Physiology and Cultivation of Algae and Cyanobacteria

6.

Culture methods

- Batch cultures
  - common, simple, low cost, closed system, volume-limited
  - any flow of nutritions & products
  - Erlenmeyer flasks, tubes, Petri dishes
  - growth curve phases – lag, acceleration, exponential, retardation, stationary, decline

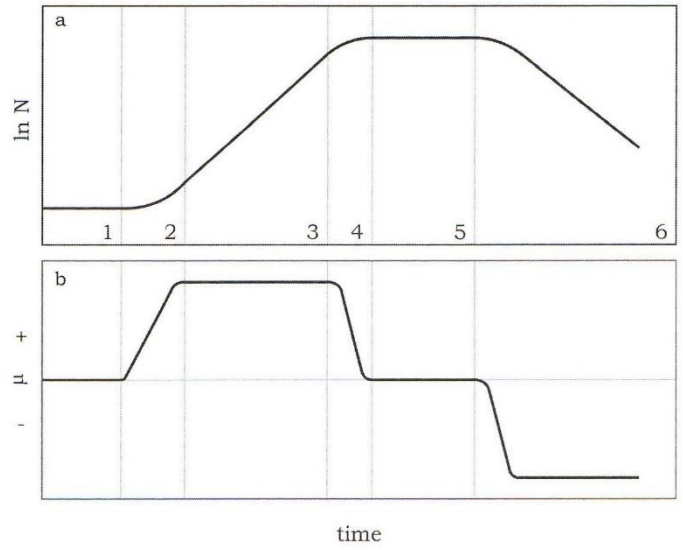


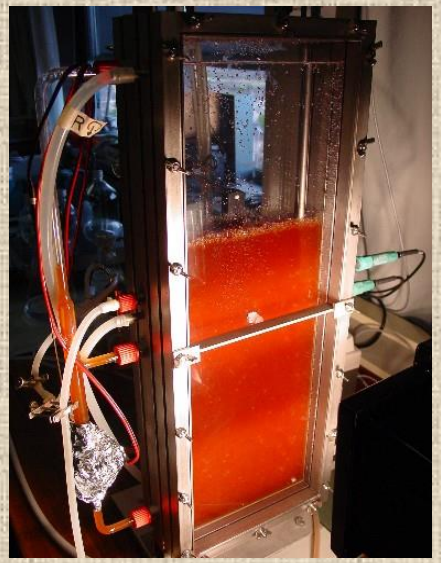
FIGURE 6.1 Growth curve of an algal population under batch culture conditions (a) and corresponding variations of the growth rate (b).

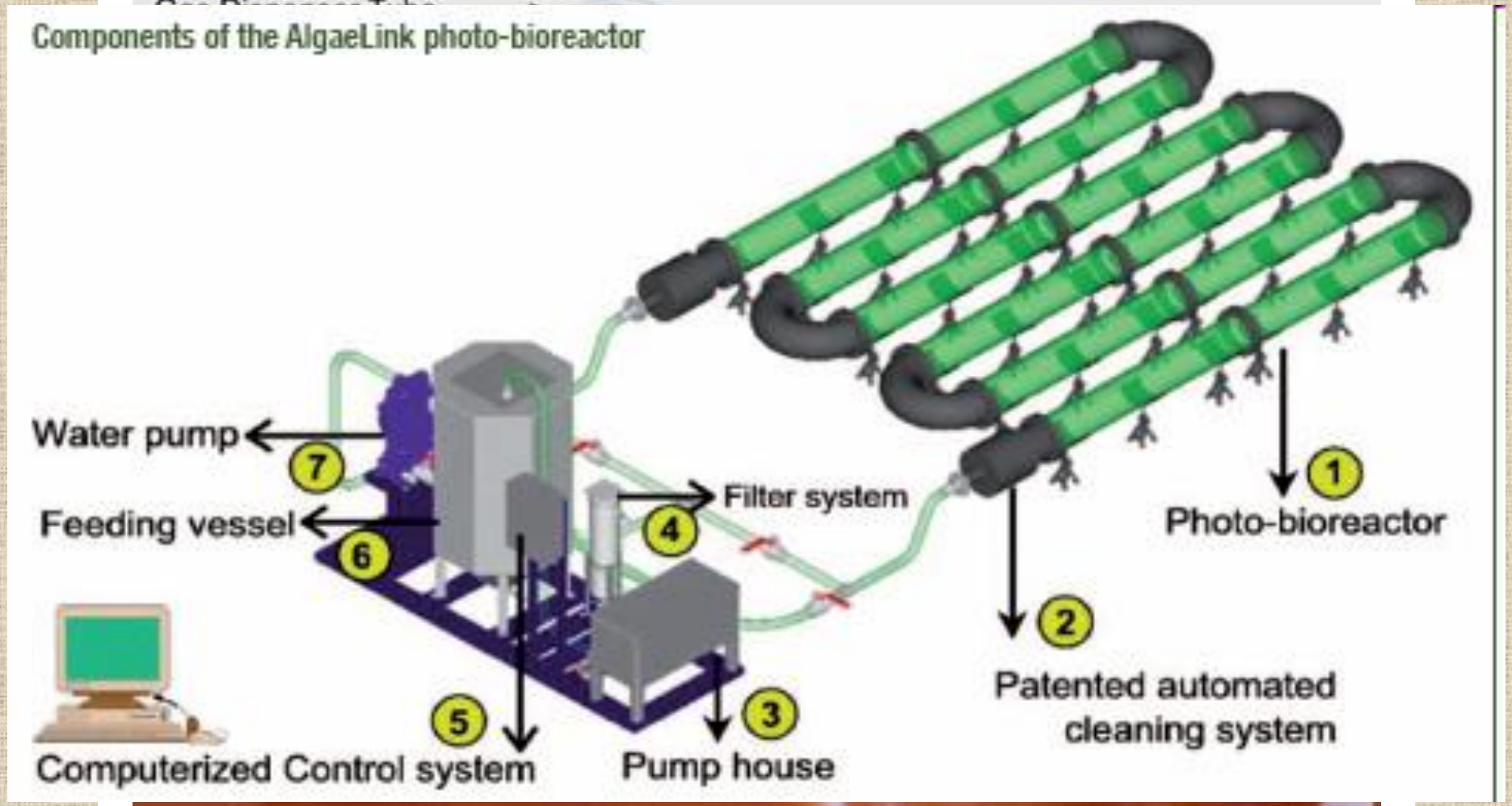
TABLE 6.19
Description of the Six Successive Phases of Growth for an Algal Population under Batch Culture Conditions

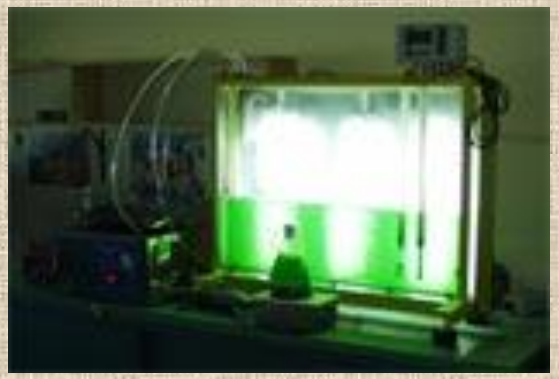
Phase	Growth	Growth Rate Interpretation	Description
1	Lag	Zero	Physiological adaptation of the inoculum to changing conditions
2	Acceleration	Increasing	Trivial
3	Exponential	Constant	Population growth changes the environment of the cells
4	Retardation	Decreasing	Effects of changing conditions appear
5	Stationary	Zero	One or more nutrients (or light) are exhausted down to the threshold level of the cells
6	Decline	Negative	The duration of stationary phase and the rate of decline are strongly dependent on the kind of organisms

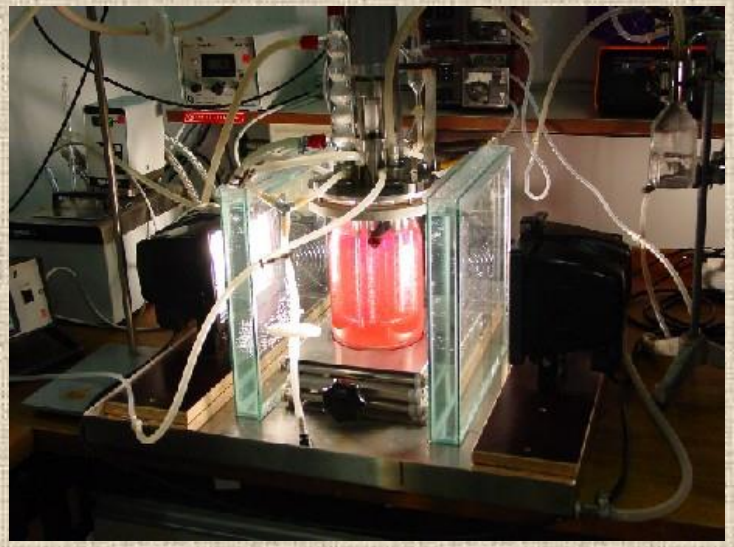
Culture methods

- Continuous cultures
  - resources are potentially infinite
  - cultures are maintained at chosen point on the growth curve by regulated addition of fresh medium
  - air pump – CO_2 source, mixing
  - categories of contin. cult.:
    - turbidostat cultures
    - chemostat cultures
- Semi-continuous cultures
  - periodic fresh medium addition & harvesting




Components of the AlgaeLink photo-bioreactor
Water pump
7
Feeding vessel
6
Filter system
4
1
Photo-bioreactor
2
Patented automated
cleaning system
5
Computerized Control system
3
Pump house





Culture methods

- Commercial-scale cultures
  - volume of cca. $10^2 – 10^9$ l
  - large open ponds, circular ponds with rotating arm, raceway ponds, large bags, tube system
  - factors to be considered:
    - biology of alga; the cost of land; labor; energy; water; nutrients; climate (if outdoors); type of product
    - light utilization efficiency (PBR & open ponds, surface-to-volume ratio 20-200 vs 5-10m^{-1}, orientation, inclination); ability to control temp.; hydrodynamic stress (mixing); oxygen accumulation; ability to maintain culture unialgal or axenic (photobioreactors *vs*. open ponds)

      -scale up ability
    - **Harvesting** (20-30%cost) – species specific
      - Flocculation -↑pH, cationic polymers (Chitosan, Zetag)
      - Centrifugation & filtration
    - **Dehydrating** – sun-drying, spray-drying, drum-drying, freeze-drying
    - Cell disruption **–** mechanical (homogenizers, bead mills, ultrasound), chemical
    - Product isolation and purification

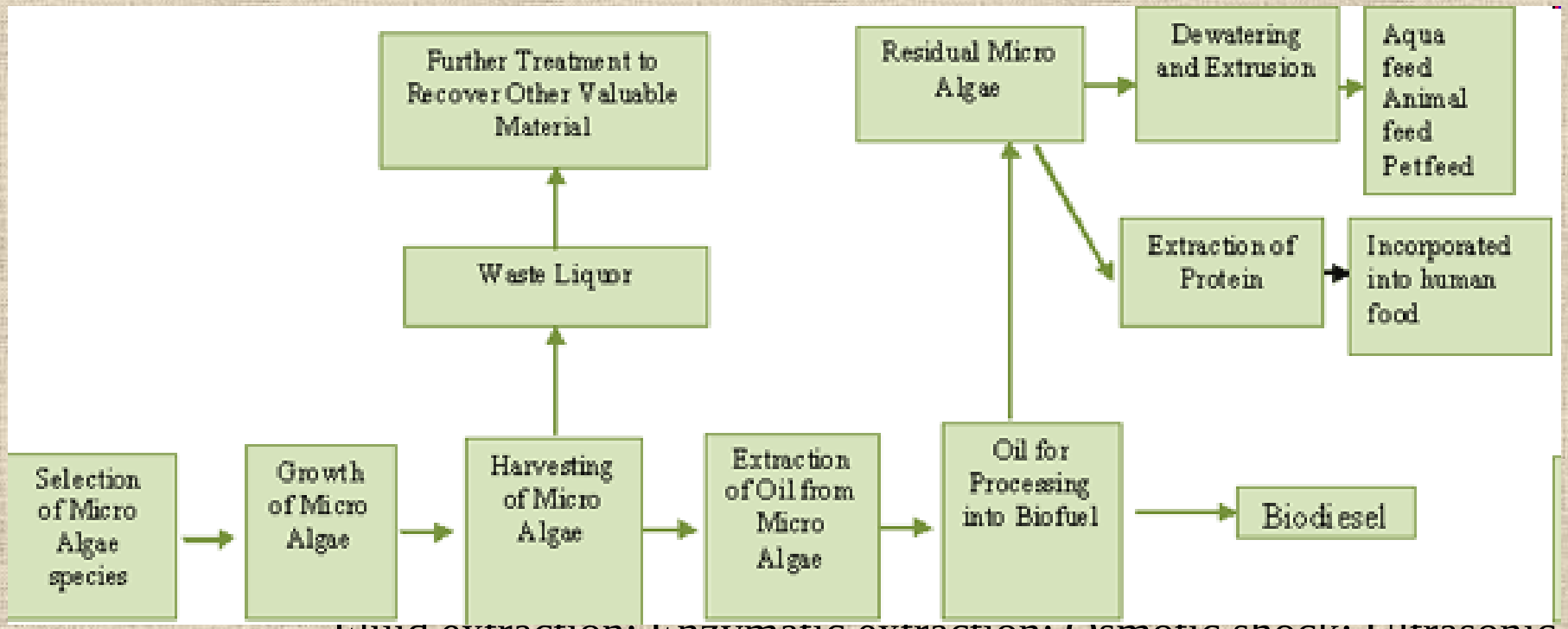
  - *Chlorella*, *Spirulina, Dunaliella*, *Nannochloropsis*

Methods used for algal culture growth evaluation

- direct
  - fresh/dry mass determination
  - counting – number of cells (colonies)
  - cell volume, PCV
  - protein content
  - calorific value
  - flow-cytometry & epifluorescence microscopy
- indirect
  - turbidity; optical density
  - chlorophyll content

Algae & Men

- macroalgae (commerce - 42 countries)
  - food
    - *Laminaria* (China, N.,S.Korea, Japan, Philipines, Chile, Norway, Indonesia, U.S., India)
    - *Porphyra*, *Kappaphycus*, *Undaria* (Wakame), *Euchema*, *Gracilaria*, *Caulerpa lentillifera* (green caviar)
    - **Nori** (*Porphyra yezzoensis*) – 13mil. t/y
- microalgae
  - carotenoids, pigmenst, proteins, vitamins, …
    - *Dunaliella*, *Haematococcus*, *Arthrospira*, *Chlorella*
    - nutraceuticals, pharmaceuticals, animal feed additives, cosmetics, fertilizers
    - N2-fixing cyano.-biofertilizers in rice fields
    - Wastewater oxidation, bioremediation
  - Microalgae – platform for recombinant proteins (*e.g.* hGH in *Chlorela*)



Fluid extraction; Enzymatic extraction; Osmotic shock; Ultrasonic-assisted Extraction

- Ethanol from algae
  - high carbohydrate content (*Sargassum*, *Glacilaria*, *Prymnesium parvum*, *Euglena gracilis*)
- Cultivation of algae for CO_2 capture
  - can absorb over 2 million tons of Co2 a year per acre

Endogenous rhythms

- Chronobiology
  - free-running vs. entrained (synchronized)
- circadian rhythms
  - molecular feedback loop ~ 24h~ environmental light-dark cycles ~ positive/negative phase shifts according to phase-response curve of particular response
  - necessity to transfer sunlight-sensitive processes into the night (cell division in night)
  - lost under certain conditions *(e.g.* constatnt light, bright light, growth of *Euglena* on organic medium)
    - phototaxis
    - timing of cell division
    - photosynthetic capacity
    - bioluminiscence
    - gene expression
    - sensitivity to UV

Endogenous rhythms

- biweekly (circa-semilunar) rhythms
  - wave action (sea may reduce gamete concentration of marine org. with external fertilization)
  - *e.g.*:brown alga Dictyota dichotoma releases eggs twice a month in the field; synchronization signal every second full-moon light
- day-length effect (photoperiodism)
  - circadian timer measures length of night and triggers photoperiodic response
  - LD (12:12~light:dark) – induce upright thali formation
  - SD (8:16)– induce reproductive organs formation
- circannual rhythmicity
  - sequence of short and long days over the years results cyclic reproductive stages formation (e.g. Ascophyllum, Laminaria)
  - signal probably temporal sequence of different physiological stages

Documetation

- Microscopy (light & fluorescence)
  - drawings
  - photography – classic & digital
- algal culture collections
  - Web pages of culture collections in the world: https://www.eccosite.org/
- macroalgae ~ herbarium