Algal Biotechnology:
Physiology of growth, and Mass Cultivation, Photobioreactors

Algae : **biology**, growth, production

- Algae formal tax. standing, polyphyletic origin, artificial assemblage of O_2 evolving photosynthetic organisms; wide range of growth form; wide range of growth strategies; wide range of reproduction strategies (vegetative, asexual, sexual); tolerance of wide range of environmental condition (*e.g.* Nutrients, pH, temp., turbidity, O_2 & CO_2 conc.)
- Aquatic – marine & freshwater – planctonic, bentic, kelps; subaerial; symbiotic
- Unicellular; colonies; coenobia; filamentous; thalloid
- Nutritional strategies
  - Autotrophy (photoautotrophy) – Heterotrophy (osmotrophy, phagotrophy) – mixotrophy (auxotrophy)

    Obligate – Facultative

- Wide range of valuable metabolites – pigments, antioxidants, toxins, alelopathic metabolites, fatty acids, phenols,

Algae : biology, **growth**, production

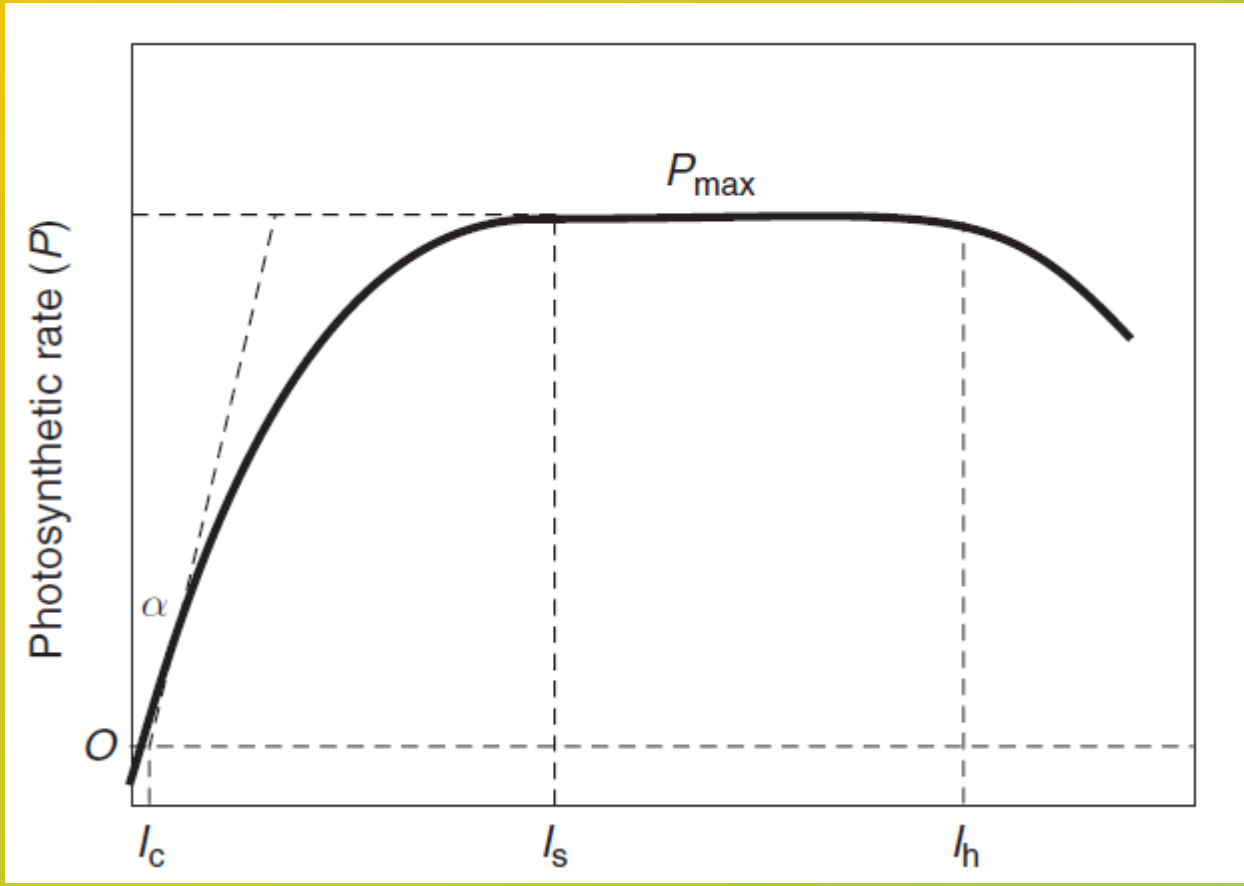
- Environment
  - Light (intensity, spectrum, photoperiod)
    - absorption, transmision, reflection, scattering, interference; environmental accessibility
  - Temperature
  - Substrate & Nutrients
    - Sources vs. Requirements
  - Environmental stability
    - nutrient flow, mechanical condions, stream, randomization

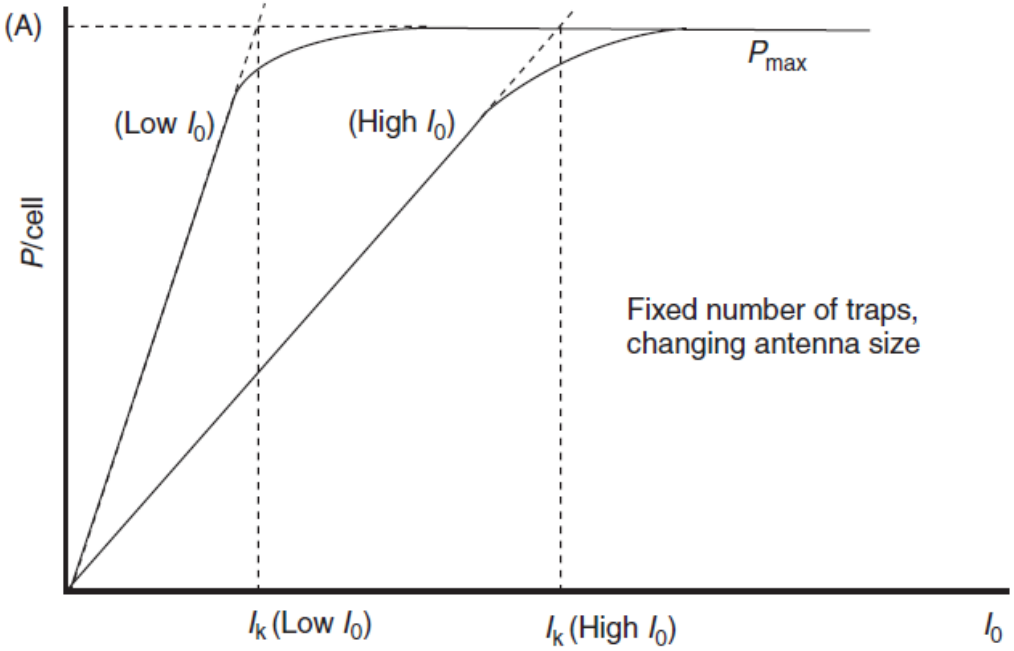
Algae : biology, **growth**, production

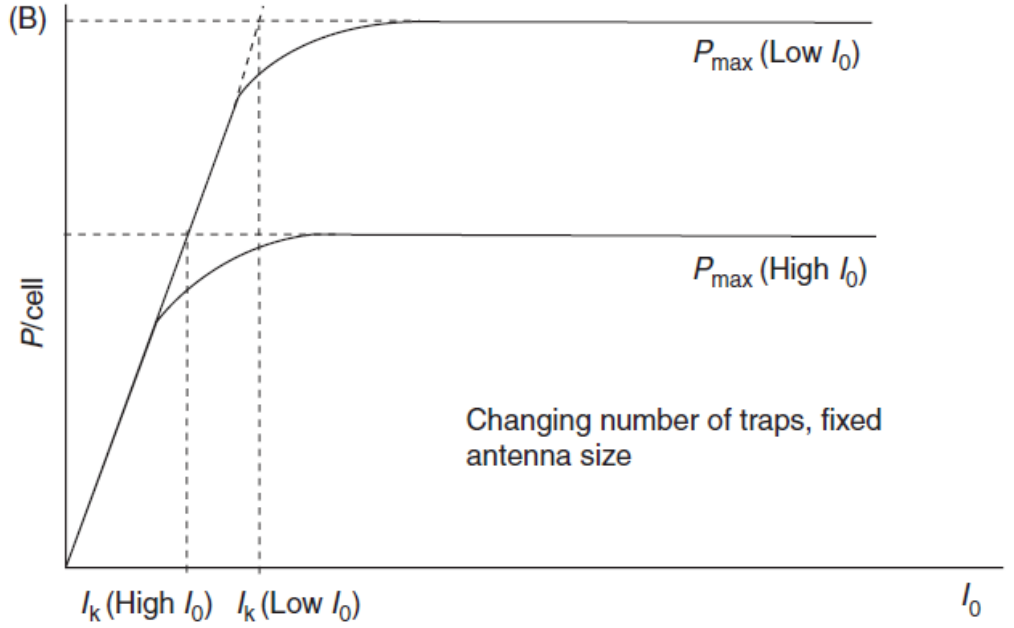
- Nutrient (N,P & CO2, HCO3) accesibility; N_2 fixation; motile stage
- Synergistic effects of combinations of chemical and physical factors
- Photosynthesis
  - Structure & function (thylacoid, chloroplast, cell; pigments – primary, accesory ; **photosystems vs. antena**)
  - Light reaction (ETR, O_2 evolution); Calvin Benson Bassham Cycle (RuBisCO activity); CCM

Algae : biology, **growth**, production

- LRC
- Pn max
- Rd
- Ic
- photoinhibition




(A)
P/cell
(Low I_0)
(High I_0)
P_{max}
Fixed number of traps,
changing antenna size
I_k (Low I_0)
I_k (High I_0)
I_0


(B)
P/cell
P_{max} (Low I_0)
P_{max} (High I_0)
Changing number of traps, fixed
antenna size
I_k (High I_0)
I_k (Low I_0)
I_0

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; pH; CO_2, O_2 conc.
  - chlorophyll content

Culture methods

- Batch cultures – small scale
  - 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

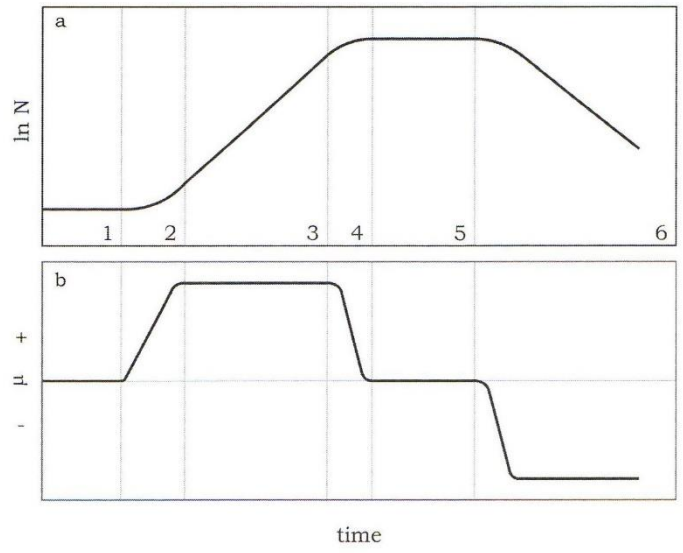


FIGURE 6.1 Growth curve of an algal population under batch culture conditions (a) and corresponding variations of the growth rate (b).

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-turbulence
  - categories of contin. cult.:
    - turbidostat
    - chemostat
    - cyclostat
- Semi-continuous cultures
  - periodic fresh medium addition & harvesting

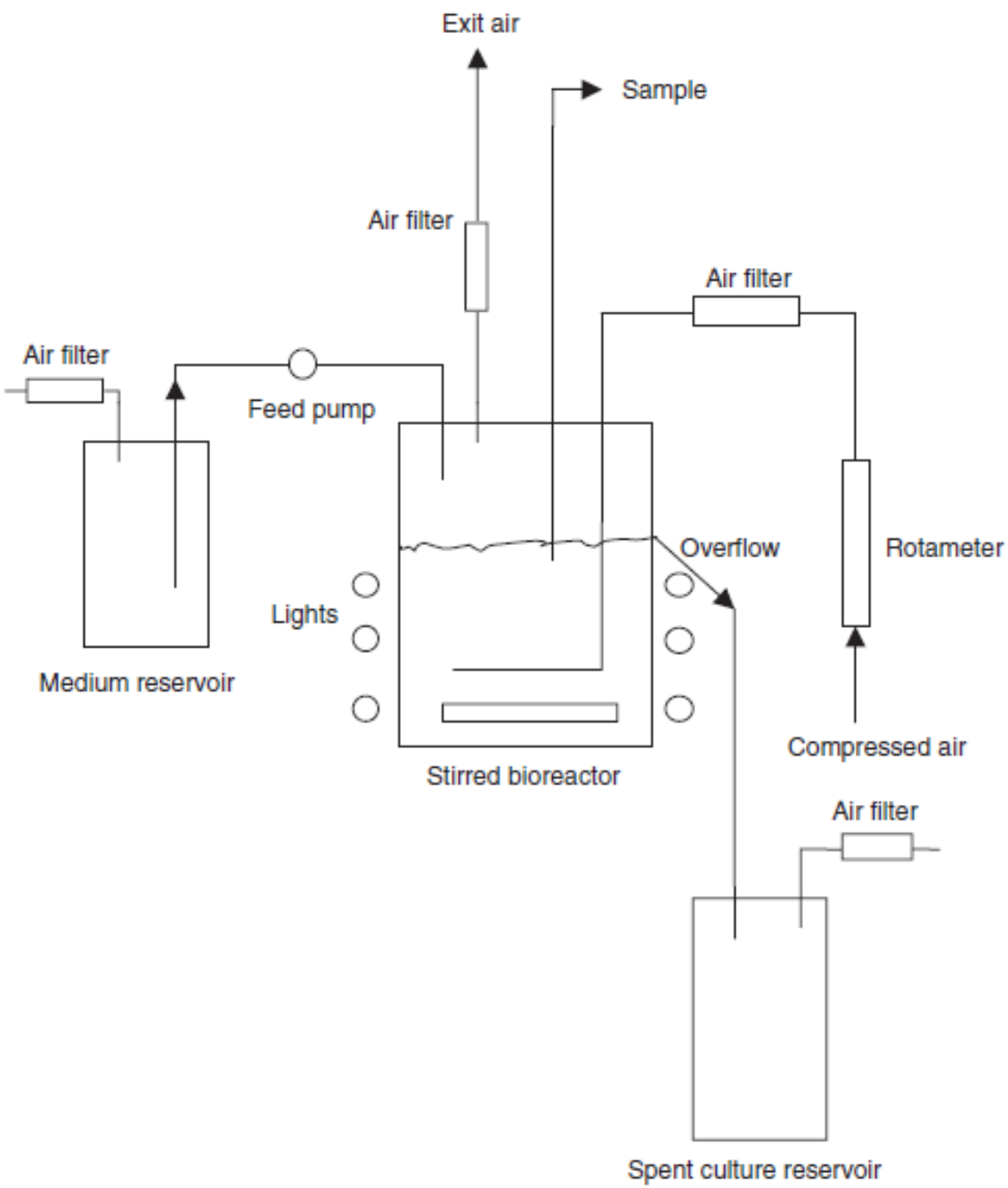


Fig. 3.1. Schematic diagram of a chemostat setup. Reprinted with permission from Kluwer Academic Publishers (*J. Appl. Phycol.*).

Mass production of Microalage

- Open ponds
  - Lakes and natural ponds
  - Inclined systems
  - Cirkular ponds
  - Raceway ponds

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Ideal
dense suspension (cells, colonies, coenobias, fillaments) cultured at low PAR/cell, high O_2 conc. And limits of anorg. C availability

Mass production of Microalage

… then growth depends on interplay of several parameters:

avg. PAR/cell

mixing

gas exchange

temperature

Effective light distribution in suspension

<>

efficiency of light conversion

> effective PBR design

> cell suspension density

> selection (gen modif.) of the culture > small antena

(to reduce excitation presure of PS units under high PAR &
maintain high efficiency of light conversion)


SPIRULINA in MYANMAR
Twyn Taung Crater Lake

Mass production of Microalage

……

- Photobioreactor
  - Tubular photobioreactors
    - Serpentine photobioreactors
    - Manifold photobioreactors
    - Helical photobioreactors
  - Flat photobioreactors
    - Flat alveolar panels
  - Vertical cylinders and sleeves

Biomass growth rate

- growth curve – exponential phase
  - doubling time

$$2^0 N_0 \rightarrow 2^1 N_0 \rightarrow 2^2 N_0 \rightarrow 2^3 N_0 \rightarrow 2^n N_0$$

N_0 = Initial number of cells
n = Number of doublings (generations)

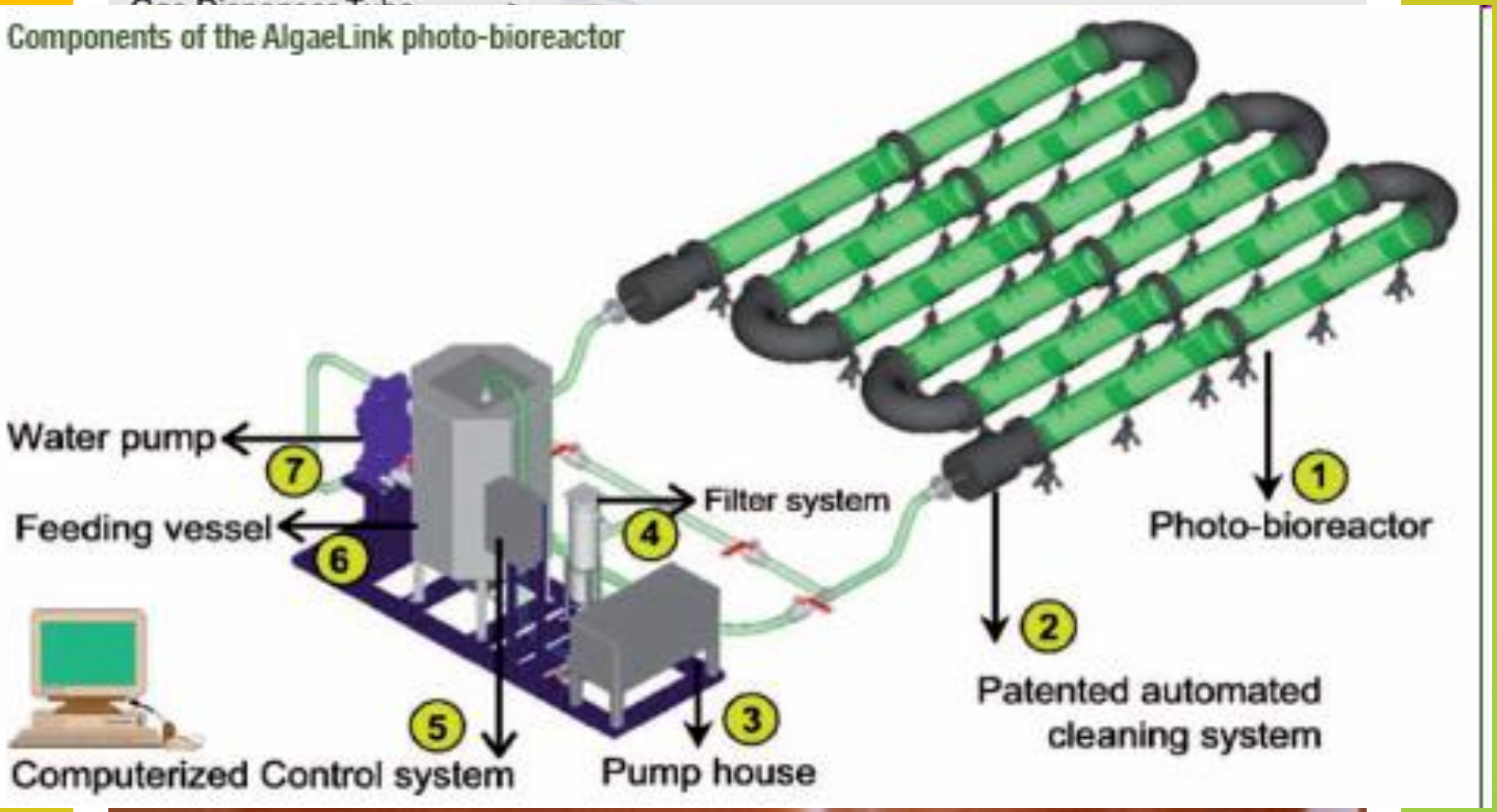
$$\ln(X_t/X_0)/t = 0.693/t_d$$

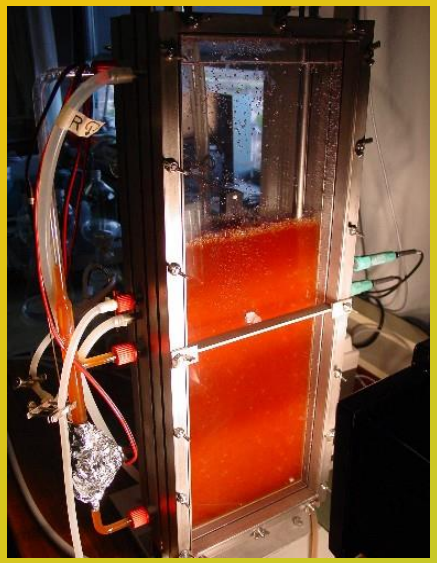
$$d(\ln X)/dt = 0.693/t_d$$

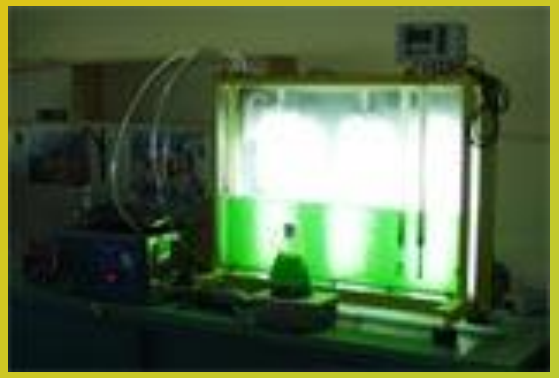
$$d(\ln X)/dX \cdot dX/dt = 0.693/t_d$$

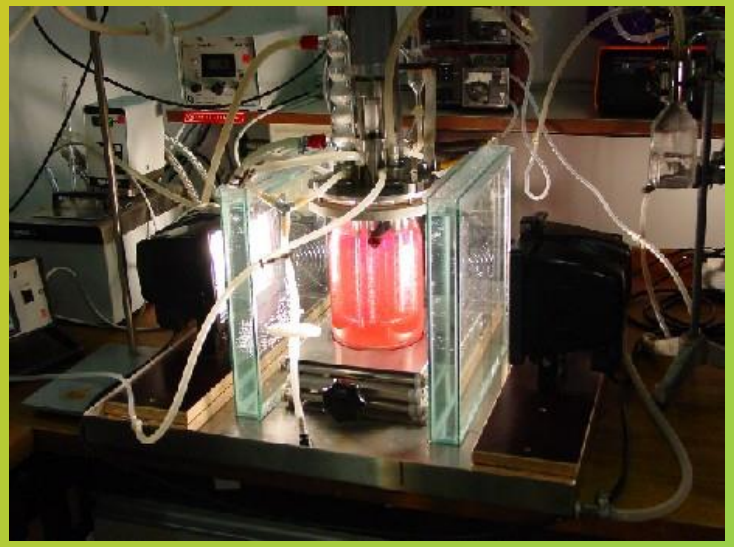
$$1/X \cdot dX/dt = 0.693/t_d$$

$$\mu = 0.693/t_d$$

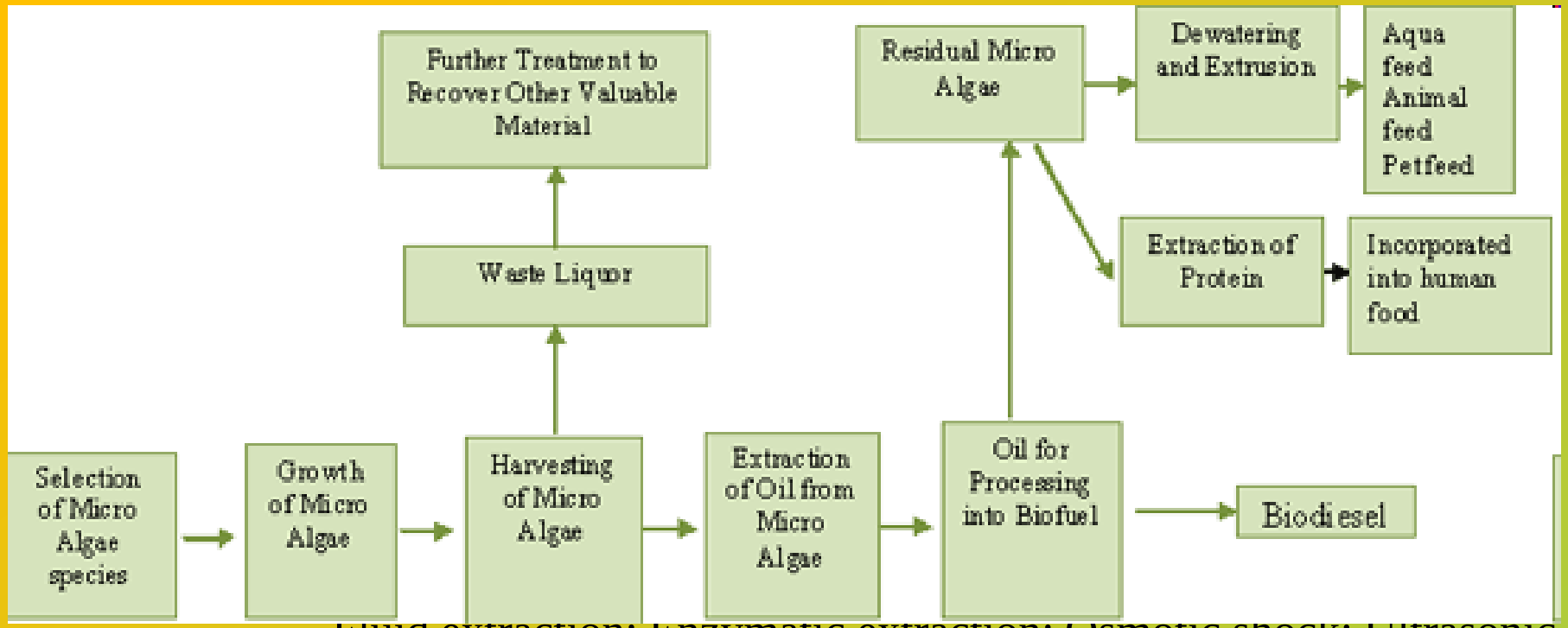

Components of the AlgaeLink photo-bioreactor
Water pump
7
Feeding vessel
6
Filter system
4
1
Photo-bioreactor
2
Patented automated
cleaning system
5
Computerized Control system
3
Pump house







- 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*



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

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*)