Bioprocesses and Downstream Processing

EPFL minor in Biotechnology - 2025

Module ChE-437

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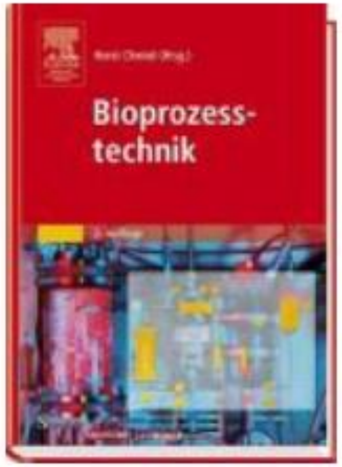
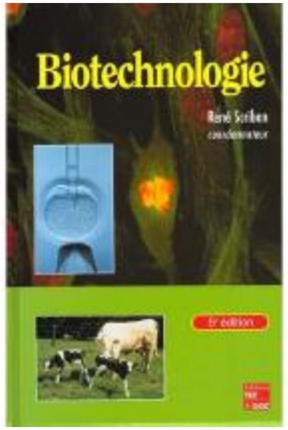
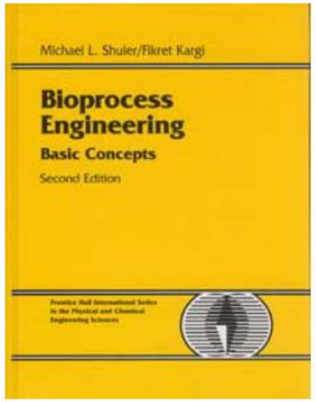
Storhas W, Bioverfahrensentwicklung
VCH ISBN 3-527-28866-X



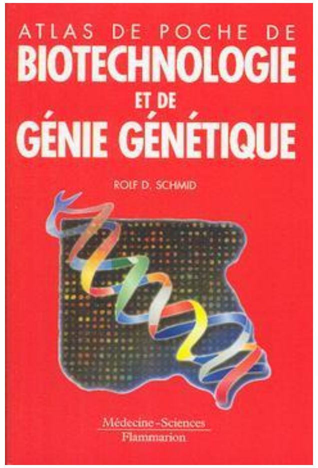
Horst Chmiel, Bioprozesstechnik
ISBN: 3827416078



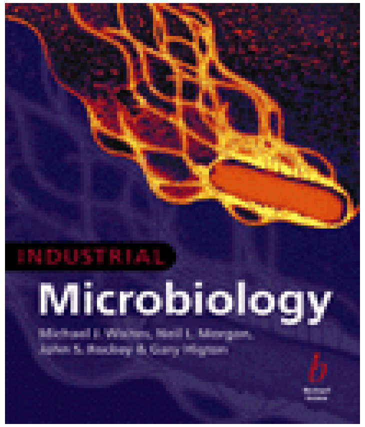
Scriban René, Biotechnologie
08-1999 - 1042p./ Lavoisier



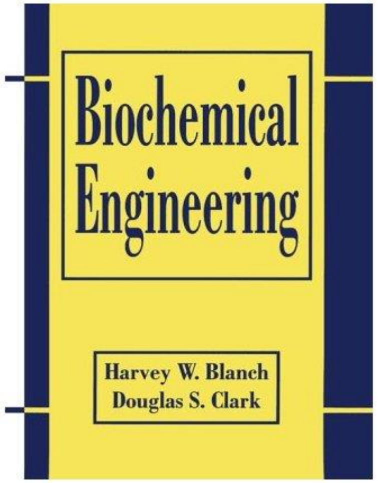
Bioprocess Engineering
Basic Concepts
Second Edition
by Michael Shuler, Fikret Kargi
ISBN: 0-13-081908-5



Atlas de poche de biotechnologie
et de génie génétique
Rolf. D. Schmid
Lavoisier

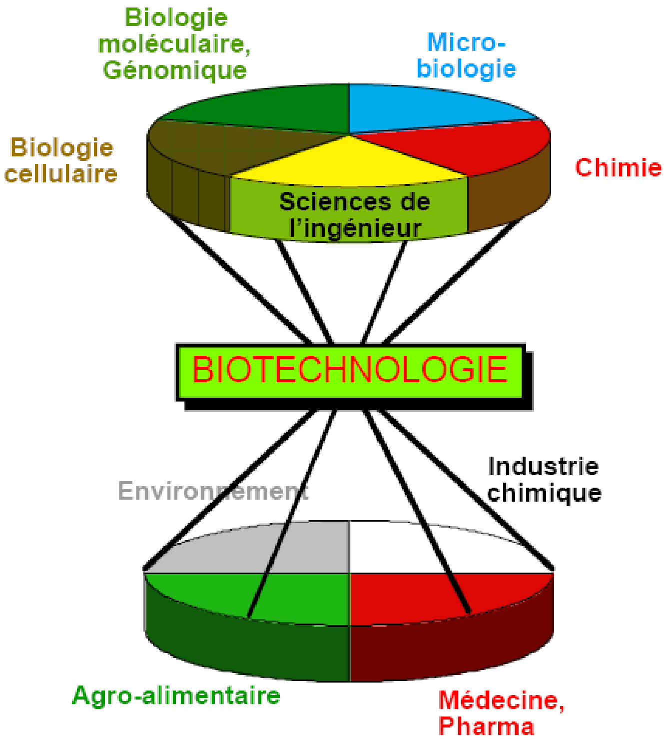


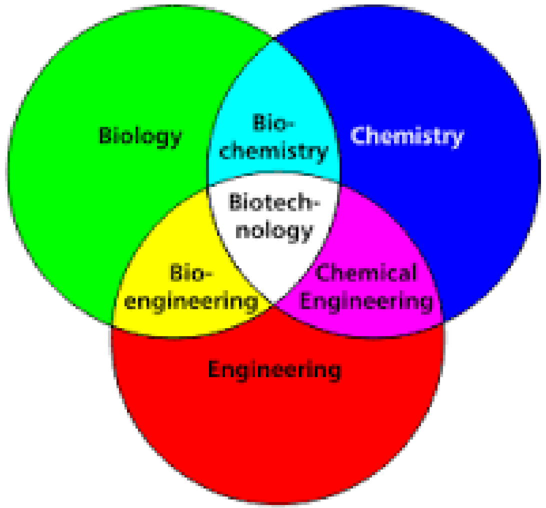
By: Mike J Waites (London South Bank University), Neil L Morgan (London South Bank University), John S Rockey (Llandovery College) and Gary Higton (South Bank University, London.)
Paperback, ISBN: 0632053070,

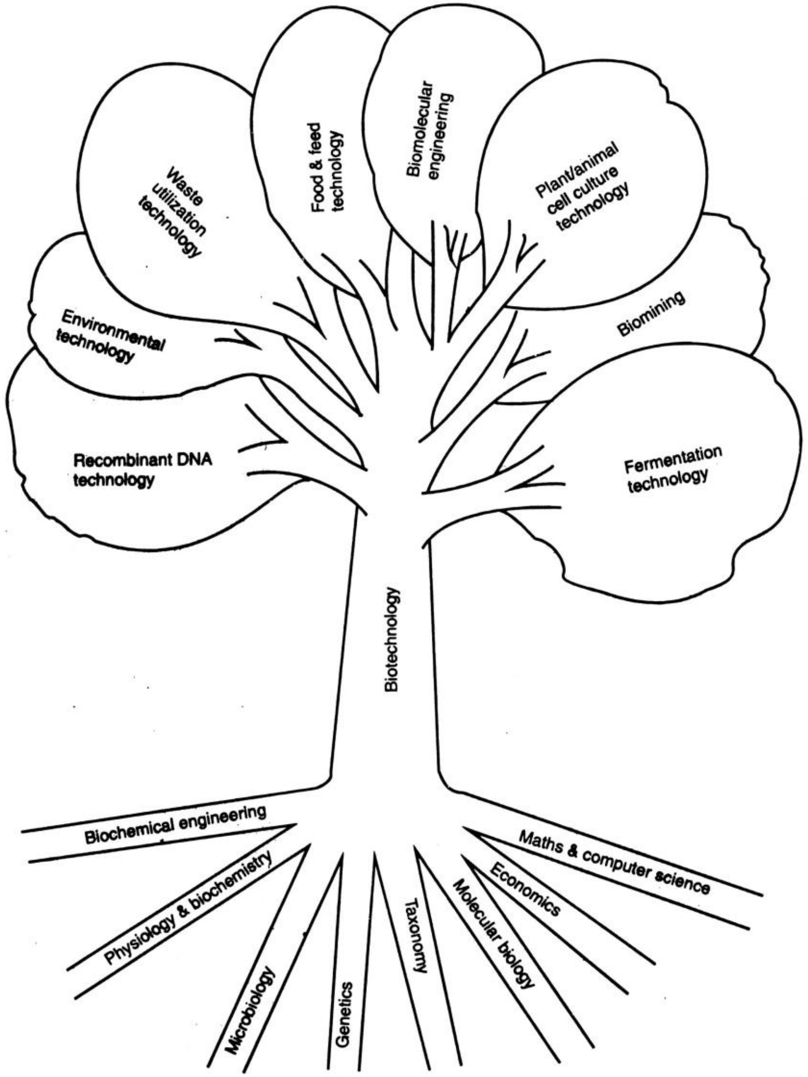


Biochemical Engineering (Hardcover)
by Blanch W. Blanch , Douglas S. Clark

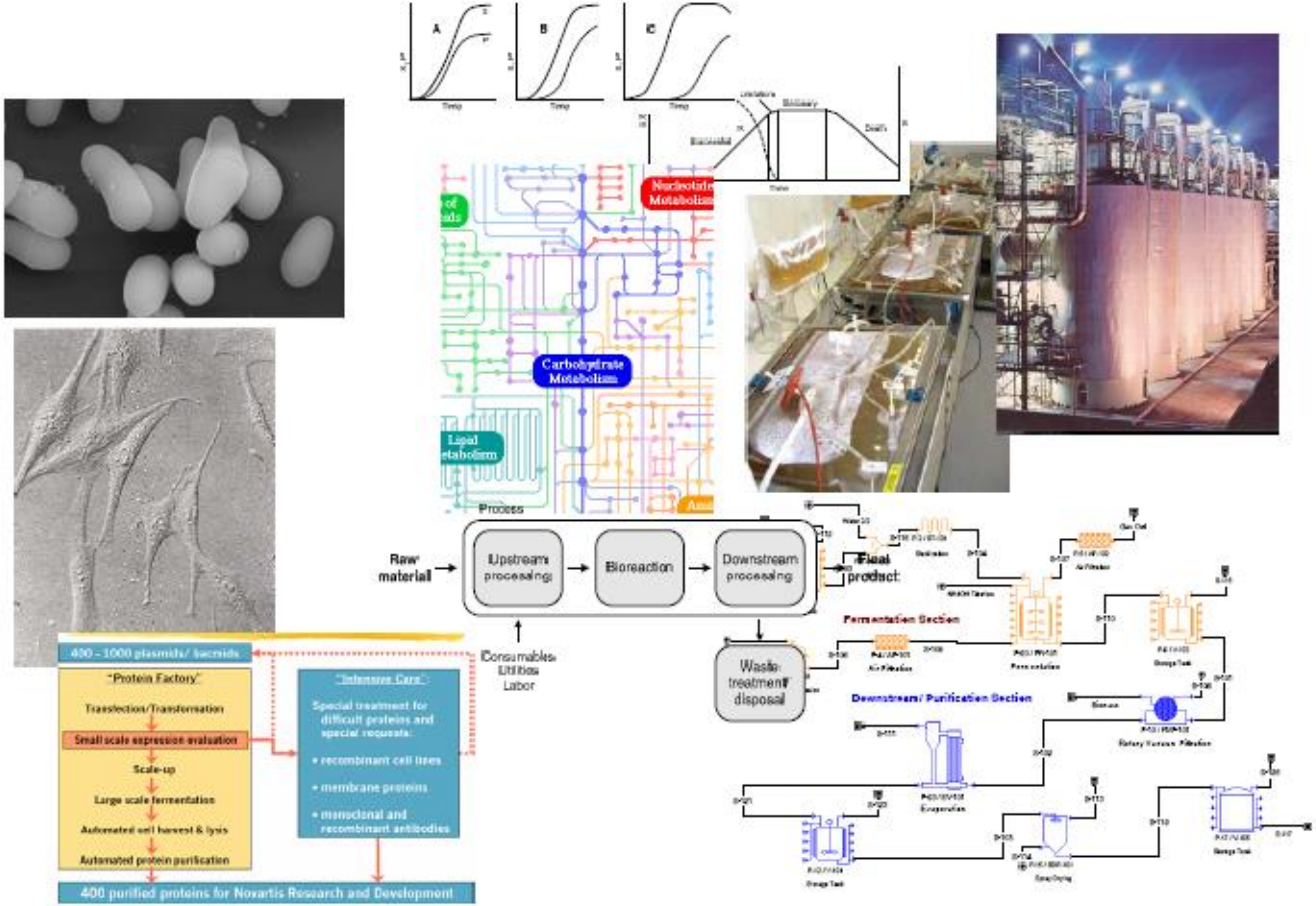
Recap


Biologie moléculaire, Génomique
Micro-biologie
Biologie cellulaire
Chimie
Sciences de l'ingénieur
BIOTECHNOLOGIE
Environnement
Industrie chimique
Agro-alimentaire
Médecine, Pharma


Biology
Bio-chemistry
Chemistry
Biotech-nology
Bio-engineering
Chemical Engineering
Engineering


Waste utilization technology
Food & feed technology
Biomolecular engineering
Plant/animal cell culture technology
Environmental technology
Biomining
Recombinant DNA technology
Fermentation technology
Biotechnology
Biochemical engineering
Physiology & biochemistry
Microbiology
Genetics
Taxonomy
Molecular biology
Economics
Maths & computer science

Recap



Nucleotide Metabolism
Carbohydrate Metabolism
Lipid Metabolism
Process
Raw material
Upstream processing
Bioreaction
Downstream processing
Final product
Consumables
Utilities
Labor
Waste treatment/ disposal
Fermentation Section
Downstream/ Purification Section
400 - 1000 plasmids/ bacmids
"Protein Factory"
Transfection/Transformation
Small scale expression evaluation
Scale-up
Large scale fermentation
Automated cell harvest & lysis
Automated protein purification
"Intensive Care"
Special treatment for difficult proteins and special requests:
• recombinant cell lines
• membrane proteins
• monoclonal and recombinant antibodies
400 purified proteins for Novartis Research and Development

What is a bioprocess ?

- Application of **natural** or **genetically manipulated** (recombinant) **whole cells/ tissues/ organs, or parts** thereof, for the production of industrially or medically important products

- **Examples**
  - Agroalimentaire food/ beverages
  - Organic acids and alcohols
  - Flavours and fragrances
  - DNA for gene therapy and transient infection
  - Antibiotics
  - Proteins (mAbs, tPA, hirudin, enzymes etc)
  - Hormones (insulin, hGH,EPO,FSH etc)

Application of Biotechnology

Area	Products or Applications
Pharmaceuticals	Antibiotics, antigens (stimulate antibody response), endorphin (neurotransmitter), gamma globulin (prevent infections), human growth hormone (treat children with dwarfism), human serum albumin (treat physical trauma), immune regulators, insulin, interferon (treat infection), interleukins (treat infectious decease or cancer), lymphokines (modulate immune reaction), monoclonal antibody (diagnostics or drug delivery), neuroactive peptides (mimic the body's pain-controlling peptides), tissue plasminogen activator (dissolve blood clots), vaccines
Animal Agriculture	Development of disease-free seed stocks healthier, higher-yielding food animals.
Plant Agriculture	transfer of stress-, herbicide-, or pest-resistance traits to crop species, development of plants with the increased abilities of photosynthesis or nitrogen fixation, development of biological insecticides and non-ice nucleating bacterium.
Specialty Chemicals	amino acids, enzymes, vitamins, lipids, hydroxylated aromatics, biopolymers.
Environmental Applications	mineral leaching, metal concentration, pollution control, toxic waste degradation, and enhanced oil recovery.
Commodity Chemicals	acetic acid, acetone, butanol, ethanol, many other products from biomass conversion processes.
Bioelectronics	Biosensors, biochips.

Product	Typical organism used
BIOMASS	
Agricultural inoculants for nitrogen fixation	*Rhizobium leguminosarum*
Bakers' yeast	*Saccharomyces cerevisiae*
Cheese starter cultures	*Lactococcus* spp.
Inoculants for silage production	*Lactobacillus plantarum*
Single-cell protein	*Candida utilis* or *Pseudomonas methylotrophus*
Yoghurt starter cultures	*Streptococcus thermophilus* and *Lactobacillus bulgaricus*
BULK ORGANICS	
Acetone/butanol	*Clostridium acetobutylicum*
Ethanol (nonbeverage)	*Saccharomyces cerevisiae*
Glycerol	*Saccharomyces cerevisiae*
ORGANIC ACIDS	
Citric acid	*Aspergillus niger*
Gluconic acid	*Aspergillus niger*
Itaconic acid	*Aspergillus itaconicus*
Lactic acid	*Lactobacillus delbrueckii*
AMINO ACIDS	
L-Arginine	*Brevibacterium flavum*
L-Glutamic acid	*Corynebacterium glutamicum*
L-Lysine	*Brevibacterium flavum*
L-Phenylalanine	*Corynebacterium glutamicum*
Others	*Corynebacterium* spp.
NUCLEIC ACID-RELATED COMPOUNDS	
5′-guanosine monophosphate (5′-GMP)	*Bacillus subtilis*
5′-inosine monophosphate (5′-IMP)	*Brevibacterium ammoniagenes*
ENZYMES	
α-Amylase	*Bacillus amyloliquefaciens*
Glucoamylase	*Aspergillus niger*
Glucose isomerase	*Bacillus coagulans*
Pectinases	*Aspergillus niger*
Proteases	*Bacillus* spp.
Rennin	*Mucor miehei* or recombinant yeast
VITAMINS	
Cyanocobalamin (B_{12})	*Propionibacterium shermanii* or *Pseudomonas denitrificans*
Riboflavin (B_2)	*Eremothecium ashbyii*
EXTRACELLULAR POLYSACCHARIDES	
Dextran	*Leuconostoc mesenteroides*
Xanthan gum	*Xanthomonas campestris*
Other	*Polianthes tuberosa* (plant cell culture)
POLY-β-HYDROXYALKANOATE POLYESTERS	
Poly-β-hydroxybutyrate	*Alcaligenes eutrophus*
ANTIBIOTICS	
Cephalosporins	*Cephalosporium acremonium*
Penicillins	*Penicillium chrysogenum*
Aminoglycoside antibiotics (e.g., streptomycin)	*Streptomyces griseus*
Ansamycins (e.g., rifamycin)	*Nocardia mediterranei*
Aromatic antibiotics (e.g., griseofulvin)	*Penicillium griseofulvum*
Macrolide antibiotics (e.g., erythromycin)	*Streptomyces erythreus*
Nucleoside antibiotics (e.g., puromycin)	*Streptomyces alboniger*
Polyene macrolide antibiotics (e.g., candidin)	*Streptomyces viridoflavus*
Polypeptide antibiotics (e.g., gramicidin)	*Bacillus brevis*
Tetracyclines (e.g., 7-chlortetracycline)	*Streptomyces aureofaciens*
ALKALOIDS	
Ergot alkaloids	*Claviceps paspali*
Taxol	*Taxus brevifolia* (plant cell culture)
SAPONINS	
Ginseng saponins	*Panax ginseng* (plant cell culture)
PIGMENTS	
β-Carotene	*Blakeslea trispora*
PLANT GROWTH REGULATORS	
Gibberellins	*Gibberella fujikuroi*
INSECTICIDES	
Bacterial spores	*Bacillus thuringiensis*
Fungal spores	*Hirsutella thompsonii*

Product	Typical organism used
MICROBIAL TRANSFORMATIONS	
D-Sorbitol to L-sorbose (in vitamin C production)	*Acetobacter suboxydans*
Steroids	*Rhizopus arrhizus*
VACCINES	
Diphtheria	*Corynebacterium diphtheriae*
Hepatitis B	Surface antigen expressed in recombinant *Saccharomyces cerevisiae*
Mumps	Attenuated viruses grown in chick embryo cell cultures
Pertussis (whooping cough)	*Bordetella pertussis*
Poliomyelitis virus	Attenuated viruses grown in monkey kidney or human diploid cells
Rubella	Attenuated viruses grown in baby hamster kidney cells
Tetanus	*Clostridium tetani*
THERAPEUTIC PROTEINS	
Erythropoietin	Recombinant mammalian cells
Factor VIII	Recombinant mammalian cells
Follicle-stimulating hormone	Recombinant mammalian cells
Granulocyte–macrophage colony-stimulating factor	Recombinant *Escherichia coli*
Growth hormones	Recombinant *Escherichia coli*
Hirudin	Recombinant *Saccharomyces cerevisiae*
Insulin and insulin analogues	Recombinant *Escherichia coli*
Interferons	Recombinant *Escherichia coli*
Interleukins	Recombinant *Escherichia coli*
Platelet-derived growth factor	Recombinant *Saccharomyces cerevisiae*
Tissue plasminogen activator	Recombinant *Escherichia coli* or recombinant mammalian cells
MONOCLONAL ANTIBODIES	
Various, including Fab and Fab_2 fragments	Hybridoma cells
THERAPEUTIC TISSUES AND CELLS	
Cartilage cells	Human (patient) chondrocytes
Skin	Human skin cells

Why bioprocesses?

- Produce 'natural' products
- Highly selective reactions e.g. stereospecific
- Operate under mild reaction conditions e.g. T, pressure, pH
- High reaction rates catalyzed by biological enzymes (catalysts)
- Used to produce molecules or catalyze reactions difficult to achieve by chemical synthesis
- Products have high biological activity (enzymes, pharmaceuticals), biodegradable (pHB), used for food (cheese, alcohols etc.) and animal feed (SCP), treatment of genetic disorders etc….

However, biological processes have some disadvantages:

1. *Complex product mixtures*: In cases of cell cultivation (microbial, animal, or plant), multiple enzyme reactions are occurring in sequence or in parallel, the final product mixture contains cell mass, many metabolic by-products, and a remnant of the original nutrients. The cell mass also contains various cell components.
2. *Dilute aqueous environments*: The components of commercial interests are only produced in small amounts in an aqueous medium. Therefore, separation is very expensive. Since products of bioprocesses are frequently heat sensitive, traditional separation techniques cannot be employed. Therefore, novel separation techniques that have been developed for analytical purposes, need to be scaled up.
3. *Contamination*: The fermenter system can be easily contaminated, since many environmental bacteria and molds grow well in most media. The problem becomes more difficult with the cultivation of plant or animal cells because their growth rates are much slower than those of environmental bacteria or molds.
4. *Variability*: Cells tend to mutate due to the changing environment and may lose some characteristics vital for the success of process. Enzymes are comparatively sensitive or unstable molecules and require care in their use.

Definition of Fermentation

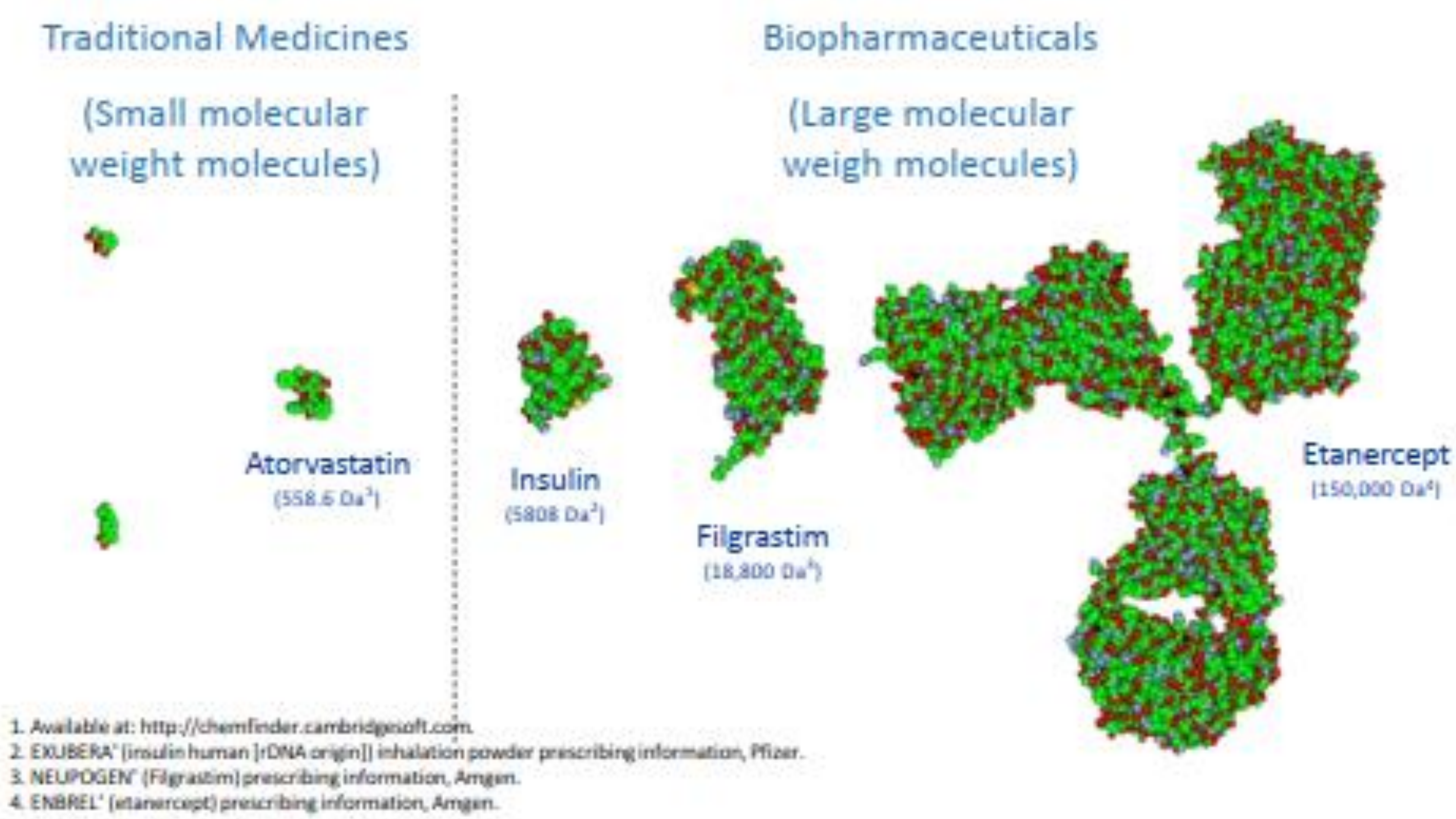
- Traditionally, fermentation was defined as the process for the production of alcohol or lactic acid from glucose ($C_6H_{12}O_6$).

$$C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2C_2H_5OH + 2CO_2$$

$$C_6H_{12}O_6 \xrightarrow{\text{enzymes}} 2CH_3CHOHCOOH$$

- A broader definition of fermentation is .an enzymatically controlled transformation of an organic compound. according to *Webster's New College Dictionary*

Biopharmacuetical vs Pharmaceutical?



Aims of a bioprocess

- To apply and optimize natural or artificial biological systems by manipulation of cells and their environment to produce the desired product

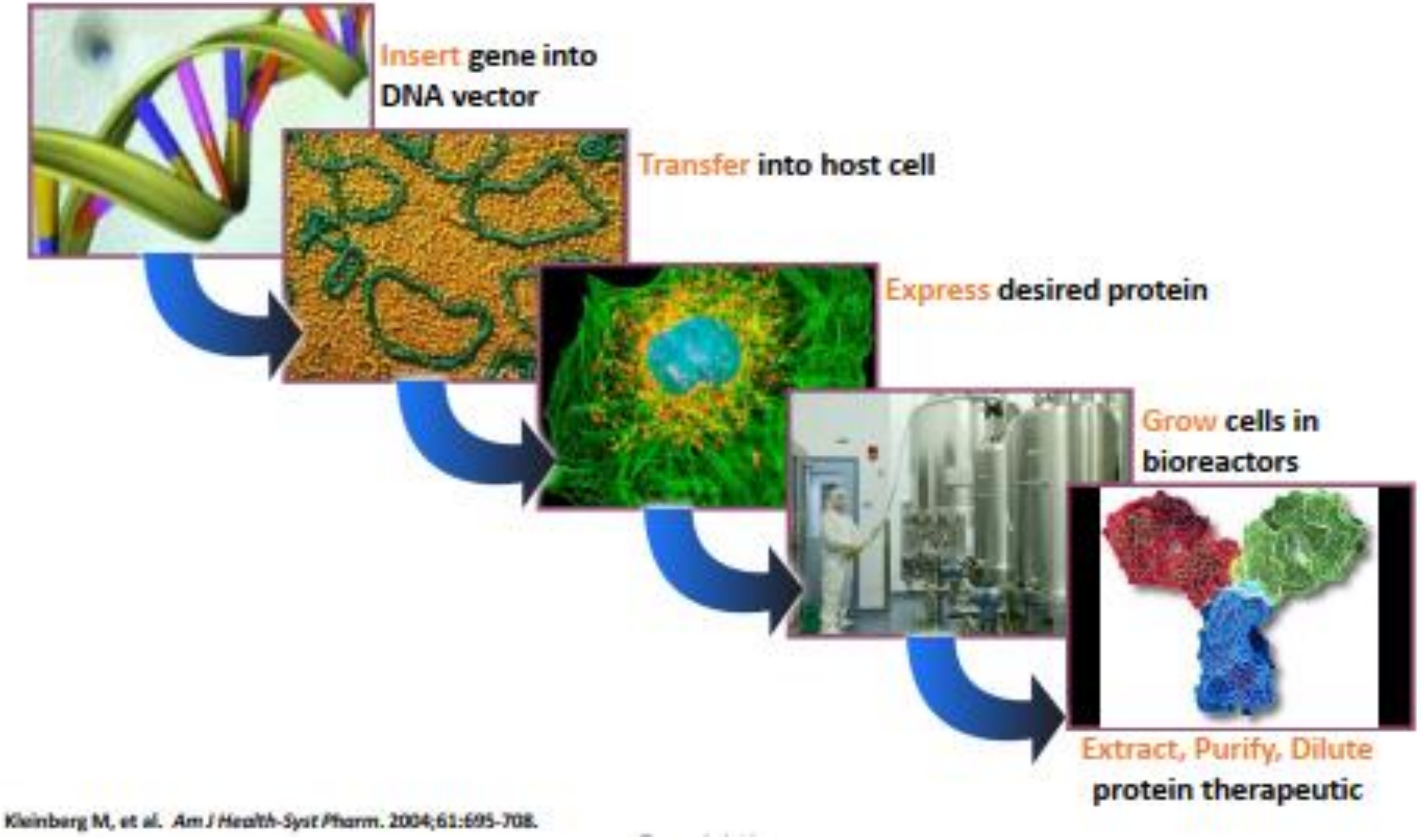
- Molecular biology (genetic engineering) is a tool to achieve this

- **Systems used include:**
  - Viruses
  - Procaryotes (bacteria, blue- green algae, cyanobateria)
  - Eucaryotes (yeasts, molds, animal cells, plant cells, whole plants, whole animals, transgenics)

Importance of bioprocess development

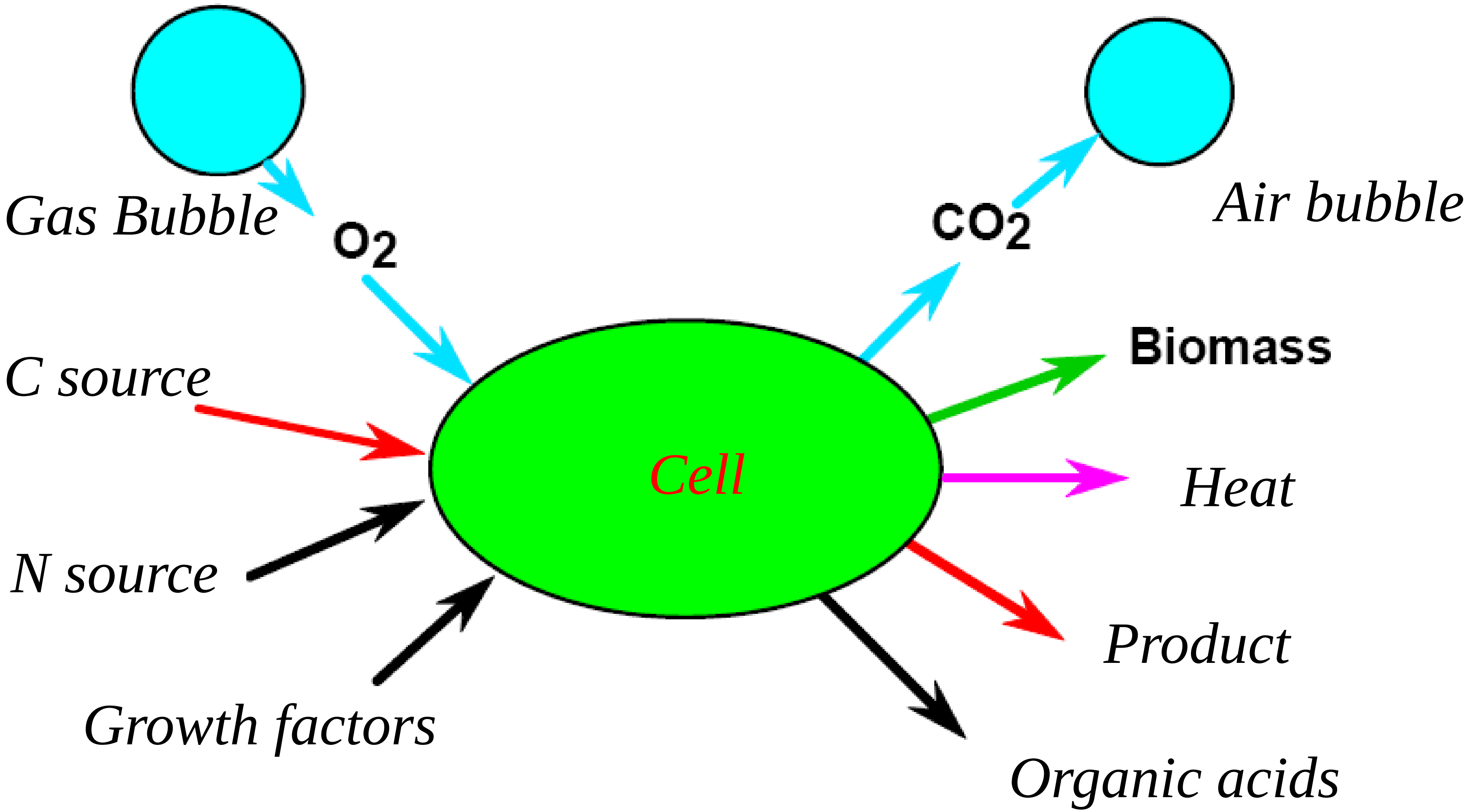
- ' Advances in genetic engineering have, over the past two decades, generated a wealth of novel molecules that have redefined the role of microbes, and other systems, in solving environmental, pharmceutical, industrial and agricultural problems.
- While some products have entered the marketplace, the difficulties of doing so and of complying with Federal mandates of safety, purity, potency, efficacy and consistency have shifted the focus from the word genetic to the word engineering.
-
- This transition from the laboratory to production- the basis of bioprocess engineering- involves a careful understanding of the conditions most favoured for optimal production, and the duplication of these conditions during scaled- up production'.
- Critical process parameters (CPP)
- Critical product attributes (CPA)

Summary of a biopharmaceutical manufacturing process



Some more details: recap

Recap



Gas Bubble
O2
Air bubble
CO2
Biomass
C source
Cell
Heat
N source
Product
Growth factors
Organic acids

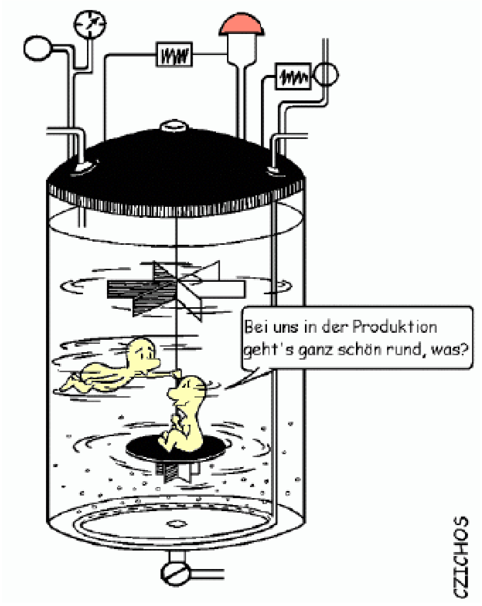
[Bioprocessing Part 1- Fermentation.mp4]

[Bioprocessing Part 2- Separation - Recovery.mp4]

[Bioprocessing Part 3- Purification.mp4]

Recap

Overview & Outlook



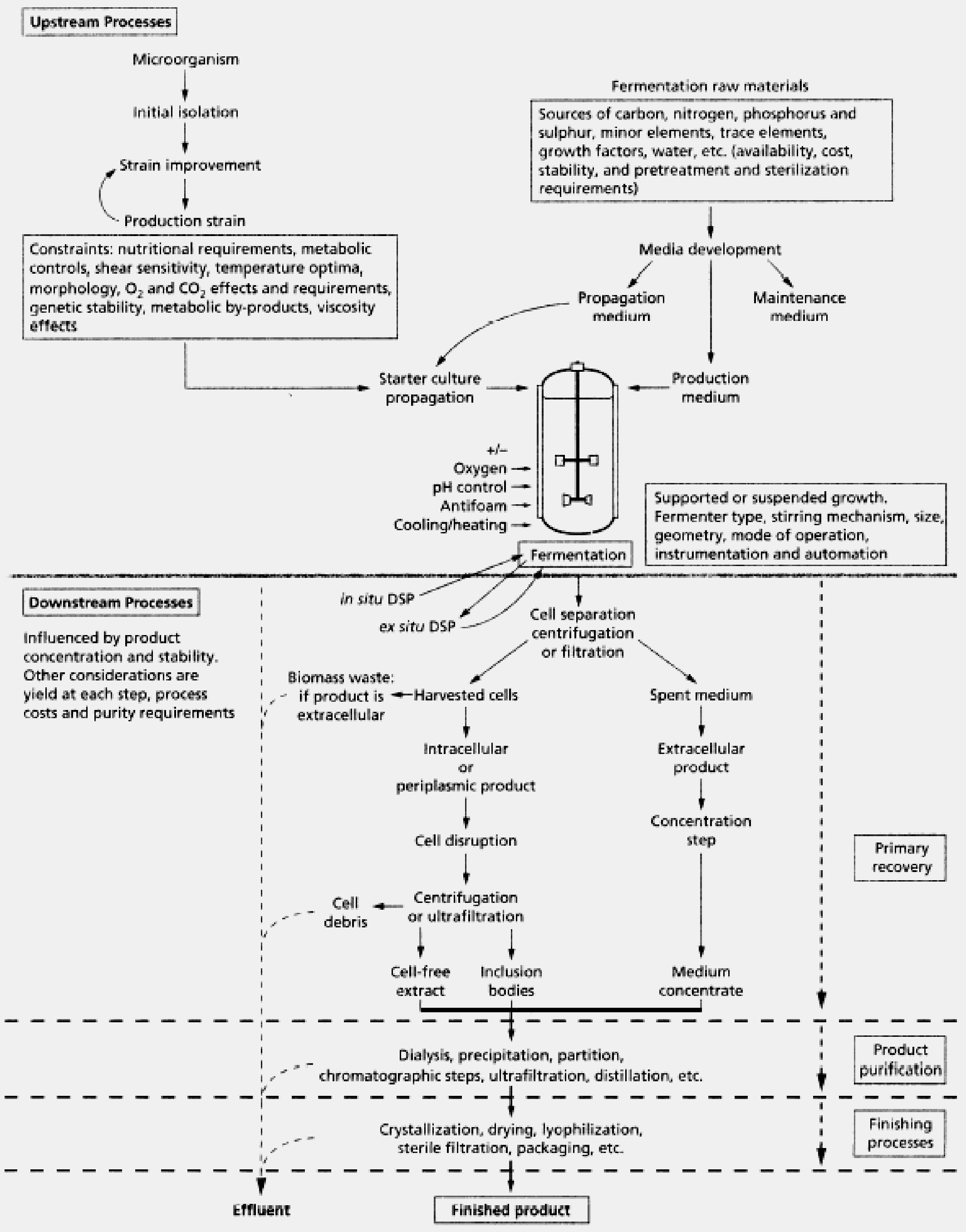
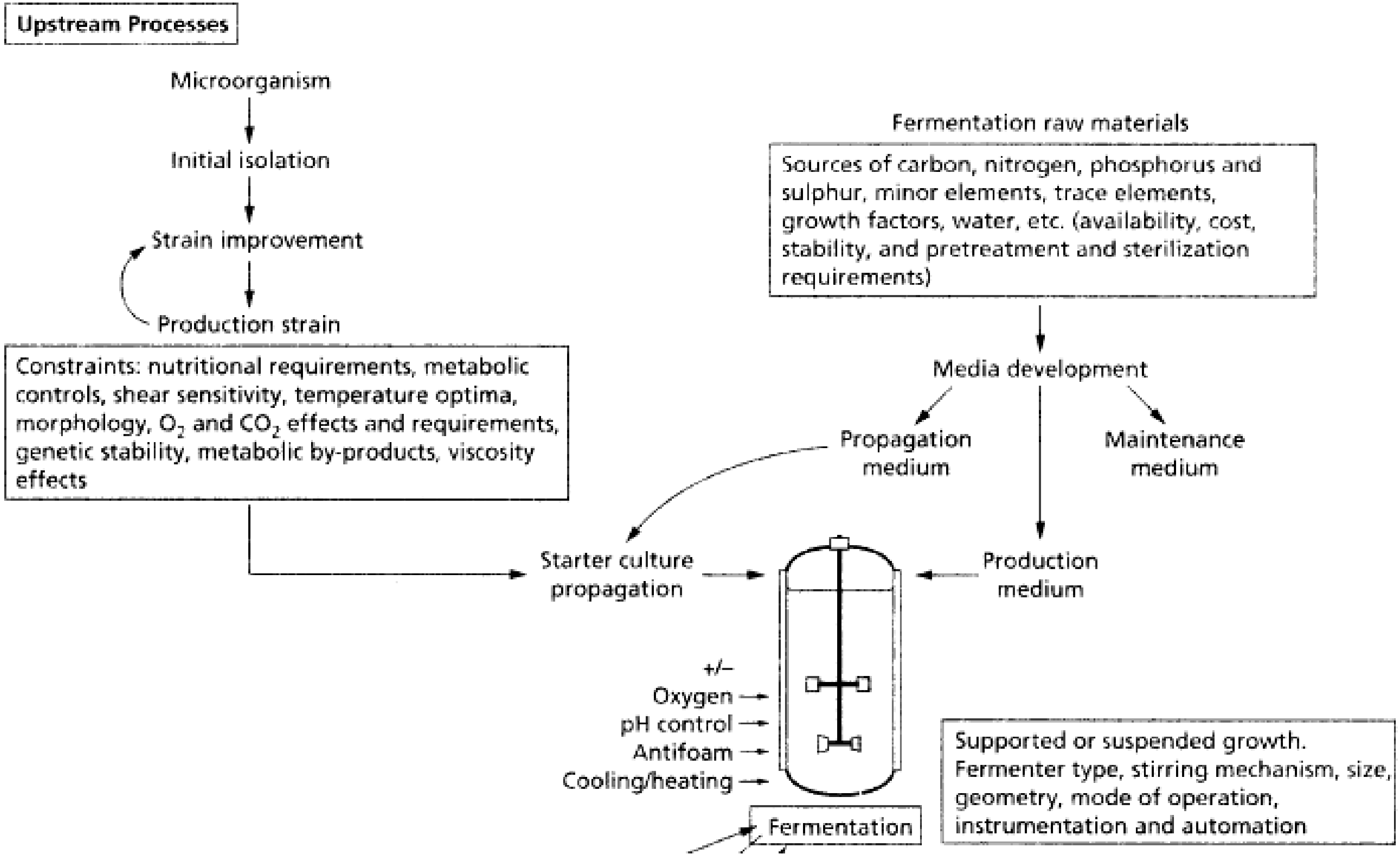
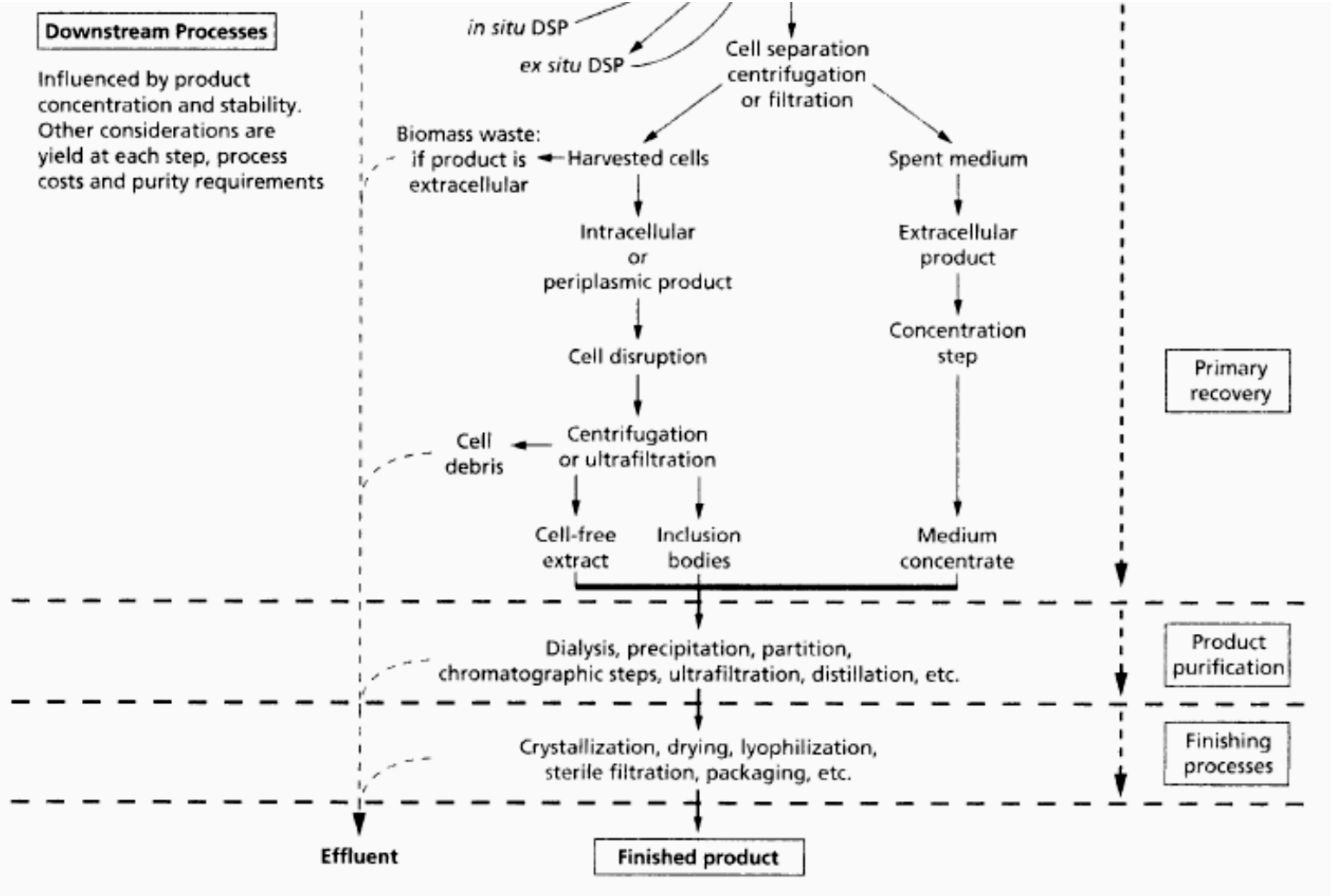
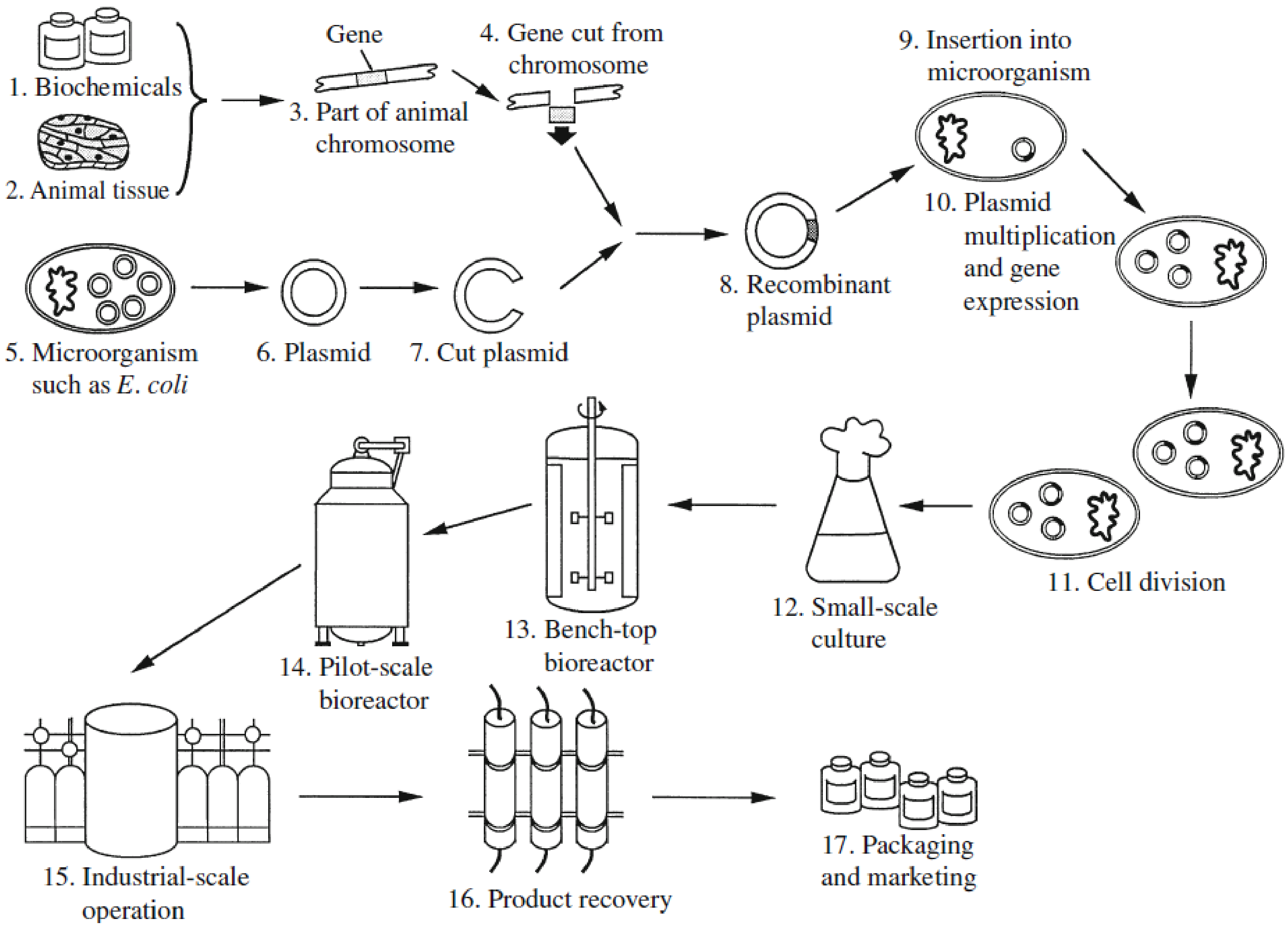


Fig. 7.1 An outline of upstream and downstream processing operations.

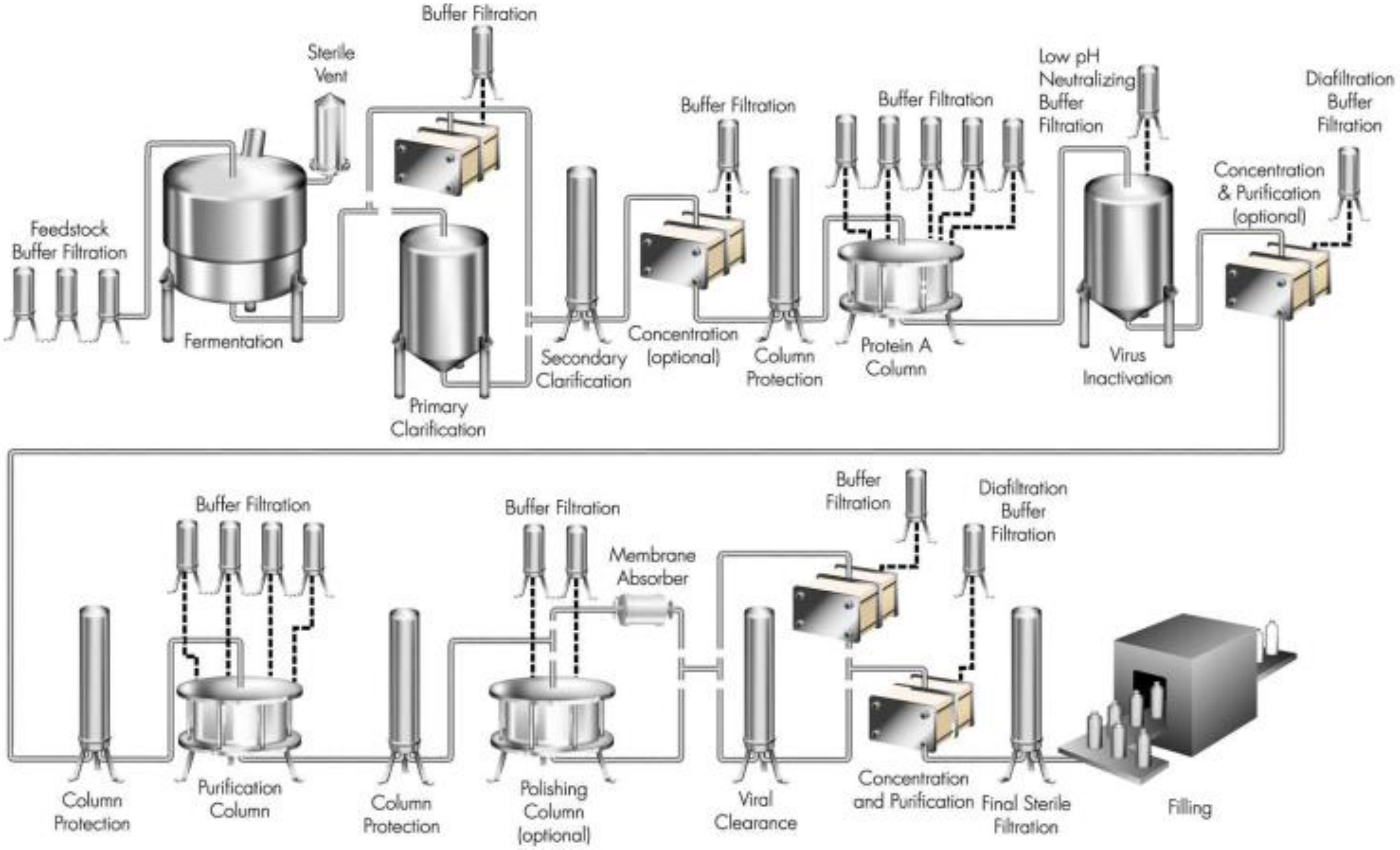

Upstream Processes
Microorganism
Initial isolation
Strain improvement
Production strain
Constraints: nutritional requirements, metabolic controls, shear sensitivity, temperature optima, morphology, O_2 and CO_2 effects and requirements, genetic stability, metabolic by-products, viscosity effects
Fermentation raw materials
Sources of carbon, nitrogen, phosphorus and sulphur, minor elements, trace elements, growth factors, water, etc. (availability, cost, stability, and pretreatment and sterilization requirements)
Media development
Propagation medium
Maintenance medium
Starter culture propagation
Production medium
+/–
Oxygen
pH control
Antifoam
Cooling/heating
Fermentation
Supported or suspended growth. Fermenter type, stirring mechanism, size, geometry, mode of operation, instrumentation and automation

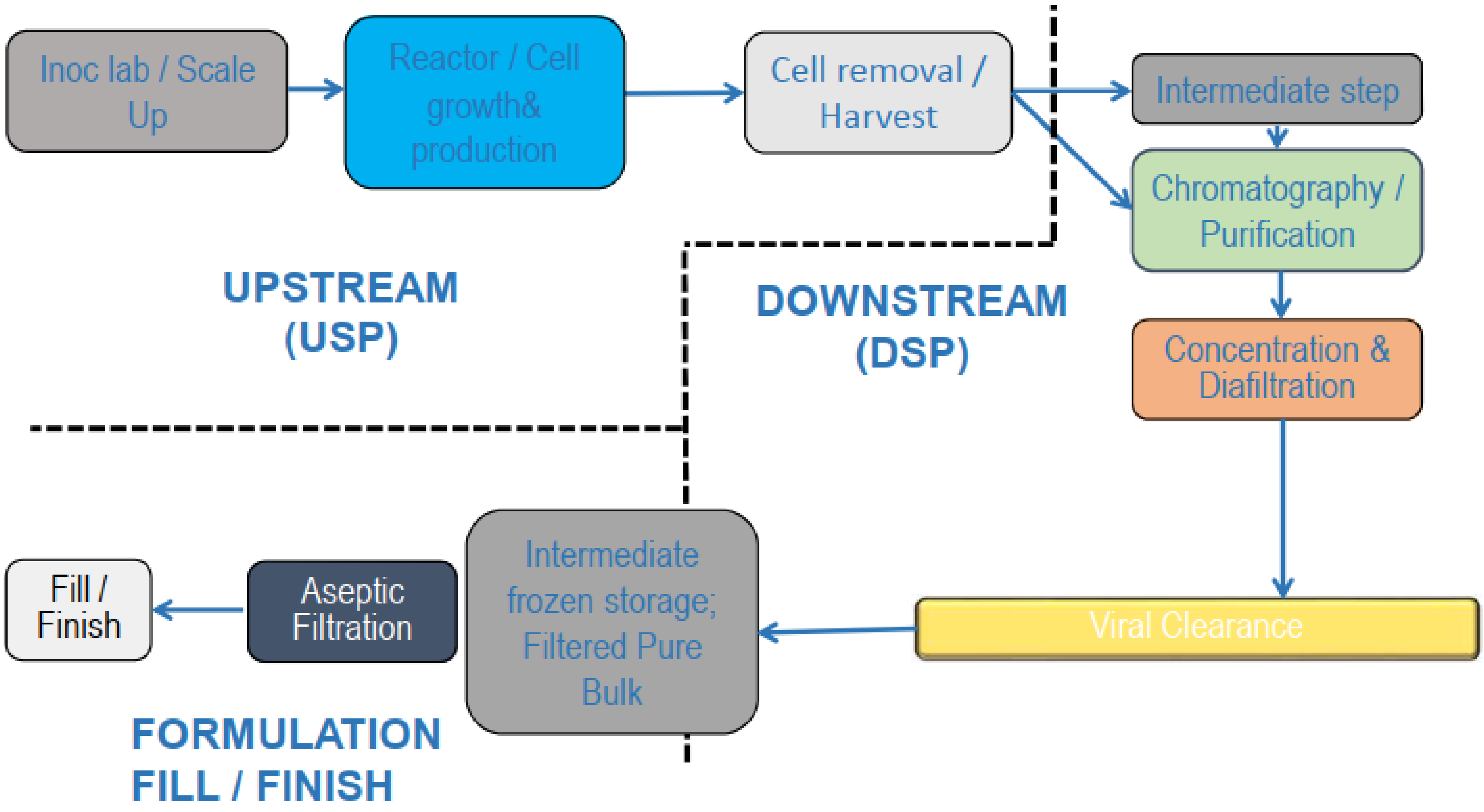

Downstream Processes
Influenced by product concentration and stability. Other considerations are yield at each step, process costs and purity requirements
in situ DSP
ex situ DSP
Cell separation centrifugation or filtration
Biomass waste: if product is extracellular
Harvested cells
Spent medium
Intracellular or periplasmic product
Extracellular product
Concentration step
Cell disruption
Primary recovery
Cell debris
Centrifugation or ultrafiltration
Cell-free extract
Inclusion bodies
Medium concentrate
Dialysis, precipitation, partition, chromatographic steps, ultrafiltration, distillation, etc.
Product purification
Crystallization, drying, lyophilization, sterile filtration, packaging, etc.
Finishing processes
Effluent
Finished product

Stages of a typical bioprocess: recombinant DNA-derived product



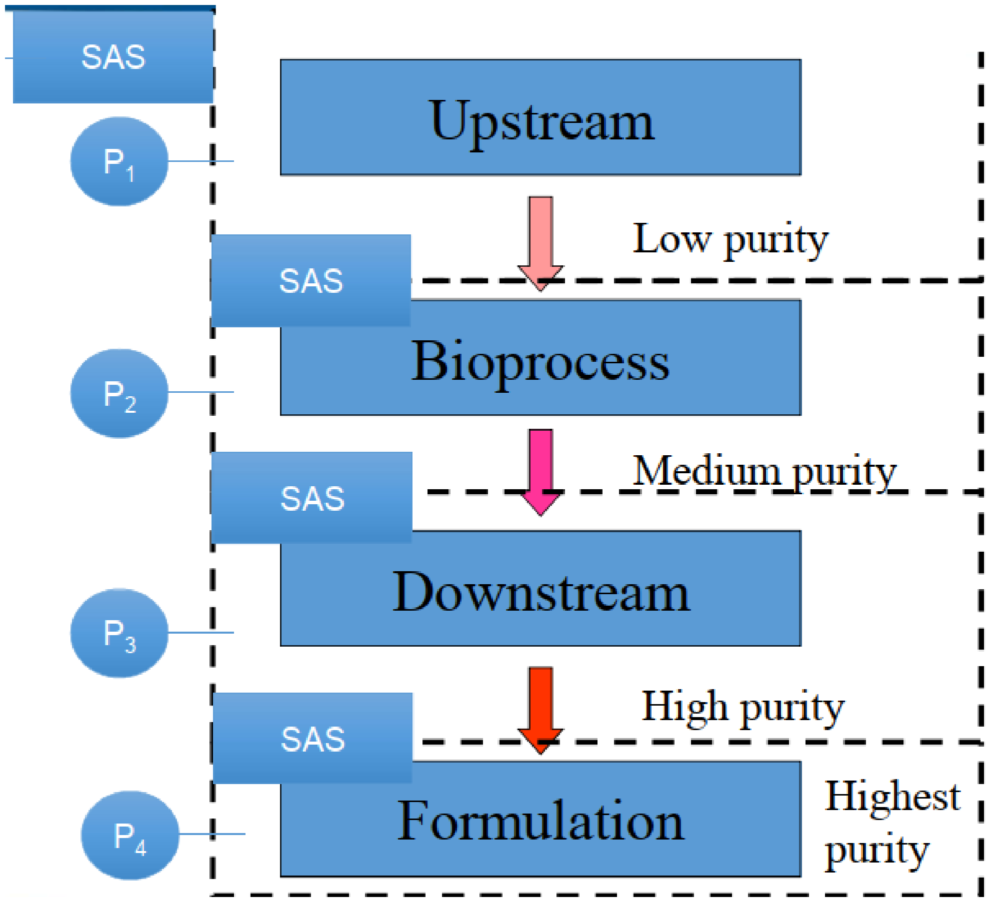
Stages of a typical bioprocess. e.g. mAbs





Inoc lab / Scale Up
Reactor / Cell growth& production
Cell removal / Harvest
Intermediate step
Chromatography / Purification
Concentration & Diafiltration
UPSTREAM (USP)
DOWNSTREAM (DSP)
Viral Clearance
Intermediate frozen storage; Filtered Pure Bulk
Aseptic Filtration
Fill / Finish
FORMULATION FILL / FINISH

Bioprocess containment / Aseptic conditions



Containment

With respect to 4 criteria

1. Plant environment (internal e.g. personnel)
2. Outside plant (external e.g. environment)
3. Process (contamination from external chemicals or biologicals)
4. Product (contamination by microorganisms, mycoplasmas, retroviruses, host cell proteins, DNA, endotoxins, pyrogens, allergens etc.)

Process validation:

GLP, cGMP, FDA, SKBS, EMA, Swiss Medic etc.

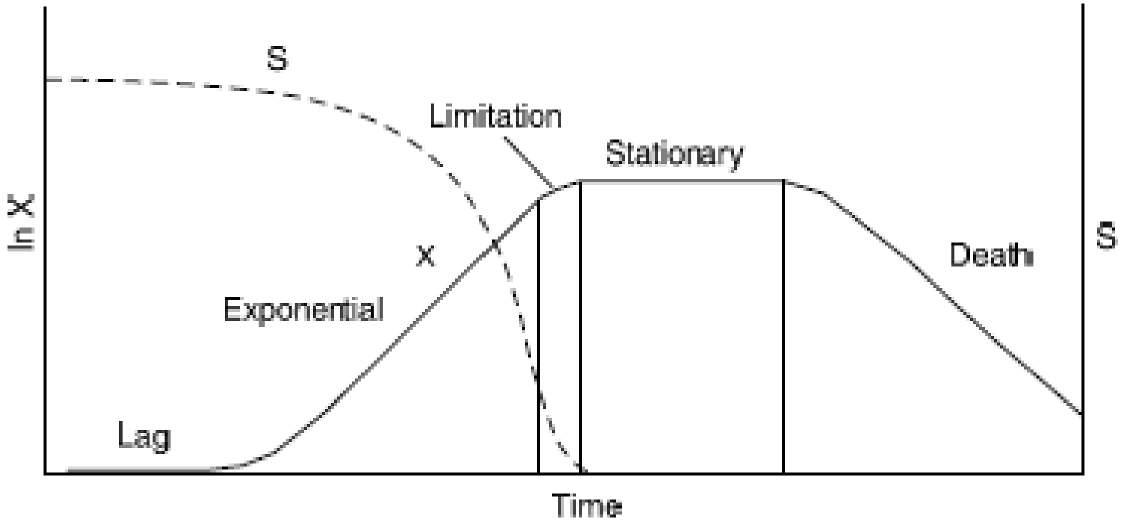
Good manufacturing practice (GMP)

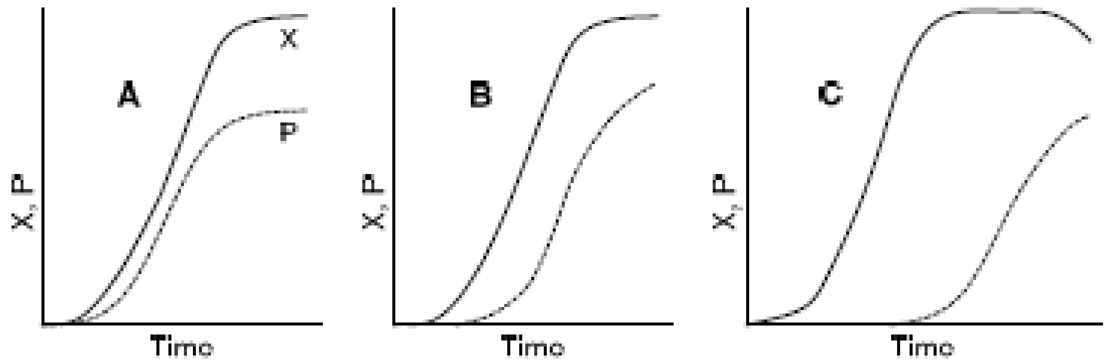
That part of the pharmaceutical quality assurance, which ensures that products are **consistently** produced
and controlled in **conformity** with quality standards appropriate for their **intended use** and as required by the product **specification**.

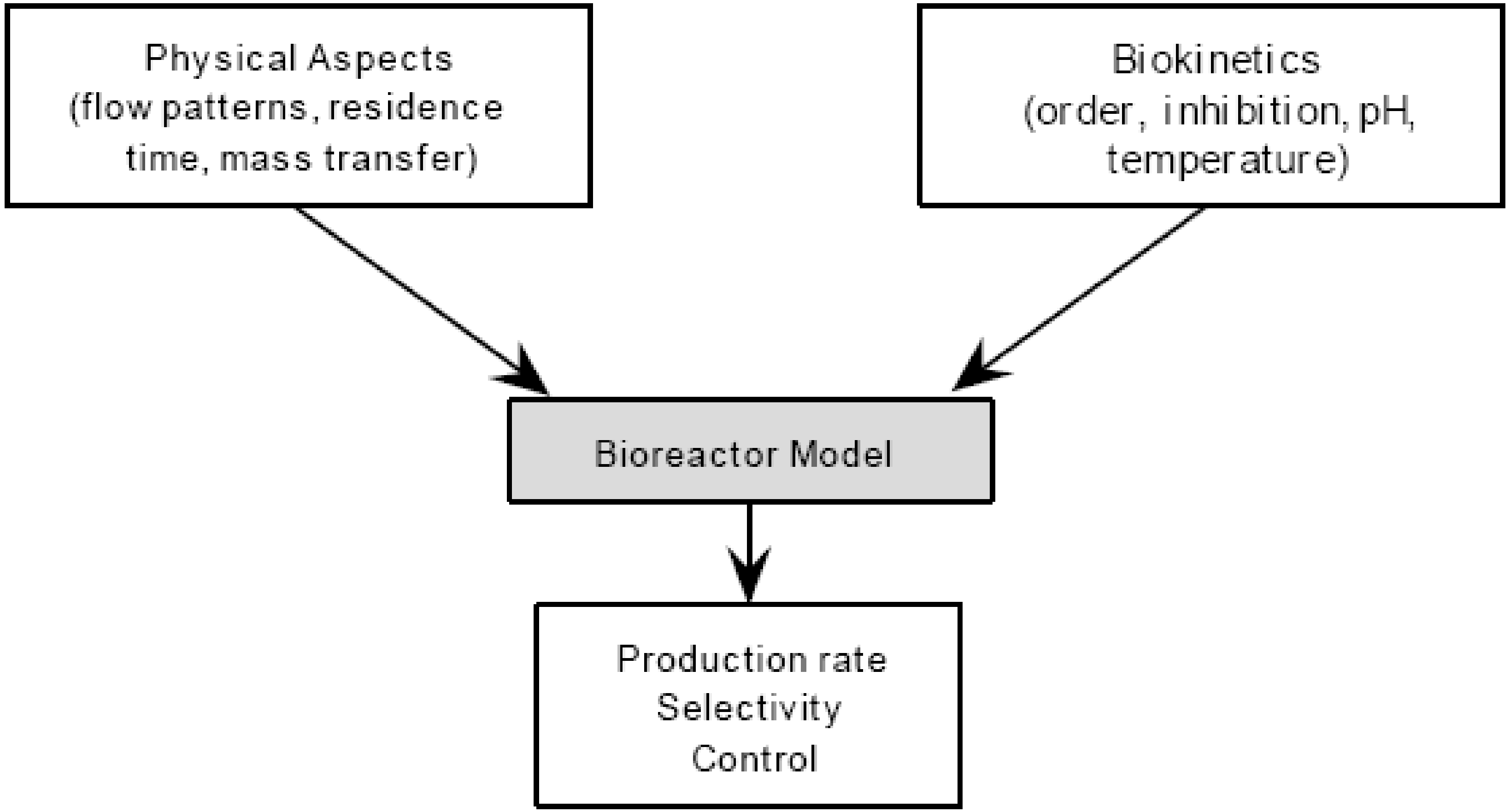
Requirements for Good manufacturing practice (GMP)

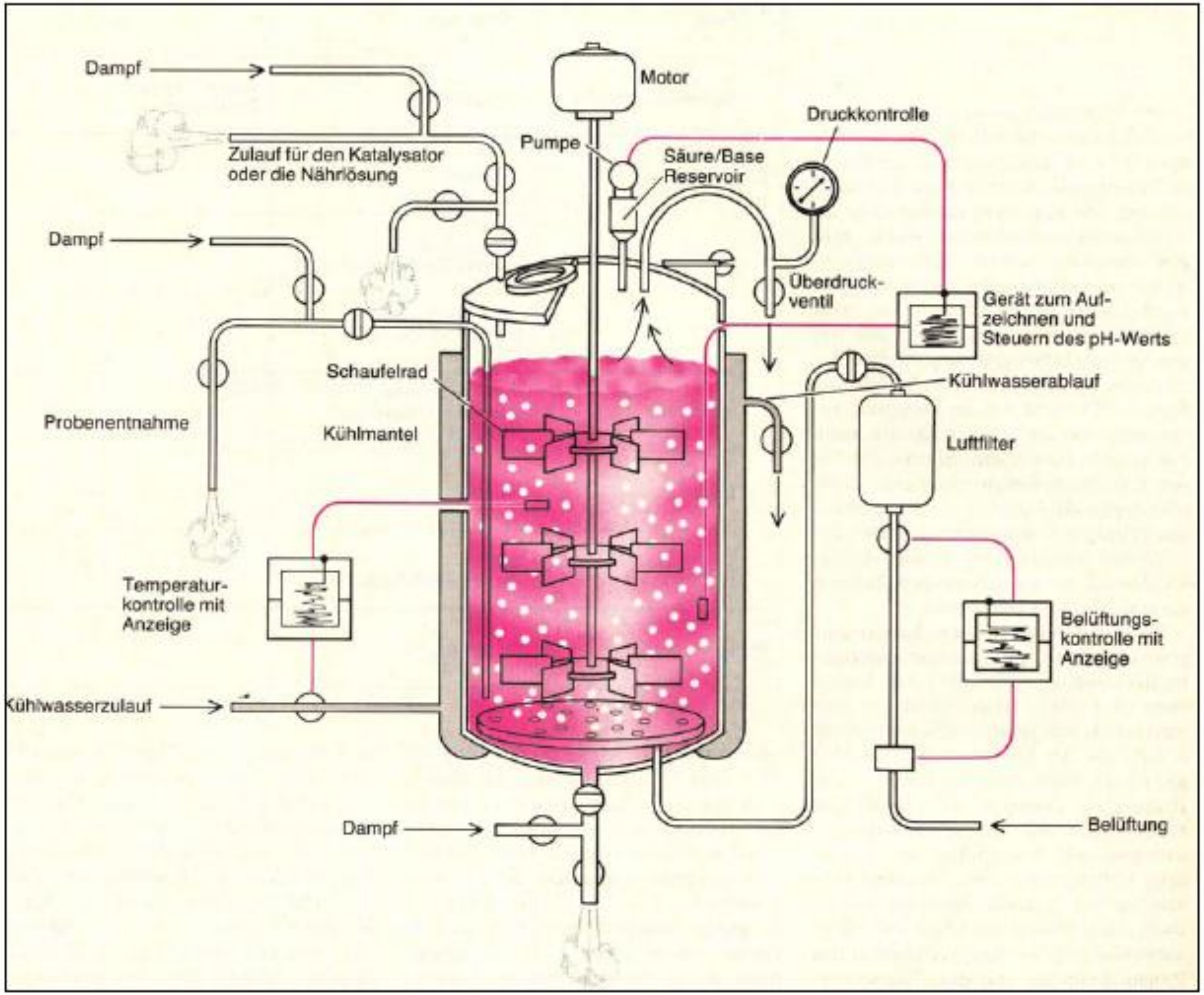
- All manufacturing processes are clearly defined, systematically reviewed and shown to be consistent.
- Critical steps and significant changes are validated.
- Procedures are written down in SOP format, all equipment qualified and all operators trained.
- Records necessary to show quantity / quality of product was as expected.
- All deviations must be fully recorded and investigated.
- Records of all manufacture including distribution retained.

Kinetics

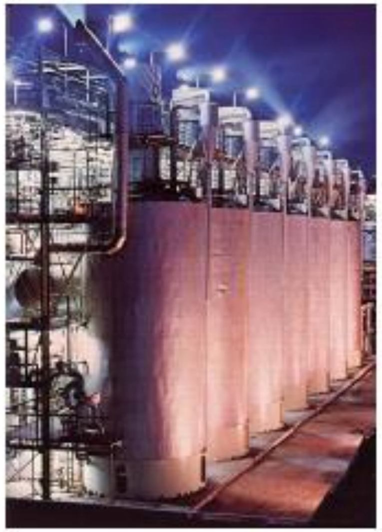

S
Limitation
Stationary
ln X
X
Exponential
Death
S
Lag
Time

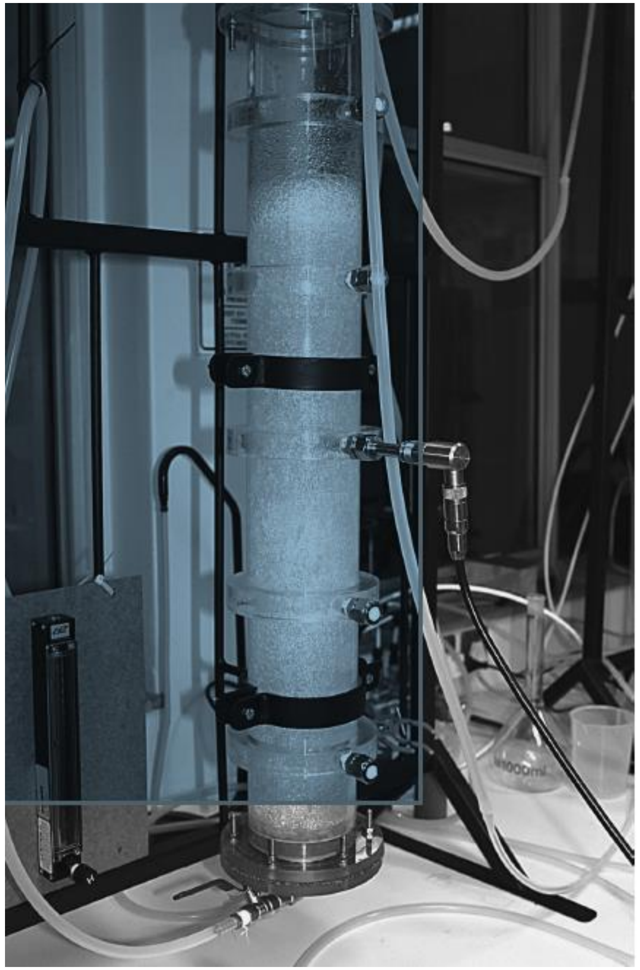

A
X
P
X, P
Time
B
X, P
Time
C
X, P
Time

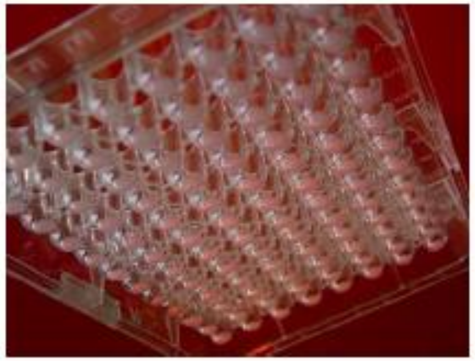

Physical Aspects
(flow patterns, residence time, mass transfer)
Biokinetics
(order, inhibition, pH, temperature)
Bioreactor Model
Production rate
Selectivity
Control


Dampf
Zulauf für den Katalysator
oder die Nährlösung
Motor
Pumpe
Säure/Base
Reservoir
Druckkontrolle
Dampf
Überdruck-
ventil
Gerät zum Auf-
zeichnen und
Steuern des pH-Werts
Schaufelrad
Kühlwasserablauf
Probenentnahme
Kühlmantel
Luftfilter
Temperatur-
kontrolle mit
Anzeige
Belüftungs-
kontrolle mit
Anzeige
Kühlwasserzulauf
Dampf
Belüftung

Bioreactors / Fermenters







SUT = Single Use Technology

Course content

- Bioreactor designing
- Classification of Bioreactor process
- Components/ Parameter of bioreactor
- Types of bioreactor
- Recent advance in bioreactor design
- Application

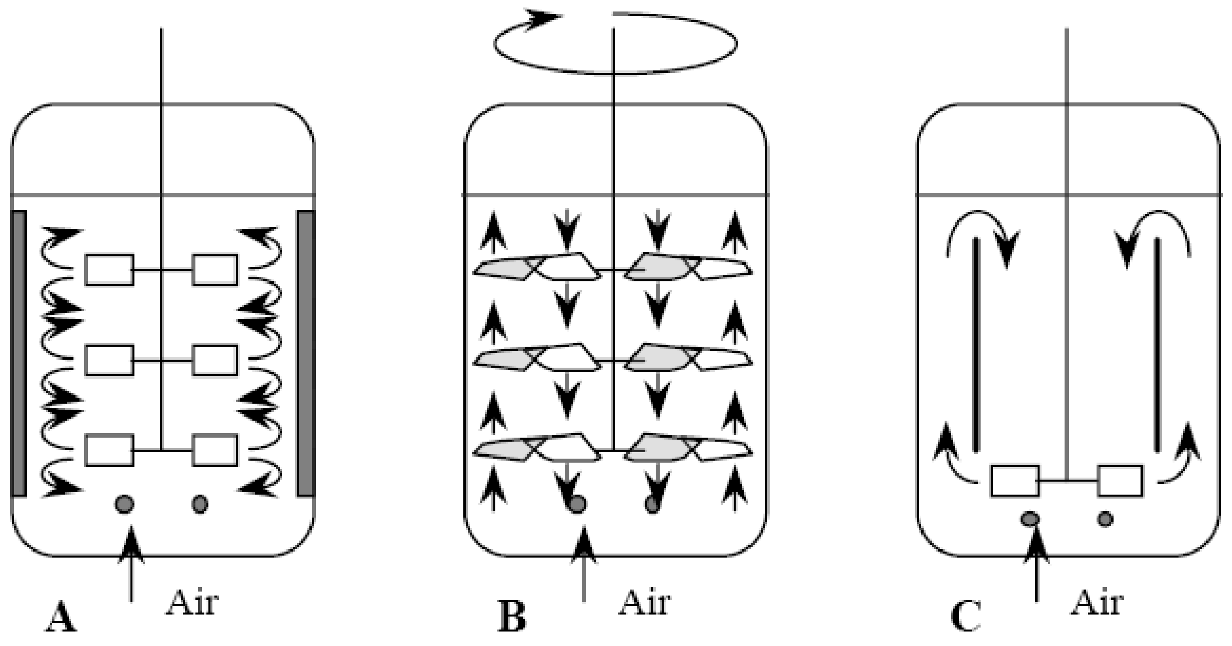
Bioreactor

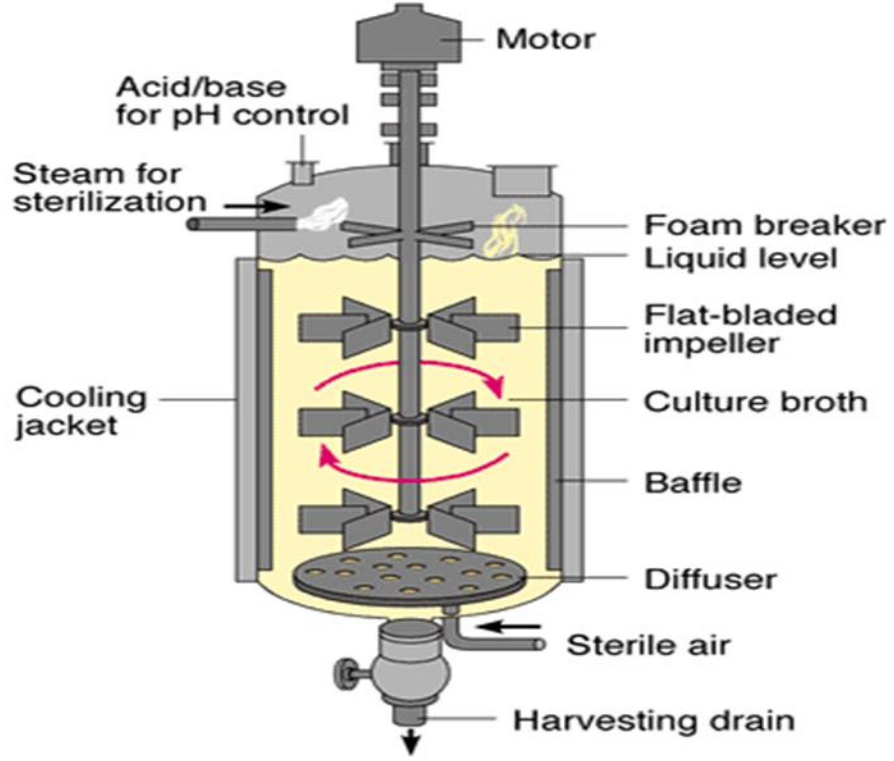
A bioreactor may refer to a device or system meant to grow animal cells or tissues in the context of cell culture. These devices are being developed for use in tissue engineering or biochemical engineering.

Fermenter

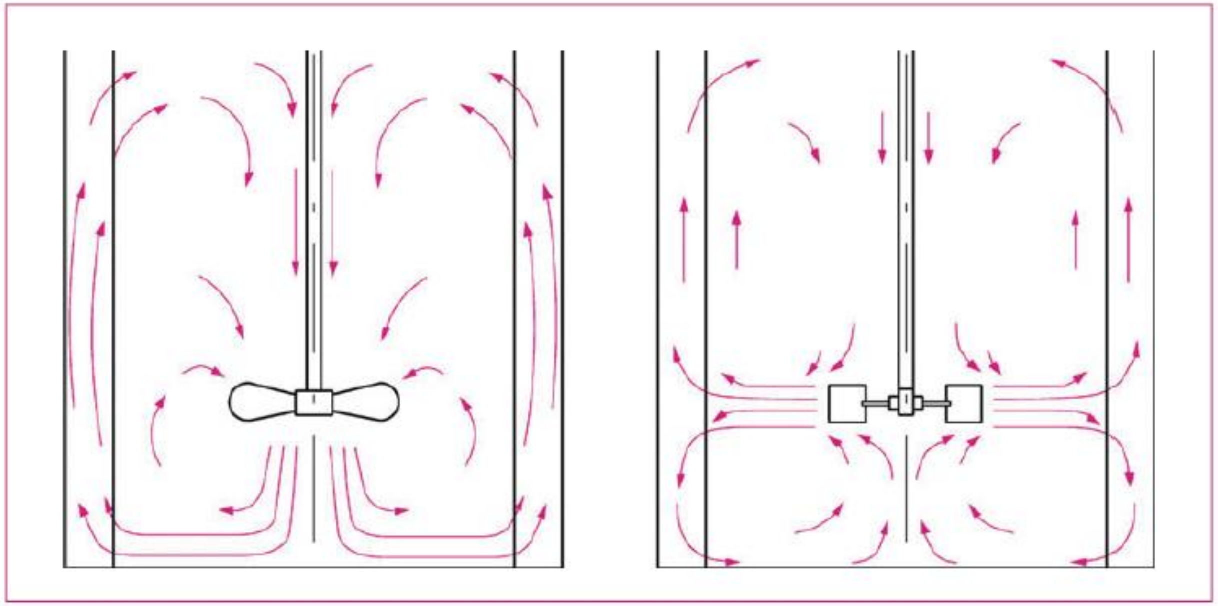
Fermenters are well established for the cultivation of microbes ,proteins ,industrial product(acetic acid, alcohol etc.) under monitored ,controlled environmental and operational conditions up to an industrial scale.

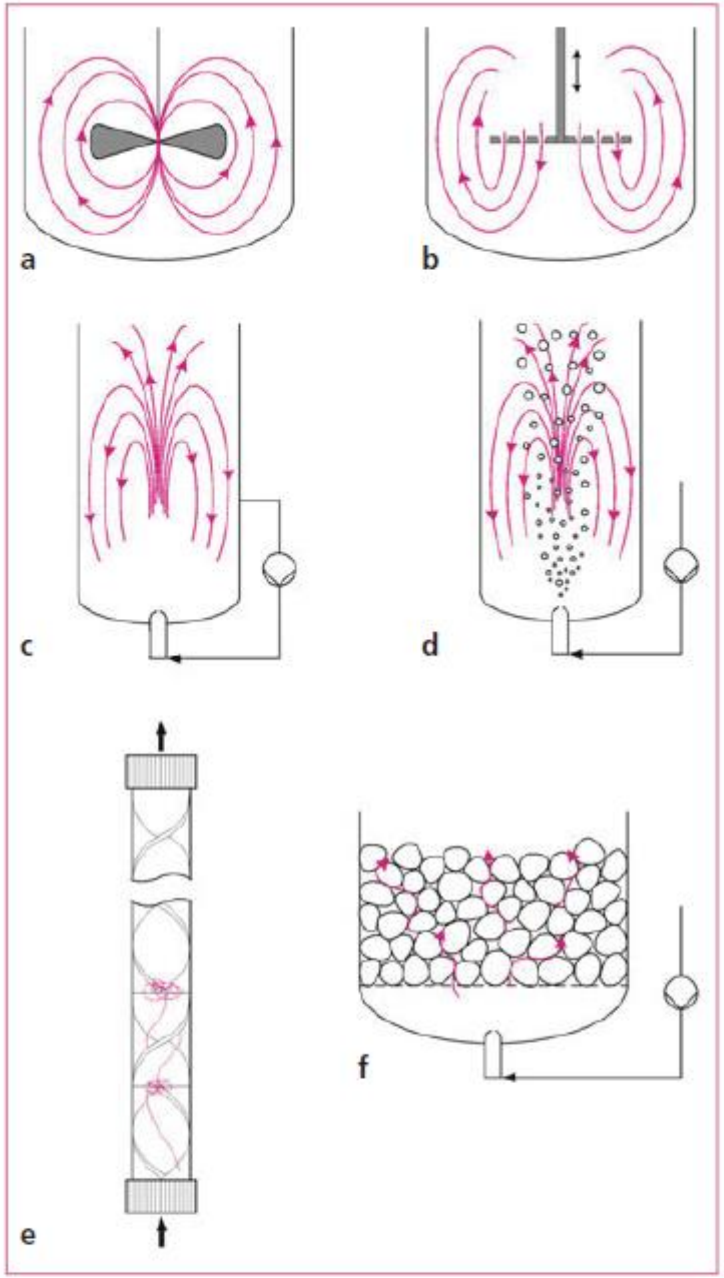
Stirred Bioreactors





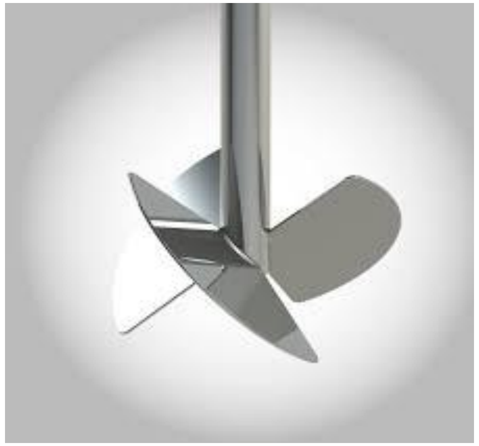
Propeller and Impeller Mixing

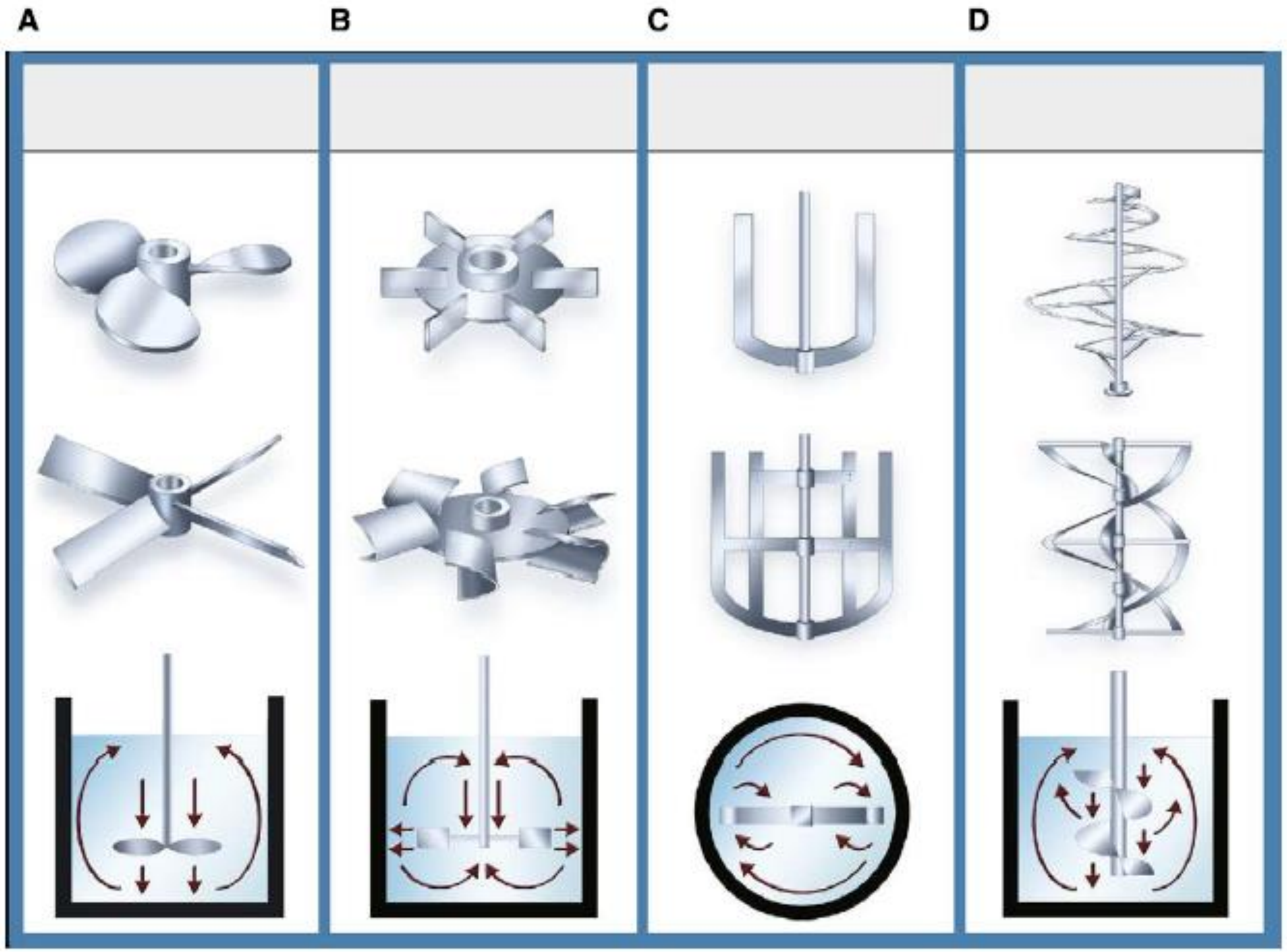




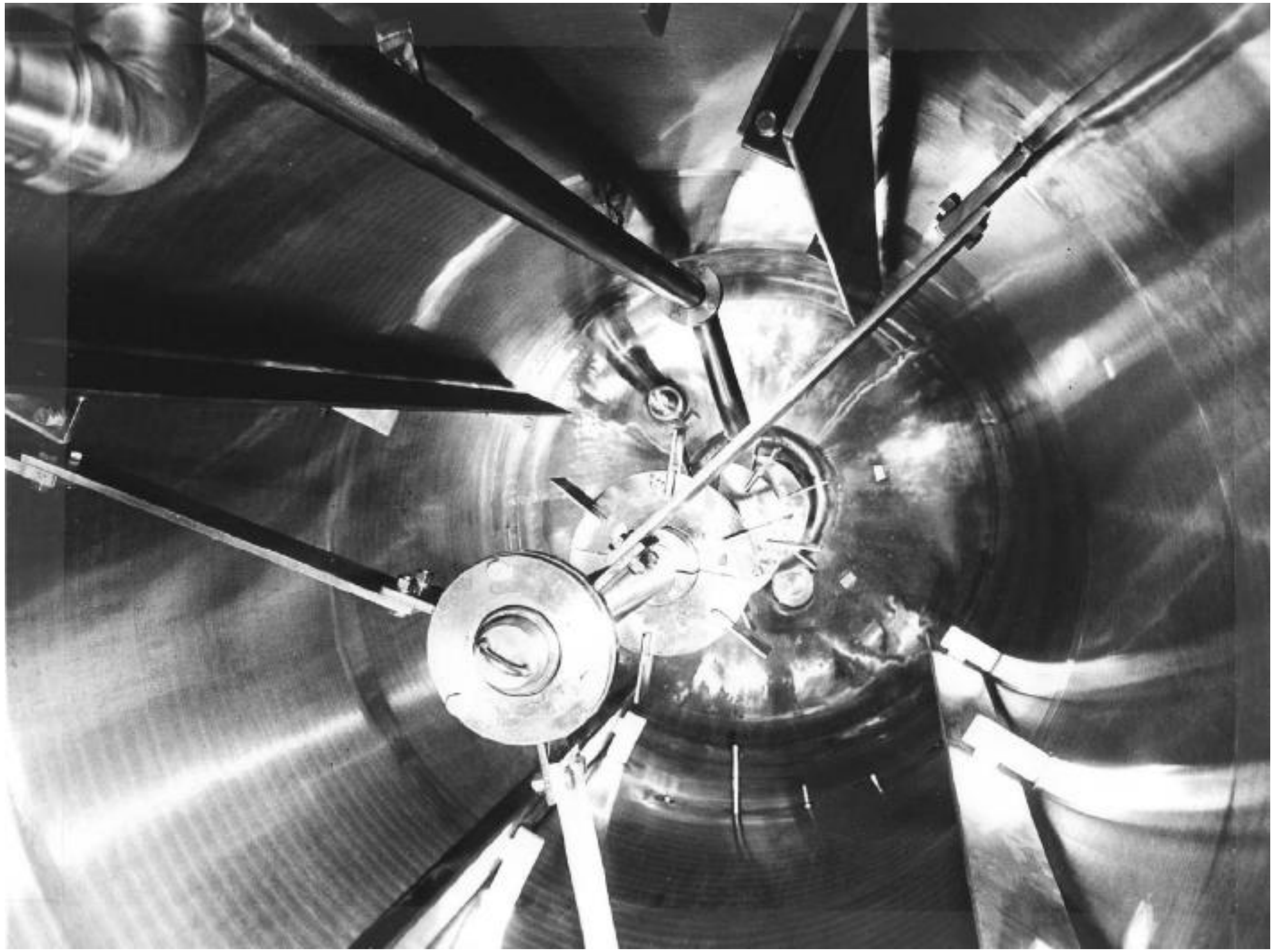


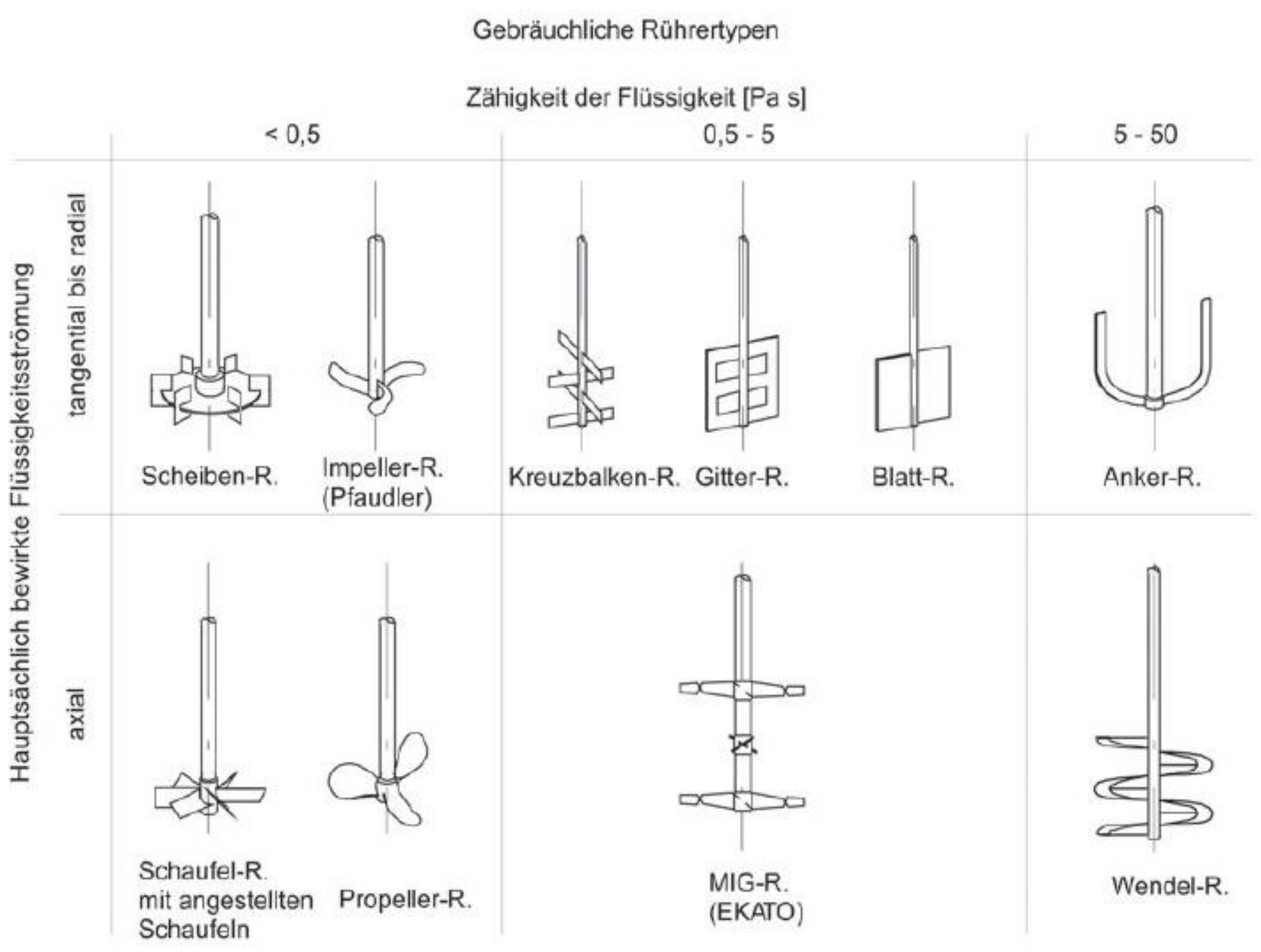




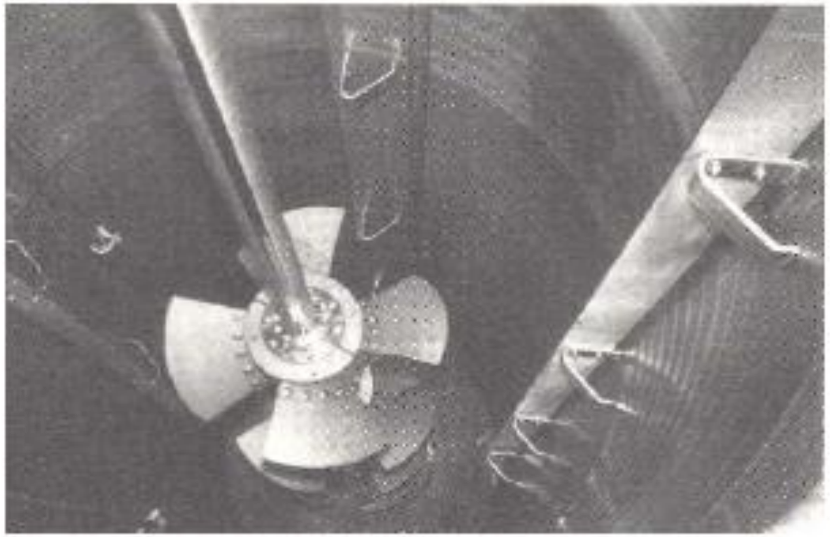



Some types of impellers employed in STBR and the predominant flow pattern induced: (A) propeller and pitched blade (axial flow); (B) turbine (radial flow); (C) anchor (tangential flow); (D) helical (axial flow).

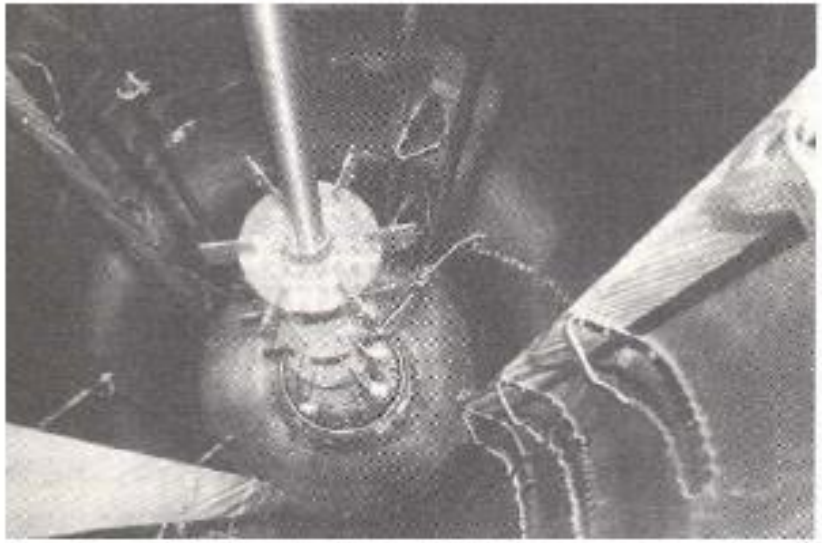



Gebräuchliche Rührertypen
Zähigkeit der Flüssigkeit [Pa s]
< 0,5
0,5 - 5
5 - 50
Hauptsächlich bewirkte Flüssigkeitsströmung
tangential bis radial
axial
Scheiben-R.
Impeller-R. (Pfaudler)
Kreuzbalken-R.
Gitter-R.
Blatt-R.
Anker-R.
Schaufel-R. mit angestellten Schaufeln
Propeller-R.
MIG-R. (EKATO)
Wendel-R.

Mixing, Mass and Heat Transfer

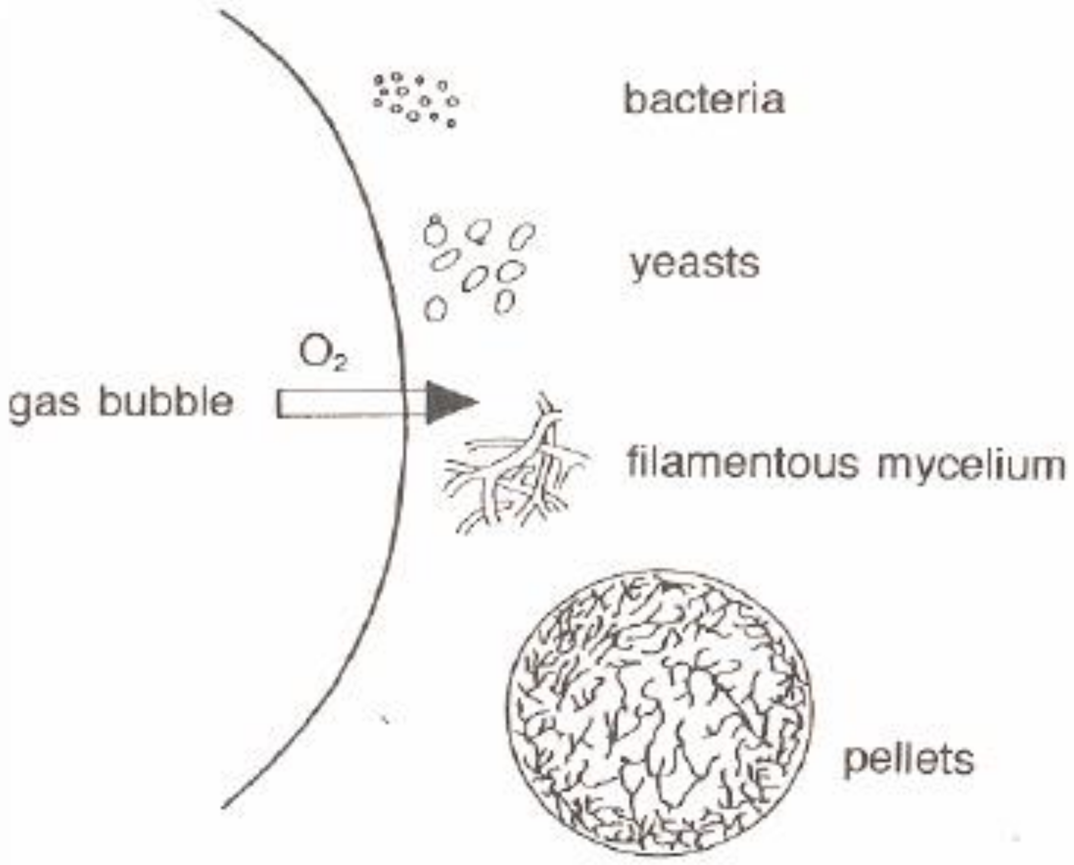


Hydrofoil impeller



Turbine impeller

Oxygen Transfer



Immobilized Biocatalyst Recycle Reactors

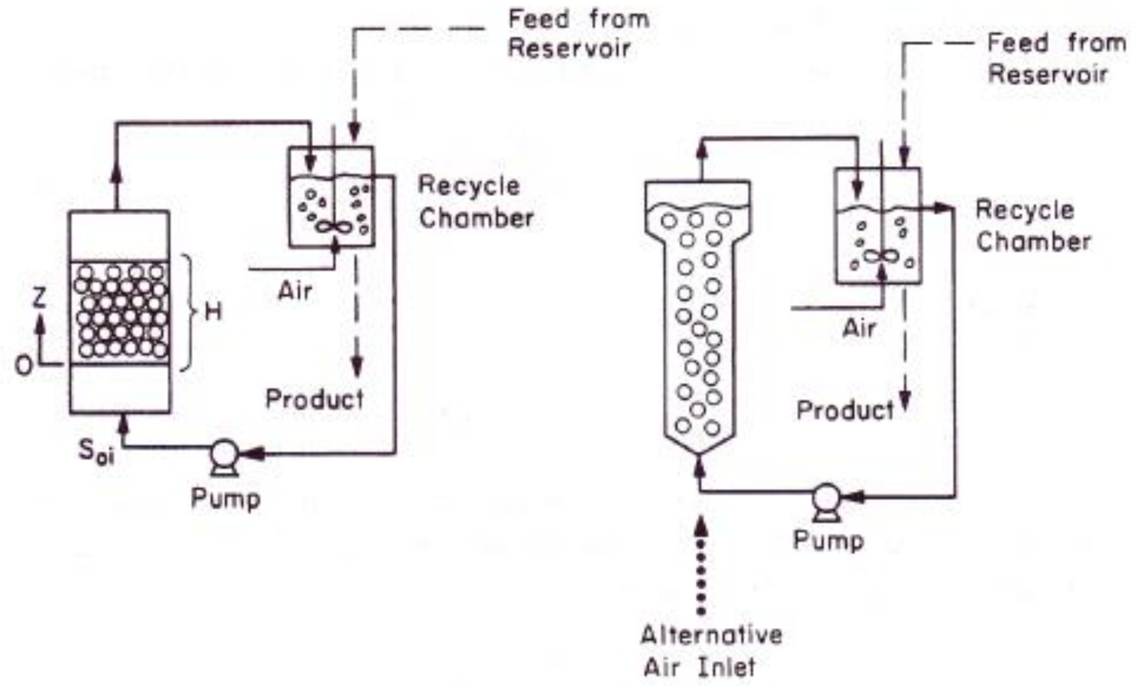


Figure 9.15. Schematics of a packed-bed and a fluidized-bed biofilm or immobilized-cell bioreactors are shown. In batch operation, only the streams with solid lines exist. In continuous operation, the streams shown by dashed lines are added. For the fluidized bed, fluidization can be accomplished by liquid recirculation only or a mixture of liquid and gas flows.

Perfusion Reactor

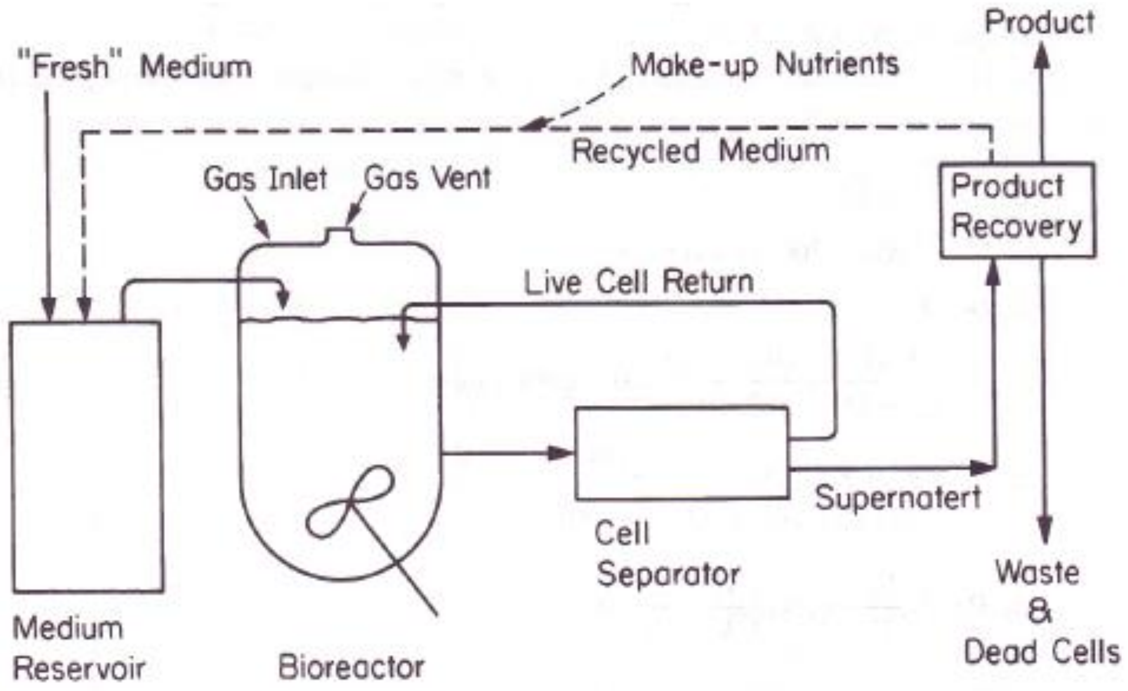
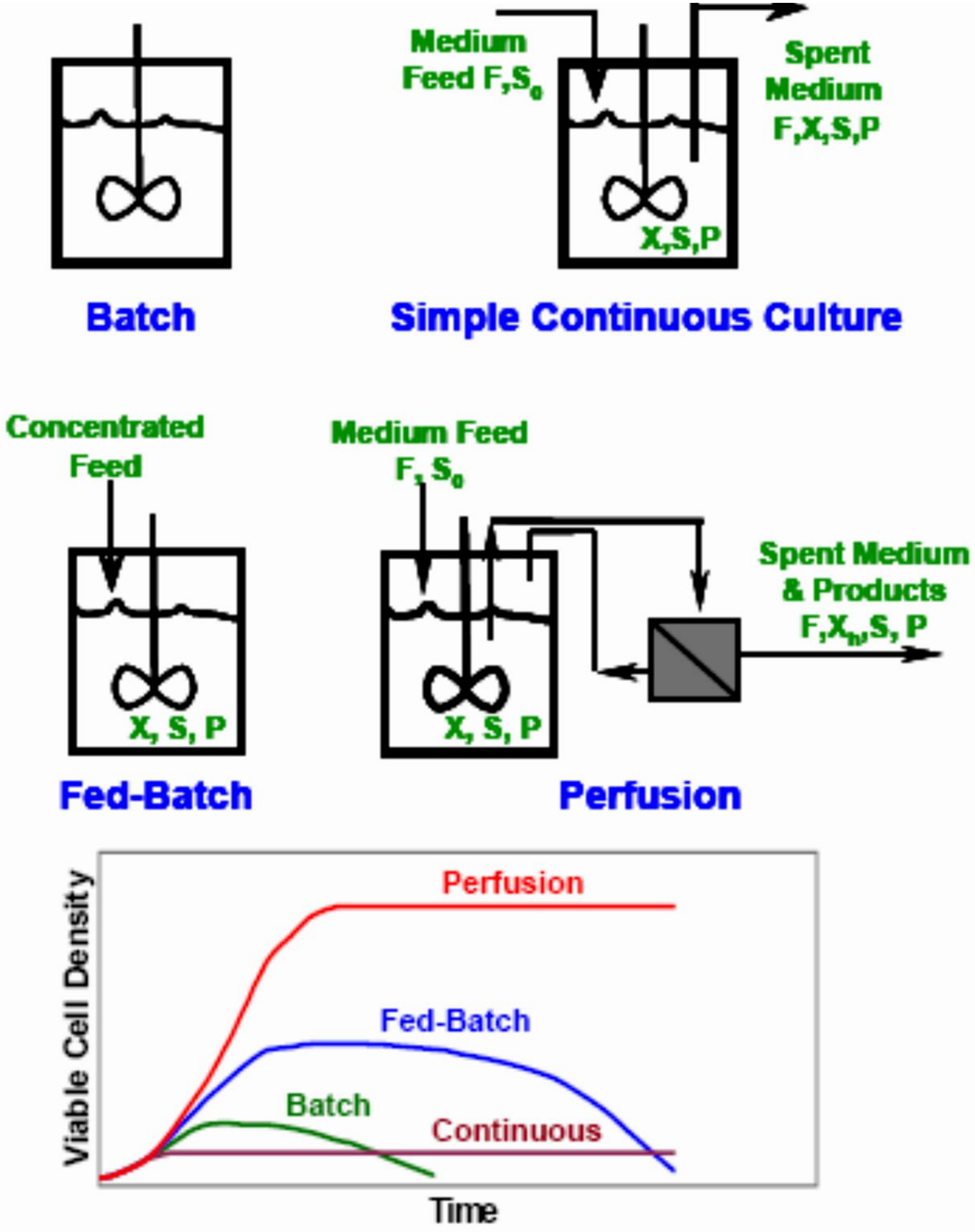
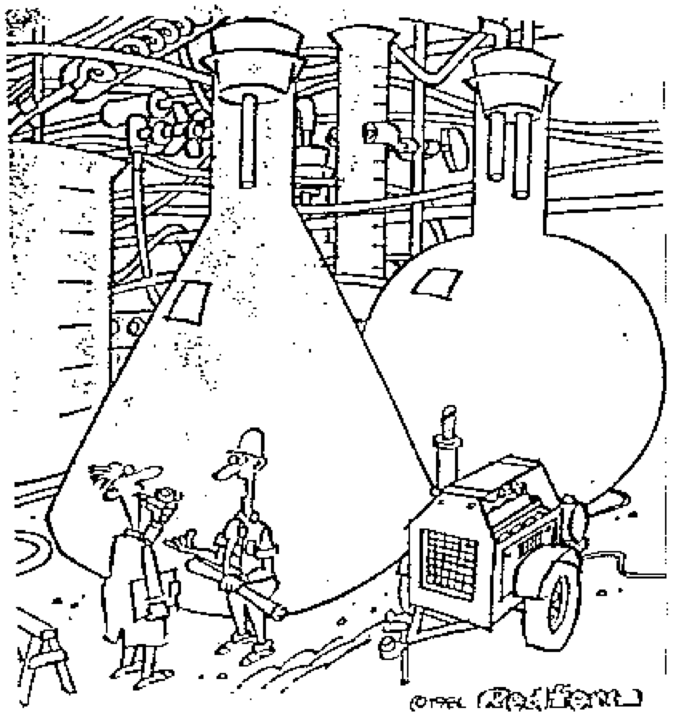


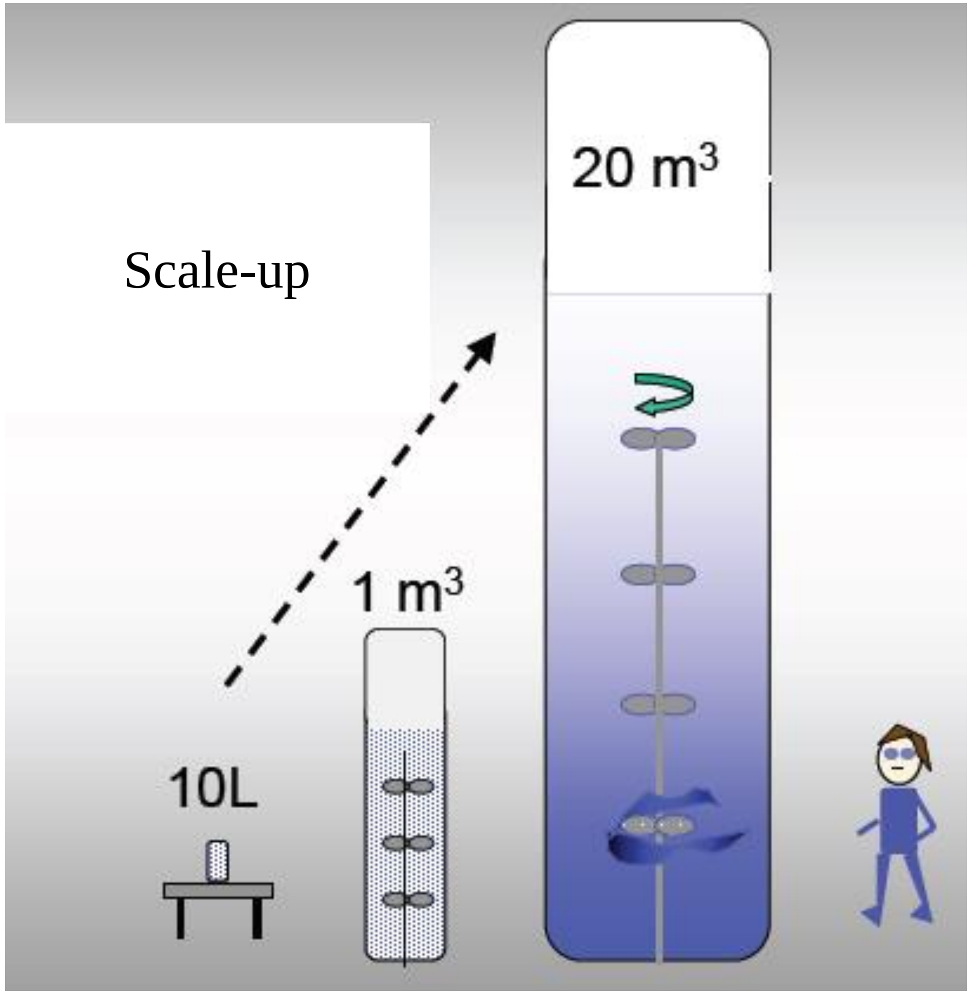
Fig. 9.10. Schematic of a perfusion system with external centrifugation and return of cells. Internal retention of cells is also possible. Return of spent medium is optional.



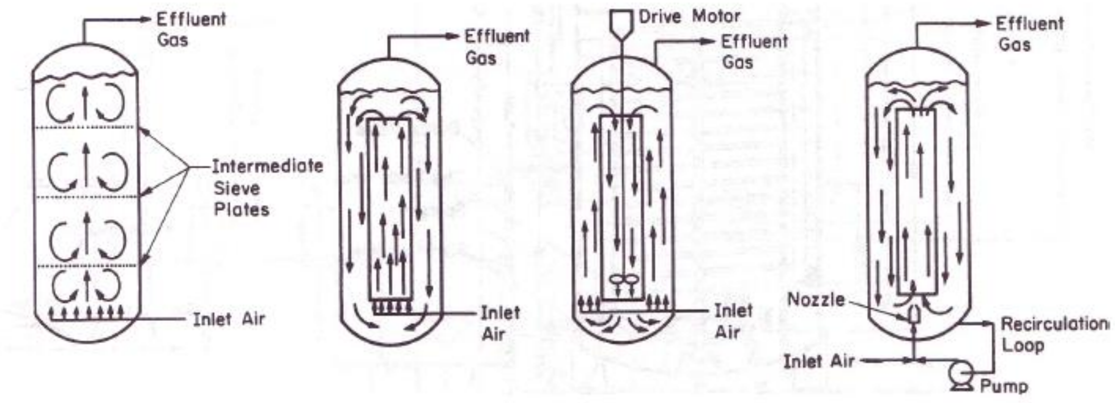
Batch
Medium Feed F,S0
Spent Medium F,X,S,P
X,S,P
Simple Continuous Culture
Concentrated Feed
X, S, P
Fed-Batch
Medium Feed F, S0
Spent Medium & Products F,Xh,S, P
X, S, P
Perfusion
Viable Cell Density
Perfusion
Fed-Batch
Batch
Continuous
Time

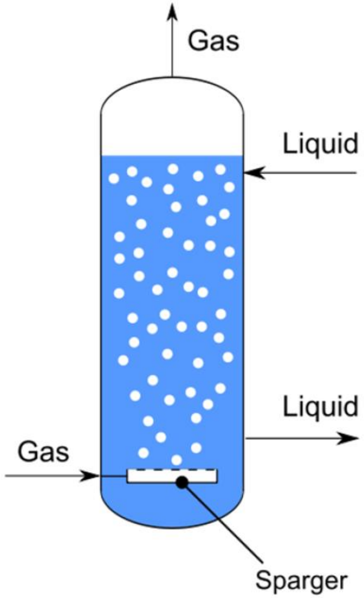


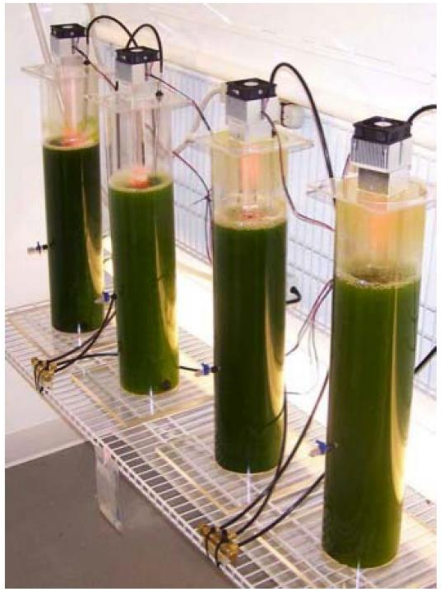
"Got a few problems going from lab scale up to full-scale commercial."

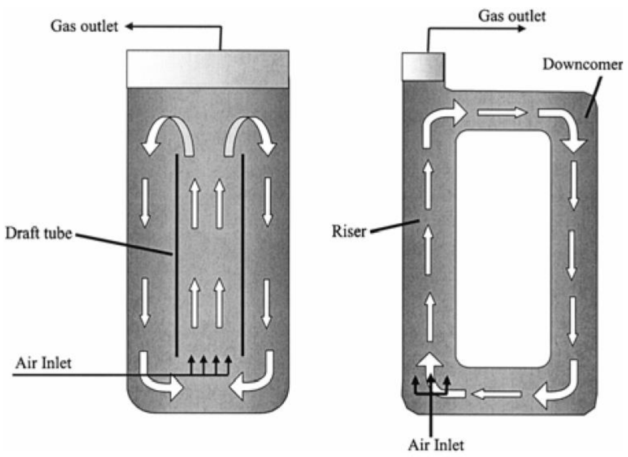


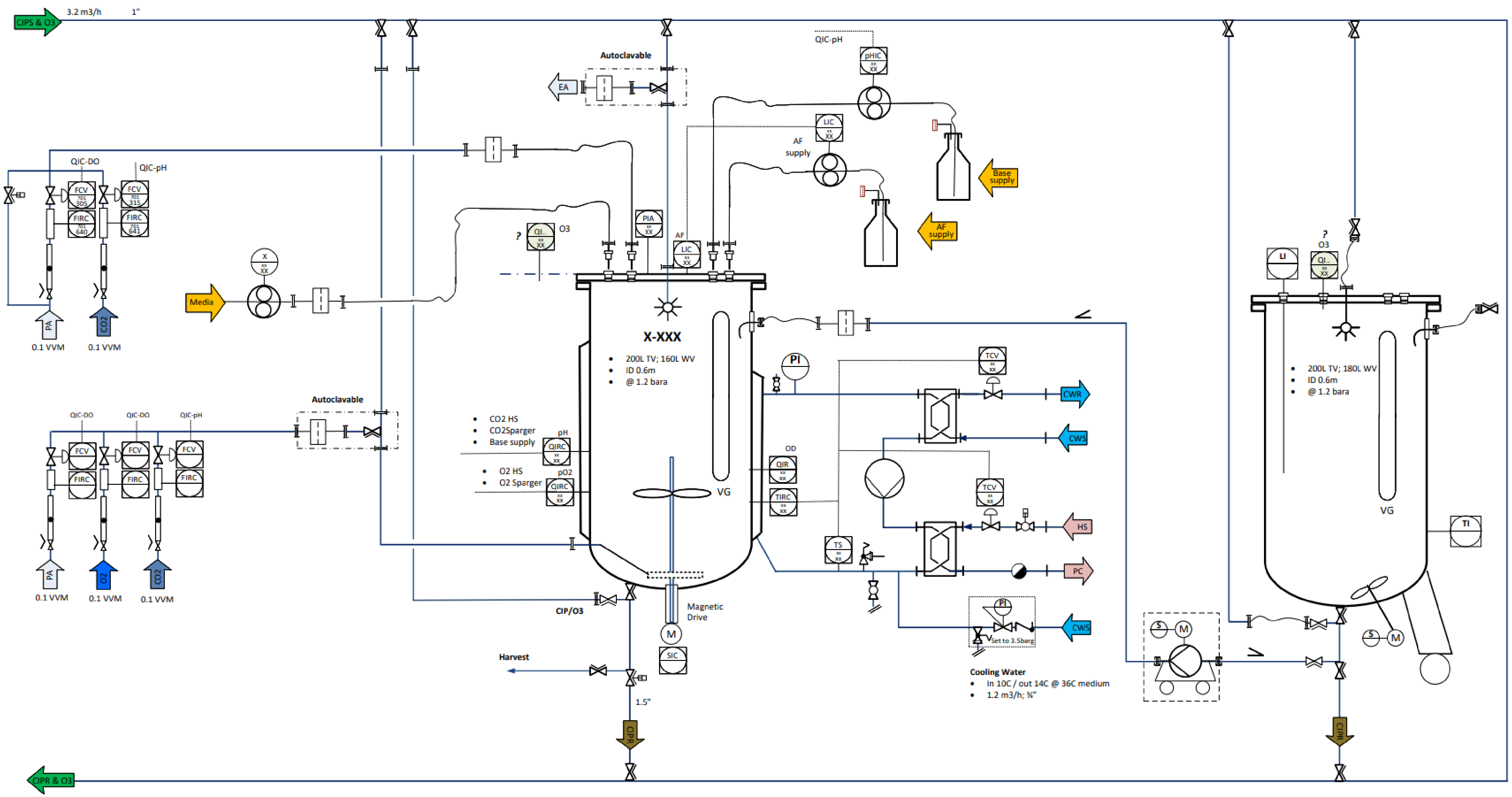
Bubble-Column and Draft-Tube Bioreactors





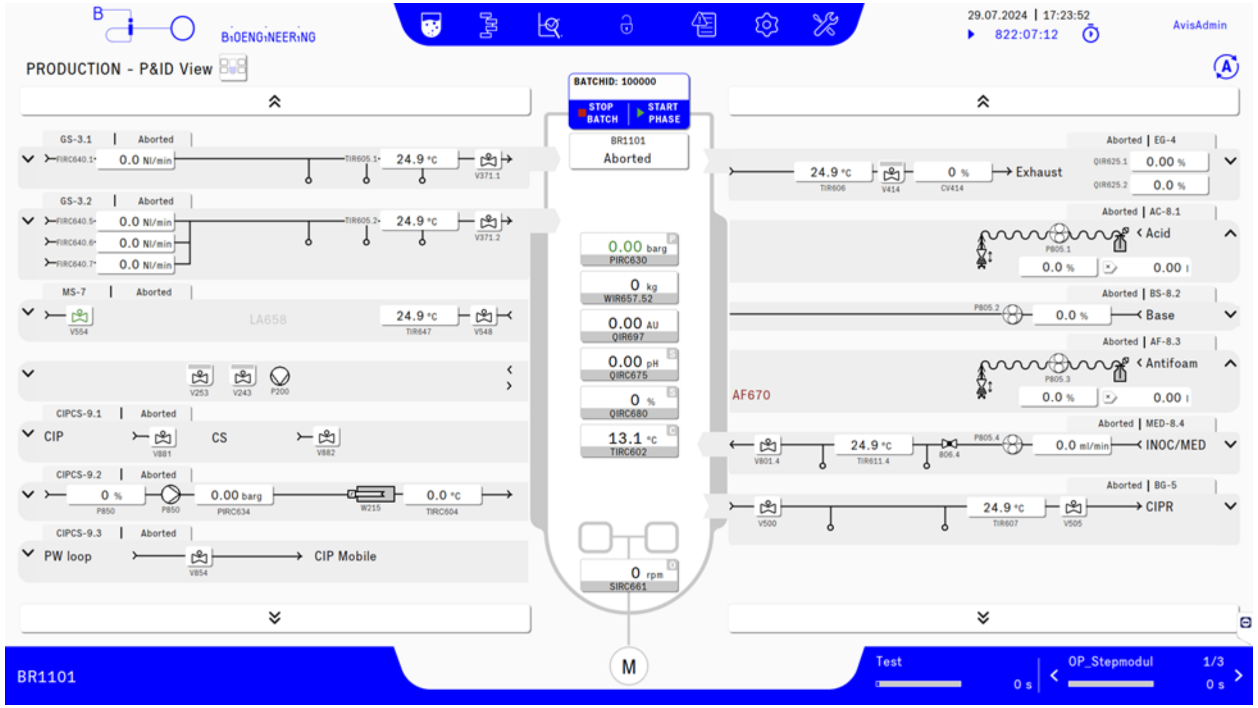






CIPS & O3
3.2 m3/h
1"
Autoclavable
EA
QIC-pH
pHIC
LIC
AF supply
Base supply
AF supply
QIC-DO
QIC-pH
FCV 701 305
FCV 701 315
FIRC 701 640
FIRC 701 641
PA
CO2
0.1 VVM
0.1 VVM
Media
X
O3
PIA
AF
LIC
X-XXX
200L TV; 160L WV
ID 0.6m
@ 1.2 bara
PI
TCV
CWR
CWS
Autoclavable
QIC-DO
QIC-DO
QIC-pH
FCV
FCV
FCV
FIRC
FIRC
FIRC
PA
O2
CO2
0.1 VVM
0.1 VVM
0.1 VVM
CO2 HS
CO2Sparger
Base supply
pH
QIRC
O2 HS
O2 Sparger
pO2
QIRC
VG
OD
QIR
TIRC
TCV
HS
TS
PC
CIP/O3
Magnetic Drive
M
SIC
Harvest
1.5"
CIPR
PI
Set to 3.5barg
CWS
Cooling Water
In 10C / out 14C @ 36C medium
1.2 m3/h; ¾"
LI
O3
QI..
200L TV; 180L WV
ID 0.6m
@ 1.2 bara
VG
TI
S
M
S
M
CIPR
CIPR & O3

Bioreactor and Control



Bioprocess Variables

Physical	Chemical/Biochemical
Temperature	pH
Pressure	Dissolved oxygen
Flow of gases and liquids	Concentration of substrates, products and other environmental factors (gas partial pressures, dissolved gases, carbohydrates, acids, alcohols, proteins, metal ions, etc.)
Volume	Biomass concentration
Power input	Intermediates (NAD/NADH, ATP/ADP/AMP, metabolites.)
Impeller speed	Enzyme activities
Density	Expression levels
Foam	Proteom
Viscosity	Metabolic fluxes and flux distributions
Turbidity	Activities of whole cells
etc.	etc.

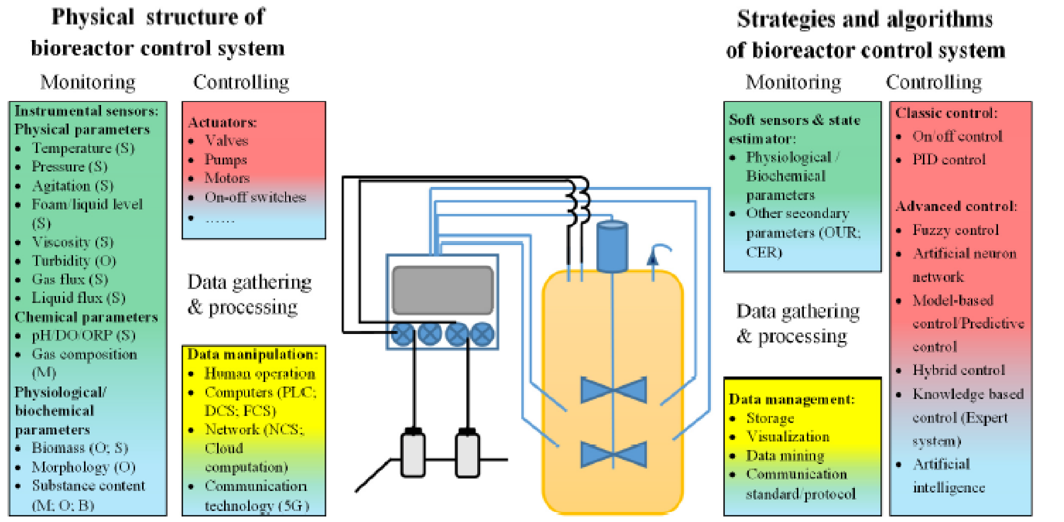


FIGURE 1 | Summary of research on bioreactor control systems. S, specific functional sensors; O, optical sensors; M, MS-based online detectors; B, biosensors; DO, dissolved oxygen; ORP, oxidation-reduction potential; PLC, programmable logic controller; DCS, distributed control system; FCS, fieldbus control system; NCS, network control system; OUR: oxygen utilization rate; CER, CO_2 emission rate; PID control, proportion-integral-differential control.

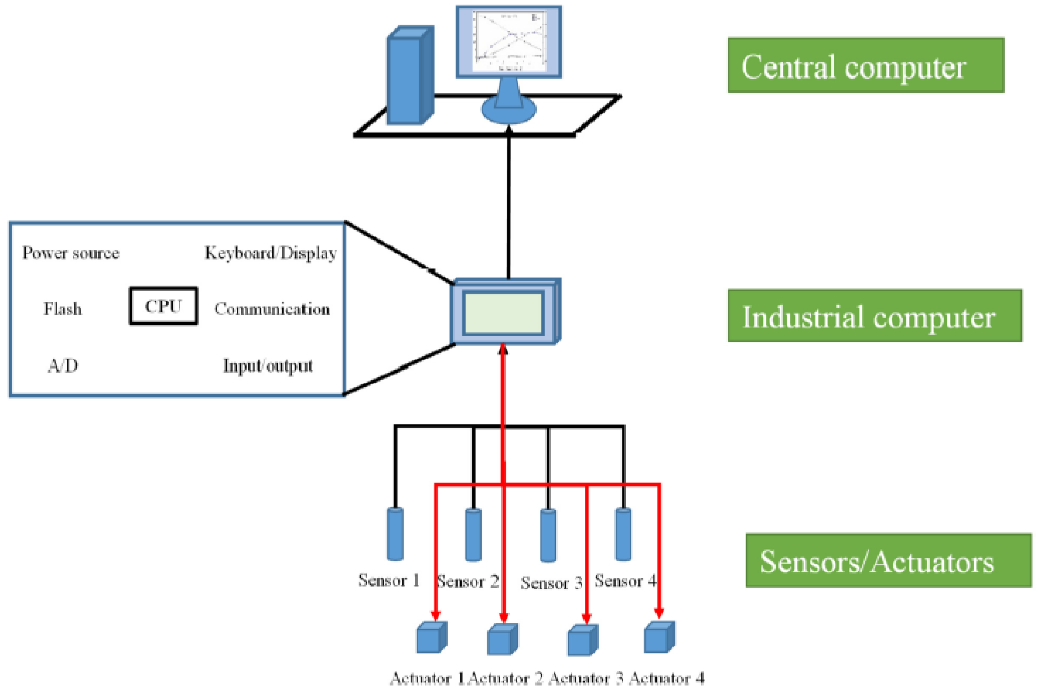


FIGURE 2 | Classic hierarchical structure control system for bioreactors.