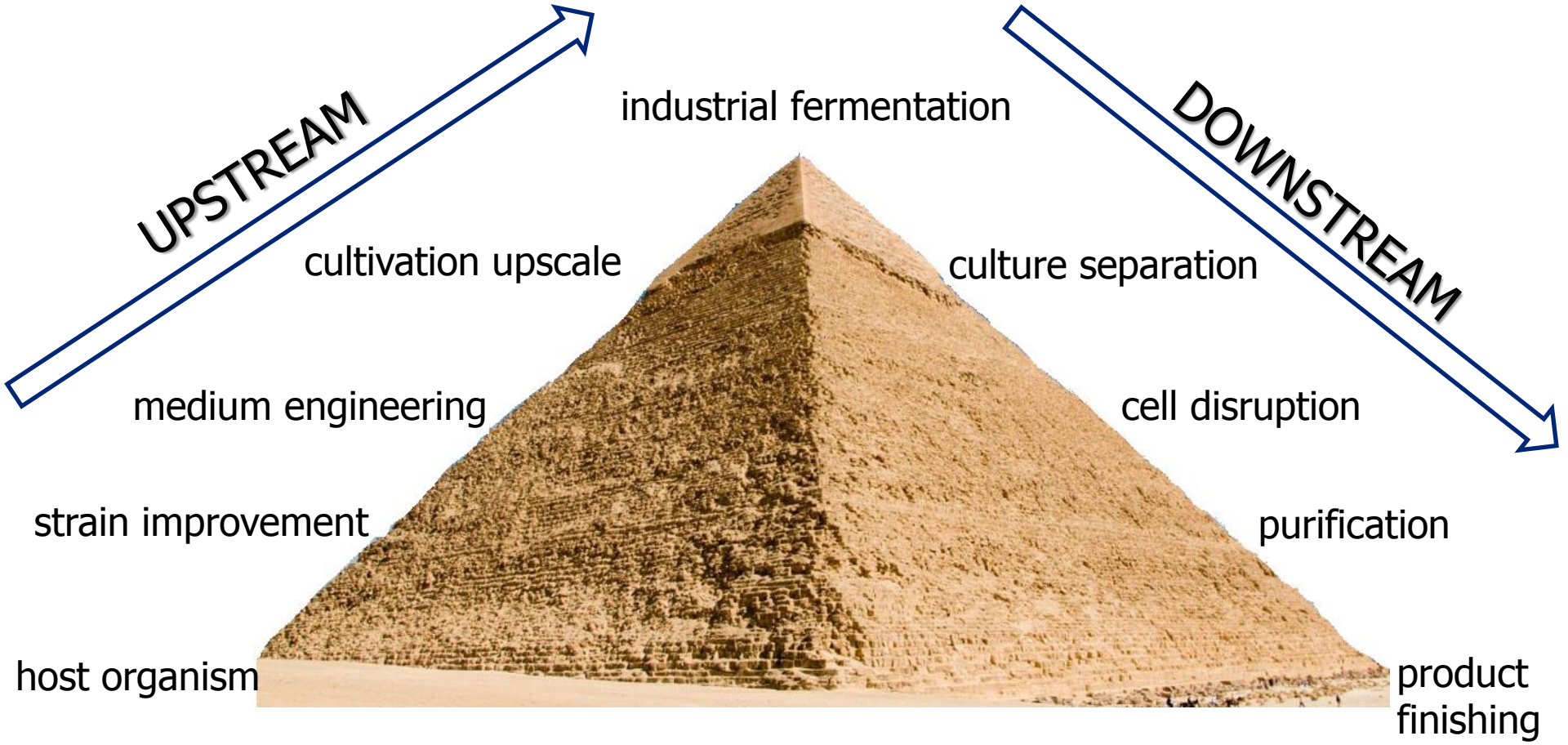
LOSCHMIDT LABORATORIES

Bi9540 Biotechnology and practical use of algae and fungi

Lecture 2 – Culturing techniques

Upstream And Downstream Processes



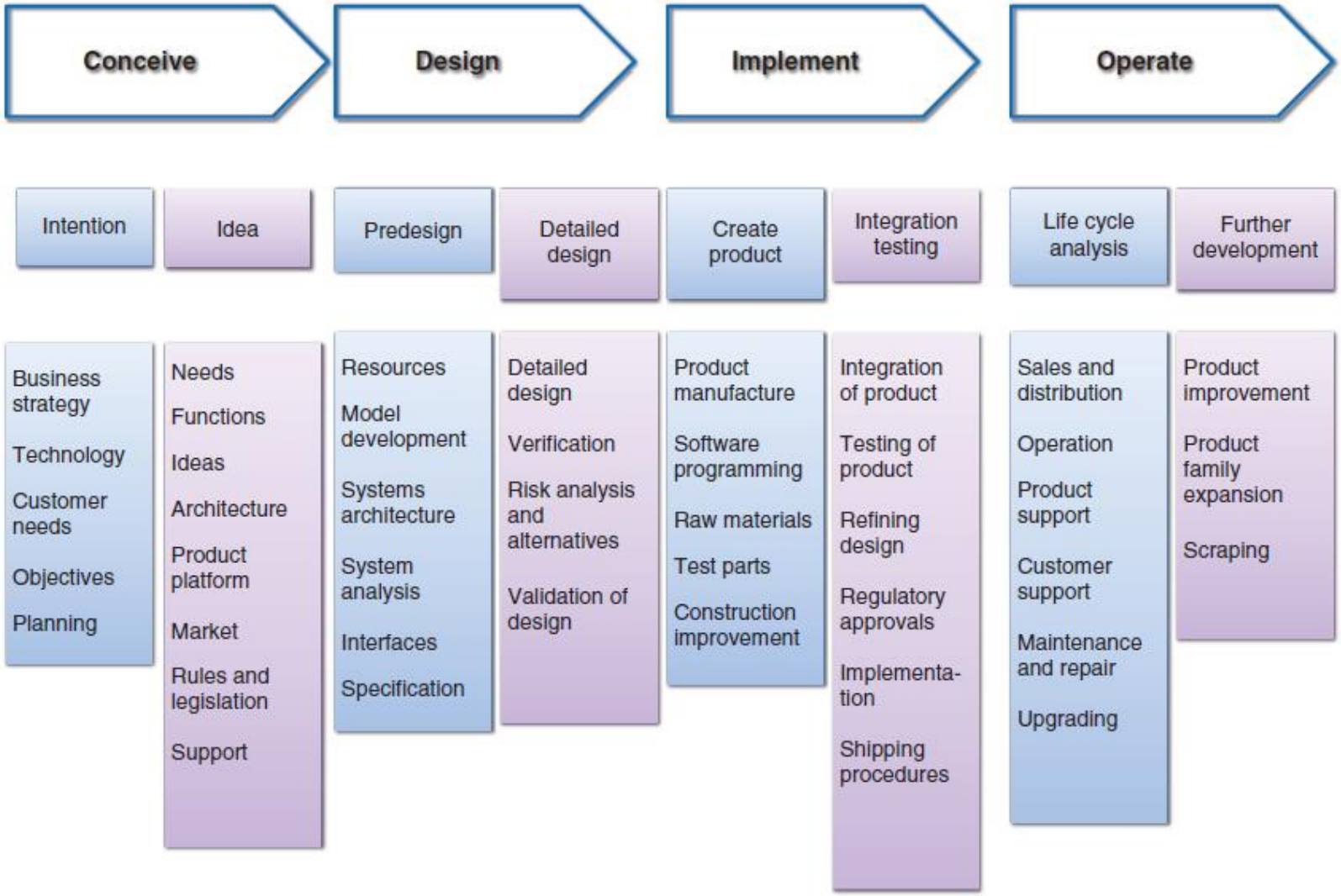


Figure 1.4 The CDIO concept: the process of developing a new product or production system is considered as a consecutive activity spanning from conceiving the product and production concept, designing the product or production system, implementing it into full-scale production, and finally operating it continuously for regular production. It is advocated that The CDIO is applicable to all industrial development work and should, therefore, be the framework for all engineering activity – from training and education till operating a process (from [18]).

FERMENTATION TECHNOLOGY

- Production of microbial biomass
- Upstream processing
- Scale
  - Laboratory (< 30 L)
  - Pilot (< 100 L)
  - Semi-industrial (100 - 5,000 L)
  - Industrial (> 5,000 L)

World largest fermenter

http://www.rpi.edu/dept/chem-eng/Biotech-Environ/FERMENT/largest.htm

WORLD LARGEST FERMENTER

- Built in 1978 in Birmingham
- Height 200 ft (61 m)
- Diam. 25 ft (7.6 m)
- Volume 736,300 gallons
  - 3 347 000 L

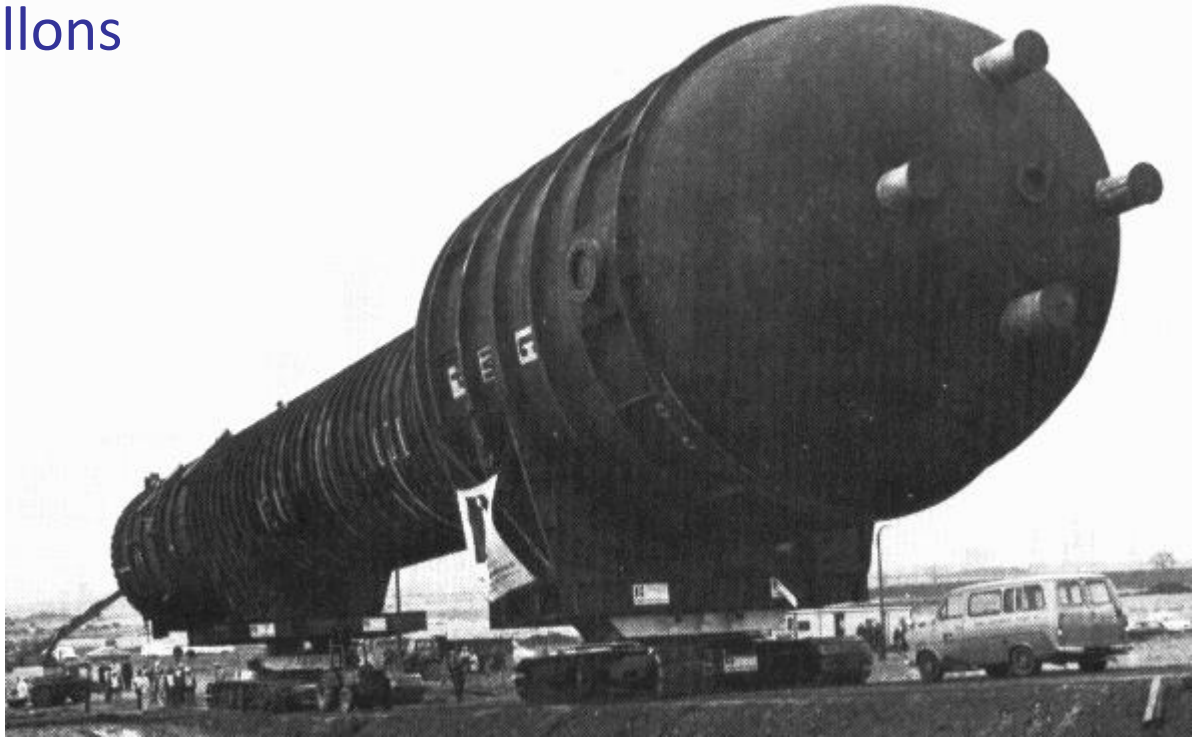
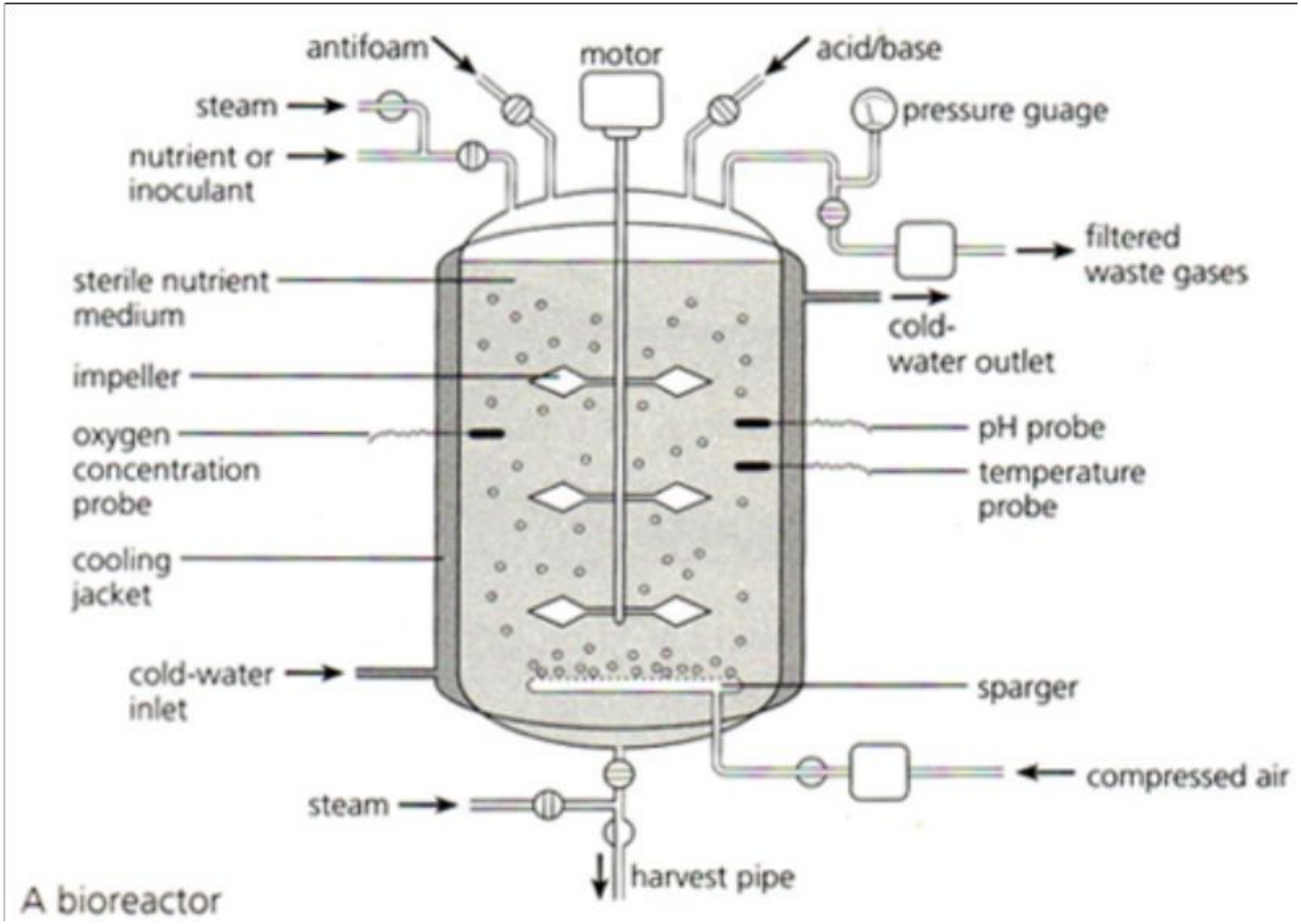


Table 1.1 Milestones in industrial biotechnology.

Major achievement	Discoverers	Year	Impact on industry applications	Implication on bioreactor design
Understanding of fermentation principles	L. Pasteur	1857	Initiating wider application of fermentation in industry	The needs for efficiency of design recognized
Anthrax bacteria	R. Koch	1876	Disease effect of bacteria and the uniqueness of a specific bacterium	
Use of antiseptics realized	I. Semmelweis	1846	Chemical control of infections	The success of large-scale microbial production and its dependency of sterility
The existence of biological catalysis	M. Traube	1877	The catalytic action of microorganisms	The optimization of the biocatalytic activity in a bioreactor
Glycerol from yeast cultures	Neuberg *et al.*	1914–1918	Need for glycerol in the war industry	Cultivation of yeast for other products than beer and wine
Acetone–butanol fermentation	C. Weizmann	1914–1918	Supply of bulk chemicals for explosives and car tires	Scale-up technology challenged to meet market demand
Penicillin discovered	A. Fleming	1929	Pharmaceutical biotechnology initiated	Strain improvement
Penicillin isolation	H.W. Florey and colleagues	1939	Product characterization	Yield improvements
Cephalosporin fermentation	Brotzu and Abraham	1948	Other microbial metabolites could act as antibiotics	Fed-batch operations
Antibiotic strain improvement	S. Waksman and others	1940s–1950s	Higher yield per volume	Process intensification

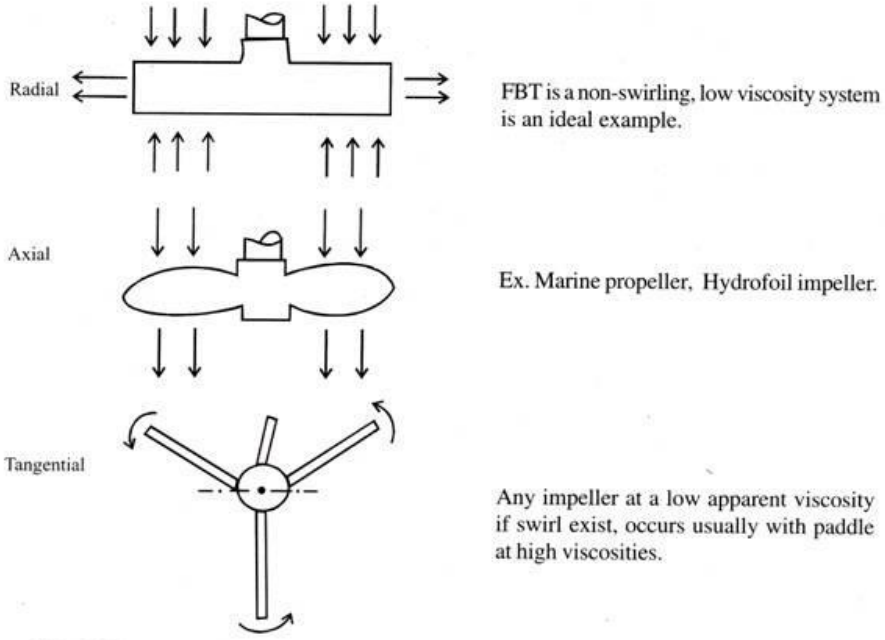
Amino acid fermentation	Kyowa Hakko Co.	1957	Metabolism in strains for amino acids is exploitable	Scale-up of microbial fermentations
Organic acid fermentation	Food industries	1940		Large-scale fermenters
Vitamin fermentation	A. Guilliermond Reichstein	1930s	Riboflavin (B2) (vitamin C)	Bioreactor processes including semisynthetic steps
Genetic engineering and recombinant DNA technique	P. Berg, D. Glaser	1971	Improved metabolism and expression in cells	Induction procedures in bioreactor
Recombinant insulin, growth hormone	H. Boyer and R. Swanson, Genentech	1978	The recombinant DNA-technique open for a biotherapeutic production	A new methodology of culturing recombinant microorganisms with induction protocols
Monoclonal antibodies in hybridoma cultures	G. Köhler, C. Milstein	1975	Diagnostics and therapeutics based on antibodies	Bioreactors to be developed for cell culture requirements, in particular, hybridoma
Recombinant DNA technique applied industrially in animal cell cultures	Pharmaceutical industries	1990s	Recombinant products in mammalian cells human-like biotherapeutics (e.g., EPO, tPA, IFN, Factor VIII)	Bioreactors to be developed for other cell cultures such as CHO, HEK, and other cell lines
Pluripotent stem cells and derived cells	S. Yamanaka	2006	Cells from hES and iPS cells the potential to become new products for cell therapy	Bioreactors must be adapted to new cultivation conditions

Construction of bioreactor



Ipellers

Rushton type impeller



FBT is a non-swirling, low viscosity system is an ideal example.

Ex. Marine propeller, Hydrofoil impeller.

Any impeller at a low apparent viscosity if swirl exist, occurs usually with paddle at high viscosities.

Fig. 5.8 Flow pattern classification of impellers

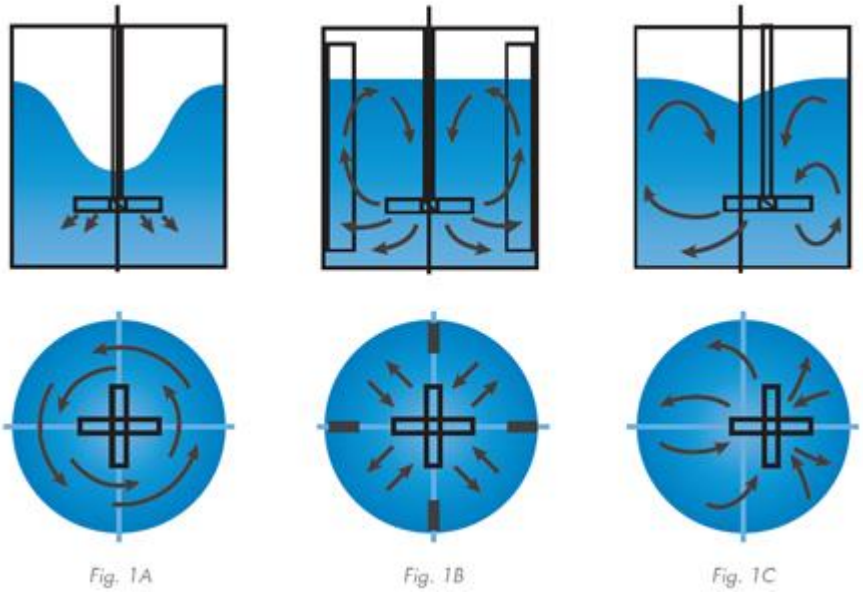


Fig. 1A Fig. 1B Fig. 1C

Mixing in propelled bioreactor

Impellers

Blade paddles

Paddles blade

Ribbon blade

Turbine vortex blade

Umbrella type blade

Flat blade turbine type

Anchor blade

Spiral Propeller blade

Ruvastar cyclo

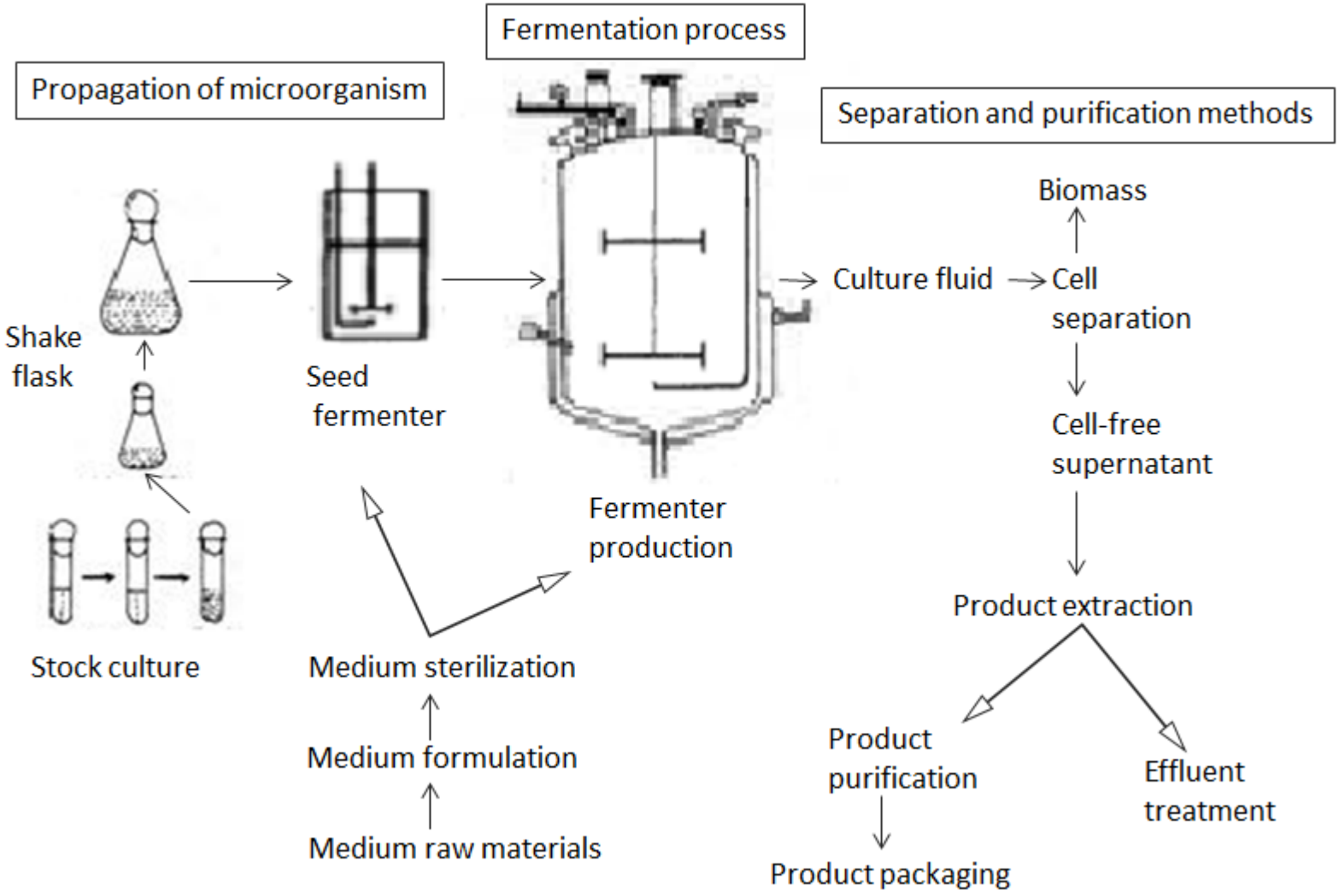
Dispersing Homogenizing blade

Open blade

High shear revers flow

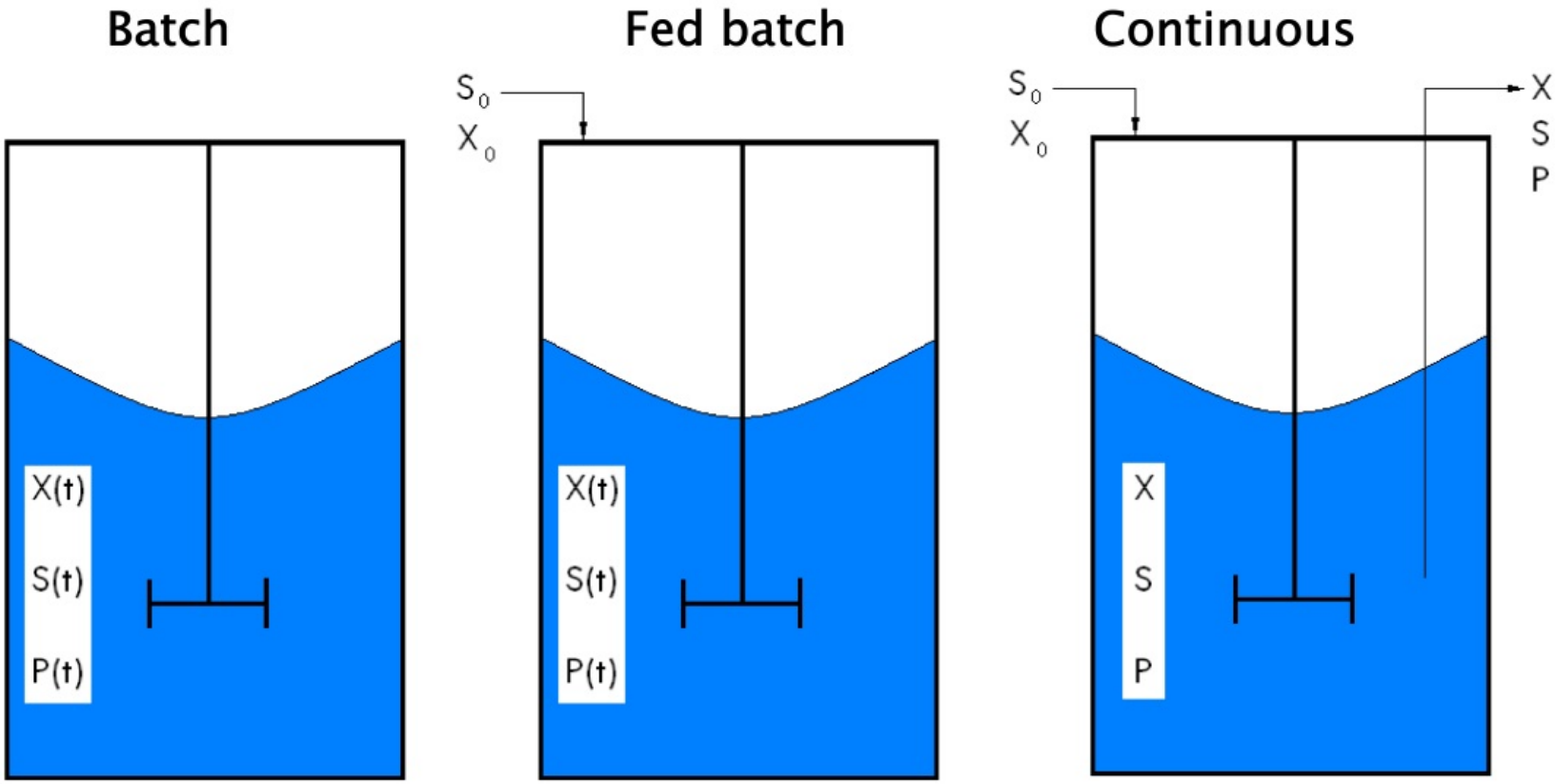
High shear homogenizer

Fermentation process

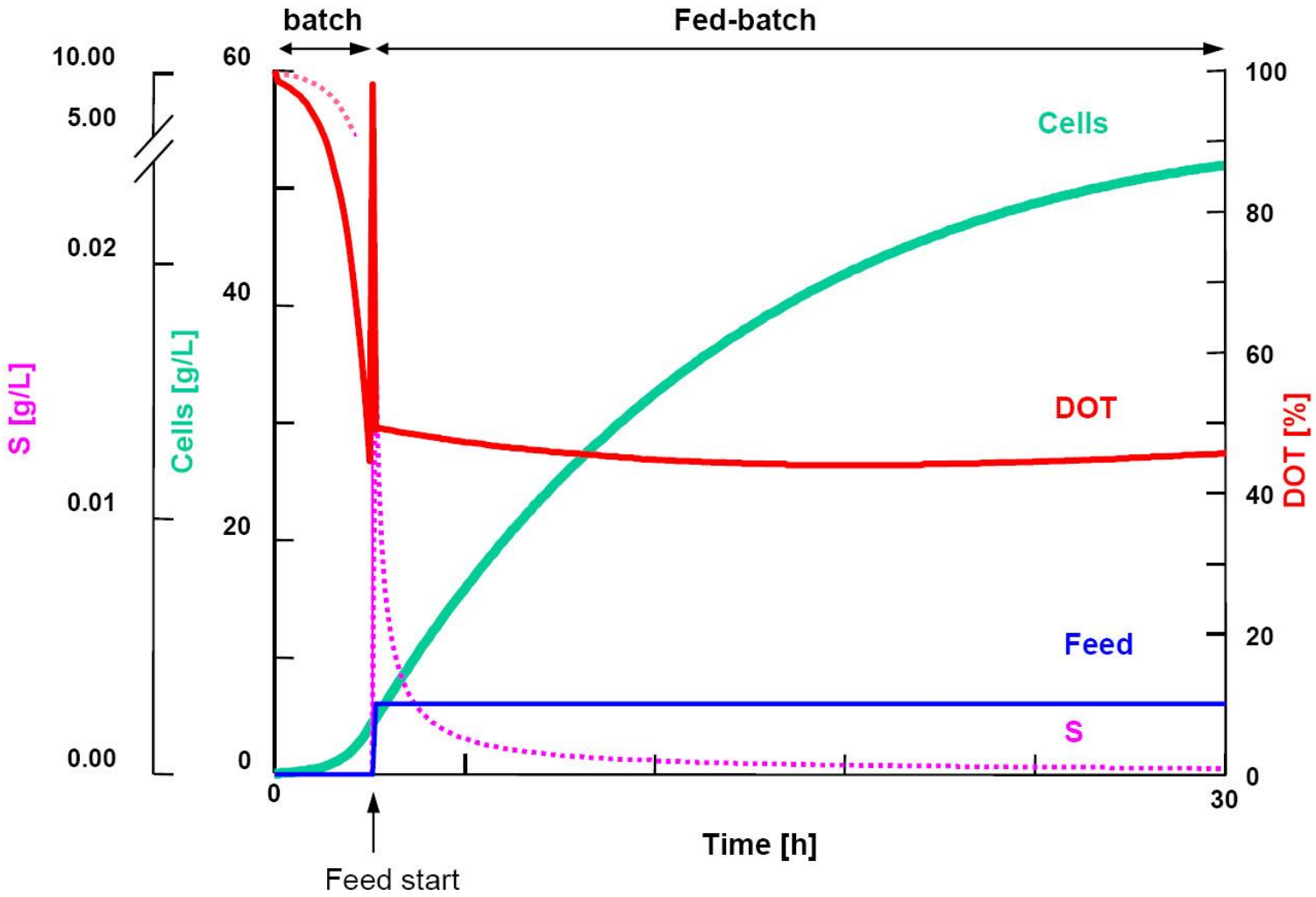


Culture types

- Batch, fed-batch, continuous culture



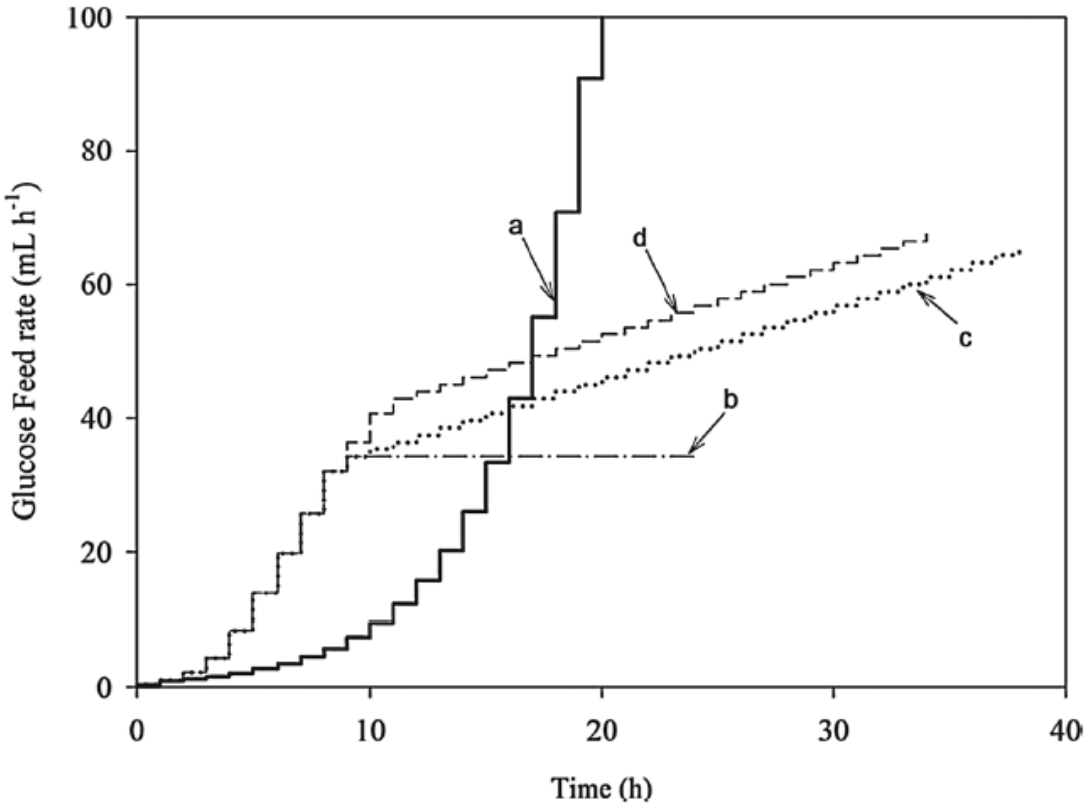
Fed-batch


batch
Fed-batch
10.00
5.00
0.02
0.01
0.00
S [g/L]
Cells [g/L]
60
40
20
0
0
30
Time [h]
Feed start
Cells
DOT
Feed
S
100
80
60
40
20
0
DOT [%]

Fed-batch

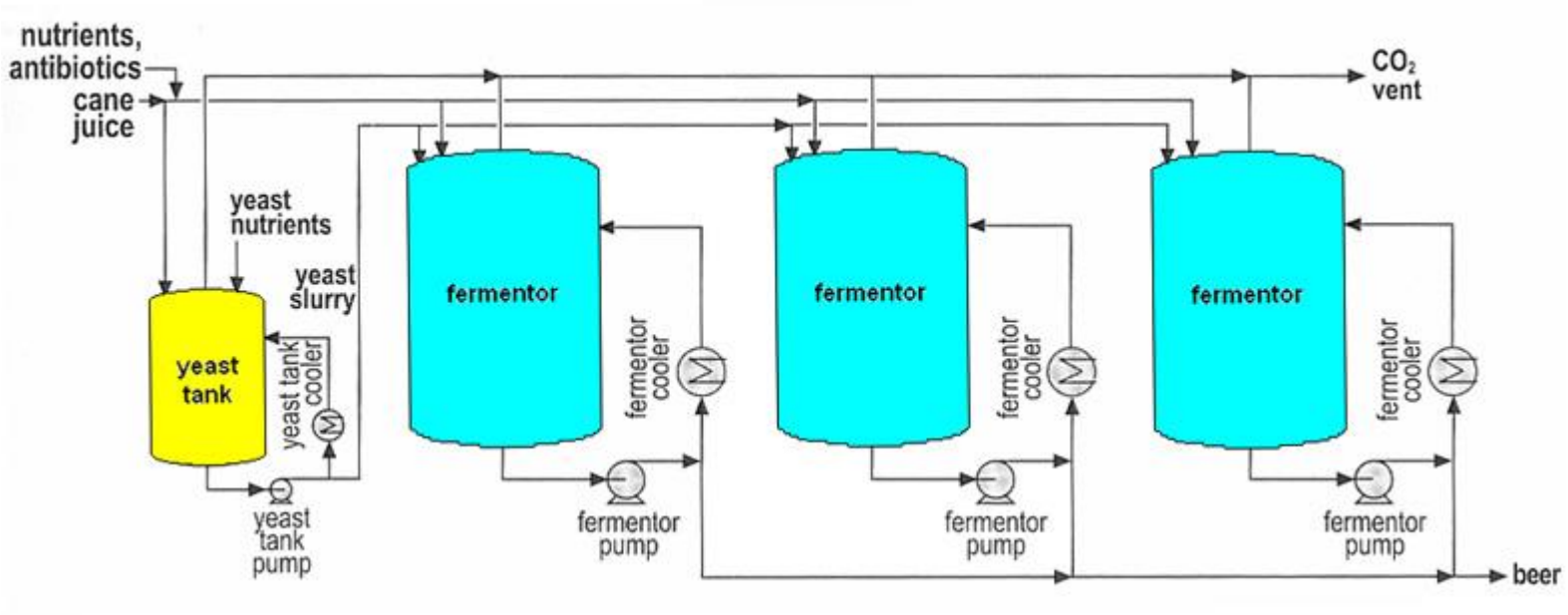
- Feeding strategy is important to avoid substrate depletion or overfeeding of the culture

$$S_t = \frac{X_t}{Y_{X/S}} = \left(\frac{X_0}{Y_{X/S}}\right) e^{(\mu t)}$$



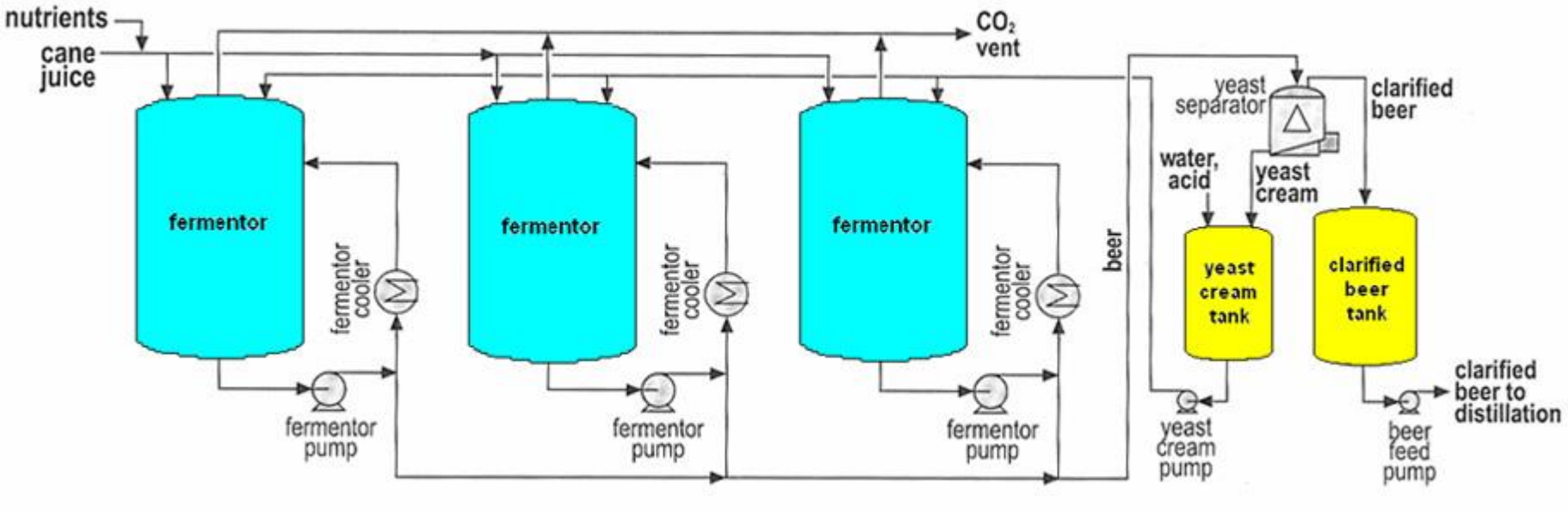
Fed-batch

- Industrial fed-batch fermentation of sugar juice



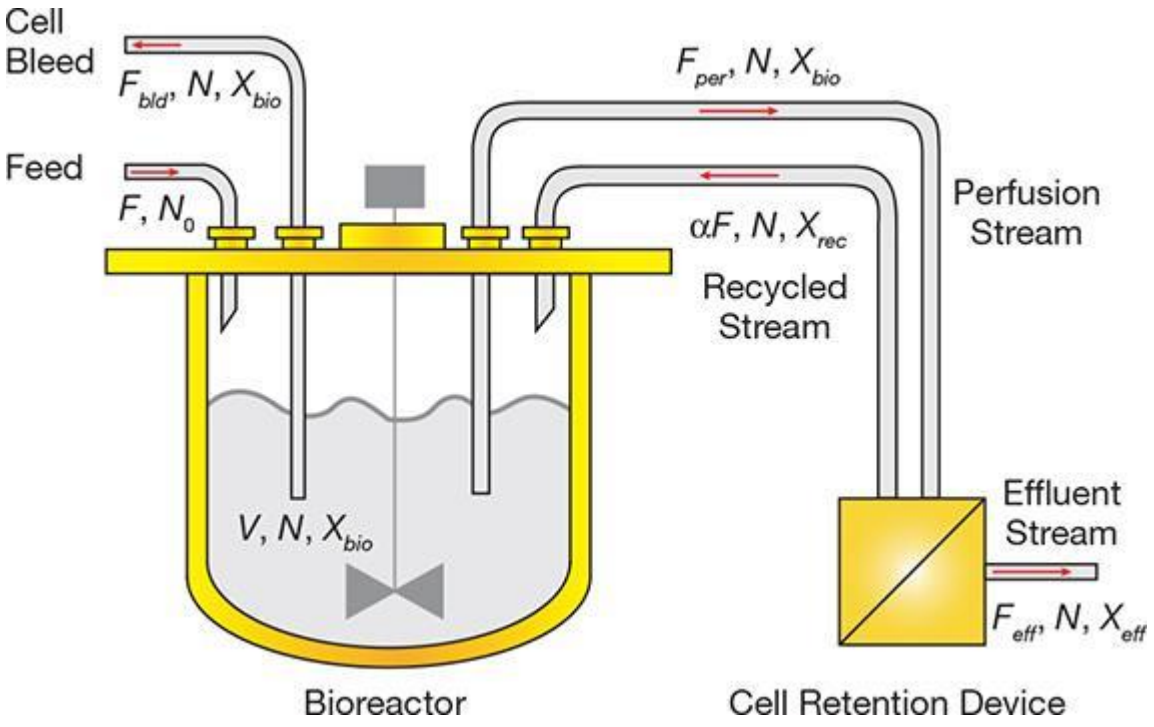
Fed-batch

- Fed-batch fermentation with yeast cell recycle



Continuous culture

- Influx of fresh medium, efflux of cell culture
- Can be operated for weeks or months



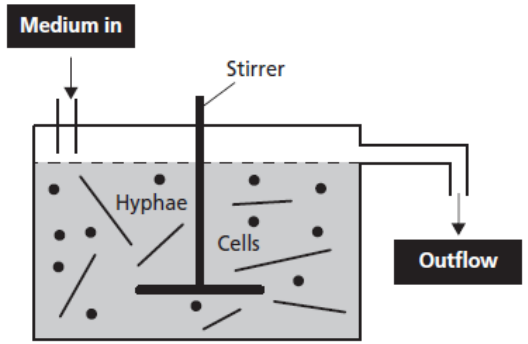
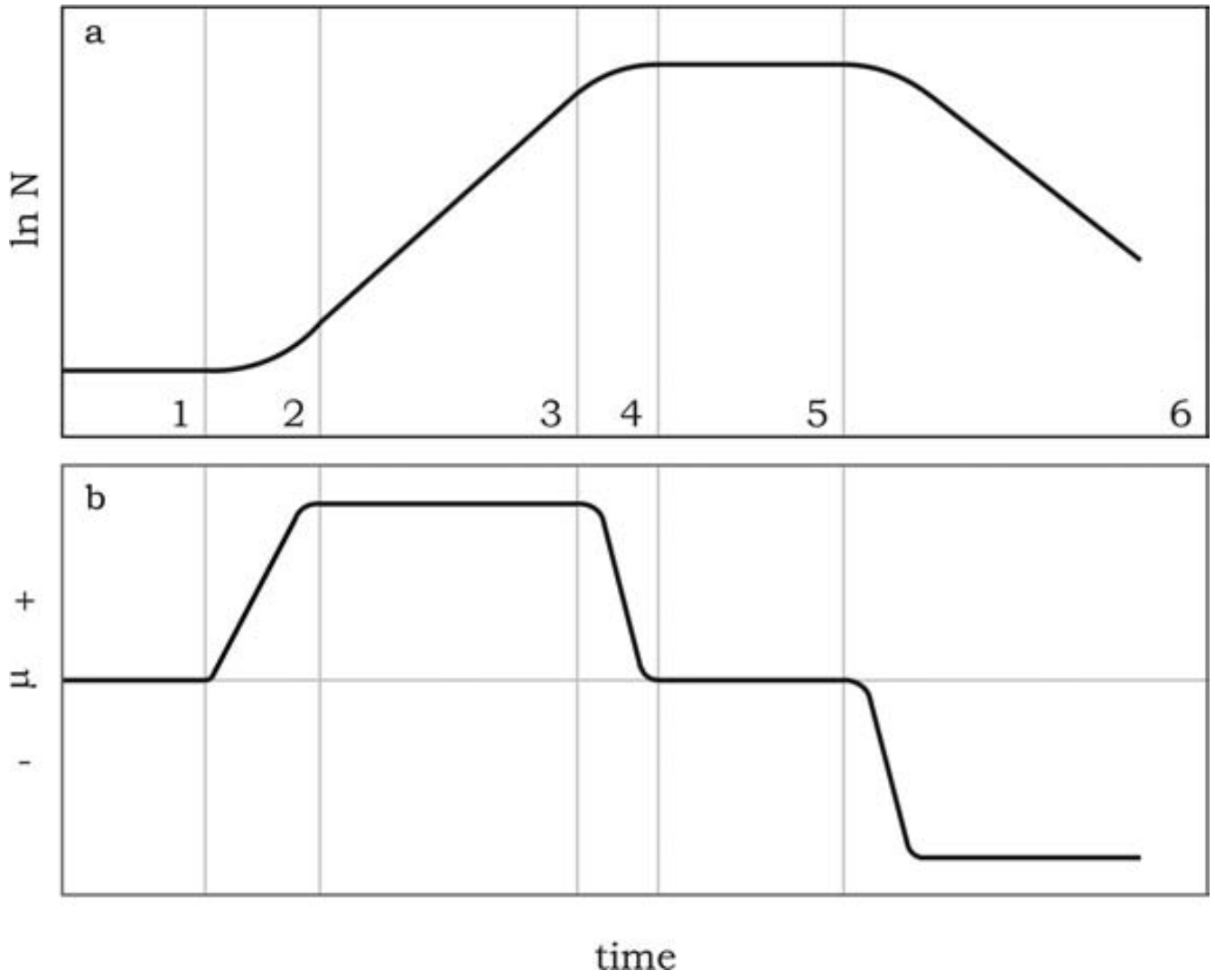


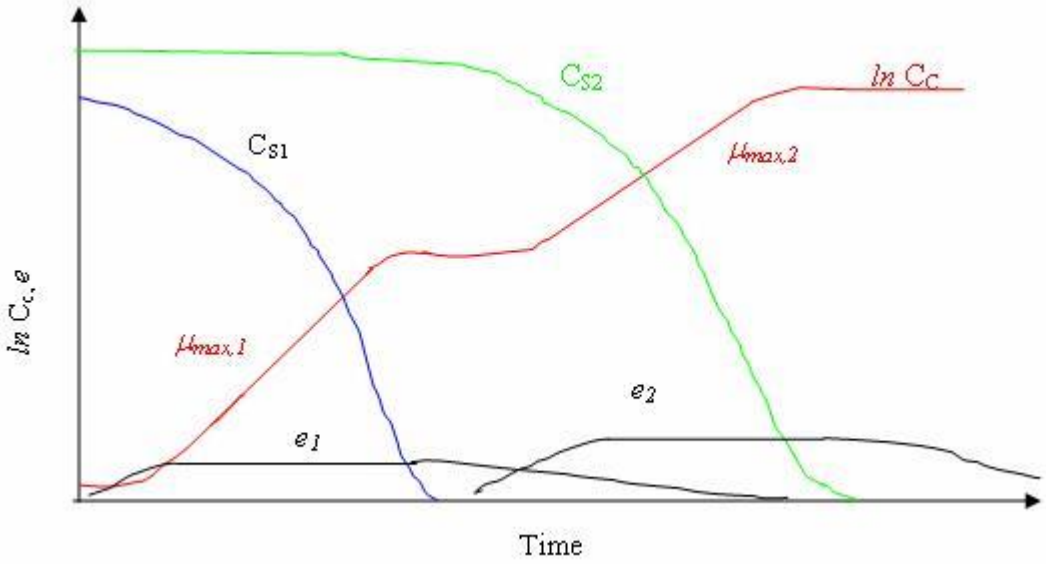
Fig. 4.16 Diagram of a continuous culture system to produce fungal biomass.

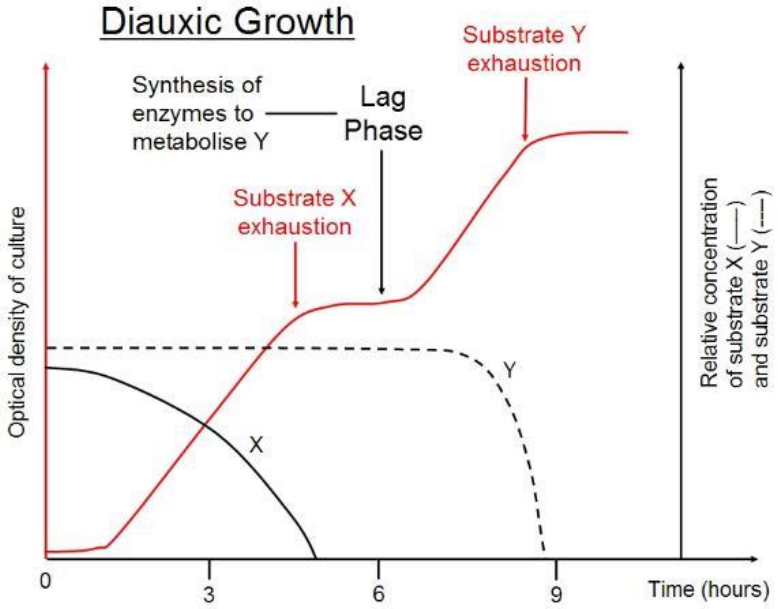
Culture growth



Diauxic growth

- Two substrates in medium
- Fungi and lactose





Microbial growth

- Growth rate

$$\mu = \mu_{\max}\left(\frac{S}{K_S + S}\right)\left(\frac{O}{K_O + O}\right)$$

- Oxygen mass transfer

$$N_{A,O2} = k_L a(C_L^* - C_L)$$

- Nutrient utilization

$$q_s = \frac{\mu}{Y_{x/s}}$$

$$q_o = \frac{\mu}{Y_{x/o}}$$

$$q_{NH4} = \frac{\mu}{Y_{x/NH4}}$$

Mass Balances

$$\frac{dX}{dt} = \mu X$$

$$\frac{dS}{dt} = -q_s X$$

$$\frac{dO}{dt} = -q_o X$$

$$\frac{dNH_4}{dt} = -q_{NH4} X$$

Potential limiting factors

- Dissolved oxygen tension
- pH
- Temperature, mixing speed
- Nutrient concentration
- Product concentration
- Secondary metabolites
- Population dynamics
- Genomic/plasmid instability

Table 1.2 Bioreactor design criteria.

Design issue	Purpose	Design means	Parameters
Gas transfer in submerged culture	Ensure high growth rate, avoiding oxygen starvation	Reactor geometry Sparger design Baffles Overpressure Impeller geometry	Aspect ratios $K_L a$ *OTR* *OUR* *CER*
Mixing efficiency	Avoiding gradients of heat, nutrients and additives, stress Reduce power	Impeller geometry Baffles Mixing analysis CFD	Aspect ratios Mixing time t Power number
Nutrient supply and addition	Efficient transfer to bioreactor volume	Feeding regime Multiple ports	Linear and exponential profile
Liquid–solid transfer	Enhance reaction rate Reduce gradients	Flow distributors Porous support	Thiele modulus
Heat transfer	Efficient removal of metabolic heat	Internal coils Recycling of media Jacket Cooling media	Dimensionless numbers

Sterility	Ensure whole unit is devoid of foreign microorganisms to avoid infection	Sterilization procedure Overpressure Barriers Containment Microfilters	Sterilization time and temperature
Strain selection	Finding strain with properties adapted to media and reactor constraints	Microbial analysis Omics	Specific rates (μ, q_P, q_S) Inhibition constants
Scale-up procedure	Ensuring same conditions at large scale	Design geometry of vessels and impellers Range of mixing	Aspect ratio Scale-up rule parameters Dimensionless numbers
Rheology		Additives affecting viscosity CFD	Reynold's number CFD data
Homogeneity of culture	Avoiding gradients for ideal reactor conditions	CFD	Zonal analysis data
Media composition	Balanced culture media	Factorial analysis Omics methods	Model fit parameters

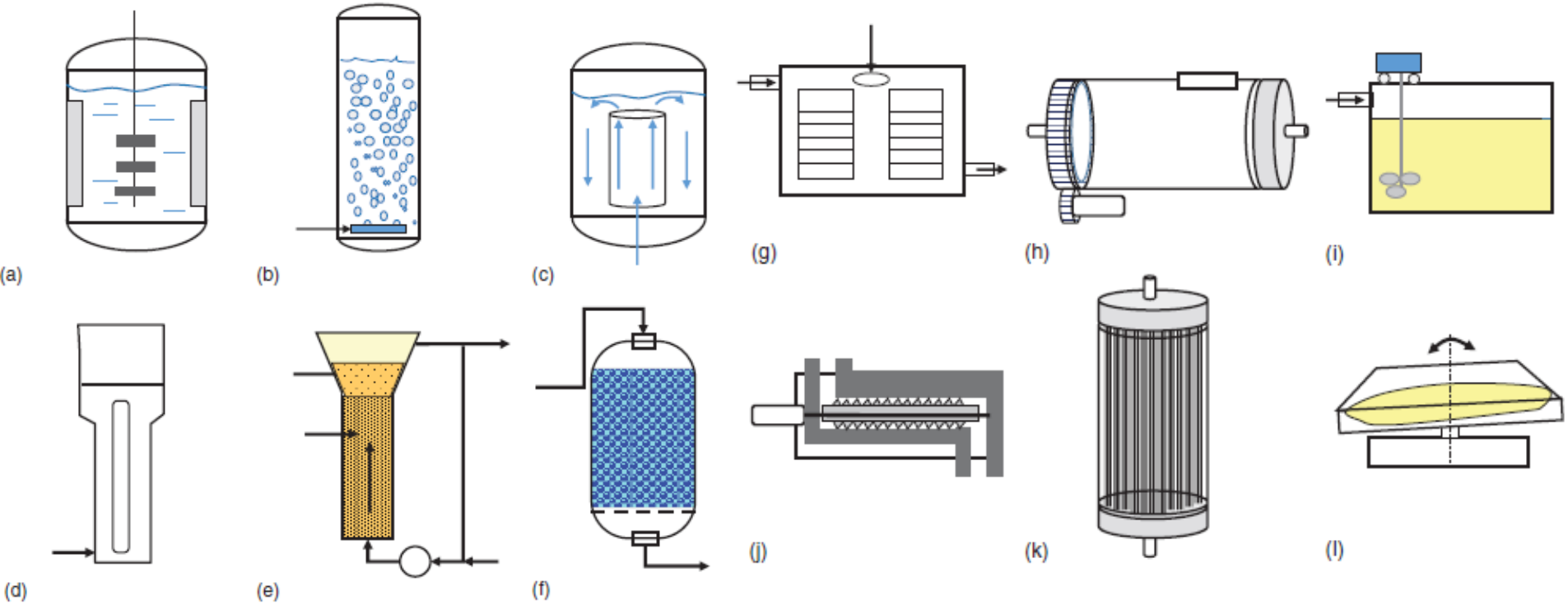
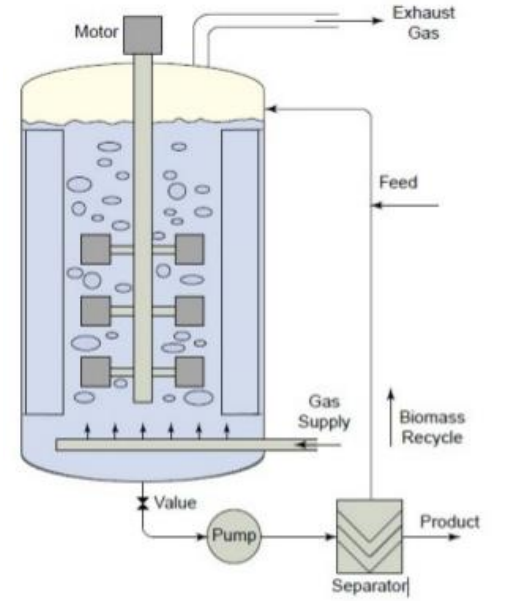


Figure 1.2 Twelve examples of bioreactor designs: (a) stirred-tank reactor, (b) bubble reactor, (c) airlift reactor, (d) loop reactor, (e) reactor with immobilized cells, (f) fluidized reactor with recycling of cells, (g) solid-phase tray reactor, (h) rotary drum bioreactor, (i) agitated-tank reactor with movable impeller, (j) continuous screw bioreactor, (k) hollow-fiber reactor, and (l) wave bioreactor

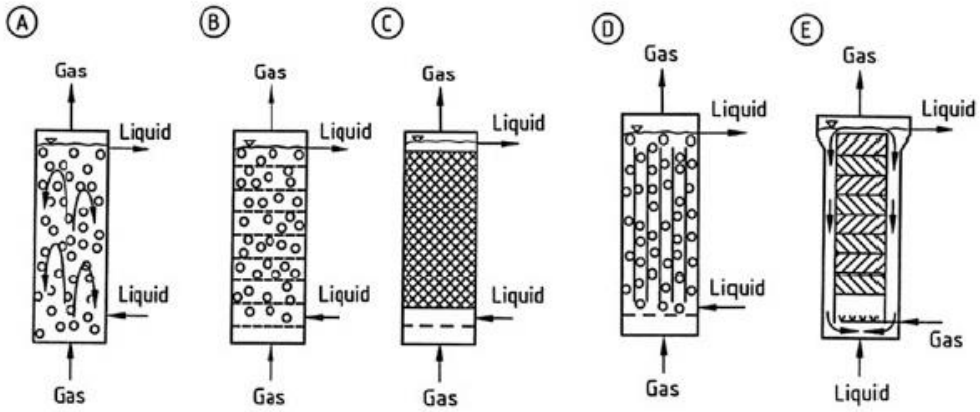
Stirred tank bioreactor

- Most common in biotechnology
- Easy cleaning and parameter control
- Well described scale-up
- Good gas transfer
- Higher investment and maintenance cost
- Mixing is not optimal
- Max volume 1,000 m^3



Bubble column

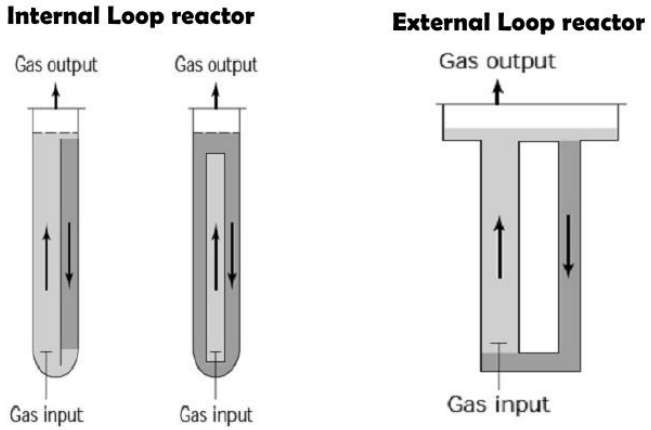
- Low investment cost, no moving parts, even over 1,000 m^3
- Easy cleaning, good gas transfer and mixing
- Foaming, difficult condition control
- Limited viscosity

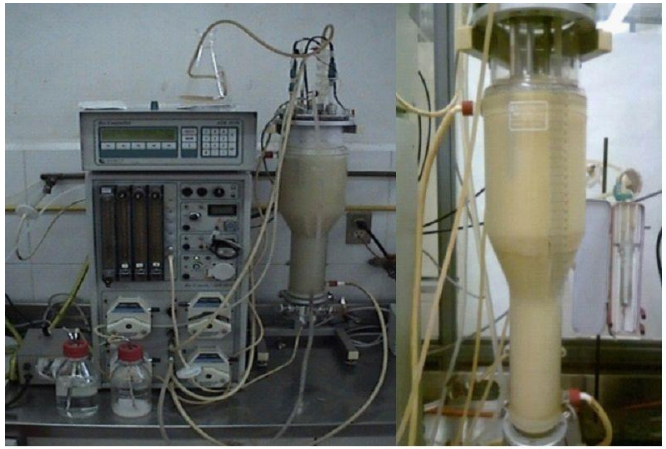


A) Simple bubble column; B) Cascade bubble column with sieve trays;
B) C) Packed bubble column; D) Multishaft bubble column;
C) E) Bubble column with static mixers

Airlift reactor

- Low investment, no moving parts, easy cleaning
- Good gas transfer
- Loop can be used for cooling
- Foaming, difficult condition control
- Poor mixing





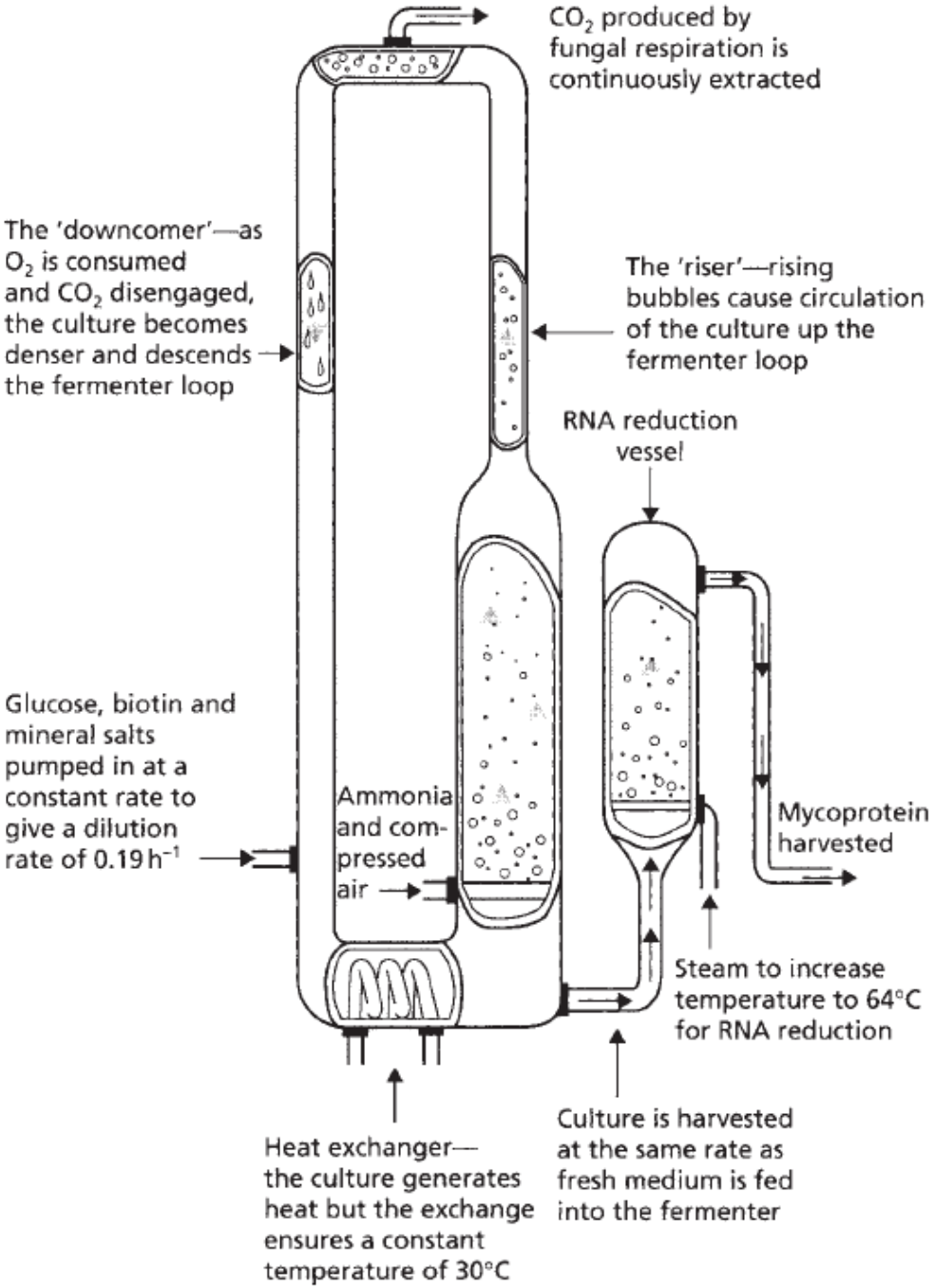


Fig. 4.17 Diagram of the air-lift fermenter used by Marlow Foods for the production of mycoprotein in continuous flow culture. (From Trinci 1992.)

Bioreactor comparison

	STR	BC	ALR
Mixing	+	+++	++
Gas transfer	+++	+	++
Heat transfer	++	+++	+++
Energy input	++	+	+
Control options	++	+	+
Handling of viscous broth	+++	+	+
Very large scale operation	+	+++	++
Ease of cleaning	++	++	++
Low maintenance	+	+++	+++

Single-use bags

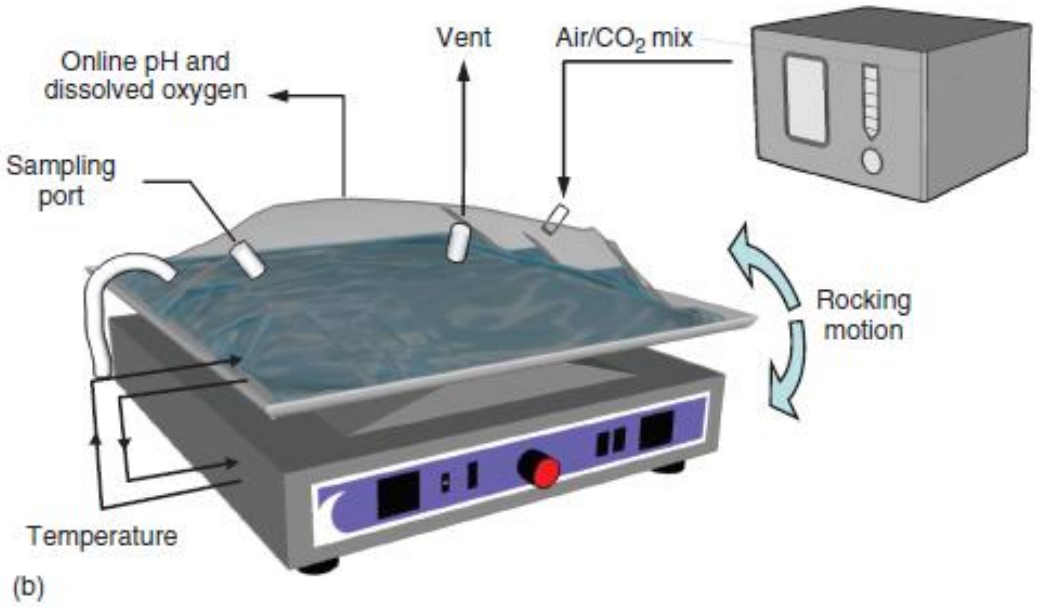


Figure 1.3 An emerging new trend is the replacement of old stainless steel fermenter with single-use wave-bioreactors. It is a striking example of how smart designs based on fabrication technology use compatible low-cost materials and new conceptual thinking lead to a leap in design (from [17], with permission).

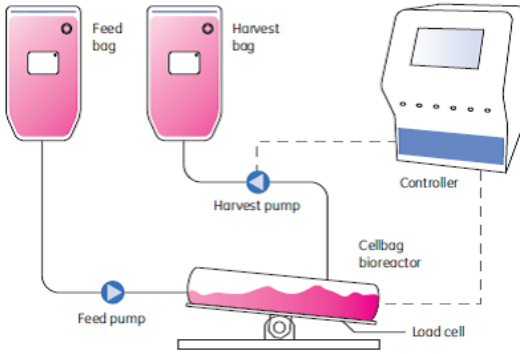
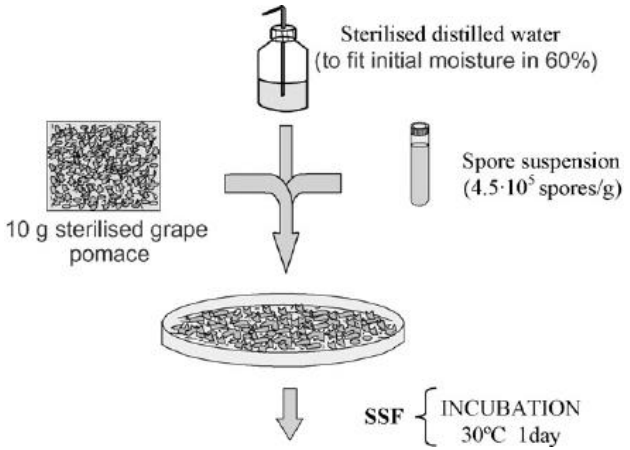


Fig 1. WAVE Bioreactor in perfusion set-up.

Solid state fermentation

- Absence of free water



Solid state fermentation

Sugar Cane Bagasse

Tea Waste

Wheat Bran

Saw Dust

Apple Pomace

Coconut oil Cake

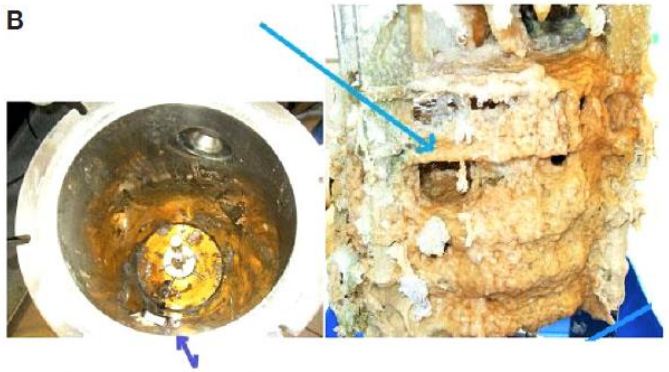
Table 2. Production of xylanase and 6-pentyl-α-pyrone by some filamentous fungi using different bioprocesses — *Production de xylanase et de 6-pentyl-α-pyrone par les champignons filamenteux en utilisant des bioprocédés variés.*

Biocompound	Production	Microorganism	Fermentation applied		Reference
Xylanase	630 IU·ml^{-1}	*Trichoderma reesei* Rut C-30	SbmF	Flask	Xiong et al., 2004
	1,350 IU·ml^{-1}	*Trichoderma reesei* Rut C-30	SbmF (Fed-batch)	Bioreactor (2 l)	Xiong et al., 2004
	14,790 IU·ml^{-1}·h^{-1}	*Aspergillus niger* KK2	SSF wheat raw	Flask	Park et al., 2002
	844 IU·ml^{-1}	*Penicillium canescens* 10-10c	SbmF	Shake flask	Gapar et al., 1997
	7,448 IU·ml^{-1}	*Penicillium canescens* 10-10c	SbmF	Shake flask	Bakri et al., 2003
	9,632 IU·g^{-1}	*Penicillium canescens* 10-10c	SSF	Flask	Bakri et al., 2003
	9,300 IU·g^{-1}	*Penicillium canescens* 10-10c	SSF	Plastic gags	Assamoi et al., 2008a
	10,200 IU·g^{-1}	*Penicillium canescens* 10-10c	SSF	Multi-layer	Assamoi et al., 2009
	18,895 IU·g^{-1}	*Penicillium canescens* 10-10c	SSF	Flask	Assamoi et al., 2009
6-pentyl-α-pyrone	455 mg·l^{-1}	*Trichoderma harzianum* Rifai	LSC	Flask (500 ml)	Kalyani et al., 2000
	474 mg·l^{-1}	*Trichoderma harzianum*	SbmF	Flask	Serrano-Carreon et al., 2004
	3,000 mg·kg^{-1}	*Trichoderma harzianum*	SSF	Flask	de Aroujo et al., 2002
	7,100 mg·l^{-1}	*Trichoderma atroviride* AG2755-5NM398	SbmF Ext-LSI	Plate (50 ml)	Shinobu et al., 2009
	230 mg·l^{-1}	*Trichoderma harzianum* IMI206040	SbmF	Shake flask (500 ml)	Rocha-Valadez et al., 2006
	230 mg·l^{-1}	*Trichoderma harzianum* IMI206040	SbmF	Stirred tank (10 l)	Rocha-Valadez et al., 2006
	5,000 mg·kg^{-1}	*Trichoderma harzianum* 4040	SSF	Flask (250 ml)	Ramos et al., 2008

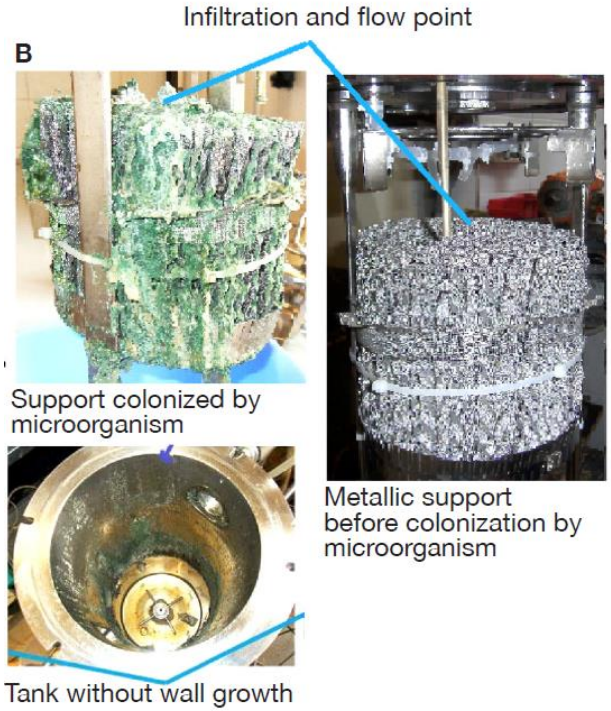
SbmF: Submerged Fermentation; SSF: Solid Substrate Fermentation; Ext-LSI: Extractive Liquid-Surface Immobilisation.

Solid state fermentation

Fungi wall growth in bioreactor at the end of fermentation



Tank without wall growth



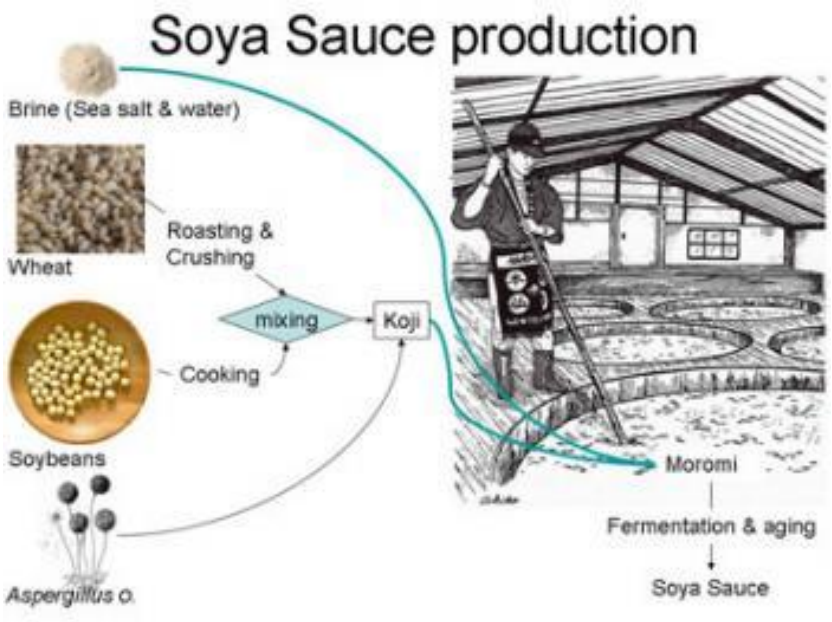
- Low-cost raw materials
- Easy down-stream processing
- Reduced waste and pollution
- Cost-effective (no water), easy control
- Resembles natural environment of the microbes

Solid state fermentation

Economic Sector	Applications	Examples
Agro-Food Industry	Traditional Food Fermentations	Koji, Tcznpch, Rae, Attickc, Fermented cheeses
	Mushroom Production & spawn	Agaricus, Pleurotus, Shn-take
	Bioconversion By-products	Sugar pulp Bagasse Composting, Detoxication
	Food Additives	Flavours. Dyestuffs.
Agriculture	Biocontrol , Bioinsecticide	Beauveria Metarhizium, Tricho derma
	PlantGrowth Hormones / Enhancers	Giberellins, Rhizobium, Trichoderma
Industrial Fermentation	Enzymes production	Amylases, Cellulases Proteases, Pectinases, Xylanases
	Antibiotic production	Pencillin, feed & Probiotics
	Organic acid Production	Citric acid, Fumaric acid,etc
	Fungal Metabolites	Alkaloids
	Ethanol Production	Malting and Brewing

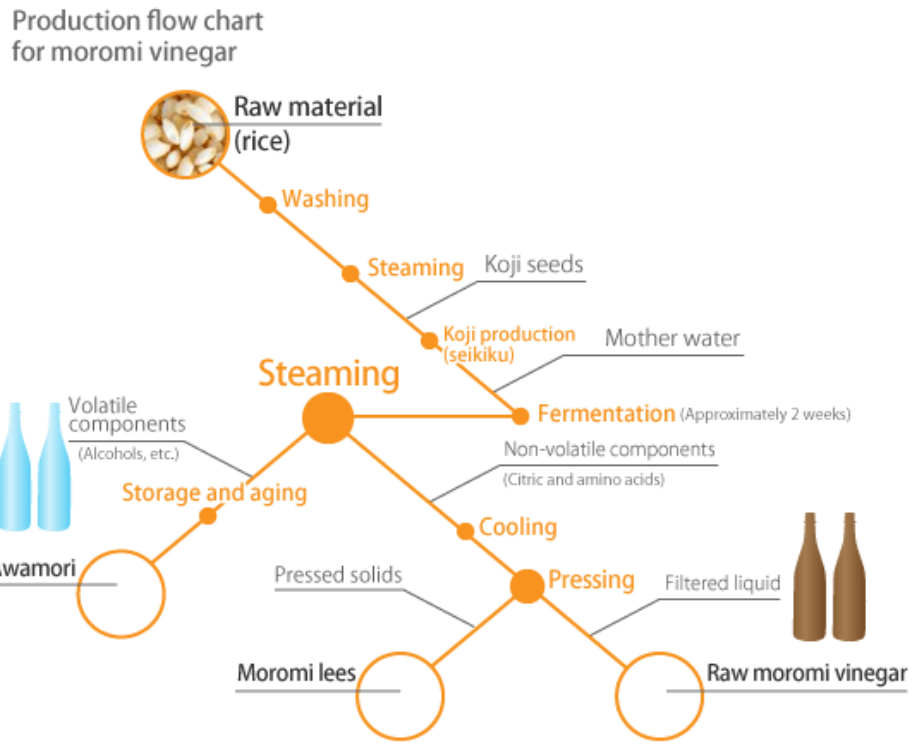
Koji fermentation

- Aspergillus oryzae = Kanji 麹
- Traditional Japanese fermentation process

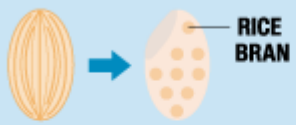


Koji fermentation

- **Shōchū** production



JAPANESE SAKE BREWING PROCESS

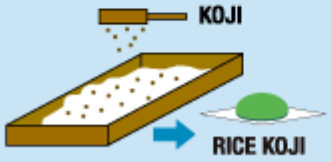


1 POLISHING

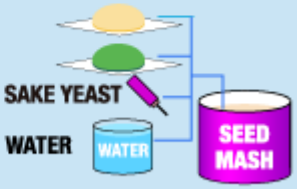
2 WASHING

3 SOAKING

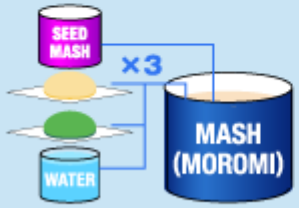
4 STEAMING



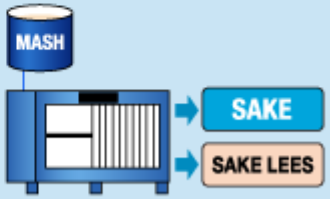
5 MOLDING (KOJI MAKING)



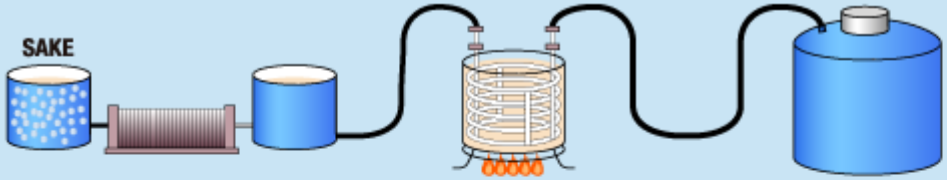
6 INCUBATION (YEAST STARTER)



7 FERMENTATION



8 PRESSING

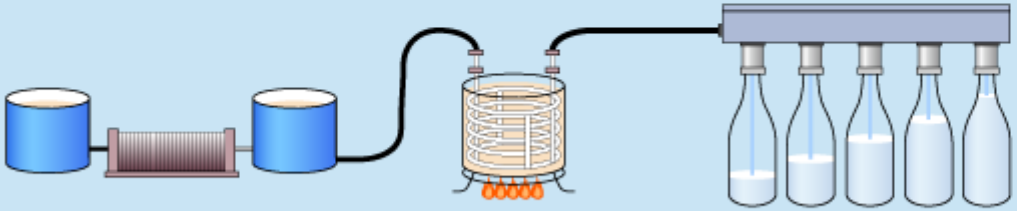


9 FILTERING

10 PASTEURIZATION

11 STRAGE

12 BLENDING



13 FILTERING

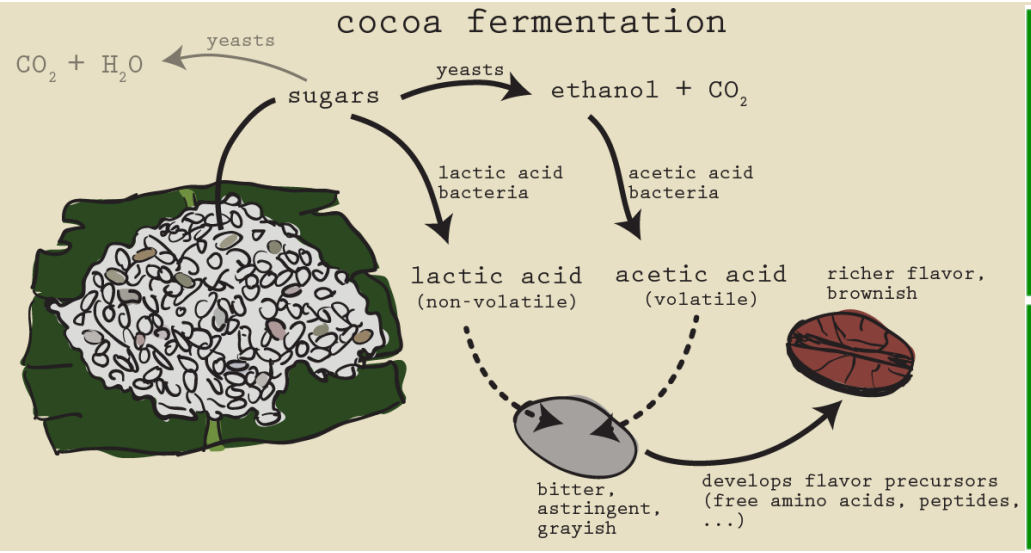
14 PASTEURIZATION

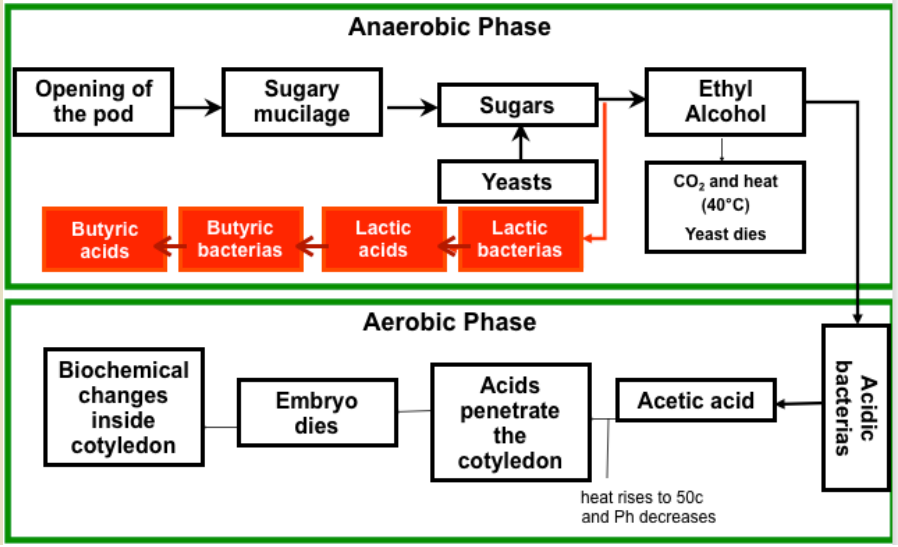
15 BOTTLING

16 CONVENTIONAL SAKE

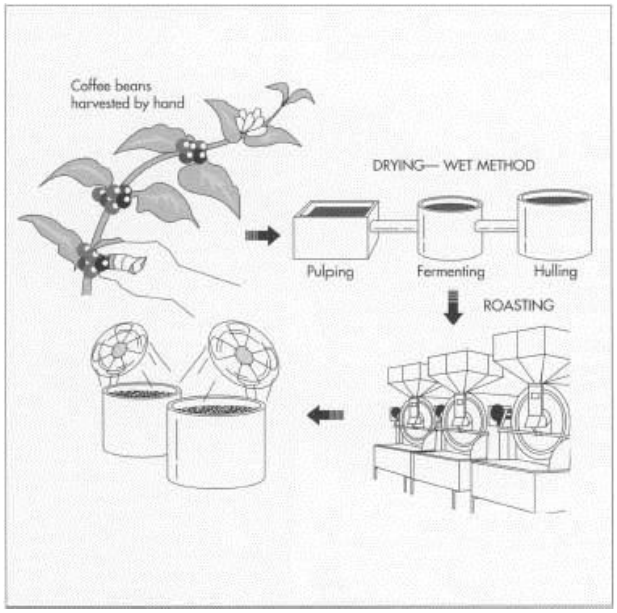
Cocoa bean fermentation

- Beans are fermented 2-7 days covered with banana leaves
- Without fermentation, there would be no chocolate





Coffee beans skin removal



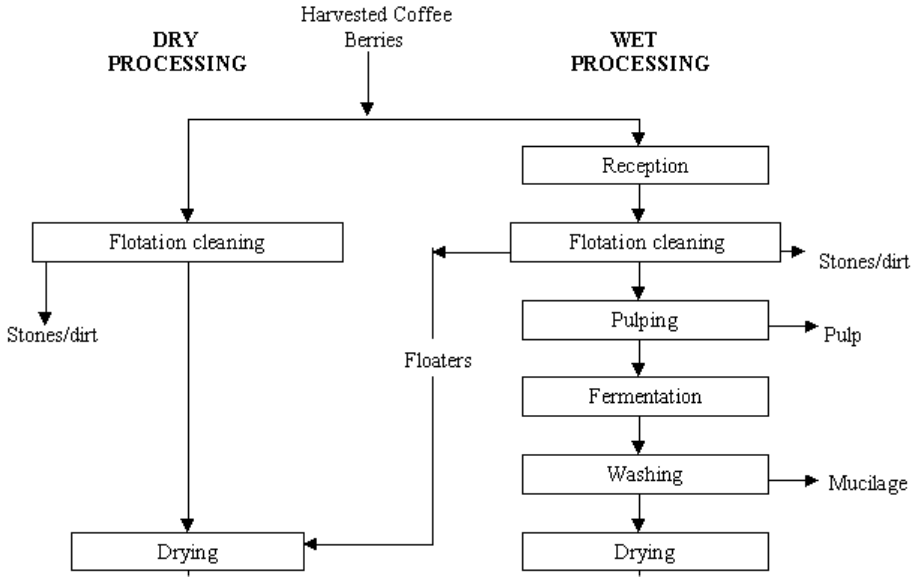


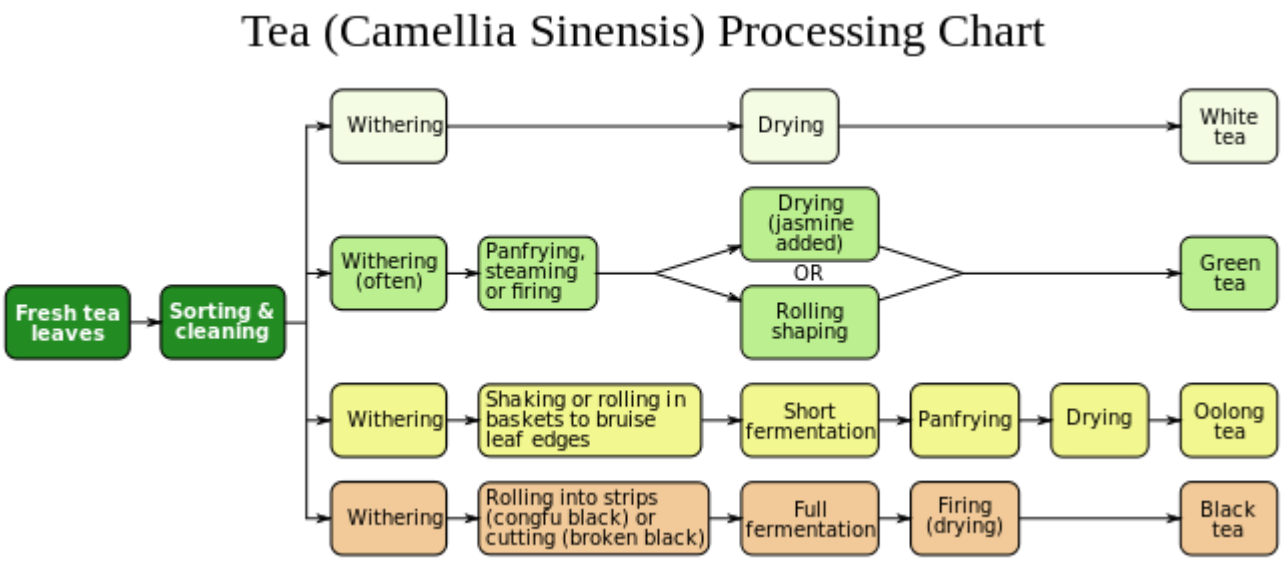
Table 1. Total counts of filamentous fungi, relative humidity and water activity of the coffee grains during fermentation and drying.

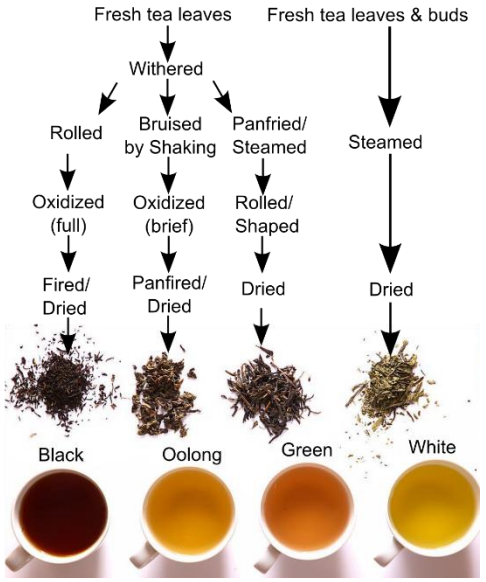
Time (days)	Counts (CFU/g)	Relative humidity (%)	Water activity
0	1.5×10^3	67.45	>0.85
2	2.8×10^3	60.83	>0.85
4	5.9×10^3	38.85	>0.85
6	7.6×10^3	29.35	>0.85
8	2.0×10^4	28.56	>0.85
12	4.0×10^4	19.72	0.82
14	6.8×10^4	19.30	0.82
16	9.0×10^4	19.70	0.82
18	1.7×10^5	15.78	0.71
20	2.0×10^5	12.90	0.63
22	1.6×10^5	11	0.52

Fermented tea

- Aspergillus niger, luchuensis
- Pu-erh and some other black teas

Tea leaves spread out on fermentation rack.



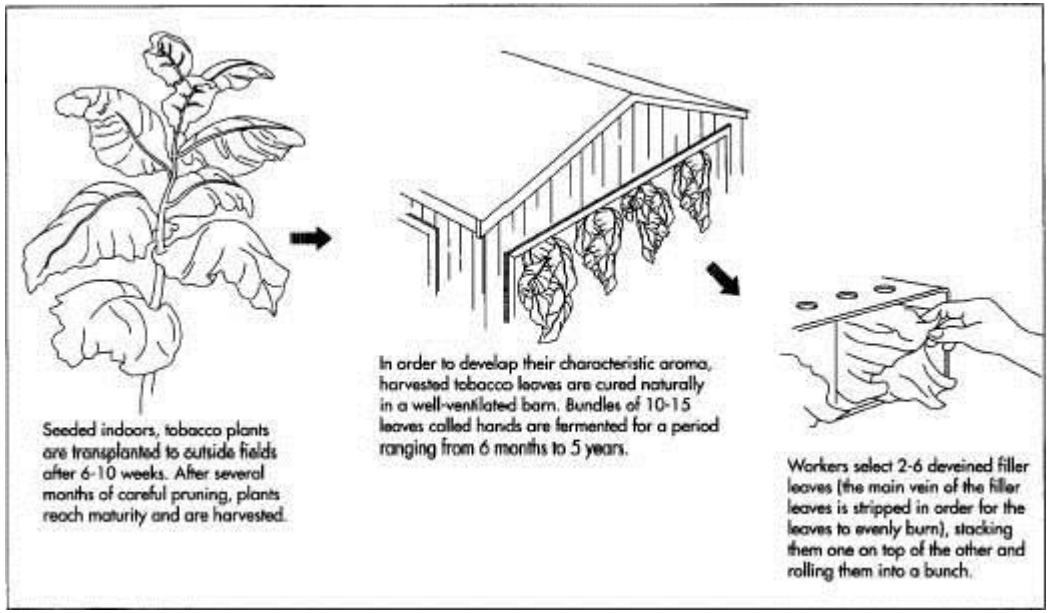


Kombucha fermentation

- Variety of fermented black or green tea
- Saccharomyces cerevisiae, Brettanomyces bruxellensis, Candida stellata, Schizosaccharomyces pombe, and Zygosaccharomyces bailii

Tobacco fermentation

- Harvested leaves are fire-cured and moistened
- Debaryomyces hansenii is predominant in early stage
- Fermentation can take from weeks to years



Photobioreactors

Feature	*Photobioreactor*	*Fermentor*
Energy source	Light	Organic carbon
Cell density/dry weight	Low	High
Limiting factor for growth	Light	Oxygen
Harvestability	Dilute, more difficult	Denser, less difficult
Vessel geometry	Dependent on light penetration	Independent of energy source
Control of parameters	High	High
Sterility	Usually sanitized	Can be completely sterilized
Availability of vessels	Often made in-house	Commercially available
Technology base	Relatively new	Centuries old
Construction costs	High per-unit volume	Low per-unit volume
Operating costs	High per-kg biomass	Low per-kg biomass
Applicability to algae	Photosynthetic algae	Heterotrophic algae

Photobioreactor vs fermentor

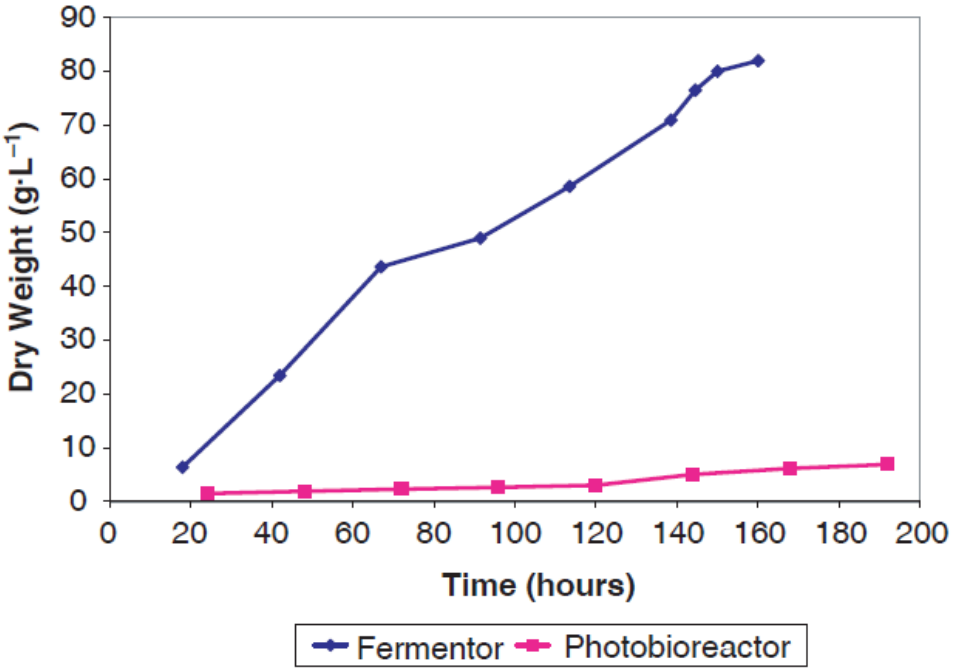
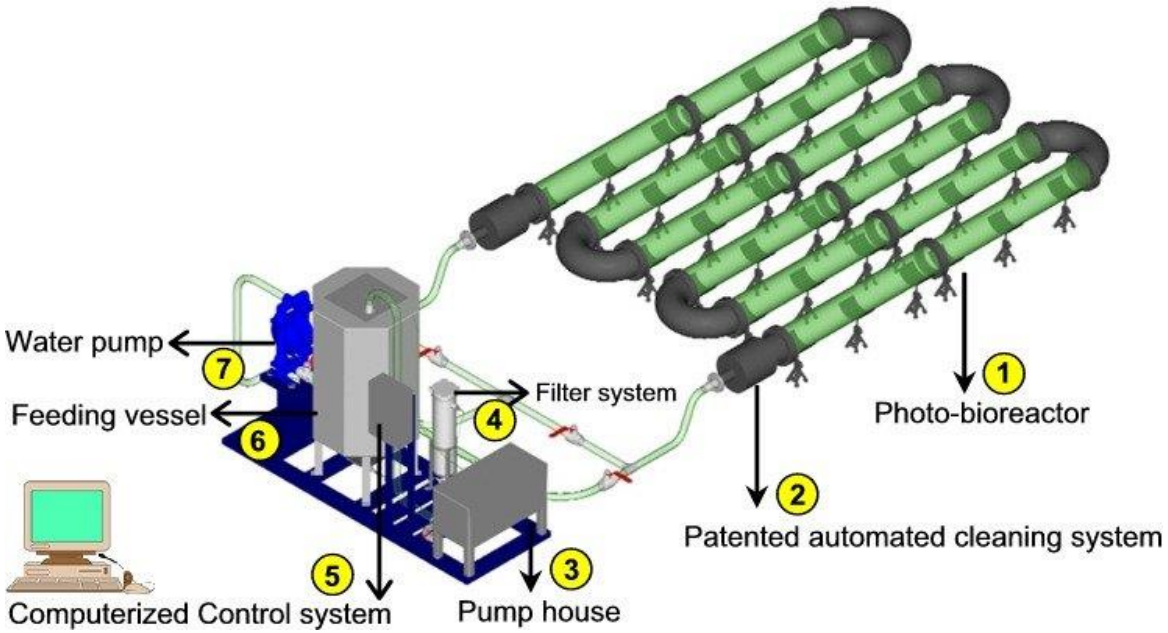


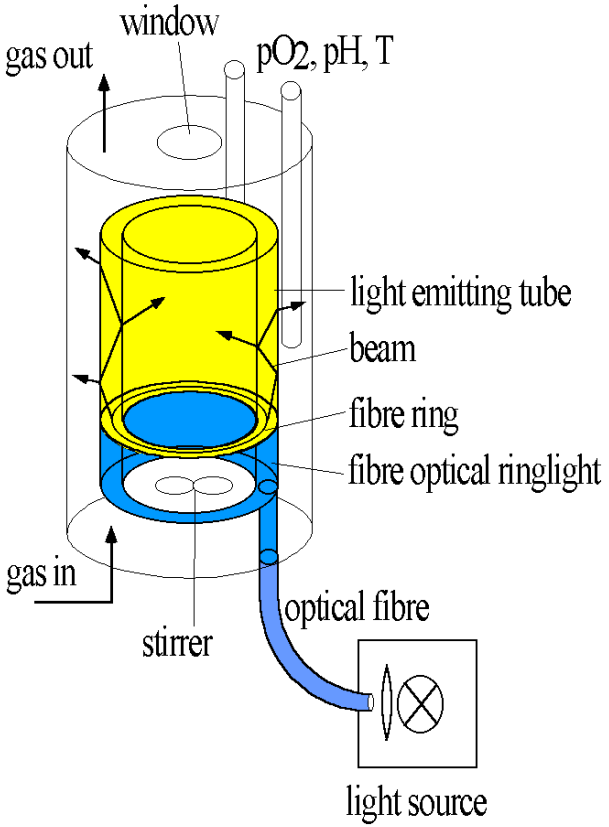
FIGURE 13.4. Comparison of *Chlorella* growth in a photobioreactor and a fermentor.

Cost factor	*Photobioreactor*	*Fermentor*
Construction method	Individually constructed	Mass produced by craftsmen
Scale	Relatively small scale	Up to 500,000 liters
Algal concentration	Dilute	High
Energy source	Light	Organic carbon

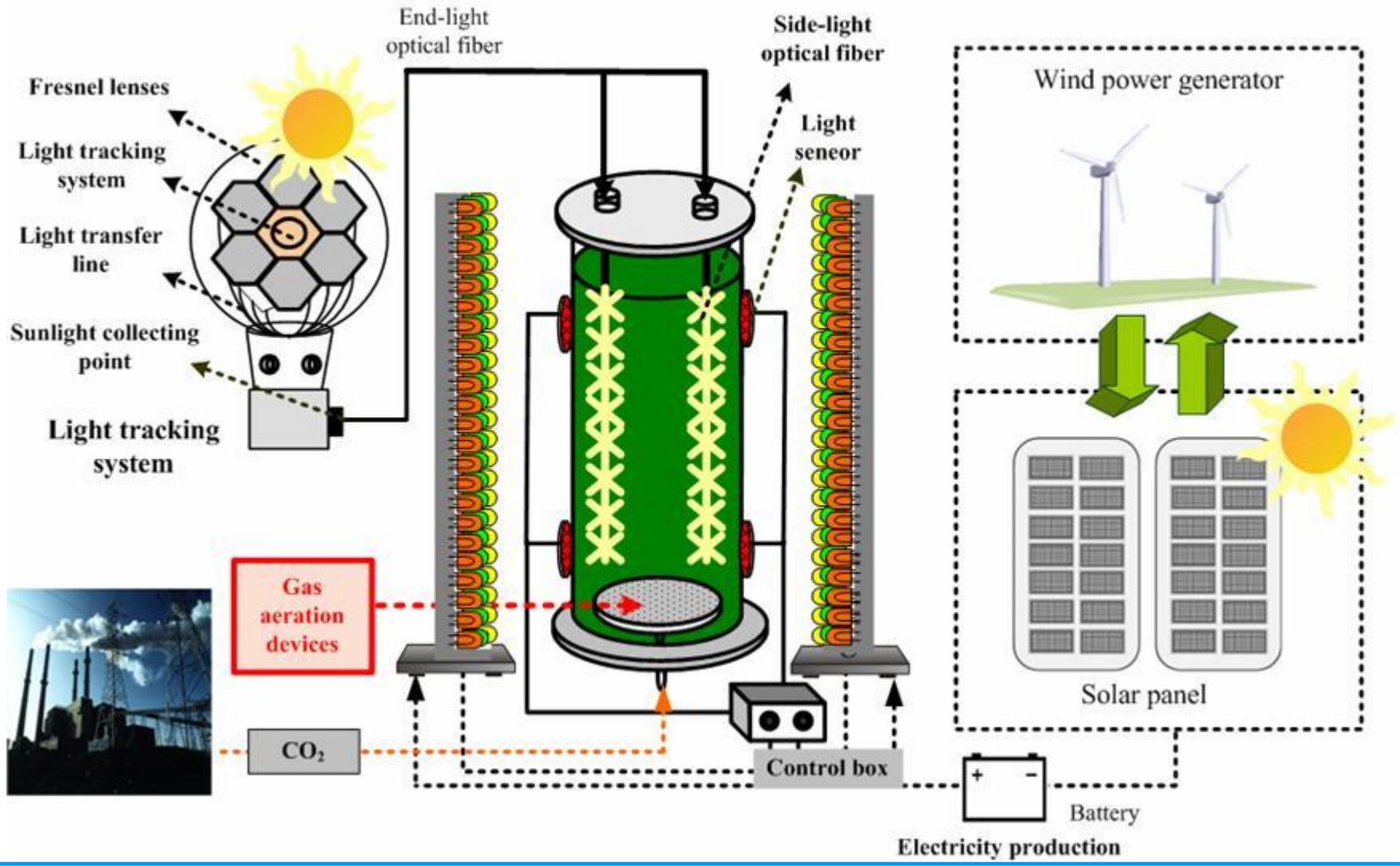
	Phototroph	*Heterotroph*
Energy source	Light	Glucose
Energy cost	$0.07/kW-hr	$0.67/kg
Estimated cost/kg of dry weight	$11.22	$0.81
Actual cost/kg of dry weight	Less than $11.22	$2.01
Productivity	0.4 g · L^{-1} · day^{-1}	5.8 g · L^{-1} · day^{-1}

Photobioreactors





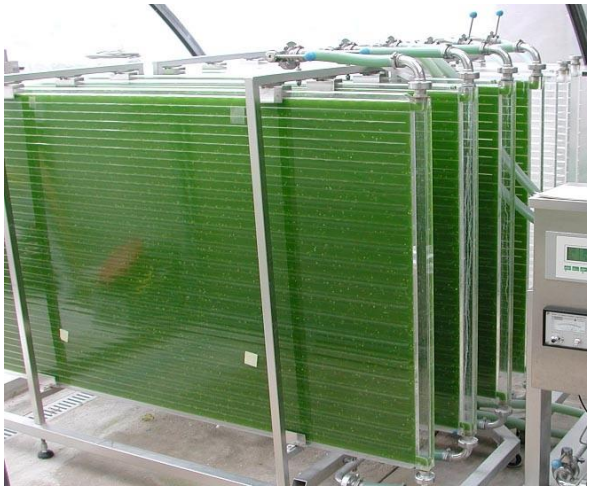
Photobioreactors



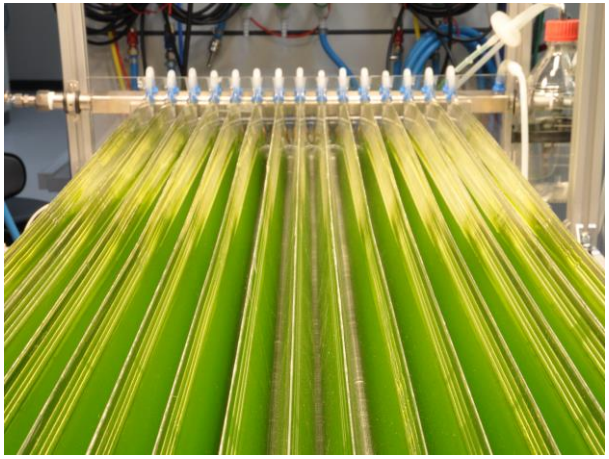
Photobioreactors

Tubular glass photobioreactor

Christmass tree photobioreactor



Plastic plate photobioreactor



Horizontal photobioreactor with zig-zag geometry

Outdoor ponds

TABLE 14.1 Summary of growth conditions for typical microalgal strains cultured in outdoor ponds; variation between strains means that some strains have optima outside the limits listed.

Species condition	*Chlorella vulgaris*	*Dunaliella salina*	*Haematococcus pluvialis*	*Phaeodactylum tricornutum*	*Spirulina platensis*
Natural habitat	Freshwater	Hypersaline brines	Freshwater	Marine	Alkaline soda lakes
Salinity, optimum (% [w/v] NaCl)[a]	0	22% (growth) 35% (carotenogenesis)	0	3%	0 to 1%
Salinity, maximum (% [w/v] NaCl)[a]	~1%	35%	~1%	~5% (?)	<3%
Temperature (°C), optimum	~25	30–40	~18–22	~18–24	30–38
pH, optimum	6.5–7.5	~9.0	~7.0	~8.0	~9.0 (–10.0)
Commonly used media[b]	Bolds basal	Modified Johnson's	Bolds basal	Guillard's f	Zarrouk

[a]For practical reasons salinity is expressed here as % (w/v) NaCl rather than the usual unit of p.s.u., given the great variability in the salt composition of algal media and the fact that salinity is generally adjusted by the addition of NaCl.

[b]References for medium composition: Borowitzka 1988, Vonshak 1997a.

FIGURE 14.1. **(a)** Extensive open ponds at the Hutt Lagoon, Western Australia, plant operated by Cognis Nutrition & Health, growing *D. salina* as a source of natural beta-carotene. The largest ponds are about 200 ha in area. **(b)** Raceway ponds used for the culture of *Spirulina* by Microbio Inc. at Calipatria, California (photo courtesy of Dr. Ahma Belay).

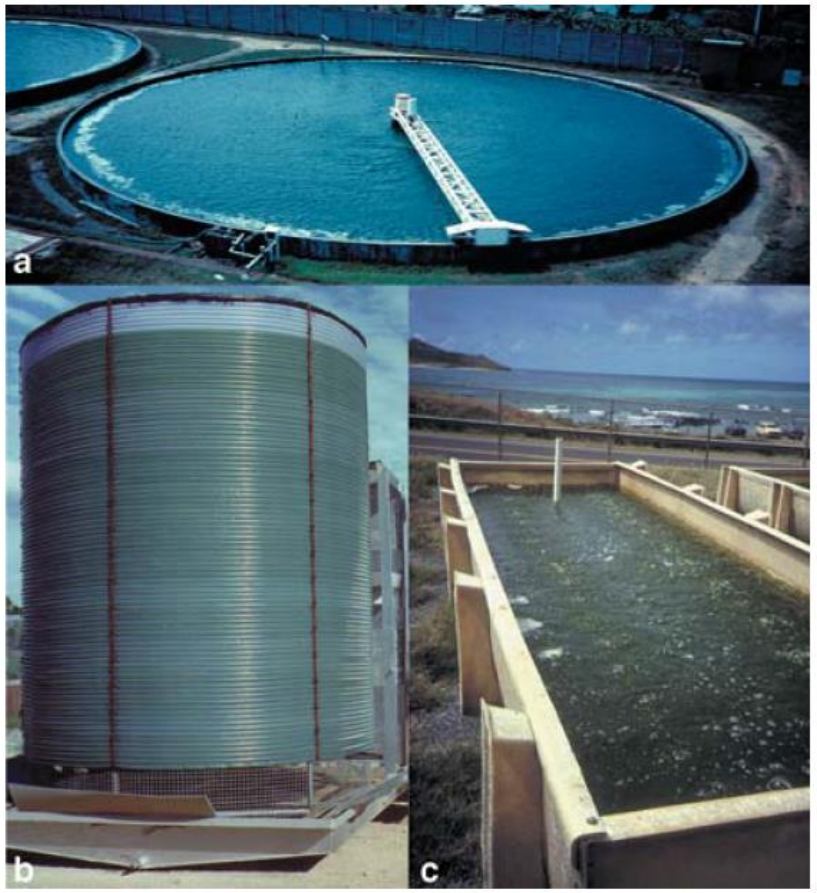


FIGURE 14.2. **(a)** Center-pivot pond used for the cultivation of *Chlorella* in Taiwan. Note the variable mixing effectiveness, as illustrated by the foaming at the pond perimeter. **(b)** Biocoil, a 1,000-liter helical tubular photobioreactor. **(c)** Deep aerated tank used for the culture of *Nannochloropsis* for aquaculture.

Maricultures of seaweed

- Marine algae can be cultured in sea water

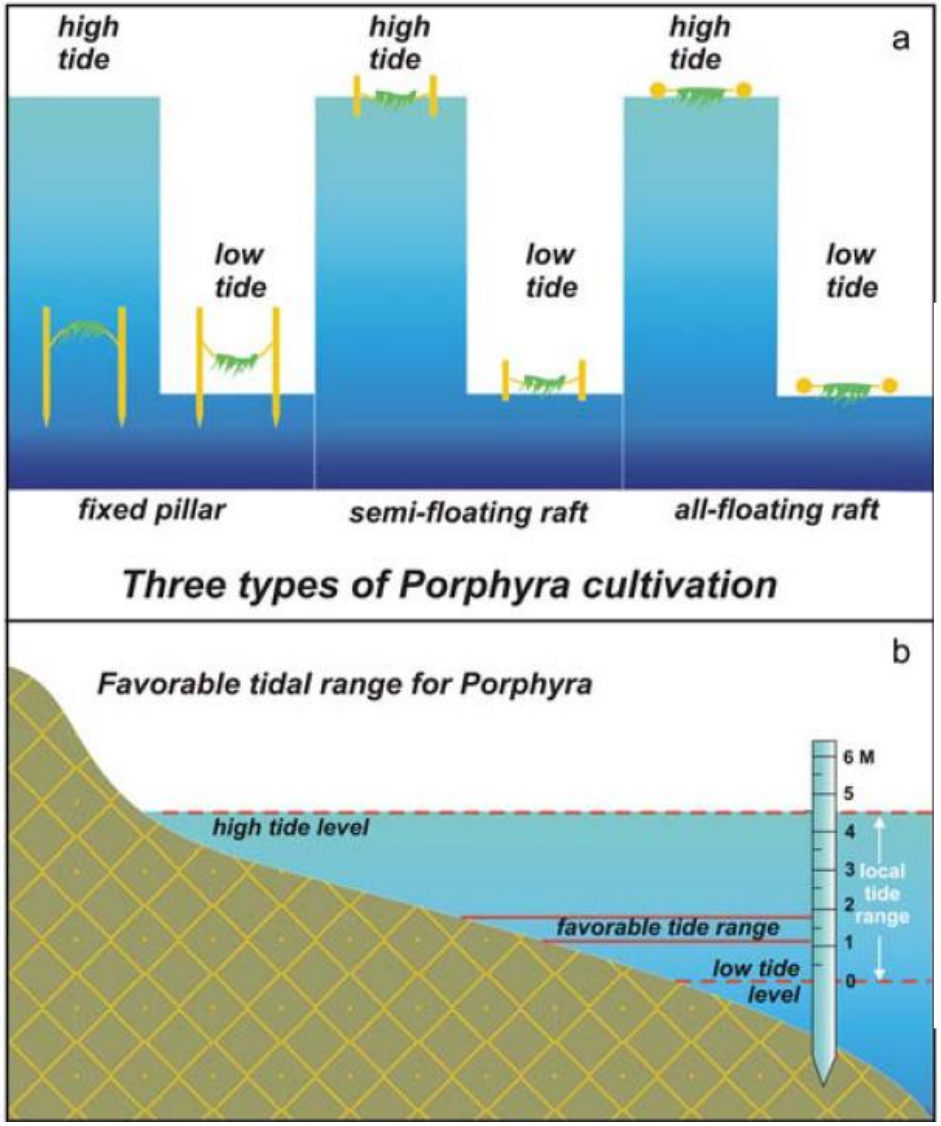
TABLE 15.1 Top cultivated seaweed genera in the world during 2000 (FAO 2003).

Taxon	*Value (10^6 U.S.$)*	*Raw material (metric tons)*	*U.S.$ per metric ton*
Laminaria	2,811	4,580,000	613
Porphyra	1,118	1,011,000	1,105
Undaria	149	311,105	480
Eucheuma and *Kappaphycus*	46	628,576	73
Gracilaria	11	12,510	879
Total	4,632	5,972,737	

Table-1. *The species of marine algae cultured and quantities harvested in (million tonnes) in Indo-Pacific region*

Name of the marine algae	Countries cultivated	Utilization	Quantities harvested (Million tonnes)
Laminaria japonica (Kombu)	China, Japan, North Korea and South Korea	Food	4.8
Undaria pinnatifida (Wakame)	China, Japan, North Korea and South Korea	Food	1.8
Gracilaria verrucosa	China, Taiwan	Food and Phycocolloid	1.15
Gracilaria spp.	Indonesia, Korea, Philippines, Vietnam	Food and Phycocolloid	0.25
Kappaphycus alvarezii	Malaysia, Myanmar, Philippines, India	Food and mainly Phycocolloid	0.24
Eucheuma spp	China, Fiji Island, and Indonesia	Food and Phycocolloid	2.0
Eucheuma cottonii	Philippines	Food and Phycocolloid	1.5
Eucheuma denticulatum	Malaysia and Philippines	Food and Phycocolloid	0.1
Porphyra tenera	Taiwan, Japan, North Korea and South Korea	Food	0.57
Porphyra spp	China	Food	0.82
Enteromorpha clathrata *Gelidium amansii*	China	Food Agar	0.12 1200 (tonnes)
Monostroma nitidum	South Korea	Food	0.80
Caulerpa lentellifera	Philippines	Food	0.43
Codium fragilis	South Korea	Food	0.12
Hizikia fusiformis	Japan and South Korea	Food	N/A
Kappaphycus alvarezii	India	Phycocolloid and Liquid Fertilizer	1500 (tonnes)

FIGURE 15.1. Culture of *Porphyra*. **(a)** Attaching oyster shells to a net before inoculation with carpospores. **(b)** Seeding oyster shells in a seeding tank. **(c)** Young conchocelis growing on oyster shells. **(d)** Net rotated in a seeding tank containing mature conchospore inoculum from the free-living conchocelis culture. **(e)** Nets raised daily to expose the young thalli to air and sun to inhibit fouling organisms; the Japanese "Ikada" system. **(f)** Floating A-frame system in China; raised and lowered to expose the young thalli, inhibiting fouling organisms. (**a–c** courtesy M. Notoya; **e** courtesy of I. Levine; **f** from X. G. Fei.)



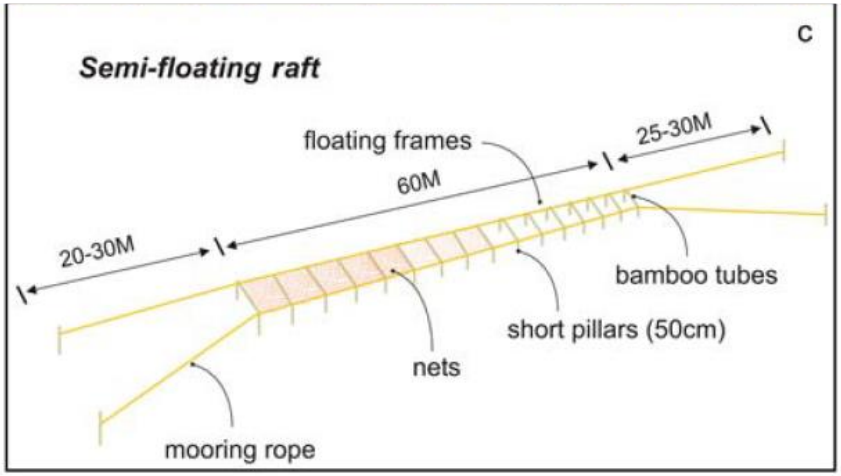


FIGURE 15.2. **(a)** *Porphyra* seedlings separated from the bundles: fixed pole, semifloating raft, and floating raft. **(b)** Determining of the best level for fixed pole intertidal *Porphyra* cultivation. **(c)** Diagrammatic illustration of a semifloating raft. (**a** after Tseng 1981; **b–c** courtesy X. G. Fei.)

FIGURE 15.3. **(a)** Attachment of *Porphyra* nets to a semifloating raft. **(b)** Korean-style all-floating rafts. **(c)** Fleet of *Porphyra* harvesting boats. Note the tubular frames that lift the nets (see **d**). **(d)** Harvesting boat with *Porphyra* net lifted from the sea. (**a** courtesy E. Hwang; **d** courtesy M. Notoya.)

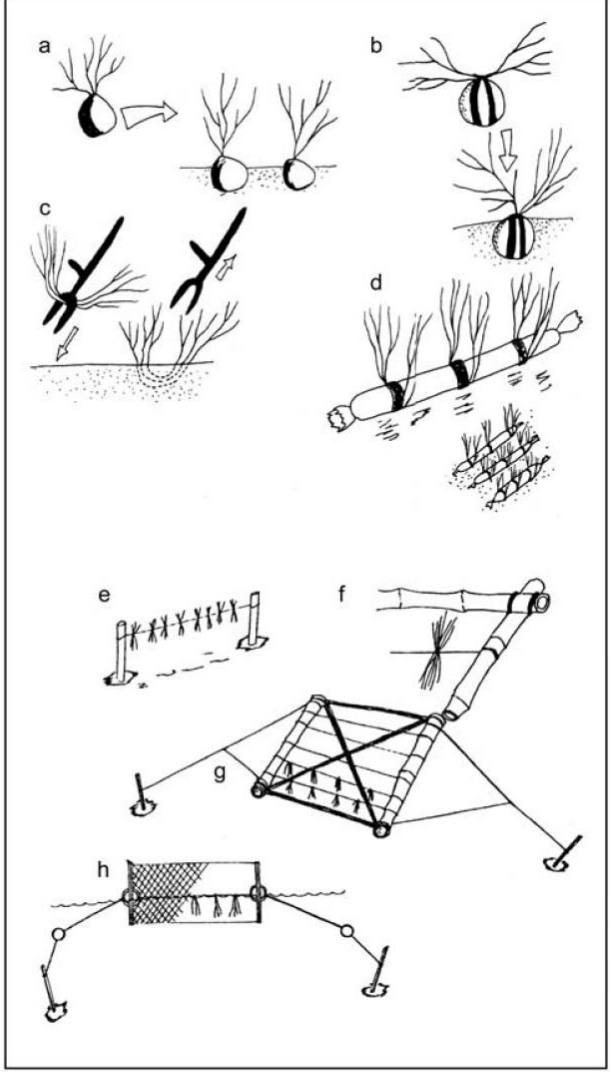


FIGURE 15.4. **(a)** Rocky substratum with attached *Gracilaria* being transplanted to a new site. **(b)** *Gracilaria* attached to rocks with rubber bands; method used for anchoring transplants in soft sediments. **(c)** Plants inserted with a fork directly into soft sediments. **(d)** Plants attached to sand-filled plastic tubes. **(e)** *Gracilaria* attached to a rope, which is stretched between two poles pushed into the sediments. **(f)** Attachment of plants for rope culture. **(g)** Bamboo floating frame with *Gracilaria* attached to ropes spanning the frame. **(h)** A floating net with *Gracilaria* attached to the meshes. (**a–d** modified from Santelices and Doty 1989, Oliveira and Alveal 1990, Critchley and Ohno 1997; **e** from Critchley and Ohno 1997.)

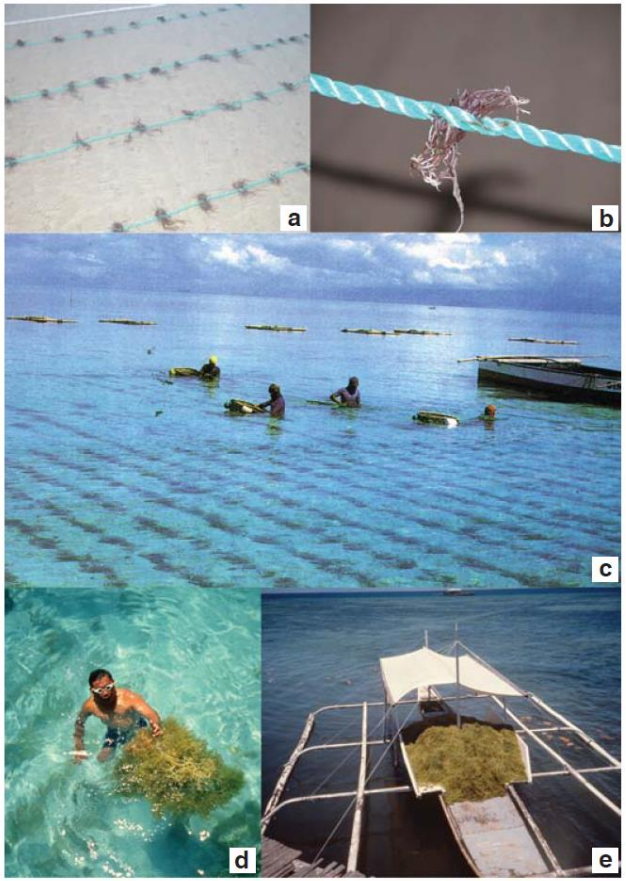


FIGURE 15.5. **(a)** Rope cultivation of *Gracilaria* using monofilament long lines. **(b)** Enlarged image showing a *Gracilaria* plant held within the twists of the lone line rope. **(c)** *Eucheuma* farmers harvesting plants from submerged long lines. **(d)** Open water cultivation and harvesting of *Kappaphycus*; diver bringing a large plant to the boat. **(e)** Typical outrigger boat used for open water harvesting. (**d** and **e** courtesy T. Chopin.)

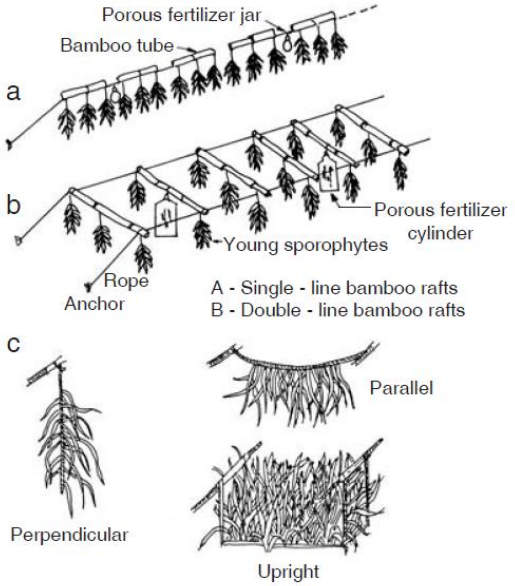
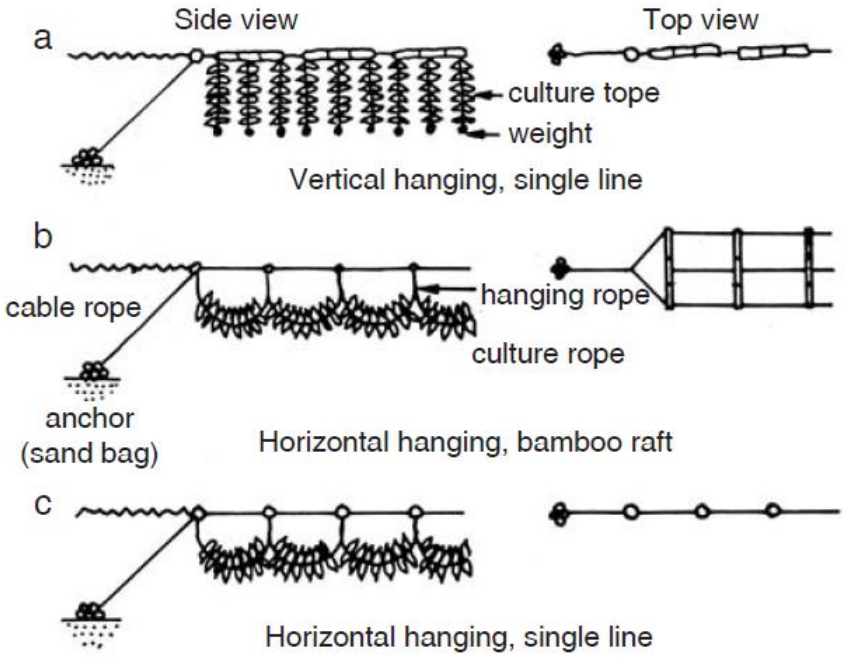


FIGURE 15.6. **(a)** A single-line bamboo raft for *Laminaria* sporelings. **(b)** A double-line bamboo raft used in Japan. **(c)** Perpendicular, parallel, and upright culture methods. **(d)** Long line with *Laminaria* after 8 months of growth (Yellow Sea, China). **(e)** Long line showing attached *Laminaria* plants (South Korea). **(f)** Young sporophytes growing on long line. (**a–c** from Cheng 1969; **e** courtesy E. Hwang.)



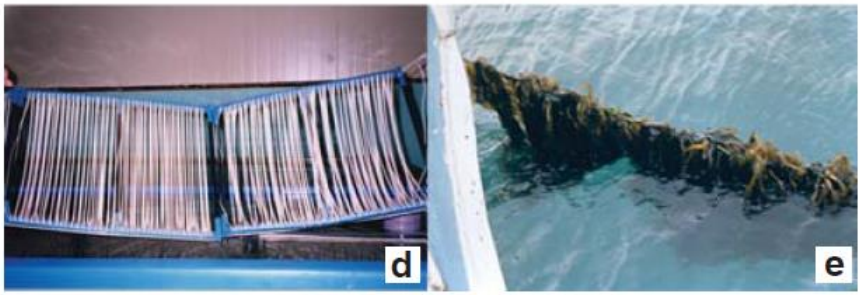
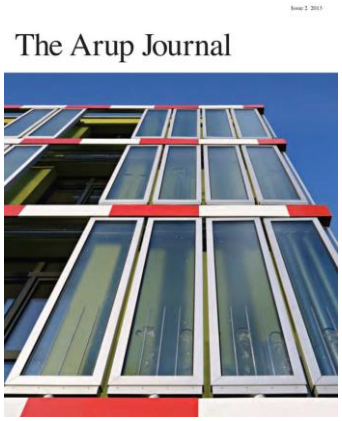
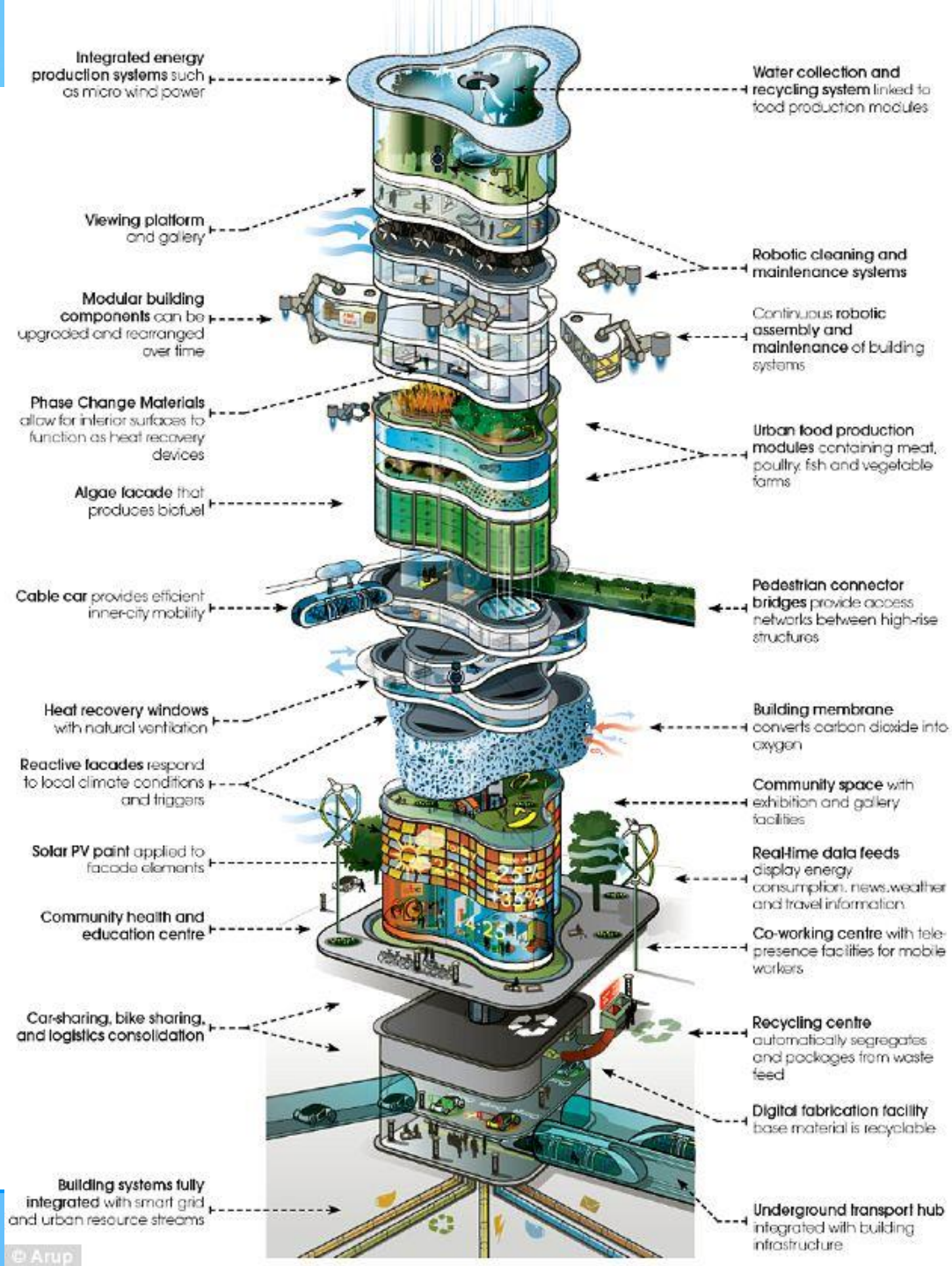


FIGURE 15.7. **(a–c)** A bamboo raft and vertical and horizontal single lines used for *Undaria* cultivation. **(d)** *Undaria* spore collector; frame is 50 by 50 centimeters and a synthetic seed string is wound around the frame. **(e)** Open water cultivation of *Undaria*. (**a–c** from Akiyama and Kurogi 1982; **e** courtesy E. Hwang.)

Algae-powered houses

- 129 bioreactors on south and south-east faces
- Hamburg, Ger




Integrated energy production systems such as micro wind power
Water collection and recycling system linked to food production modules
Viewing platform and gallery
Robotic cleaning and maintenance systems
Modular building components can be upgraded and rearranged over time
Continuous robotic assembly and maintenance of building systems
Phase Change Materials allow for interior surfaces to function as heat recovery devices
Urban food production modules containing meat, poultry, fish and vegetable farms
Algae facade that produces biofuel
Cable car provides efficient inner-city mobility
Pedestrian connector bridges provide access networks between high-rise structures
Heat recovery windows with natural ventilation
Building membrane converts carbon dioxide into oxygen
Reactive facades respond to local climate conditions and triggers
Community space with exhibition and gallery facilities
Solar PV paint applied to facade elements
Real-time data feeds display energy consumption, news, weather and travel information
Community health and education centre
Co-working centre with tele-presence facilities for mobile workers
Car-sharing, bike sharing, and logistics consolidation
Recycling centre automatically segregates and packages from waste feed
Digital fabrication facility base material is recyclable
Building systems fully integrated with smart grid and urban resource streams
Underground transport hub integrated with building infrastructure
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