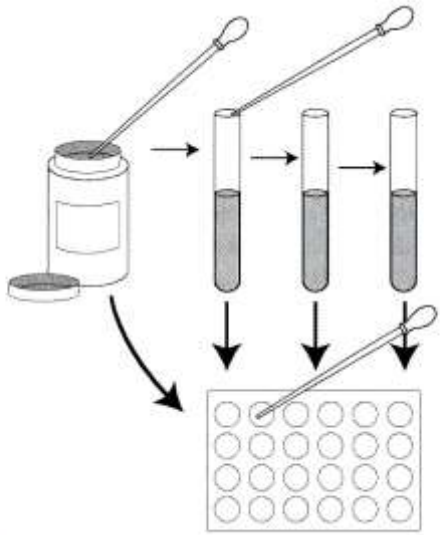


FIGURE 6.6. An illustration of the dilution technique. An aliquot is removed from the sample jar (*left*) and placed into a test tube containing sterile medium. After mixing, one aliquot is removed from the test tube and dispensed into multiwells containing sterile medium, and a second aliquot is removed and added to the middle test tube. After mixing, the process is repeated (i.e., dispensed into multiwells and added to the test tube on the right). Each cycle dilutes the original sample and increases the probability of single-cell isolation; the cycle stops when it is probable that no cell will be transferred.

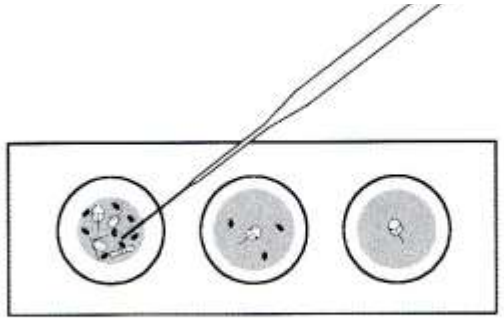


FIGURE 6.4. A pipette is used to remove other small cells (*left, middle*), leaving the target organism free of contamination (*right*). This procedure limits the handling of the target organism.

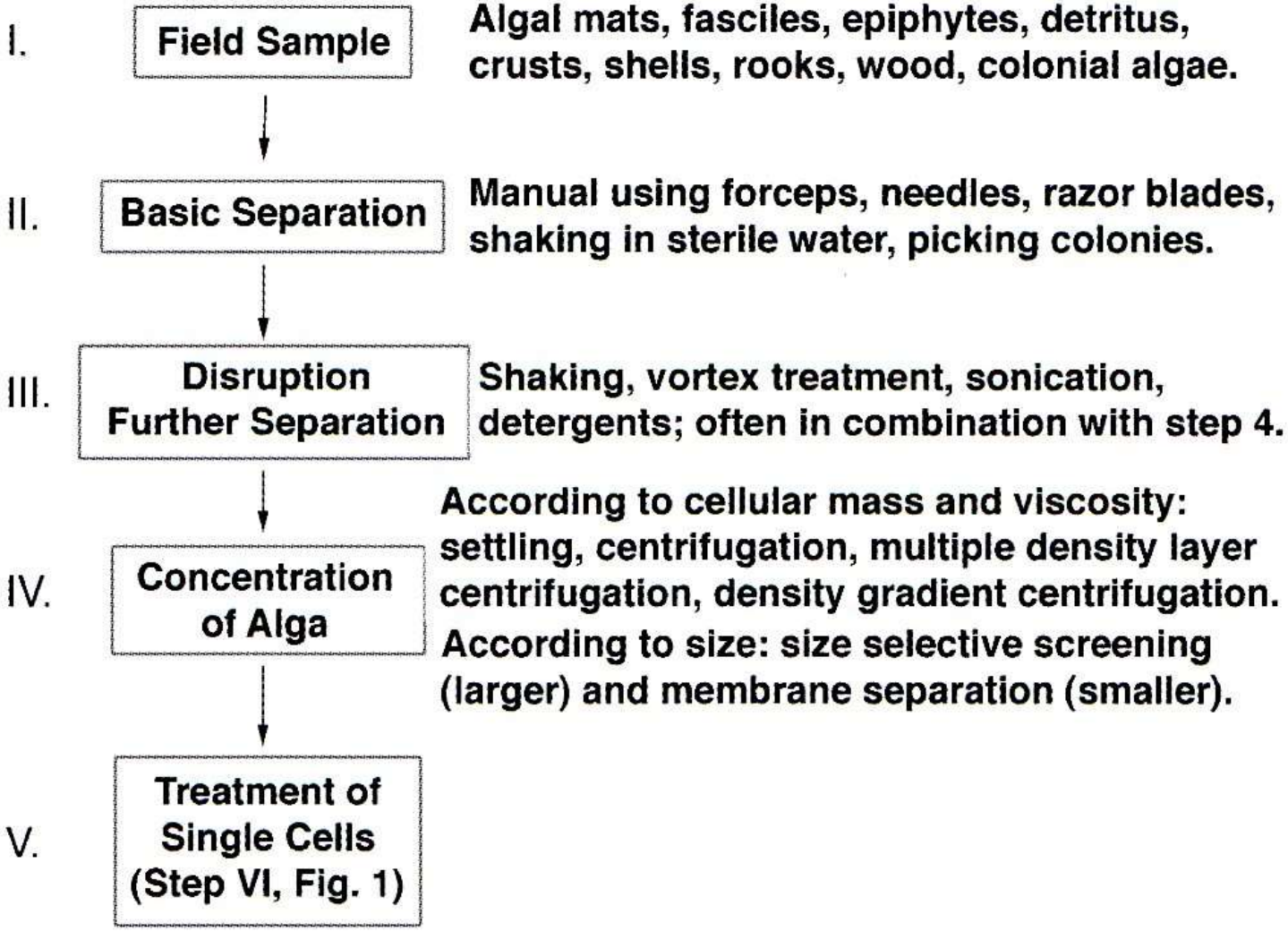


FIGURE 8.2. Flow diagram of purification steps for attached or colonial algae.

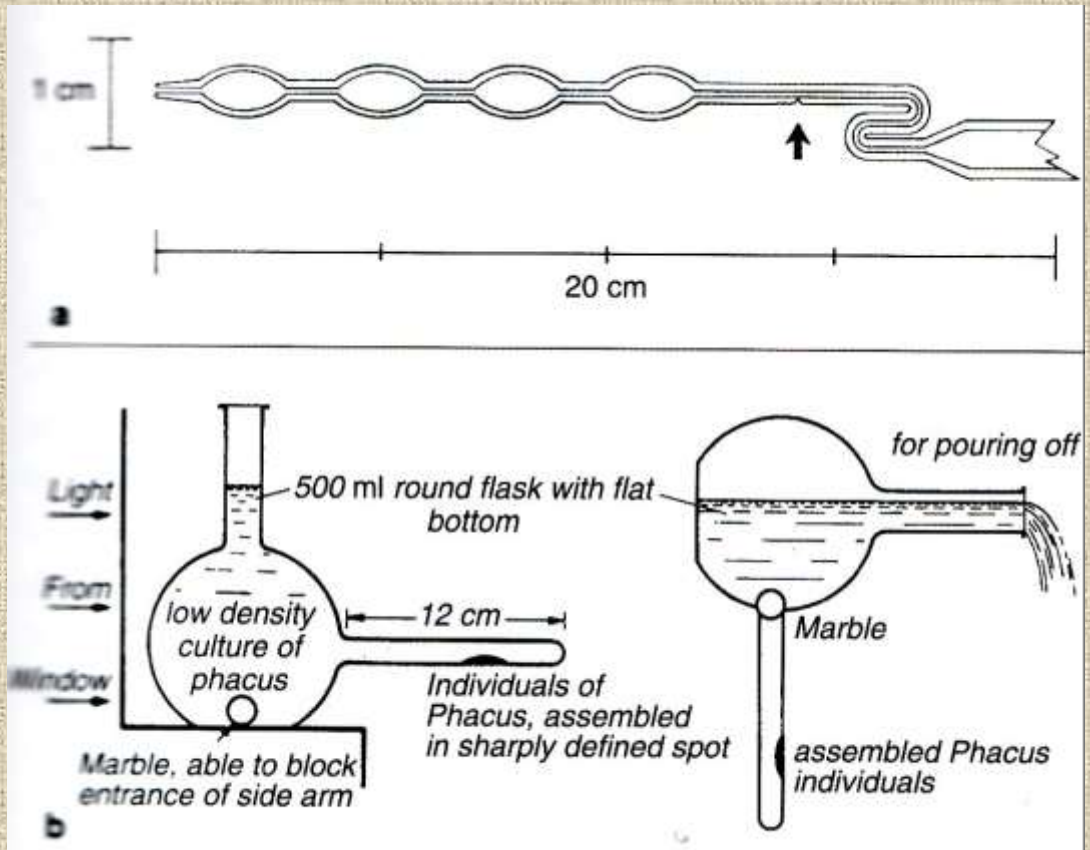


FIGURE 6.7. Phototaxis apparatuses. **(a)** Phototactic flagellates drawn into the tip of the pipette and then allowed to migrate through the cavities and into the pipette itself. The tip is broken at the arrow and discarded, and the cells in the pipette are then discharged into sterile culture medium (e.g., test tube, flask, or multiwell) (Paasche 1971, modified from Guillard 1973). **(b)** Negatively phototactic flagellates concentrated with bright light in the narrow arm of the flask (*left*) and then retained by decanting the original sample while the target cells are trapped in the arm by a glass bead (from Meeuse 1963).

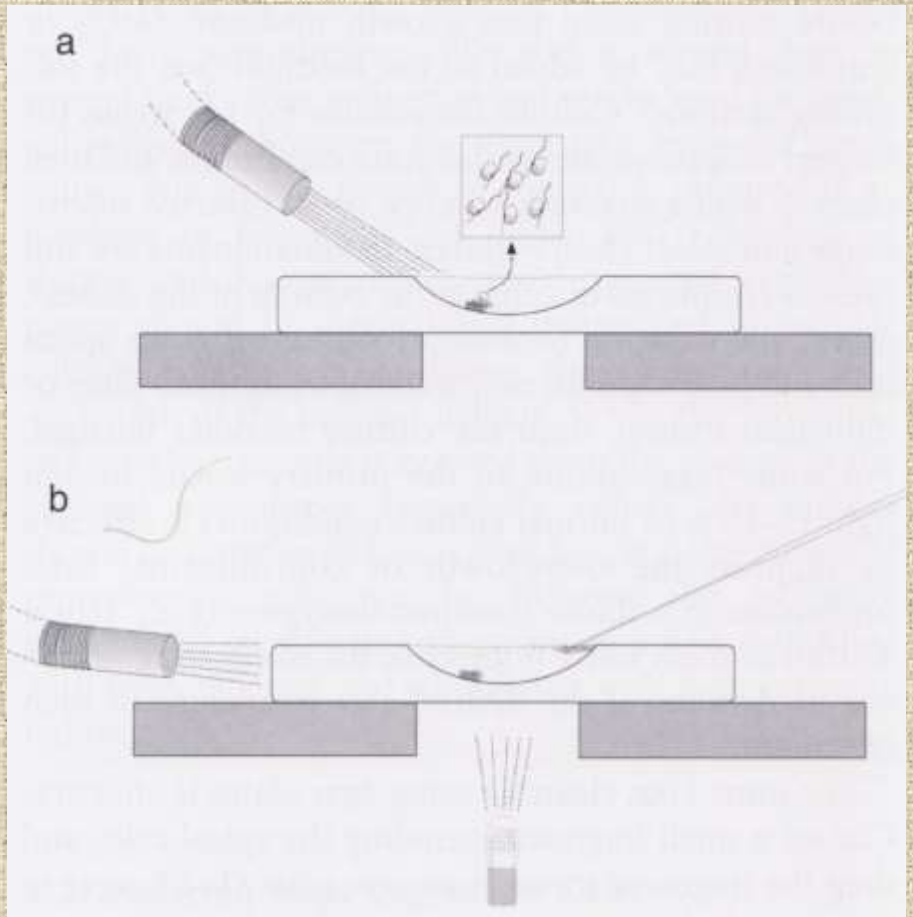


FIGURE 9.10. Isolation of swimming zooids using phototaxis. **(a)** Unilateral illumination from a fiber-optic light source to stimulate zooid release; **(b)** simultaneous lateral and bottom illumination to accumulate zooids at one upper edge of the depression slide well.

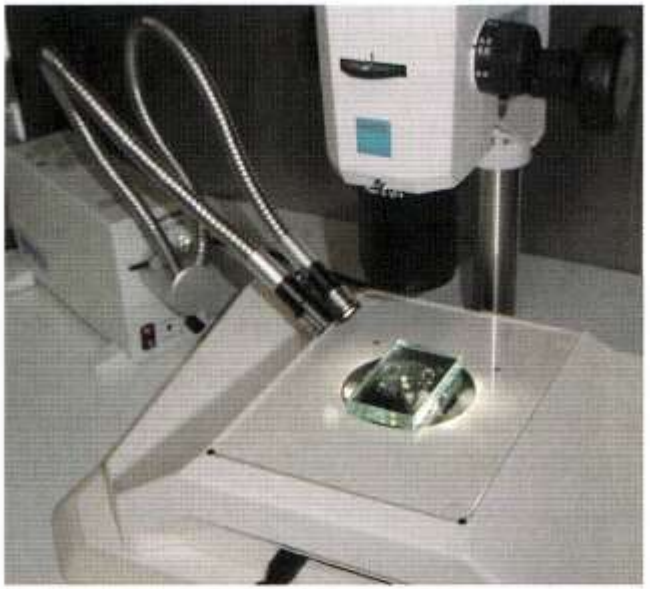


FIGURE 9.8. Isolation of phototactic zooids under observation with a stereomicroscope: Fertile algal tissue is placed at the bottom of a depression slide and the release of zooids is induced by intense illumination from a fiber-optic light source. Released zooids are accumulated at the surface of the medium (lighting from the upper side when they are positively phototactic, and from the lower side when negatively phototactic), and distant from the algal tissue.

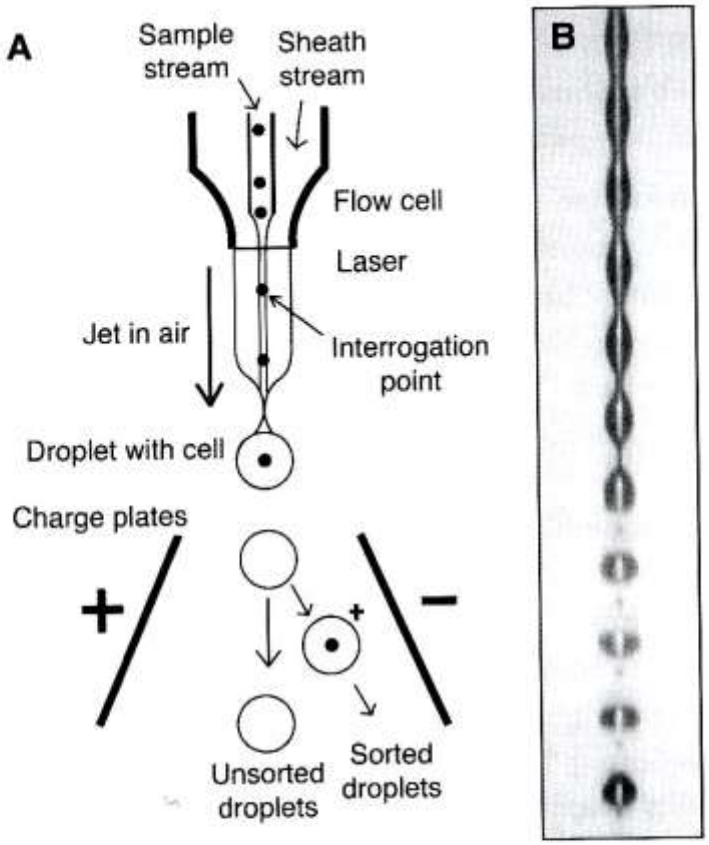


FIGURE 7.1. **(a)** Schematic diagram of droplet cell sorting, showing the joining of the sample stream and sheath fluid stream in the flow cell, forming the jet-in-air flow stream with the cells in single file. The laser intercepts the cells at the interrogation point, where measurements are made. If a cell meets the criteria of the sort logic, then the flow stream is charged just before the break-off point of the droplet containing the target cell. The droplet retains the charge and is deflected by the charge plates toward the collection tube or plate. **(b)** Image of flow stream at the droplet break-off point with use of a 70-μm tip.

Culturing techniques

- chemicals
- equipment – balances, ph-meter, autoclave, filtration, ultrasonic washer, refrigerator, cultivator
- conditions – ph, temp, irradiation, photoperiode,..
- glasware – E. flask, reagent bottles, pipetes, flask, tubes, P. dishes, spatulas, funels, filter holder, syringe, ..
- water
- agar
- soil

Media

- stock solutions
  - macronutrients
  - trace elements
  - vitamins
  - chelators
  - soil extracts
- preparation of media
- synthetic media
- enriched media
- soil water media
- solidified media – agar
- Freshwater media
- Seawater media
    - natural & artificial