# THIRUTHANGAL NADAR COLLEGE

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**NAME OF THE DEPARTMENT: PLANT BIOLOGY AND PLANT BIOTECHNOLOGY**

**SUBJECT : ALGAL BIOTECHNOLOGY**

**TOPIC : CULTUTRE METHODS OF ALGAE**

**STAFF NAME : DR.I.ISAIVANI**

# THIRUTHANGAL NADAR COLLEGE

# CULTURE METHODS OF ALGAE

Dr. I. ISAIVANI
DEPT. OF PLANT BIOLOGY & PLANT BIOTECHNOLOGY

# Algal culture techniques

- The terminology used to describe the type of algal culture include:
- **Indoor/Outdoor**. Indoor culture allows control over illumination, temperature, nutrient level, contamination with predators and competing algae, whereas outdoor algal systems make it very difficult to grow specific algal cultures for extended periods.
- **Open/Closed**. Open cultures such as uncovered ponds and tanks (indoors or outdoors) are more readily contaminated than closed culture vessels such as tubes, flasks, carboys, bags, etc.
- **Batch, Continuous, and Semi-Continuous**. These are the three basic types of phytoplankton culture which will be described later

**Table 2.6. Advantages and disadvantages of various algal culture techniques (modified from Anonymous, 1991).**

| Culture type | Advantages | Disadvantages |
|---|---|---|
| Indoors | A high degree of control (predictable) | Expensive |
| Outdoors | Cheaper | Little control (less predictable) |
| Closed | Contamination less likely | Expensive |
| Open | Cheaper | Contamination more likely |

| | | |
|---|---|---|
| Continuous | Efficient, provides a consistent supply of high-quality cells, automation, highest rate of production over extended periods | Difficult, usually only possible to culture small quantities, complex, equipment expenses may be high |
| Semi-continuous | Easier, somewhat efficient | Sporadic quality, less reliable |
| Batch | Easiest, most reliable | Least efficient, quality may be inconsistent |

# Batch culture

- 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 and the larger culture volumes are then brought to a maximum density and harvested. The following consecutive stages might be utilized: test tubes, 2 l flasks, 5 and 20 l carboys, 160 l cylinders, 500 l indoor tanks, 5,000 l to 25,000 l outdoor tanks

Production scheme for batch culture of algae (Lee and Tamaru, 1993).

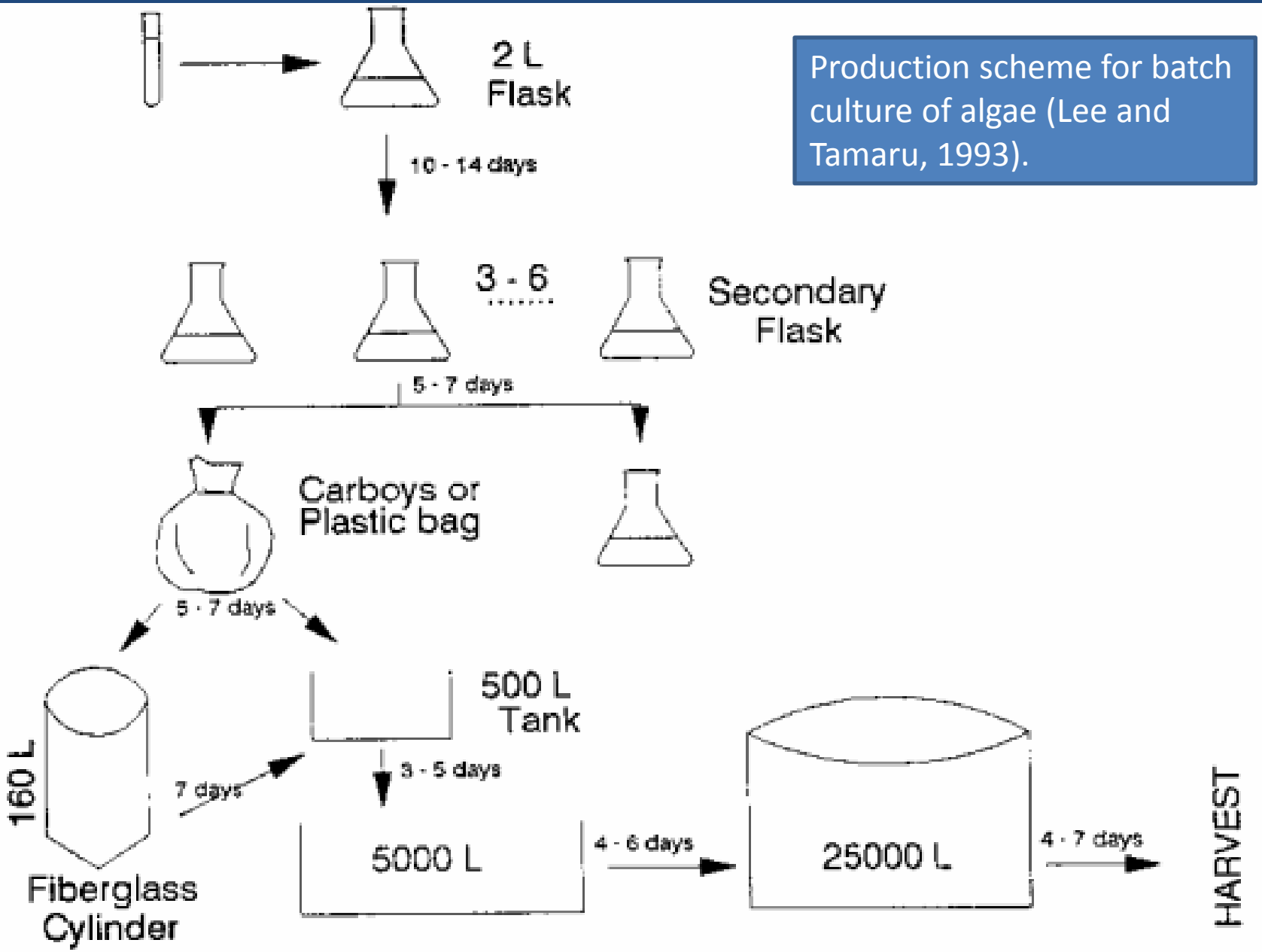
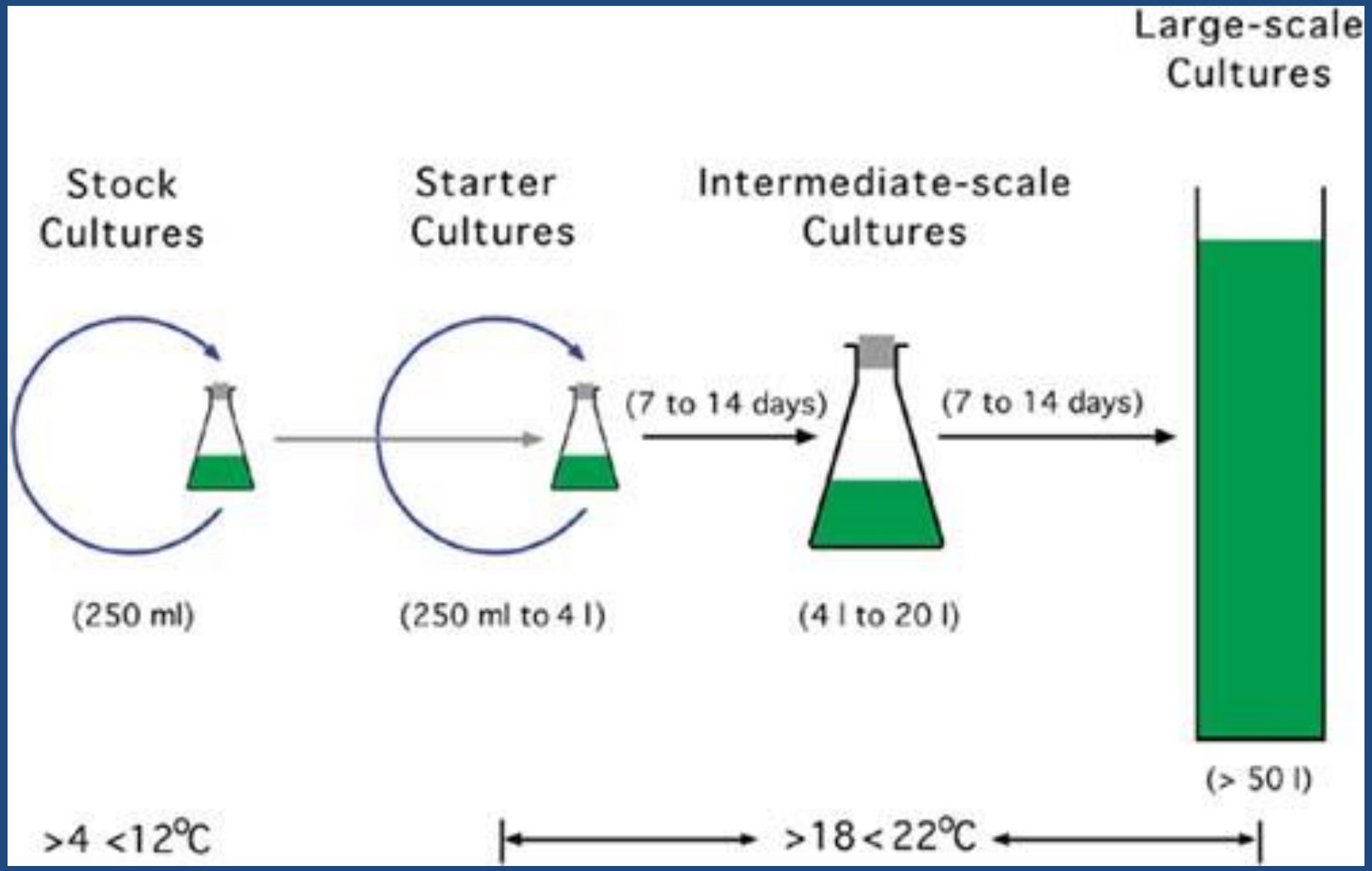



FIGURE 5. Progression of algal production at The Oceanic Institute.

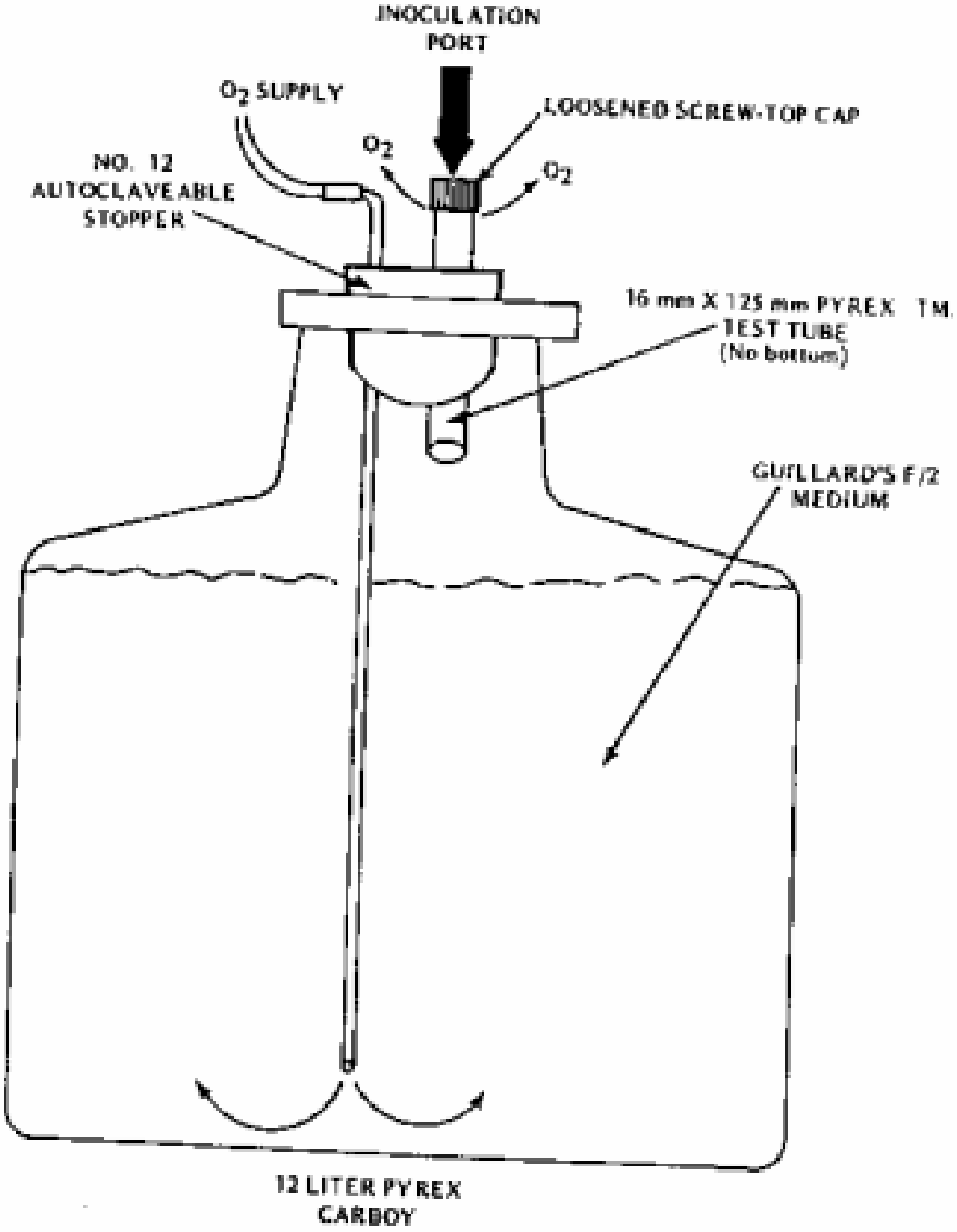

Large-scale Cultures
Stock Cultures
Starter Cultures
Intermediate-scale Cultures
(7 to 14 days)
(7 to 14 days)
(250 ml)
(250 ml to 4 l)
(4 l to 20 l)
(> 50 l)
>4 <12°C
>18<22°C

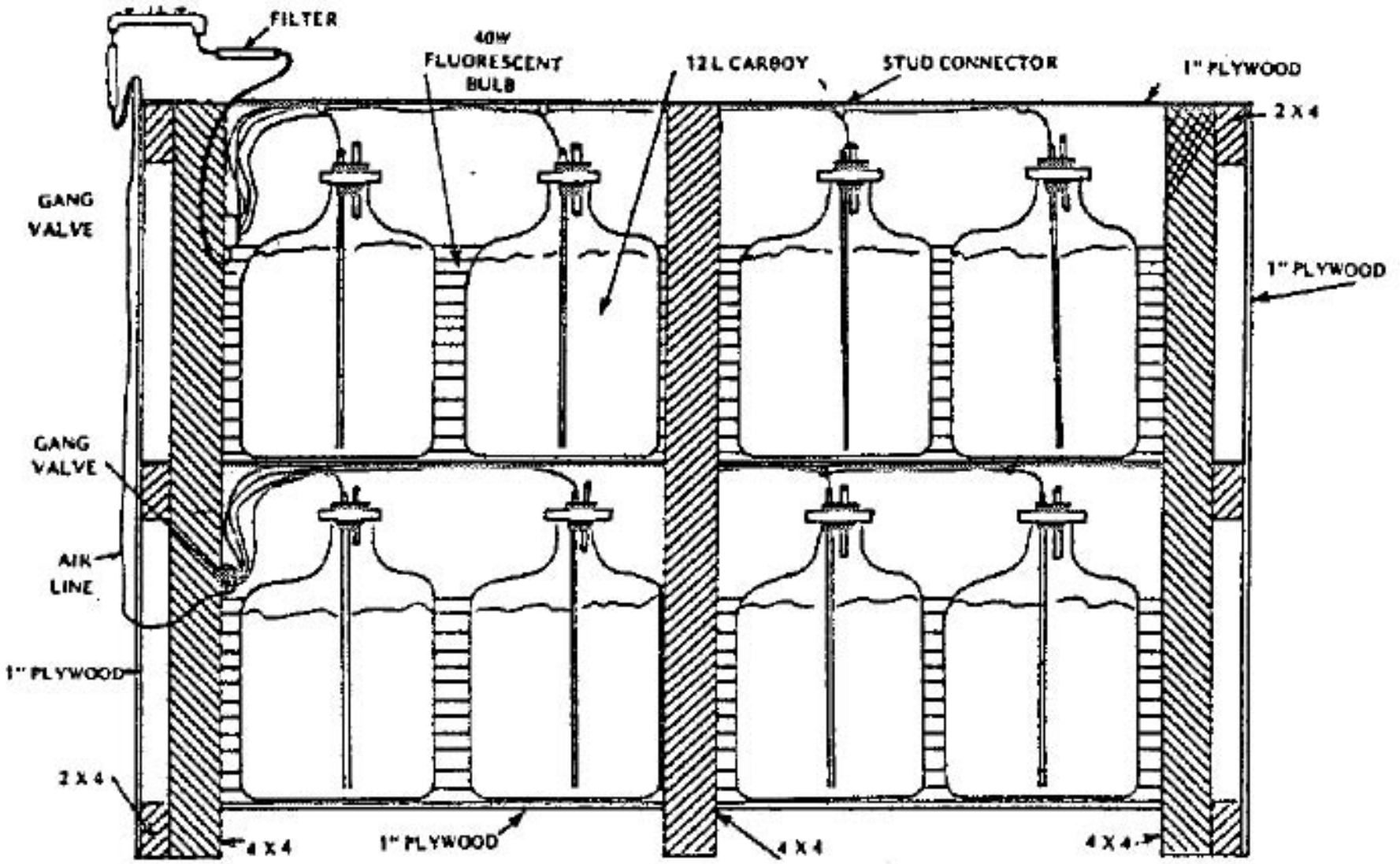
- Batch culture systems are widely applied because of their simplicity and flexibility and  often considered as the most reliable method,
- However, the quality of the harvested cells may be less predictable than that of continuous systems and for example vary with the timing of the harvest (time of the day, exact growth phase).
- Another disadvantage is the need to prevent contamination during the initial inoculation and early growth period. Because the density of the desired phytoplankton is low and the concentration of nutrients is high, any contaminant with a faster growth rate is capable of outgrowing the culture.
- Batch cultures also require a lot of labour to harvest, clean, sterilize, refill, and inoculate the containers.


Batch culture systems for the mass production of micro-algae in 20,000 l tanks .

Batch culture systems for the mass production of micro-algae in 150 l cylinders.

Carboy culture apparatus (Fox, 1983).






Carboy culture shelf (Fox, 1983).


A


B

# The continuous culture method

A culture in which a supply of fertilized seawater is continuously pumped into a growth chamber and the excess culture is simultaneously washed out, permits the maintenance of cultures very close to the maximum growth 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 rate and not the cell density is kept constant.

# Continuous culture

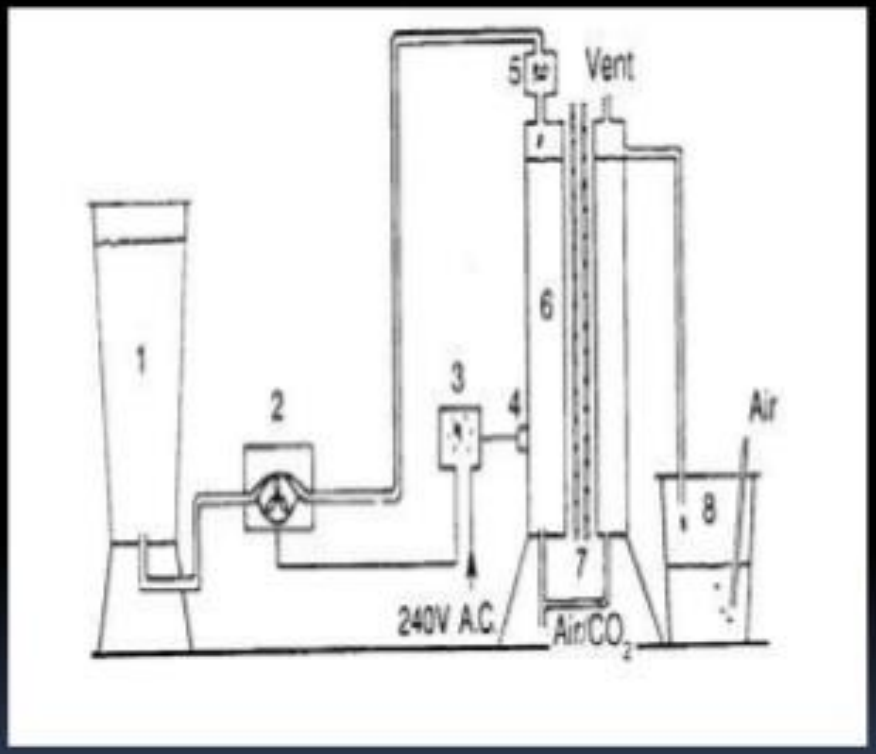
## turbidostat culture

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

## chemostat culture

- 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
- in this way the growth rate and not the cell density is kept constant.

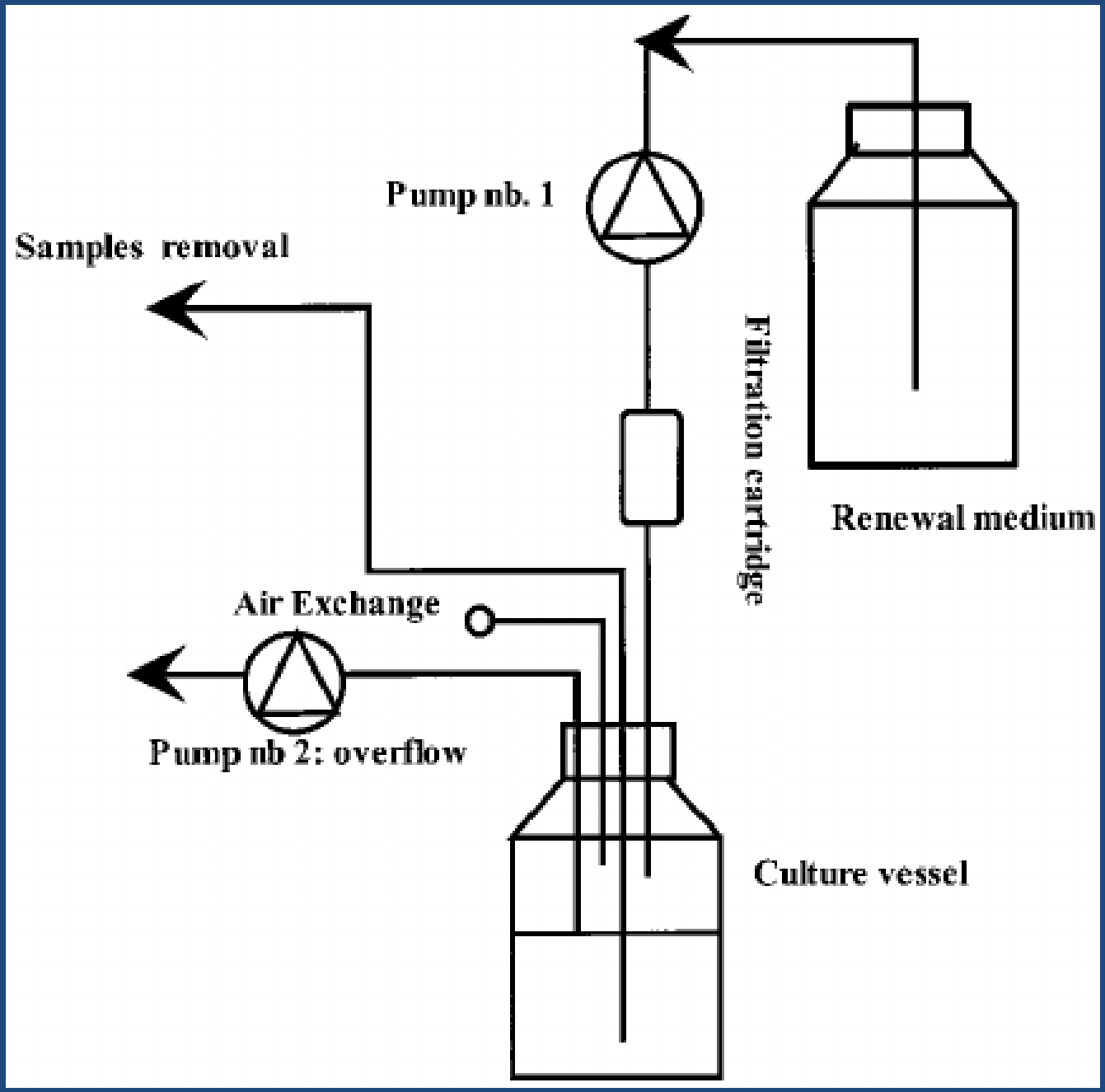
# Continuous culture




- Diagram of a continuous culture apparatus (not drawn to scale):
- (1) enriched seawater
- medium reservoir (200 l);
- (2) peristaltic pump;
- (3)resistance sensing relay (50-5000 ohm)
- (4) lightdependent
- resistor (ORP 12);
- (5) cartridge filter (0.45
- μm);
- (6) culture vessel (40 l);
- (7) six 80 W fluorescent tubes (Laing, 1991).

In continuous cultures, cultures are maintained at a chosen point on the growth curve by the regulated addition of fresh culture medium. In practice, a volume of fresh culture medium is added automatically at a rate proportional to the growth rate of the alga, while an equal volume of culture is removed. This method of culturing algae permits the maintenance of cultures very close to the maximum growth rate, because the culture never runs out of nutrients. Air is pumped into this medium vessel.

A peristaltic pump is most suitable for delivery of medium into the culture, for the mechanical parts are not in direct contact with the medium. 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 (exit air).


Pump nb. 1
Samples removal
Filtration cartridge
Renewal medium
Air Exchange
Pump nb 2: overflow
Culture vessel

As fresh medium is added to the culture vessel the level of the liquid in the culture vessel rises. The old culture medium and cells could be removed by either another peristaltic pump, or through an overflow located at the side of the culture vessel into a waste flask. There is another glass tube in the culture vessel, the sample port, which is used to draw a sample from the culture vessel when needed. Air is pumped into the culture vessel through a sterile filter. This bubbling air has three effects: it supplies $CO_2$ and $O_2$ to the culture, aids in circulation and agitation of the cultures. The magnetic stirrer help to prevent the cells from collecting in the bottom of the culture vessel.

The rate of flow of medium into a continuous culture system is known as the “dilution rate.” When the number of cells in the culture vessel remains constant over time, the dilution rate is said to equal the rate of cell division in the culture, as the cells being removed by the outflow of medium are being replaced by an equal number through cell division in the culture. On the other hand, if the dilution rate exceeds the maximum cell division rate, then cells are removed faster than they are produced and total washout of the entire cell population eventually occurs.

Advantages • Continuous cultures have the advantages of producing algae of more predictable quality. • They are amenable to technological control and automation, which in turn increases the reliability of the system and reduces the need for labor Disadvantages The disadvantages of the continuous system are • Its relatively high cost and complexity. • The requirements for constant illumination and temperature mostly restrict continuous systems to indoors and this is only feasible for relatively small production scales.

The disadvantages of the continuous system are its relatively high cost and complexity. The requirements for constant illumination and temperature mostly restrict continuous systems to indoors and this is only feasible for relatively small production scales. However, continuous cultures have the advantage of producing algae of more predictable quality. Furthermore, they are amenable to technological control and automation, which in turn increases the reliability of the system and reduces the need for labour.

SEMI-CONTINUOUS CULTURES In a semi-continuous system the fresh medium is delivered to the culture all at once, by simply opening a valve in the medium delivery line. Fresh medium flows into the culture vessel, and spent culture flows out into a collecting vessel. Once the required medium has entered the culture, the valve is closed, and the culture is allowed to grow for 24 h, when the procedure is repeated. The semicontinuous technique prolongs the use of large tank cultures by partial periodic harvesting followed immediately by topping up to the original volume and supplementing with nutrients to achieve the original level of enrichment.

The culture is grown up again, partially harvested, etc. Semi-continuous cultures may be indoors or outdoors, but usually their duration is unpredictable. Competitors, predators, or contaminants and metabolites eventually build up, rendering the culture unsuitable for further use. As the culture is not harvested completely, the semi-continuous method yields more algae than the batch method for a given tank size.