MICROBIOLOGY

Bioreactors

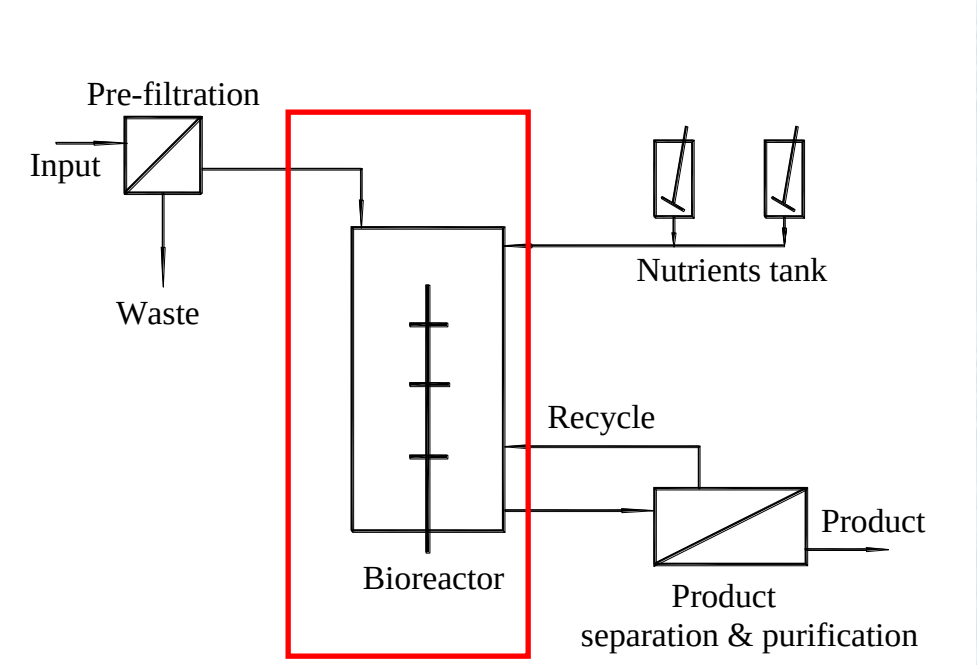
Structure of presentation

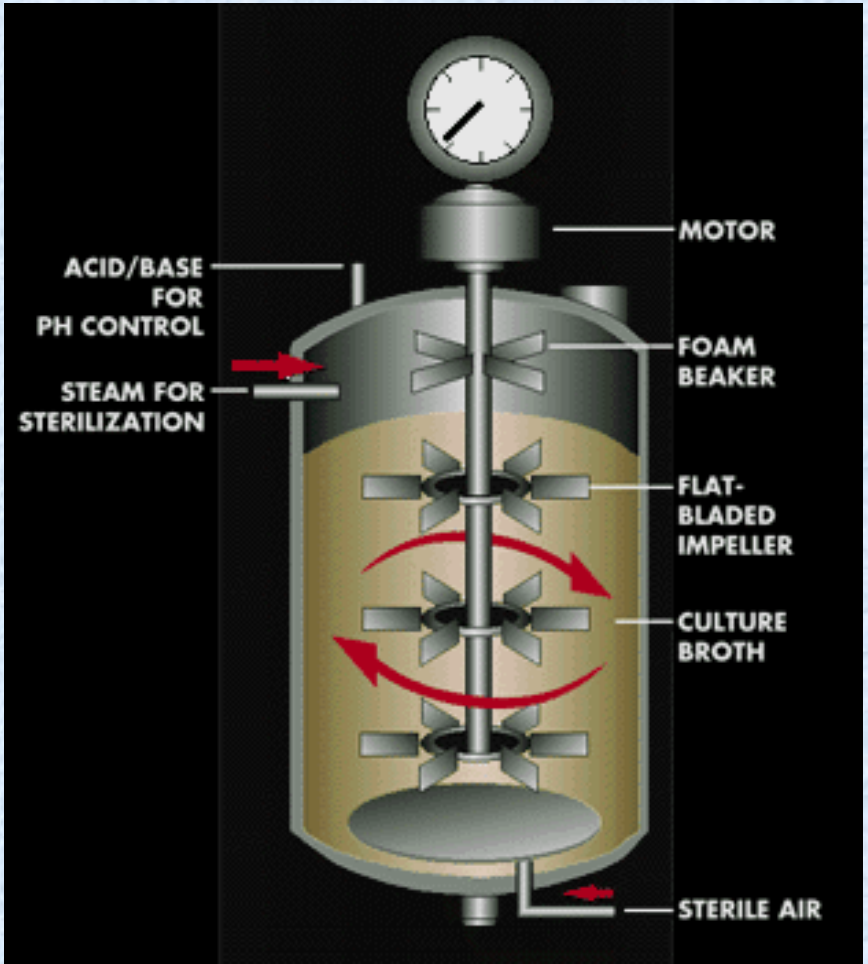
1. Brief description of bioreactors
2. Bioreactor parameterization
3. Modes of operation of the bioreactor
4. Practical considerations for bioreactor design

What bioreactor is?

Bioreactor: An apparatus, a vessel, used to apply the action of a biological catalyst to bring about the desired chemical modification

Fermenter: A bioreactor in which the biocatalyst is a living cell





What bioreactor is?

temperature recorder

motor

pH recorder

inoculation

spin recorder

product

air

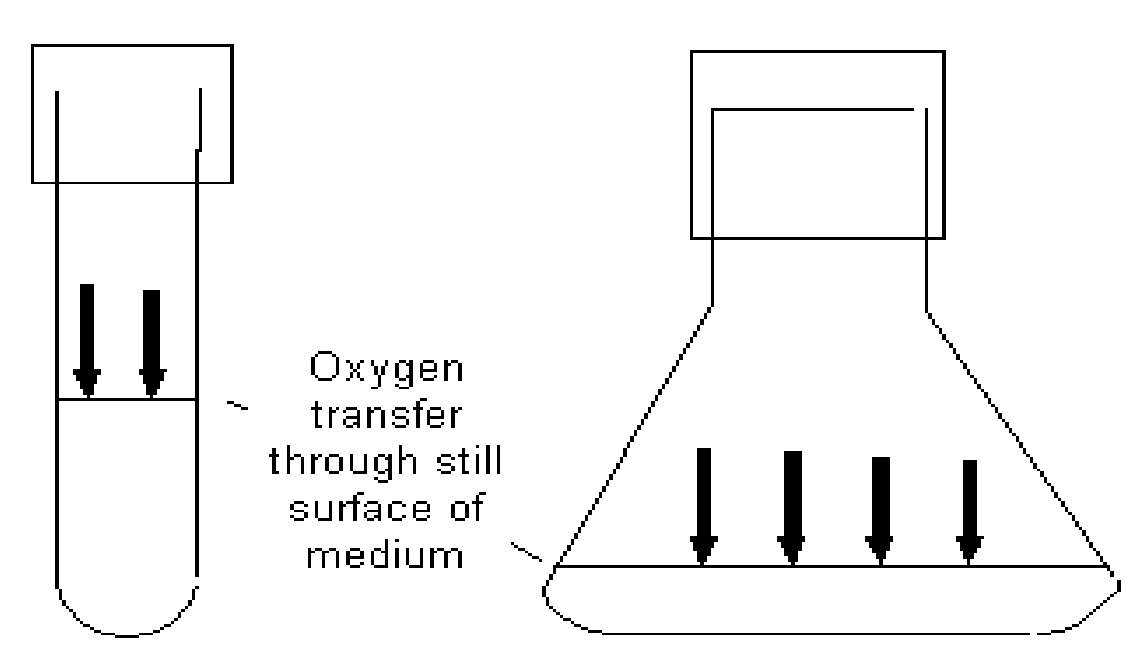
pH electrode

stirrer

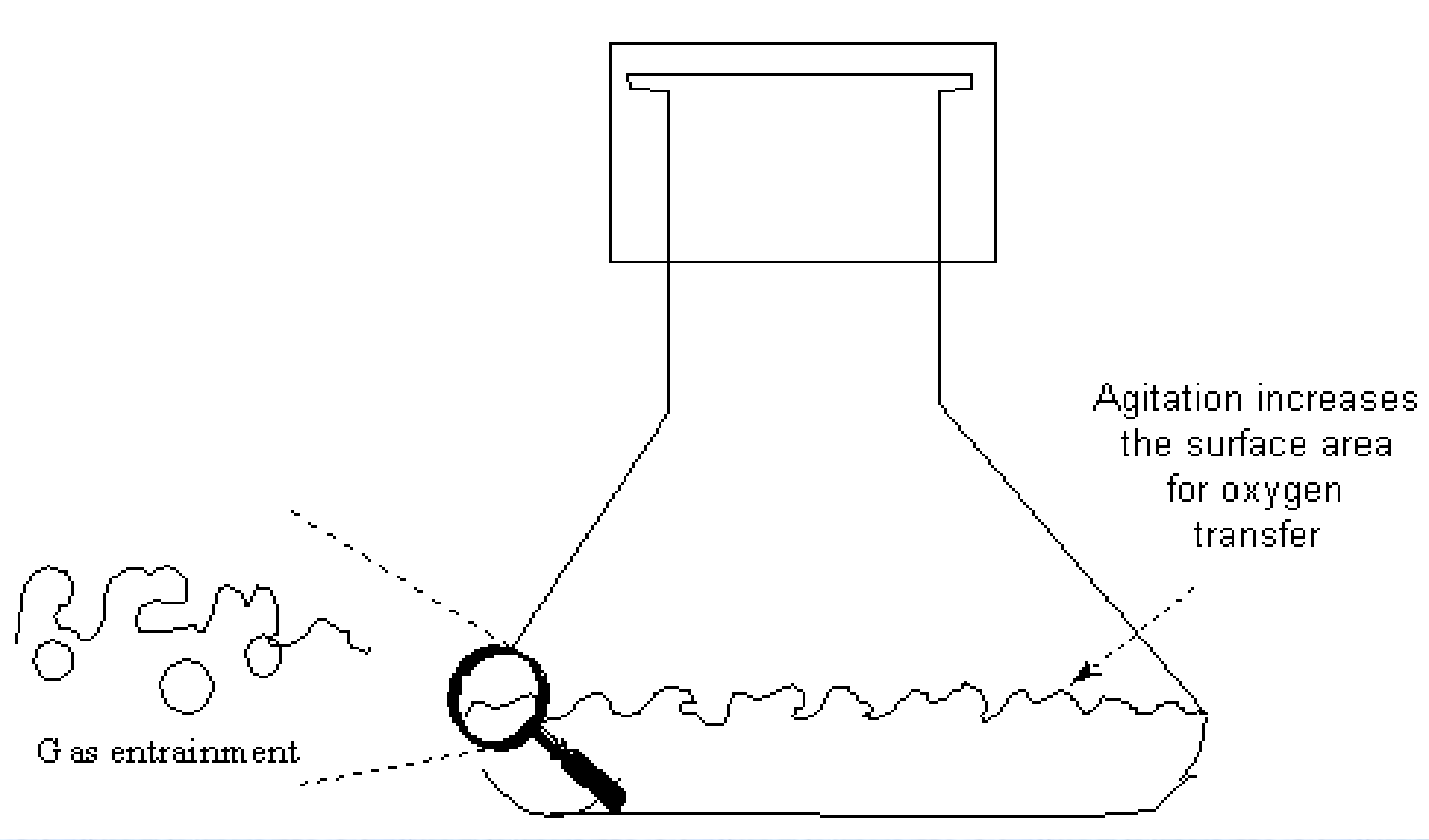
vessel

Unagitated cultivation

- Little or no aeration
- Oxygen transfer is achieved through surface

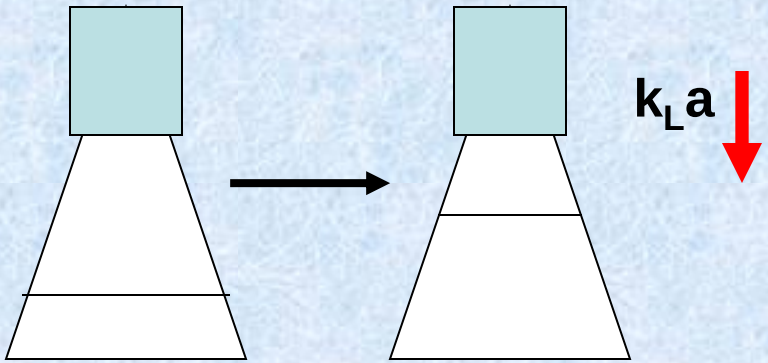


Agitated bottles

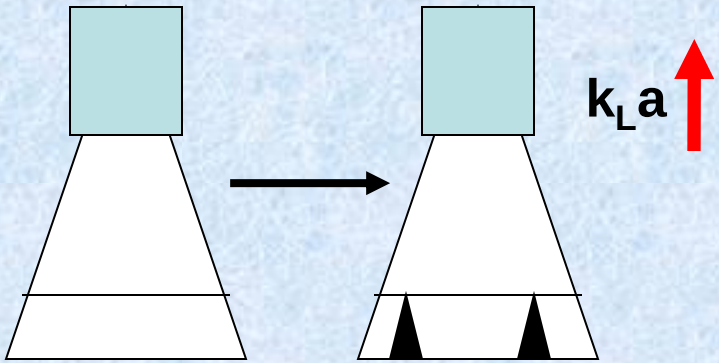


O_2 transfer in agitated bottles

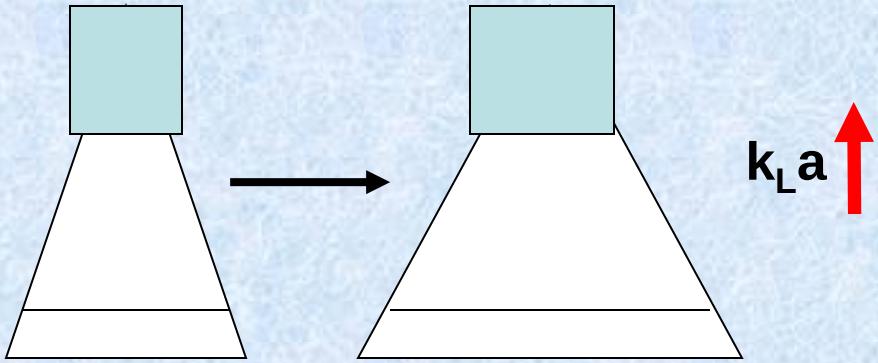
k_La: decreases with volume



k_La: higher when baffles are used



k_La

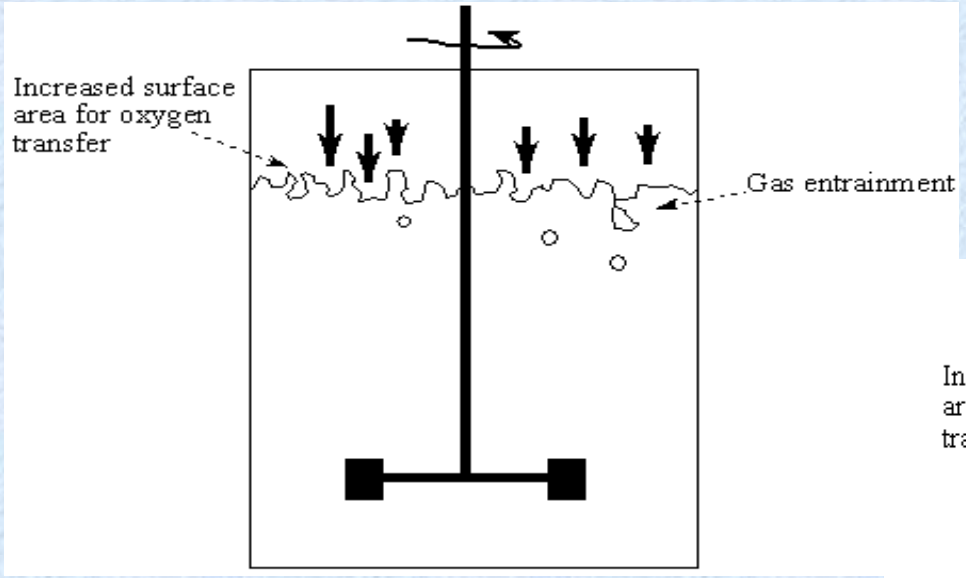


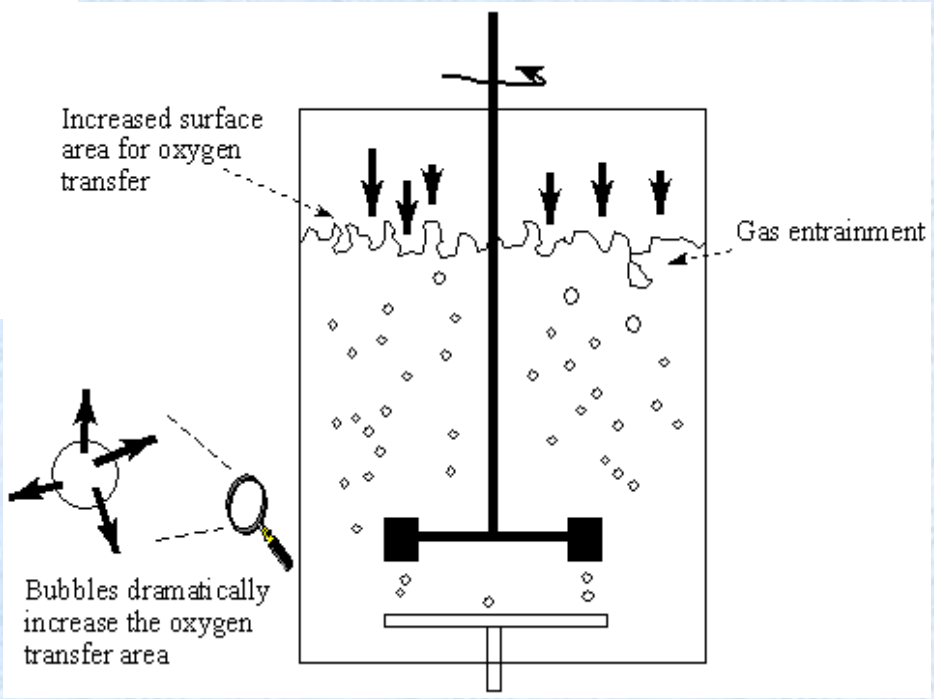
k_La: increases with liquid surface

Unbaffled flask

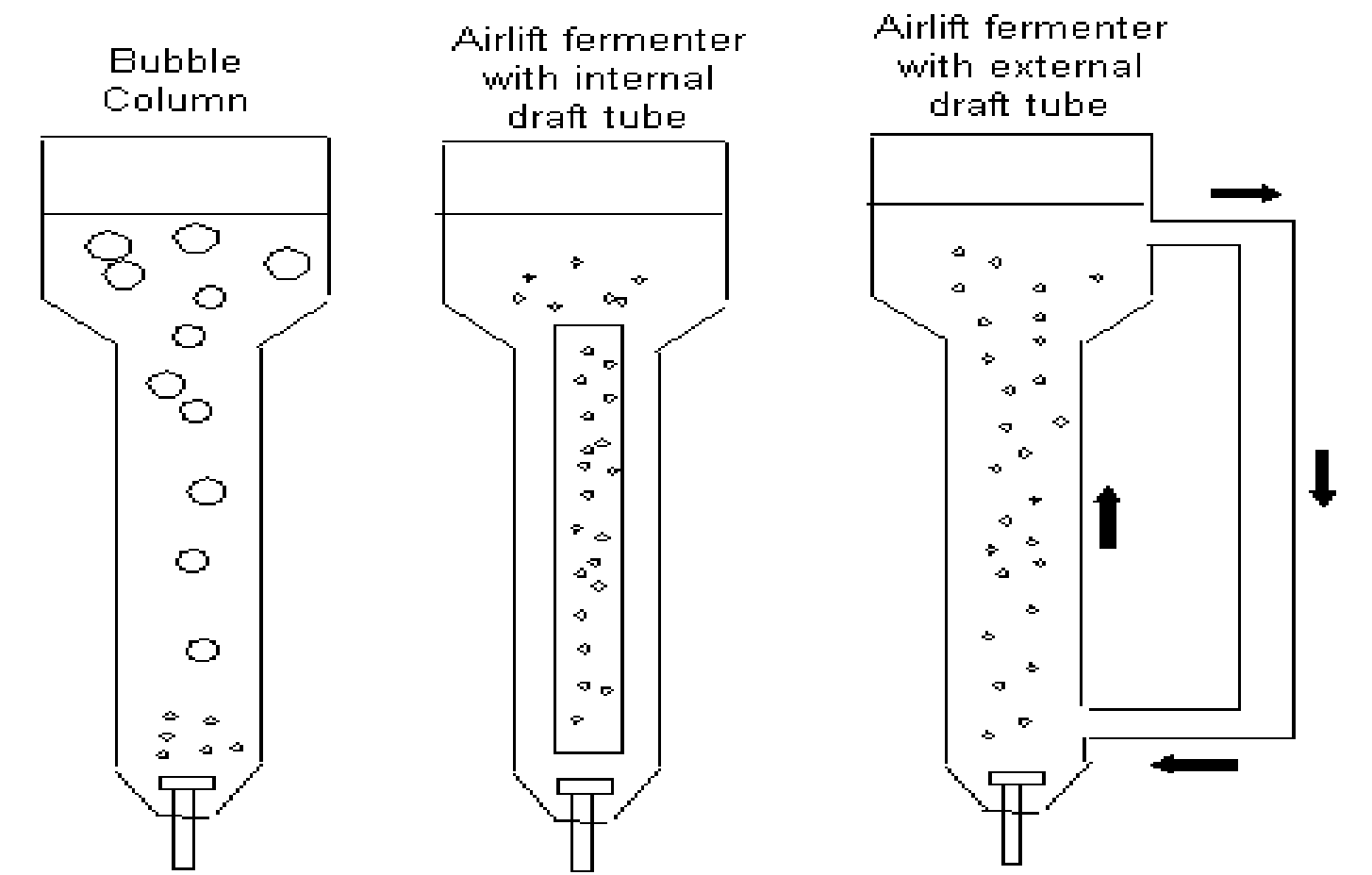
Baffled flask

Bioreactors with mechanical agitation

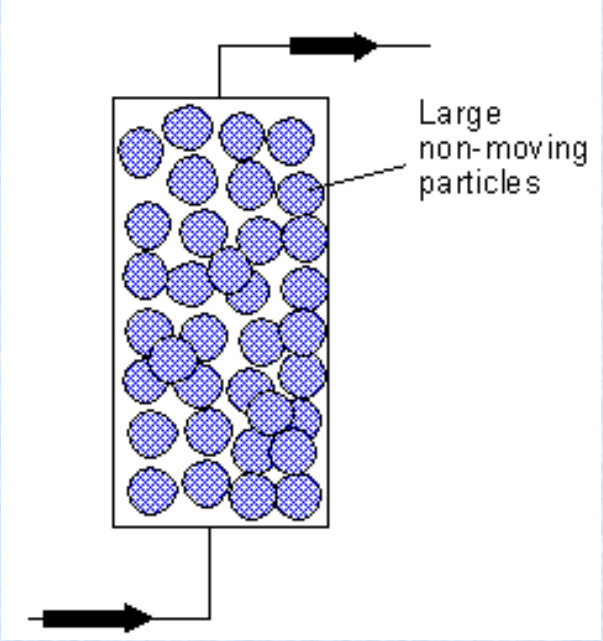


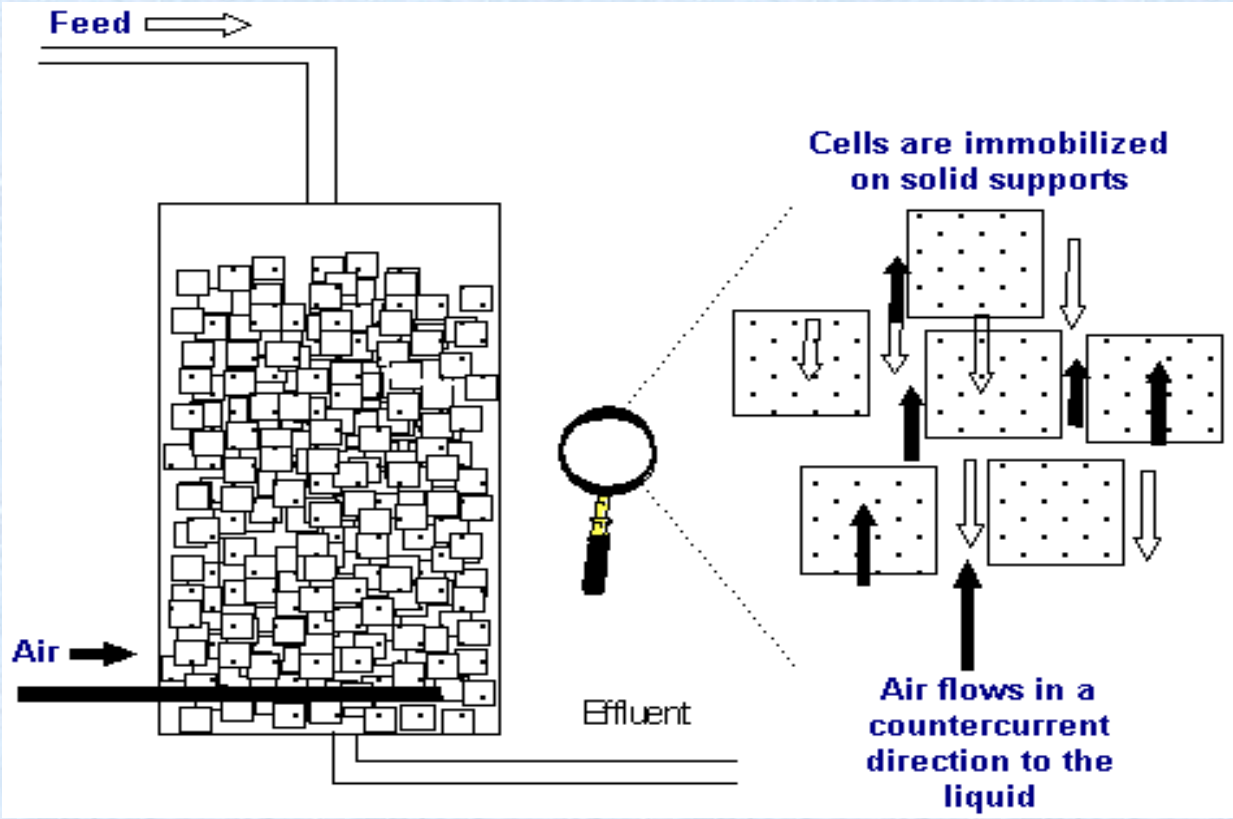


Bubble bioreactor



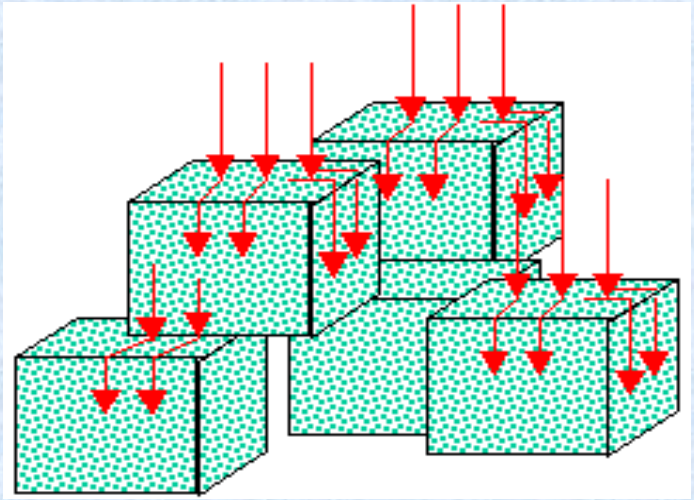
Bed and tear flow bioreactors

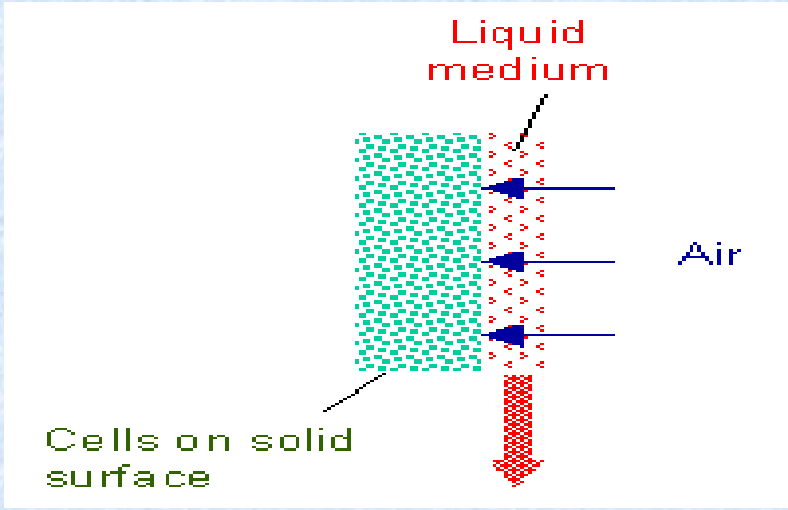




Tear flow bioreactors

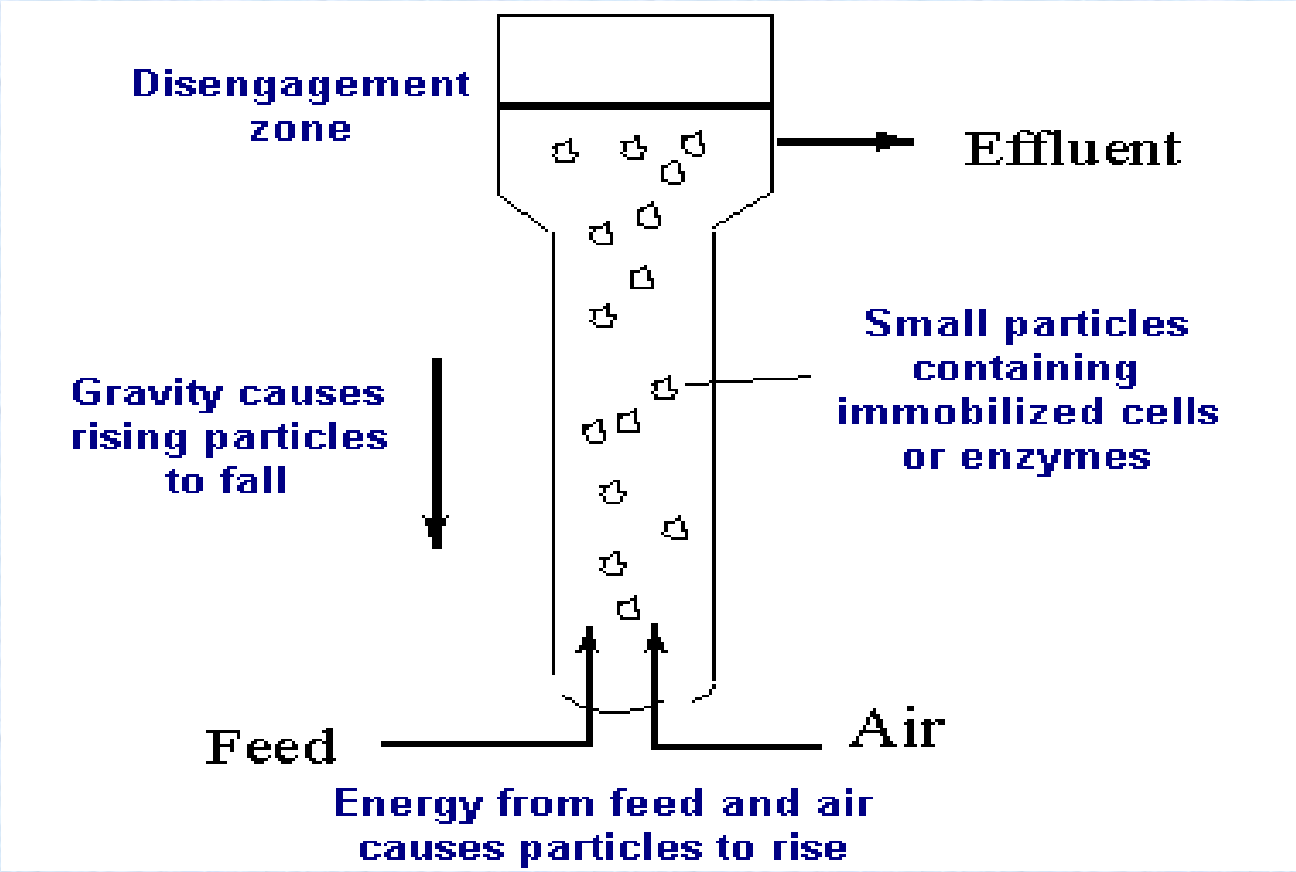
- The culture medium flows (drips or tears) onto the solid particles, in which the cells are immobilized
- The particles are not immersed in the liquid

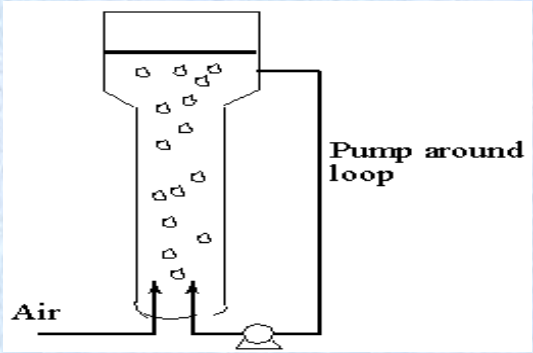




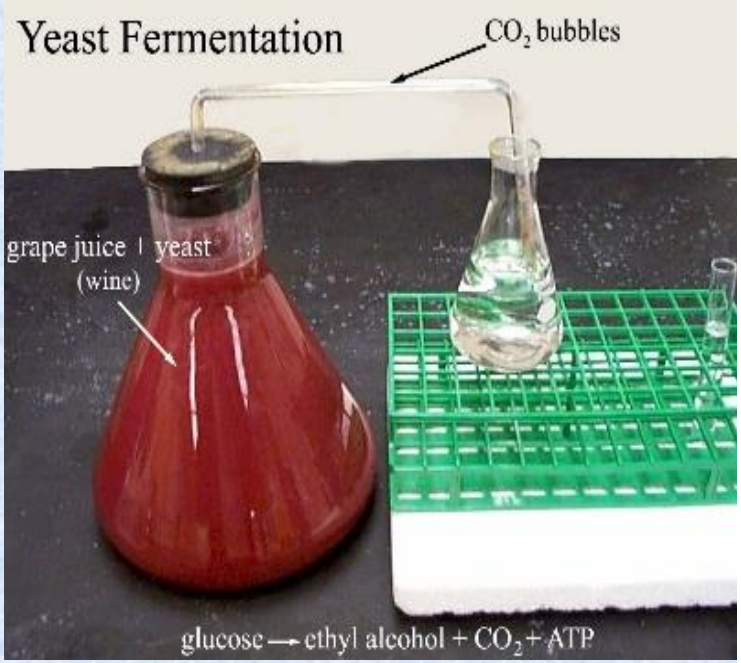
It is widely applied in aerobic digestion of sludge

Fluidized bed bioreactors





Cell culture fermenter



Shake flask fermenter

laboratory fermenter

Pilot fermenter

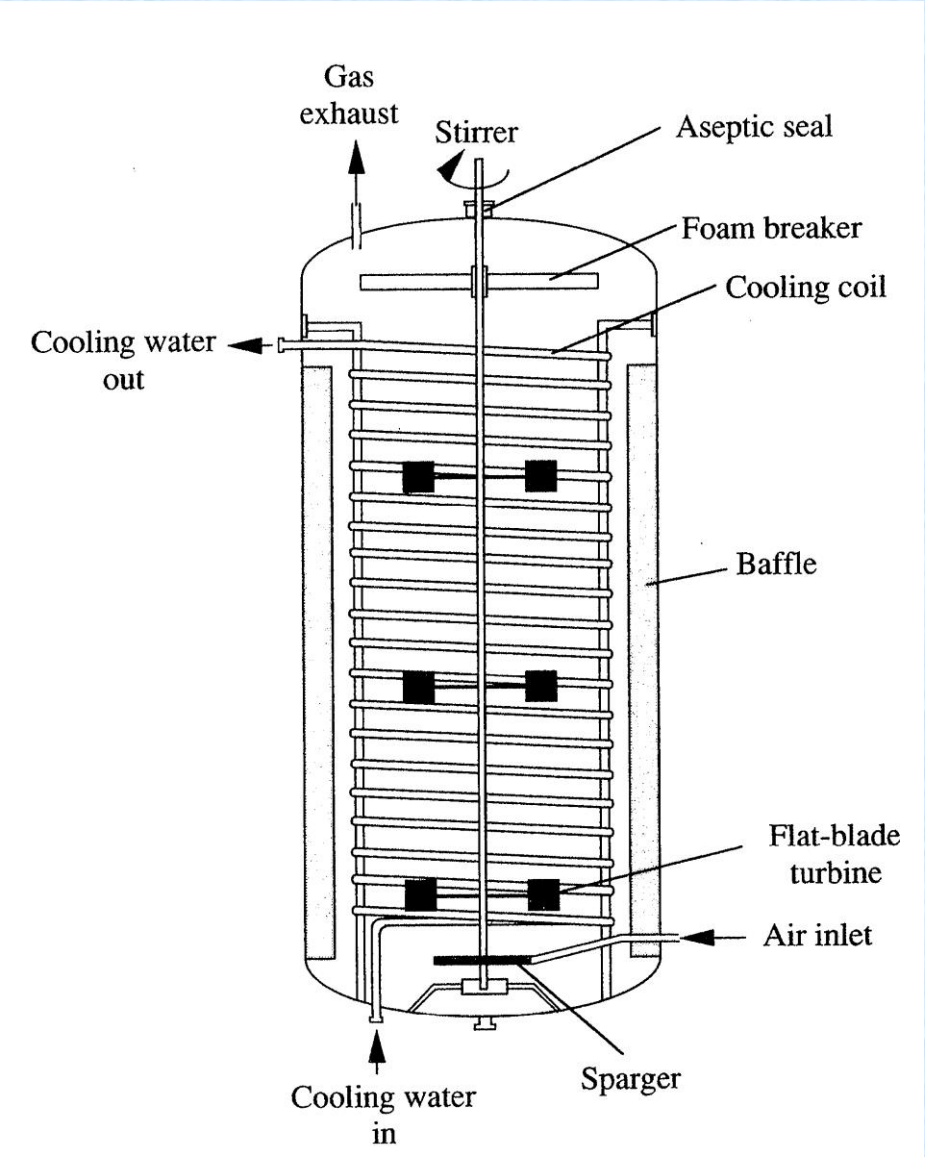
Plant fermenter

Actions in bioreactor design

1. **Aerobic bioreactor: proper mixing and aeration required**
2. **Anaerobic bioreactor: no agitation by shaking or bubbling required**

Bioreactor parameterization

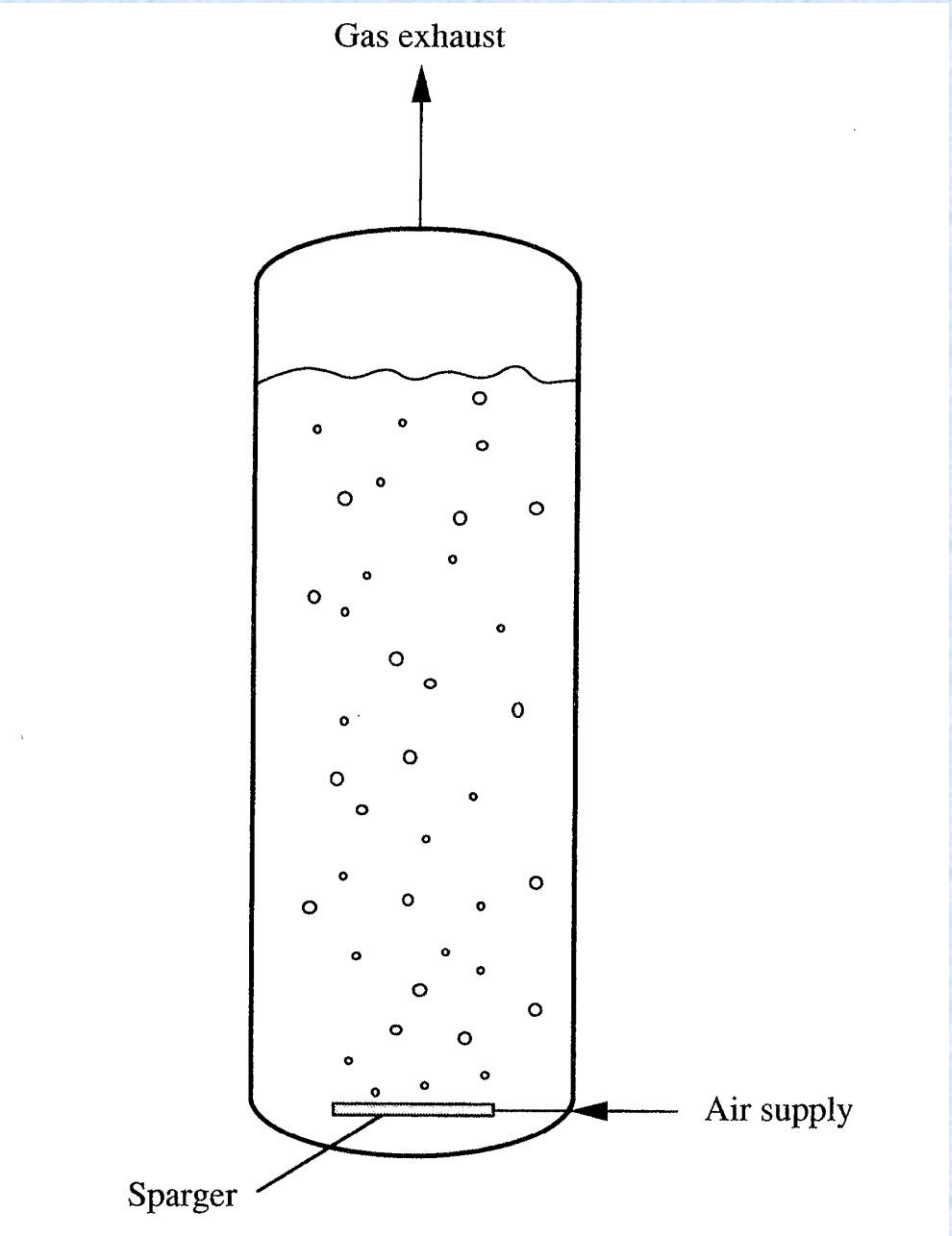
1. Full mixing tank



Mixing method: Mechanical stirring

- Baffles are usually used to reduce turbulence
- Applications: immobilized cells
- High shear stresses can damage cells
- High energy expenditure is required

Bioreactor parameterization
2. Bubble tank



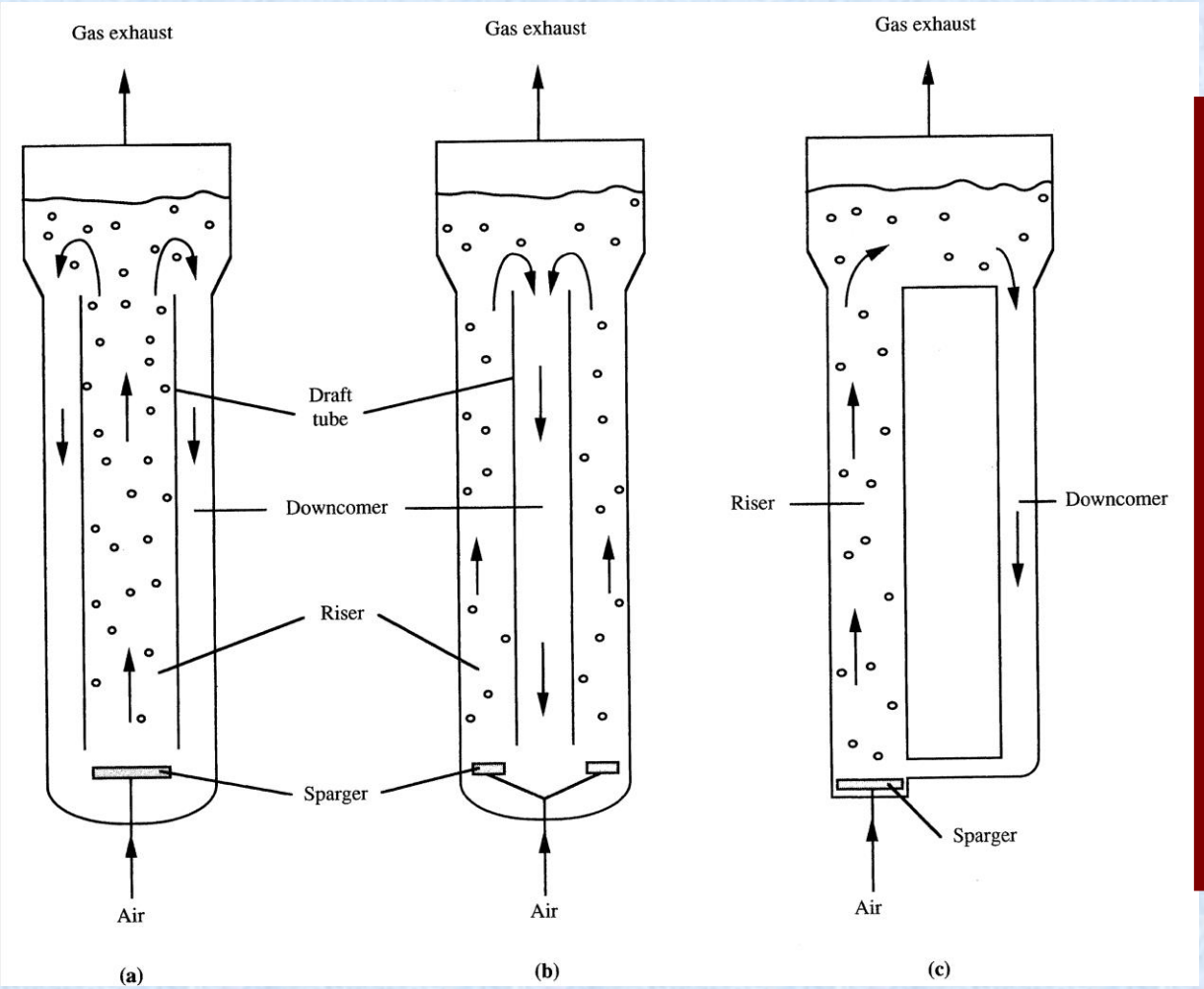
Stirring method: Air bubbler

- Simple design
- Very good mass and heat transfer
- Low energy consumption

Gas-liquid transfer coefficients depend mainly on the diameter of the bubbles and the dissolution of air in the culture medium

Bioreactor parameterization

3. Airlift/loop tank

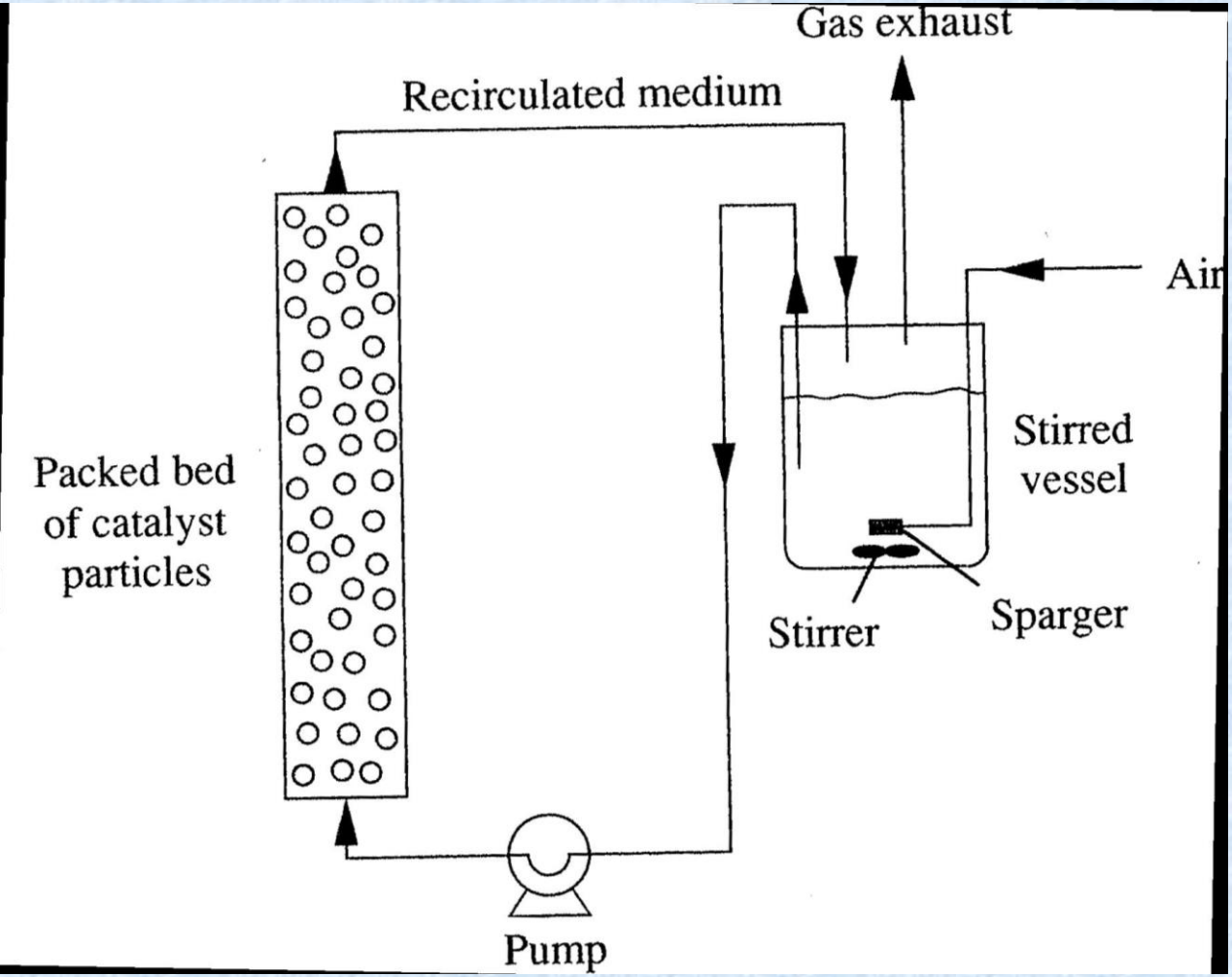


Mixing method: Air transport

- In this type there are two liquid streams, one anodic and one cathodic
- The liquid circulates due to the different density of the anode and cathode currents

Bioreactor parameterization

4. Packed bed reactor



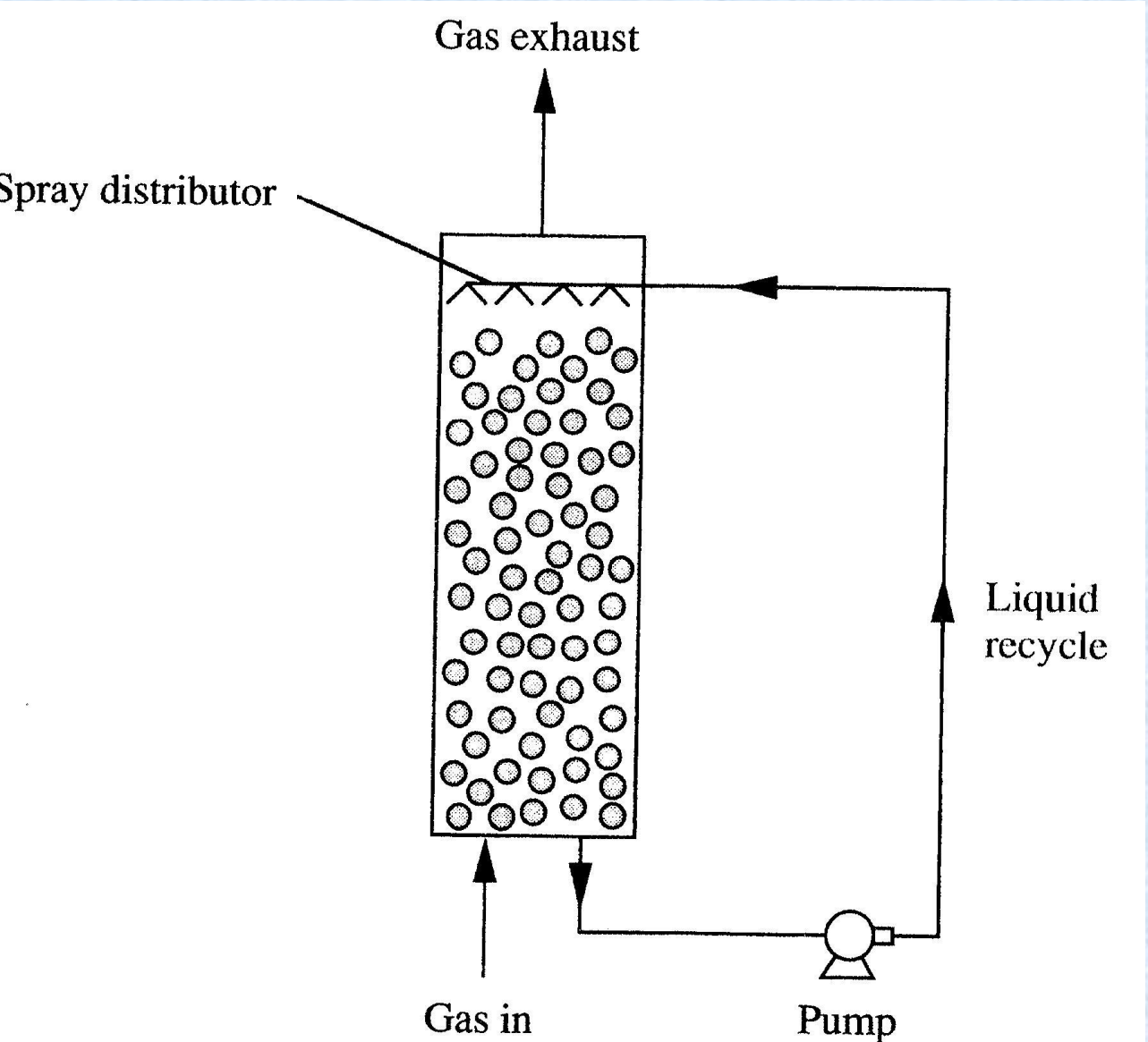
Bed reactors are used when the catalyst is in cellular or immobilized form

This is a continuous flow reactor

The culture medium is fed from either the top or the bottom

Bioreactor parameterization

5. Tearing bed reactor

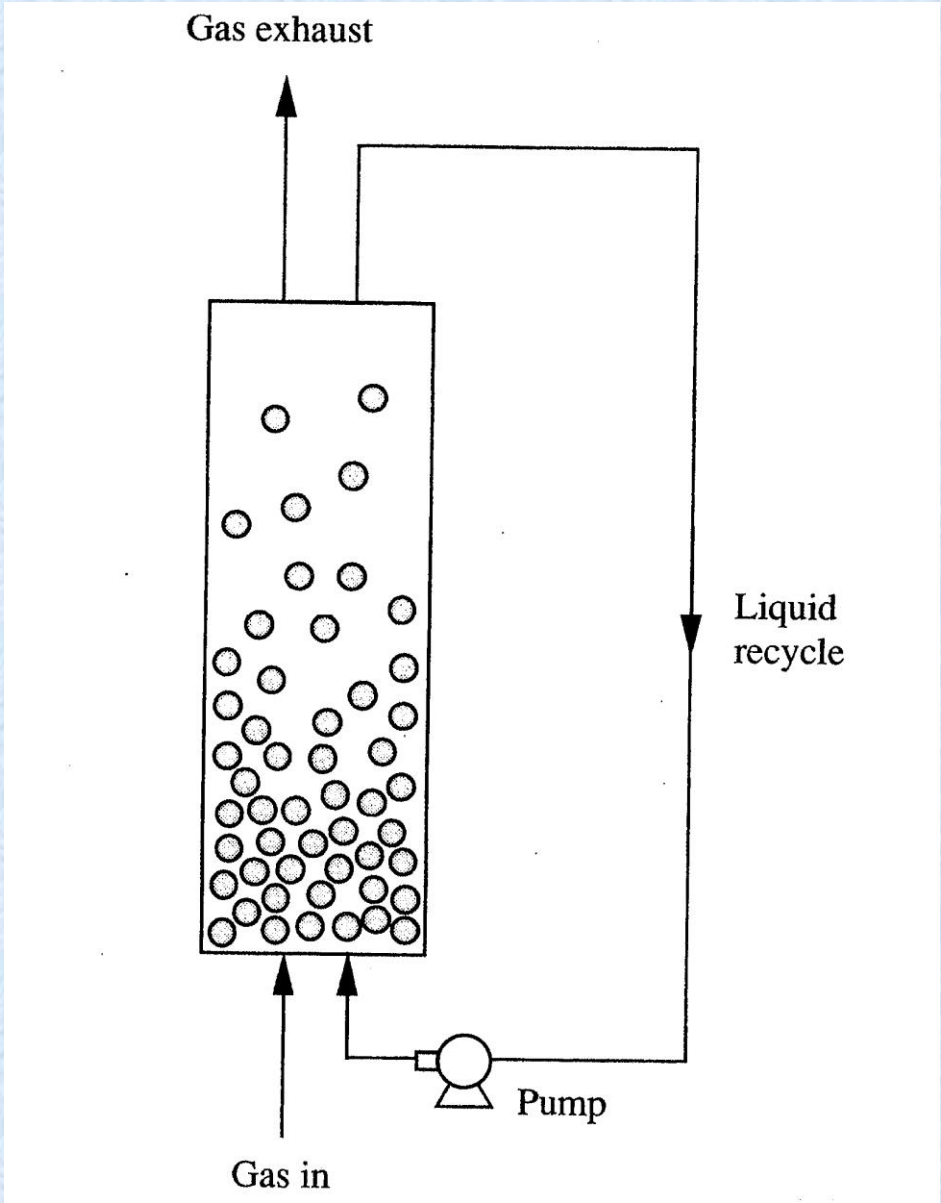


This is an alternative form of the packed bed reactor

Liquid is fed to the top of the reactor as a spray and then flows into the bed

Bioreactor parameterization

6. Fluidized bed reactor



When the packed bed reactor is fed from the bottom, the bed floats and expands, especially at high flows

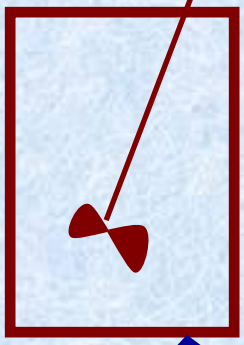
Modes of operation of the bioreactor

1. Batches

The batch bioreactor usually has a stirring system to mix the solution

The pH is maintained using either a buffer or a pHstat

Defoamer is usually installed to break up the foam



Batch operation under agitation

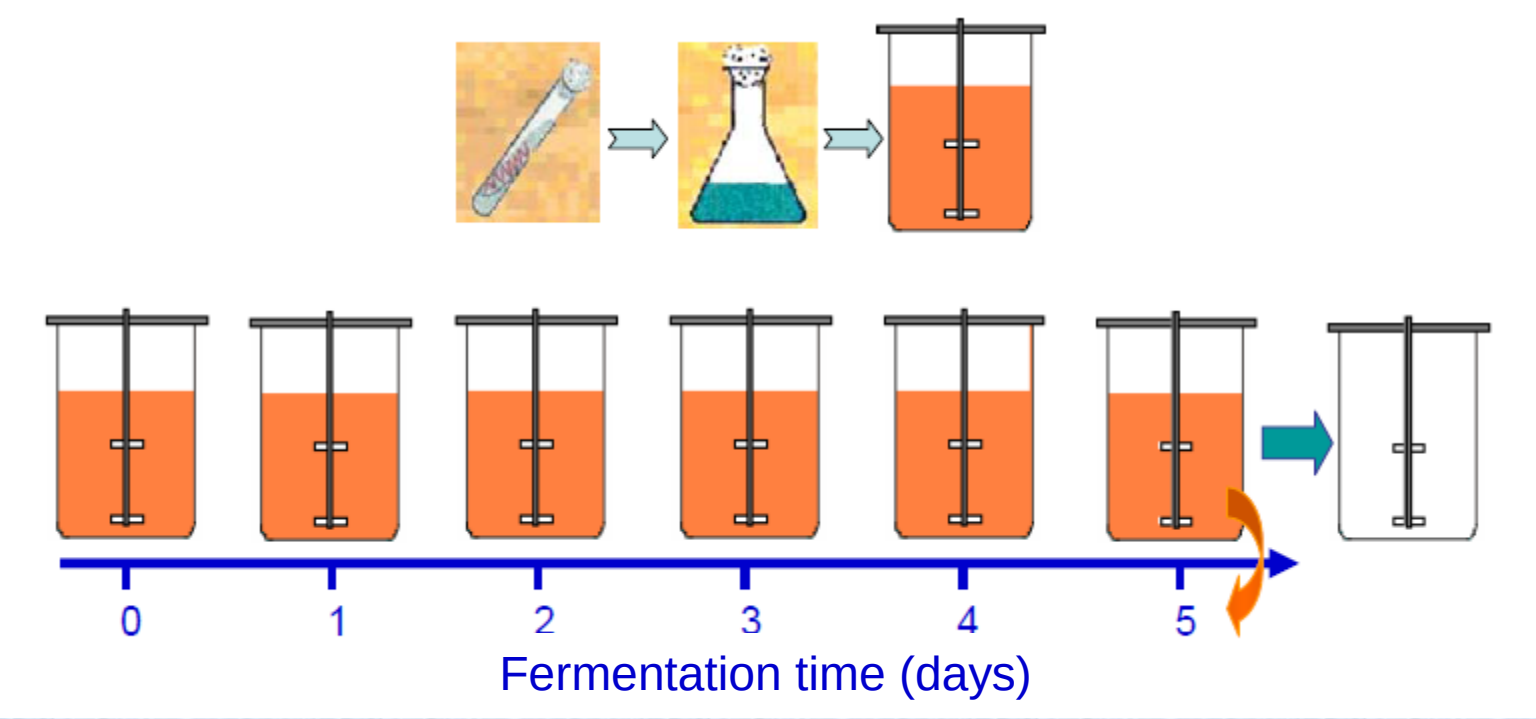
$$r = \frac{dC_s}{dt} = \frac{r_{\max} C_S}{K_m + C_S}$$

Variation of Cs with t

$$K_m \ln \frac{C_{s0}}{C_s} + \left(C_{s0} - C_s\right) = r_{\max} t$$

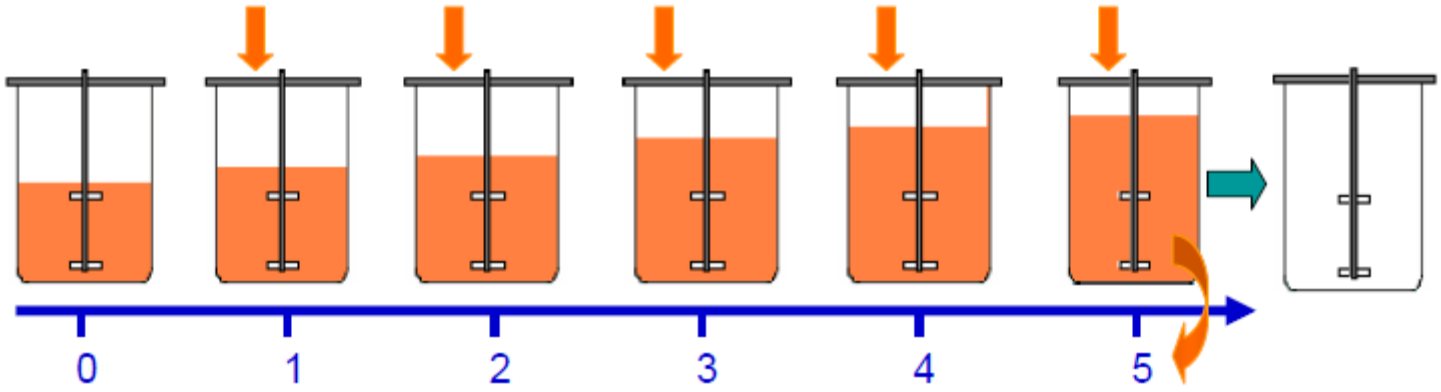
Modes of operation of the bioreactor

1. Batches



Semi-batch bioreactor

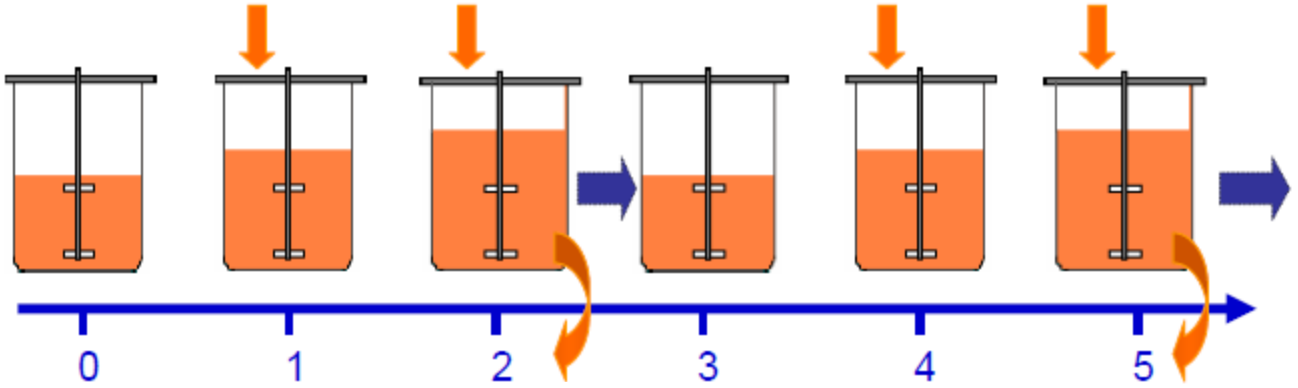
Nutrients (sterilized) are added in selected intervals or continuously



Fermentation time (days)

Advantages: increase in biomass production
and neutralization of catabolic repression

Repetitive batch bioreactor



Fermentation time (days)

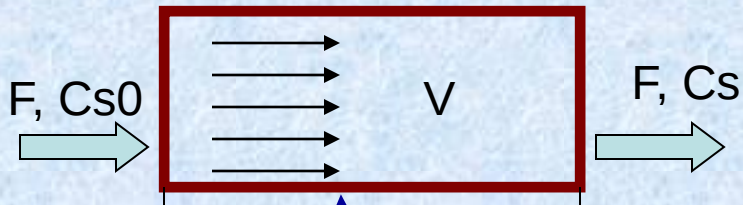
A batch fermentation is transformed to a semi-continuous fermentation
The volume of nutrients solution, the feeding and the specific growth rate are subjected to cyclic changes
When changes occur at fixed time intervals, the culture goes through equilibrium conditions that can be accompanied by high rates of product production

Modes of operation of the bioreactor

2. Plug flow

The culture medium enters at one end (of a cylindrical tube containing the cells) and the product exits at the other end

The ideal reactor is a long tube filled with cells



t = 0

$$\tau = \frac{V}{F}$$

Residence time

Continuous operation without stirring

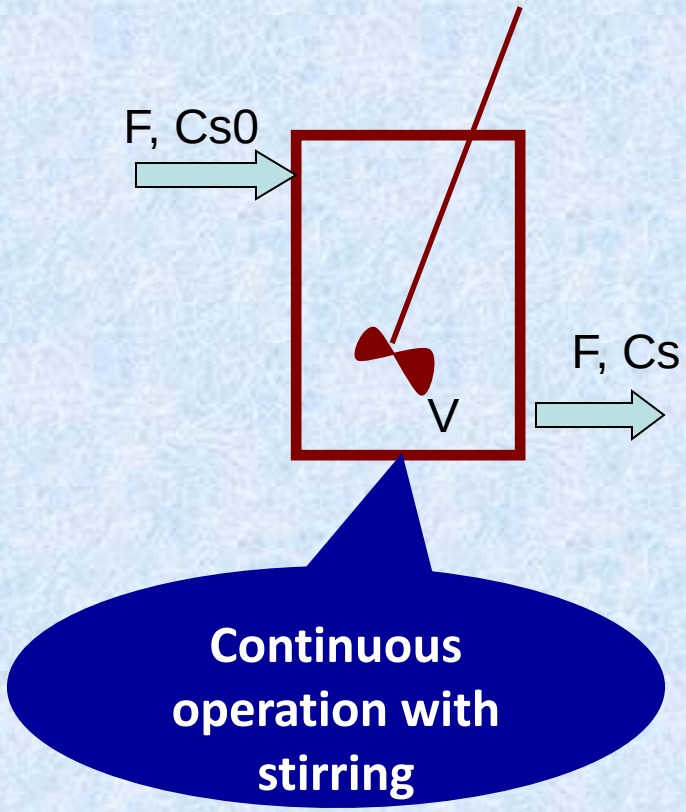
$$K_m \ln \frac{C_{s0}}{C_s} + \left(C_{s0} - C_s\right) = r_{\max} t$$

Modes of operation of the bioreactor

3. Continuous operation with stirring

A continuous flow reactor (CSTR) is the ideal reactor

It relies on very good mixing of the reactants



Modes of operation of the bioreactor

3. Continuous operation with stirring

This open type of fermentation operates under:

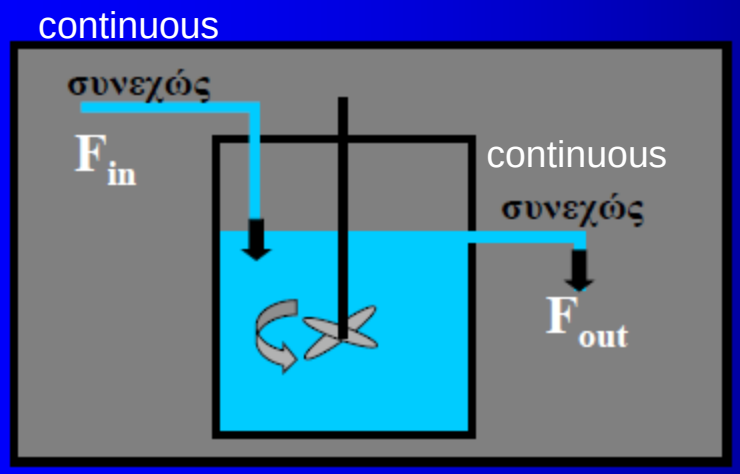
Constant volume of nutrients
Constant specific growth rate of microorganism
New nutrient is introduced to the bioreactor under constant rate and aseptic conditions
On the same time, equal amount of cultured material is removed

The process is characterized according to:

The nature of the final product (biomass or a metabolite)
The type of process or operation (homogenous/heterogenous, single/multiple, with/without cell reuse)
Control mode of bioreactor (i.e., chemostat, when the process is controlled by the addition of nutrients)

Modes of operation of the bioreactor

3. Continuous operation with stirring



$$D = \frac{F}{V}$$

ΤΑΧΥΤΗΤΑ ΑΡΑΙΩΣΗΣ

(Dilution rate)

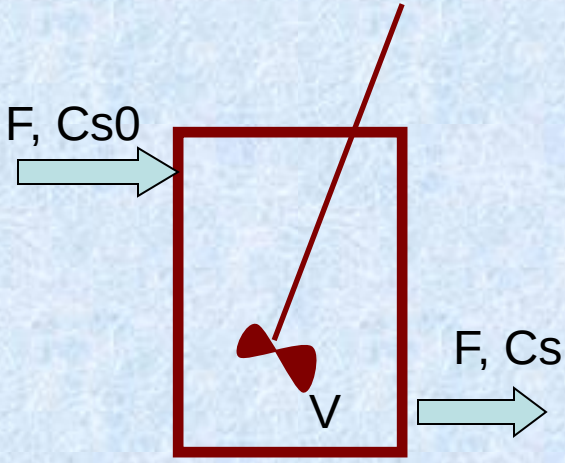
Constant volume $F_{in}=F_{out}$

Concentrations in output = concentrations in bioreactor

Constant concentrations

Modes of operation of the bioreactor

3. Continuous operation with stirring



Substrate mass balance

input-output-consumption=accumulation

$$FC_{s0} - FC_s - r_s V = V\frac{dC_s}{dt}$$

Stable situation

$$\frac{dC_s}{dt} = 0$$

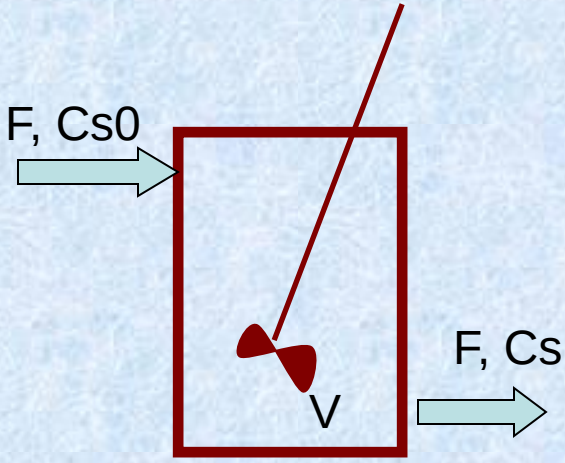
Michaelis-Menten rate

$$r = \frac{r_{\max} C_S}{K_m + C_S}$$

$$FC_{s0} - FC_s - V\frac{r_{\max} C_s}{K_m + C_s} = 0$$

Modes of operation of the bioreactor

3. Continuous operation with stirring



Substrate mass balance

$$FC_{s0} - FC_s + V\frac{r_{\max}C_s}{K_m + C_s} = 0$$

$$\frac{F}{V} = \frac{r_{\max}C_s}{(C_{s0} - C_s)(K_m + C_s)}$$

$$\frac{F}{V} = \frac{1}{\tau}$$

$$C_s = -K_m + \frac{r_{\max}C_s\tau}{C_{s0} - C_s}$$

Modes of operation of the bioreactor

3. Continuous operation with stirring

Continuous operation process advantages over batch process

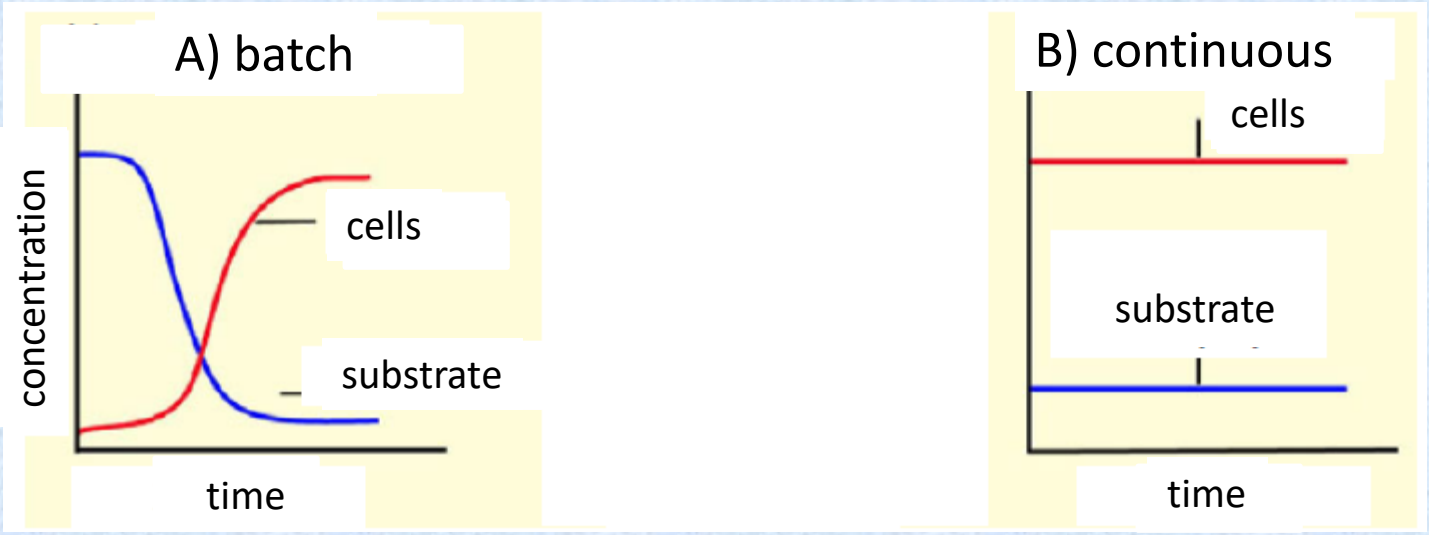
High volumes of fermentation with better financial result
More complete control of the process
Decreased loss of microorganism (due to constant conditions of operation)
Mathematical modeling of process

Continuous operation process disadvantages

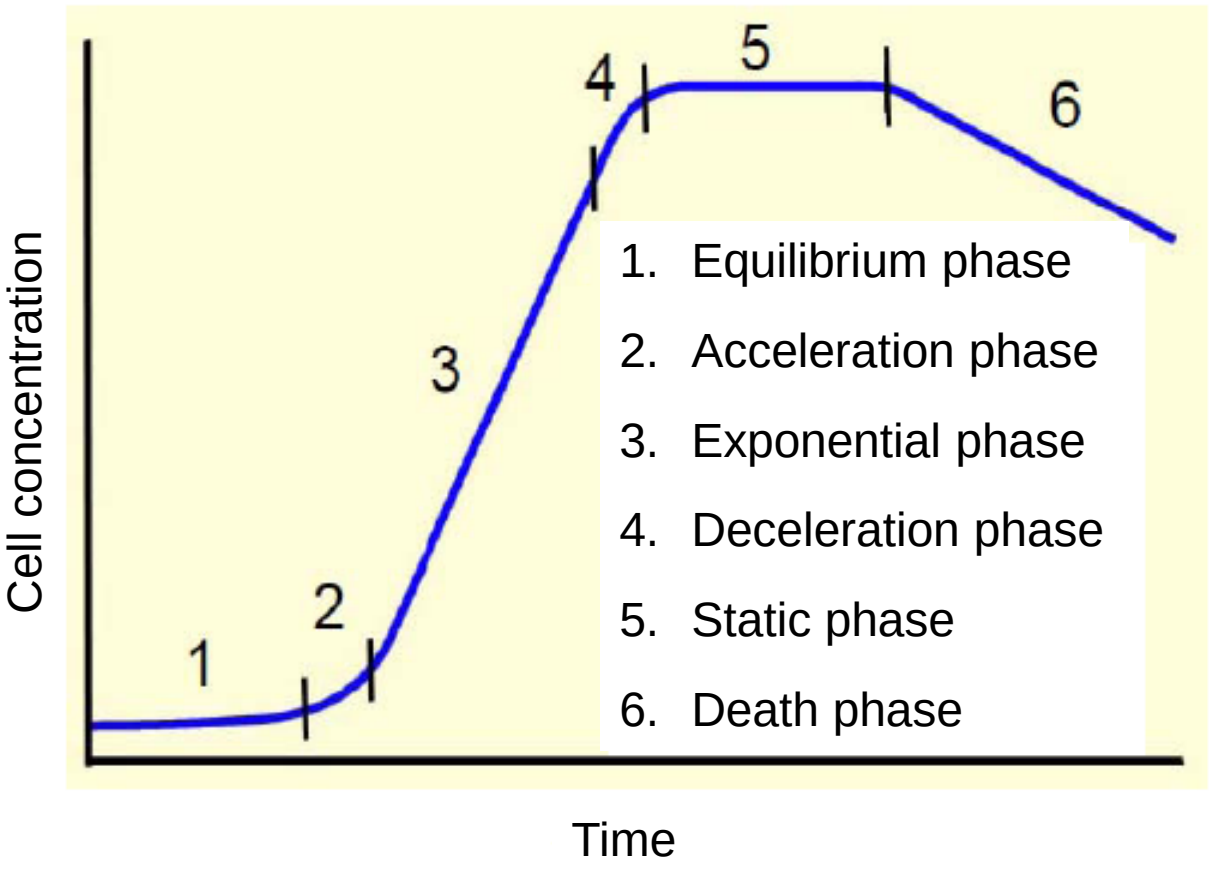
Difficulty in maintaining aseptic conditions
Limited use for different products production from the same bioreactor
Mostly bioindustries (biopharmaceutical) are small in scale

Main applications are:
Anaerobic fermentation for biogas (methane) production
Waste treatment

Microbial growth



Microbial growth in batch processes



Exponential phase

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

μ: Specific growth rate

Monod equation

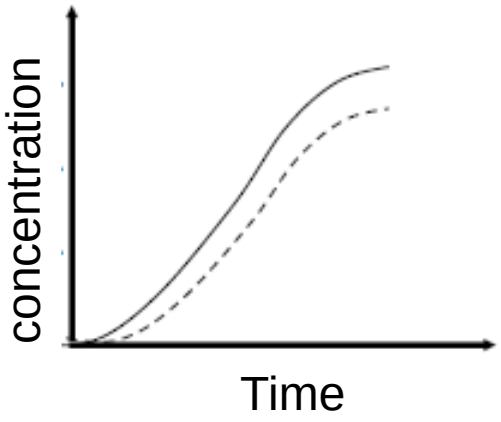
$$\mu = \frac{\mu_{max} S}{K_s + S}$$

S: Substrate concentration

Models of microbial products production

PRIMARY METABOLITES | **SECONDARY METABOLITES**

Related to cellular growth

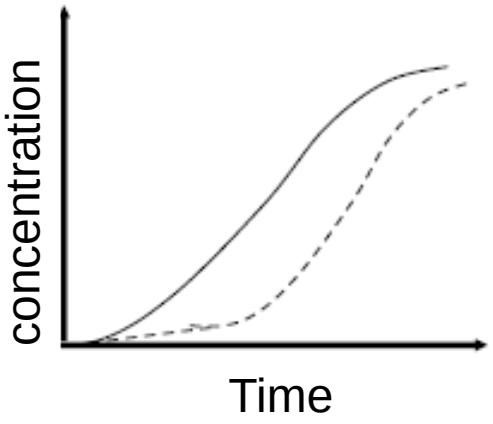


$$\frac{dP}{dt} = a * \frac{dx}{dt}$$

Brown - Vass

$$\frac{dP}{dt} = a * \frac{dx(t-t_m)}{dt}$$

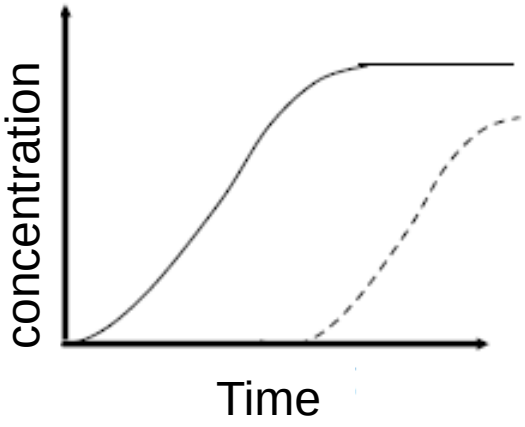
Mixed model



Leudeking-Piret

$$\frac{dP}{dt} = \left(a * \frac{dx}{dt}\right) + (b * x)$$

Non-Related to cellular growth



$$\frac{dP}{dt} = b * x$$

Practical aspects of bioreactors

Thermal load: Determined by energy balances

Heat generation rate

$$\dot{q} = V \cdot \mu \cdot C \cdot \frac{1}{Y_{kcal}}$$

Popular method

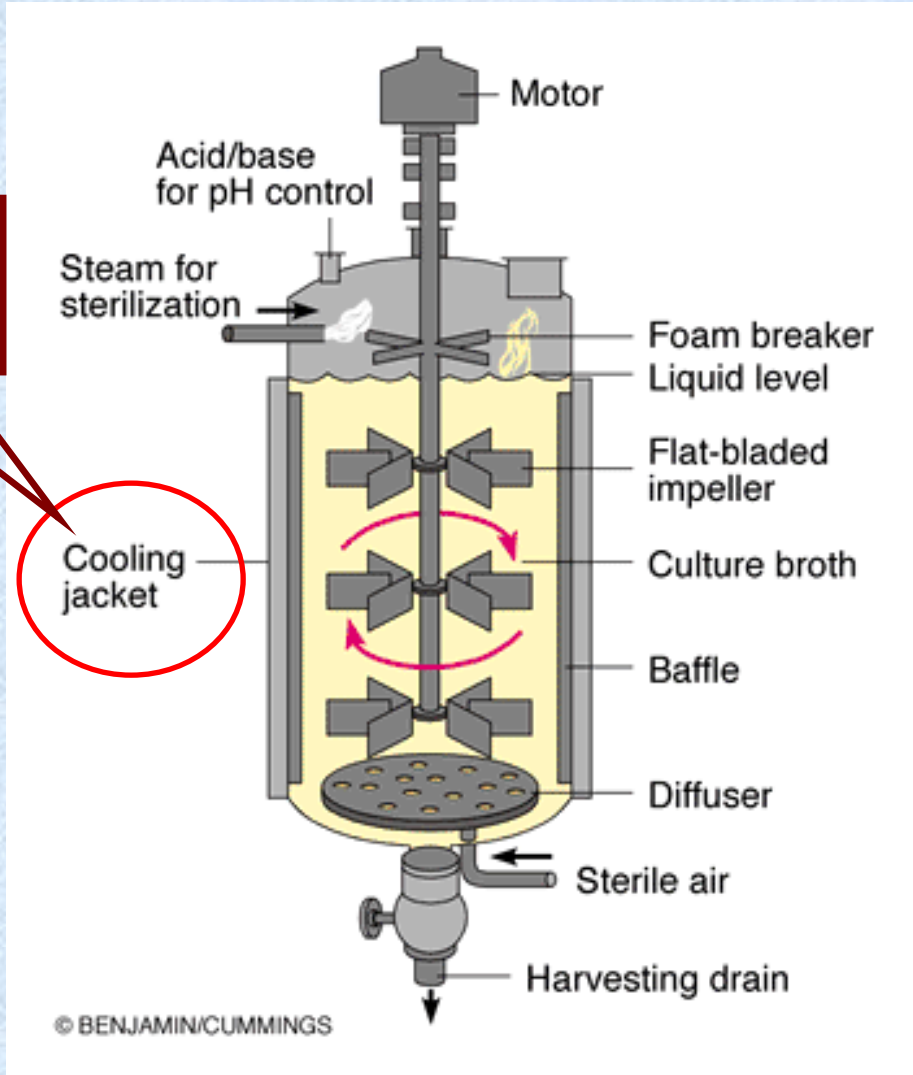
$\dot{q}$: Heat generation rate, *kcal/l·s*

V: volume of liquid in the reactor, *l*

μ: specific growth rate, s^{-1}

C: biomass concentration*(g/l)*

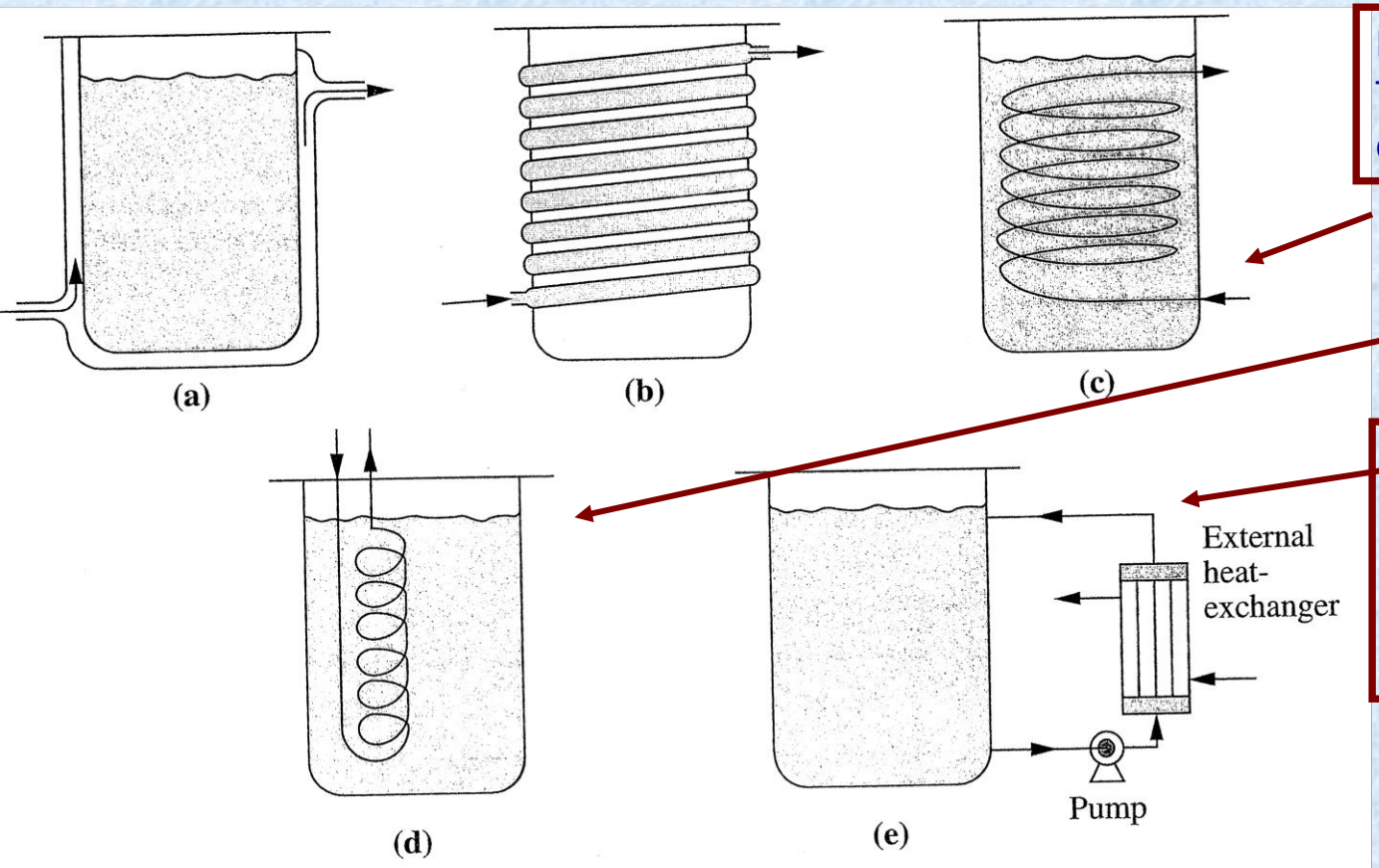
Y_{kcal}: efficiency factor, which corresponds to grams of cells per kcal of energy expended, *g cells/kcal*



Practical aspects of bioreactors -Temperature control (heat transfer)

Heat exchange surface:

1. Low in the outer (a) casing and (b) coil in small reactors
2. High internal thread (c) helical and (d) adjustable in large reactors
3. Adaptable in case of (e) external exchanger



Difficulty in cleaning
They are easily stained by cells growing on their surface

Easy cleaning

- Sterilization requirement
- Shear stresses in cells
- Loss of oxygen

Practical aspects of bioreactors
-Agitation (transport of gases)

All biological reactions are three-phase (gas-liquid-solid)

Mass transfer between phases is critical (e.g. oxygen feed in aerobic digestion)

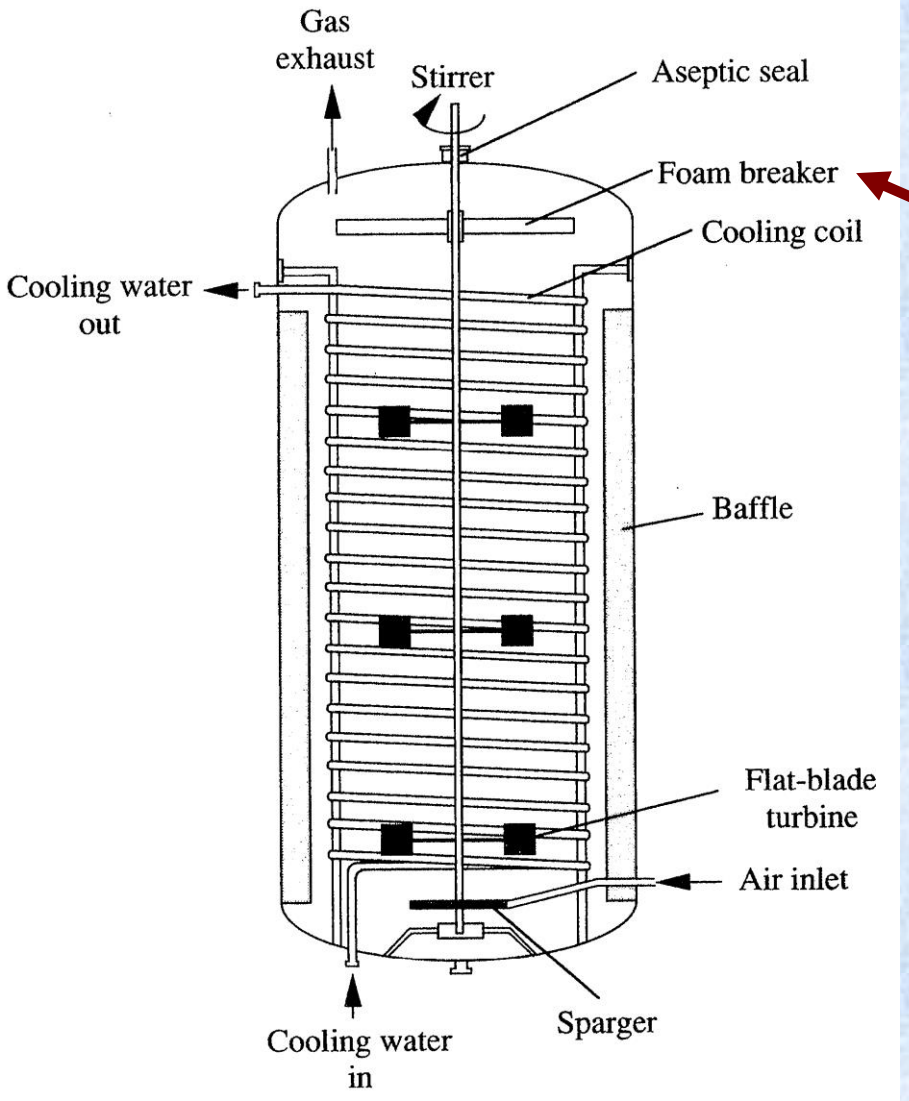
The equation that determines the oxygen transfer rate is:

$$J_A = K_l\left(C_A^* - C_{A_g}\right) \qquad C_A^* = P_{A_g} / H$$

Agitation:

- Mechanical stirring (in small reactors and/or viscous liquids, at low heat of reaction)
- Air driven stirring (in large reactors and/or high heat of reaction)

Practical aspects of bioreactors
- Removal of foam



1. **Mechanically (adding a special breaker)**
2. **Chemical defoamers (may reduce oxygen transfer rate)**

Practical aspects of bioreactors - Others

1. Aseptic operation (3-5% of industrial-scale fermentations are lost due to sterilization failure)
2. Materials of construction (glass for small bioreactors, e.g. < 30 liters and stainless steel for large ones)
3. Cell addition mode (three designs: porous, orifice, nozzle)
4. Control of evaporation due to dry air supply

According to operation mode

BATCH

- Simplicity and lower costs
- Lower risk of infections
- Lower risk of mutations
- Flexibility in planning production
- Less sensitivity to disturbances

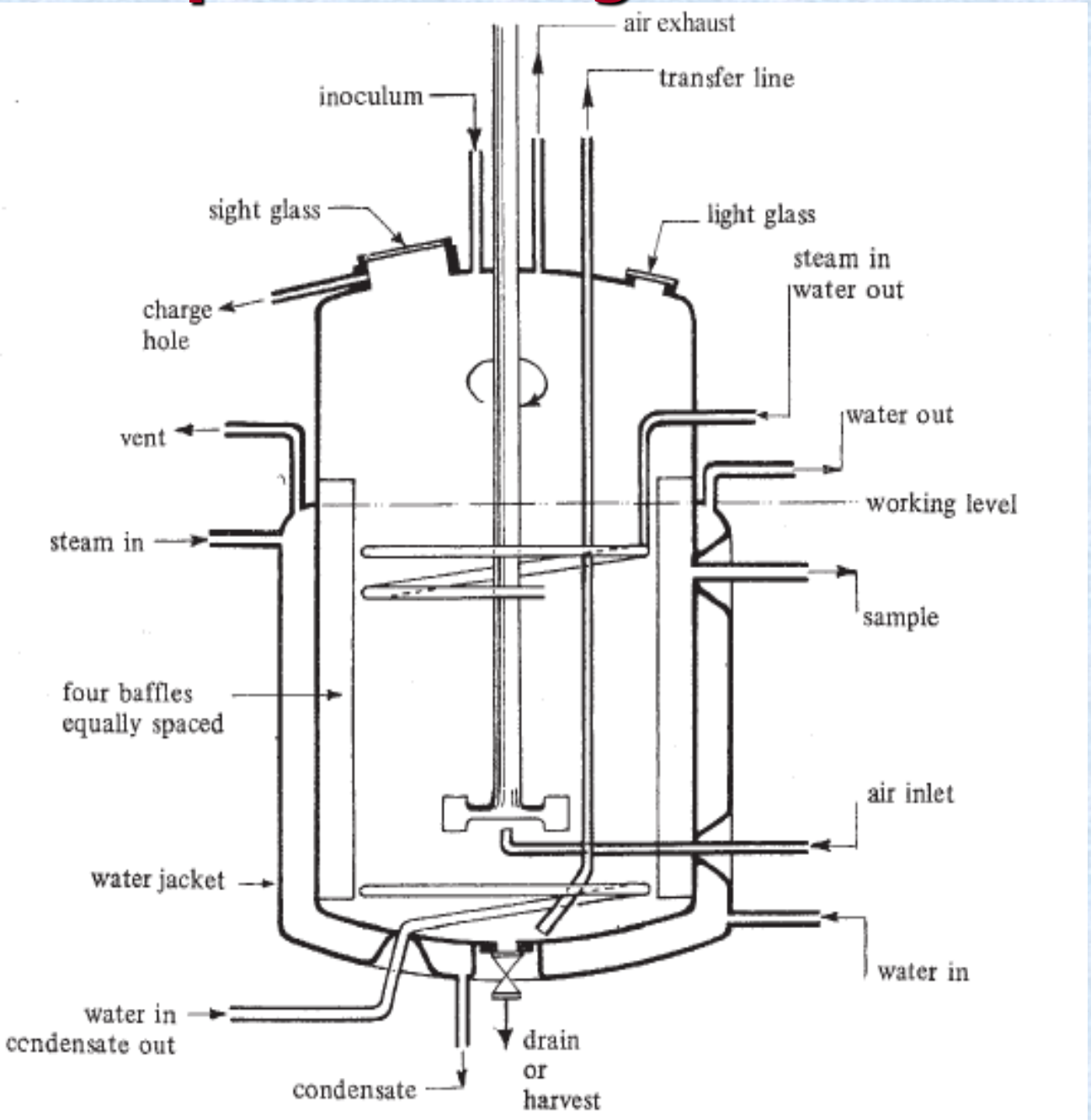
- Bigger bioreactors
- Decreased productivity due to adjustment (equilibrium) time

CONTINUOUS

- Minimum time consumption in non-productive processes
- Homogenous product
- Optimum regulation of growth rate
- Increase in productivity of product purification

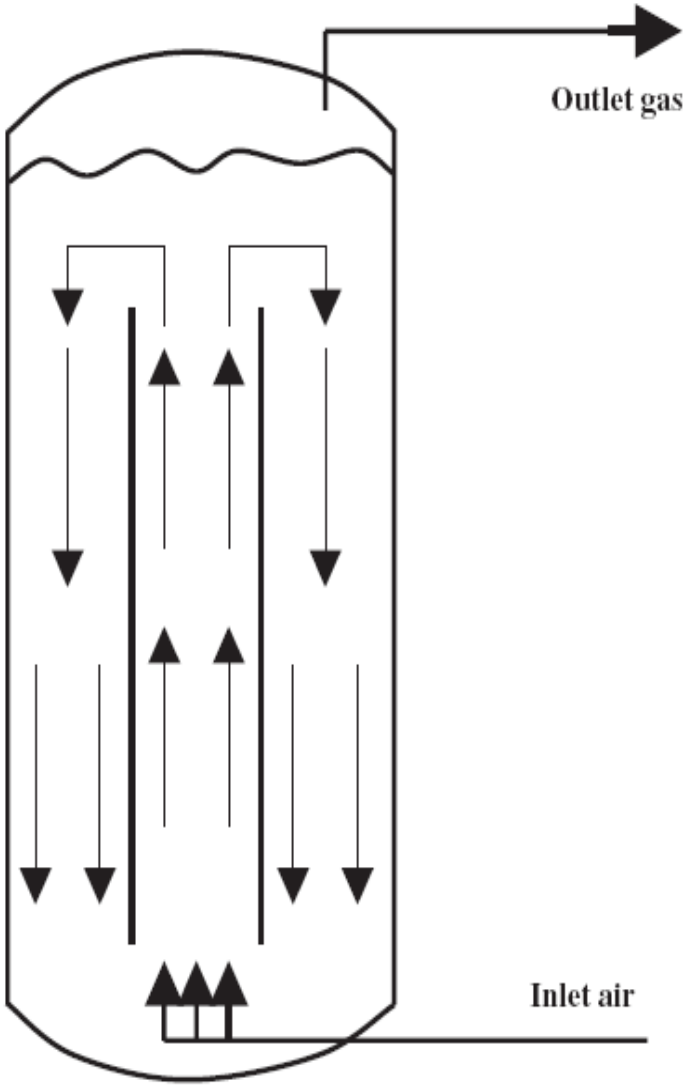
- Difficulties in maintaining aseptic conditions
- Difficulties in quality demands (different batches)
- Decrease in productivity (plasmid loss)
- Unsatisfactory products in some cases
- Changes in final yield (due to possible changes in cell and nutrients supply)

Complete mixing bioreactor

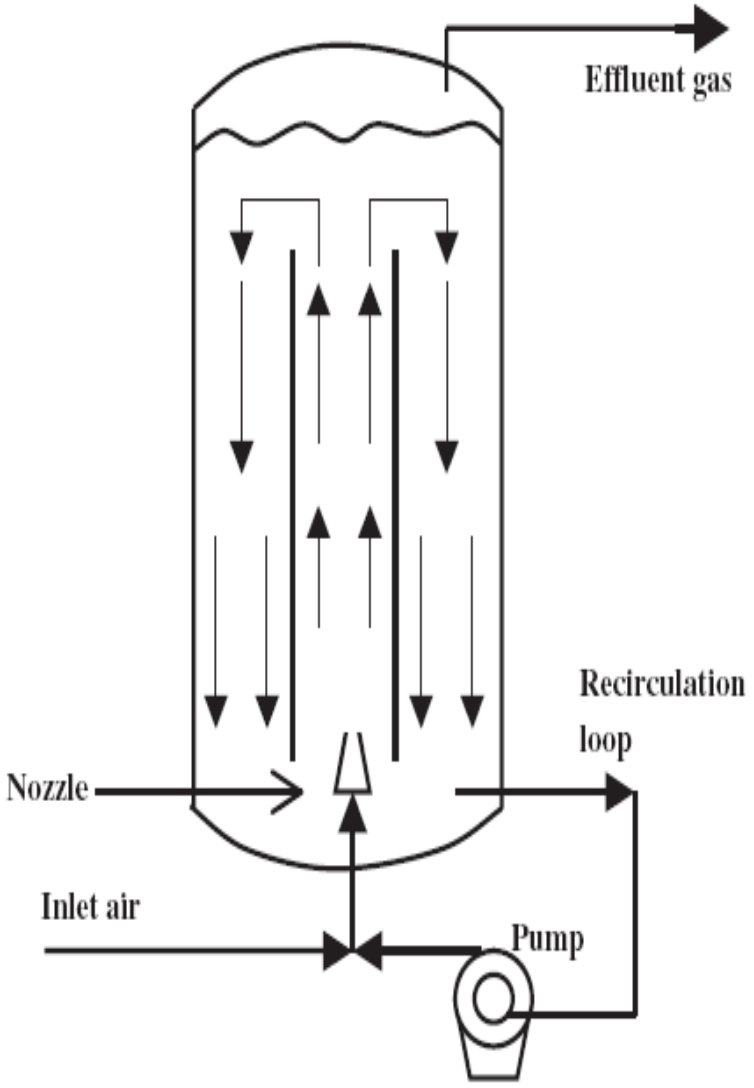


Loop bioreactor

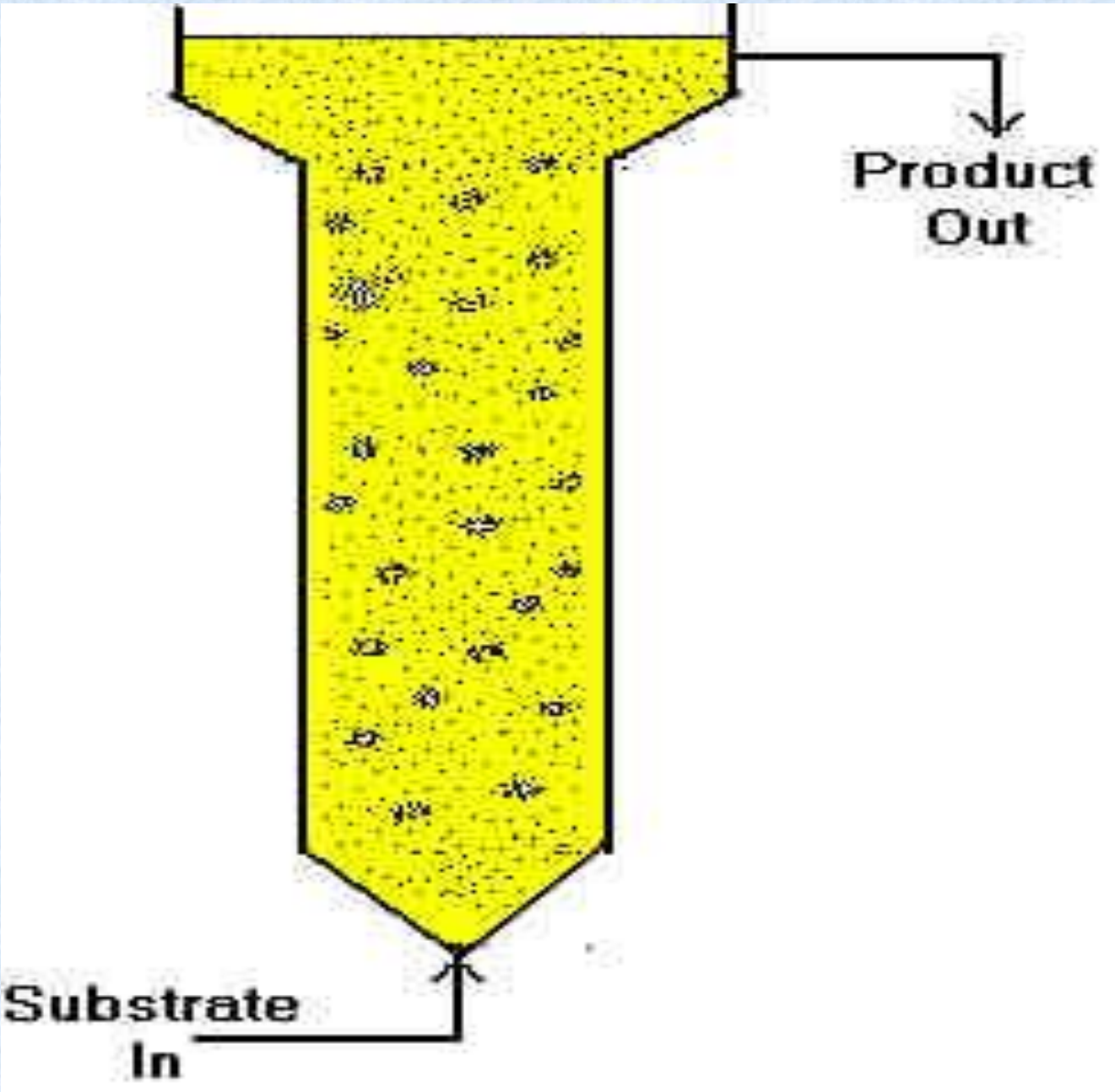
Inner loop



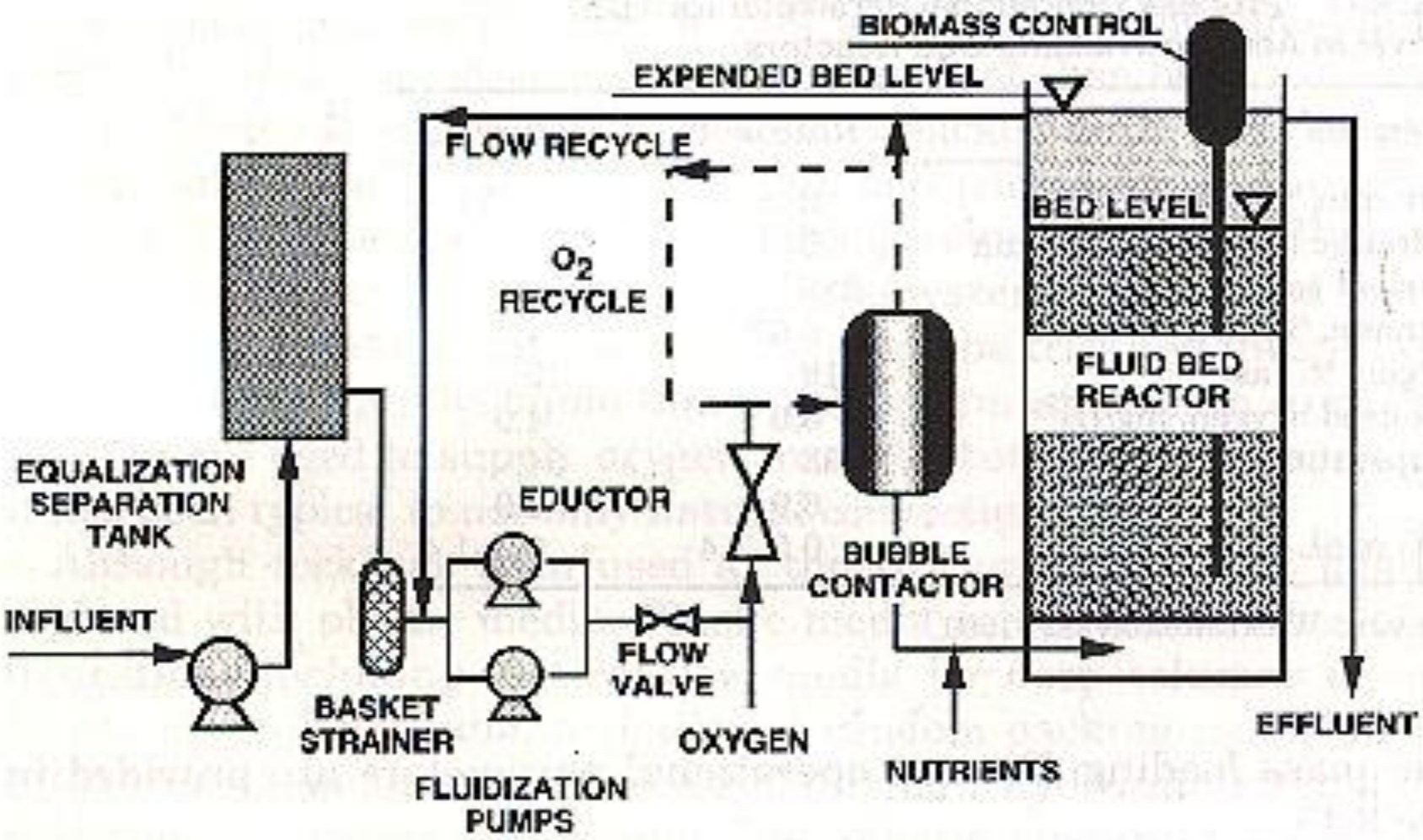
Outer loop



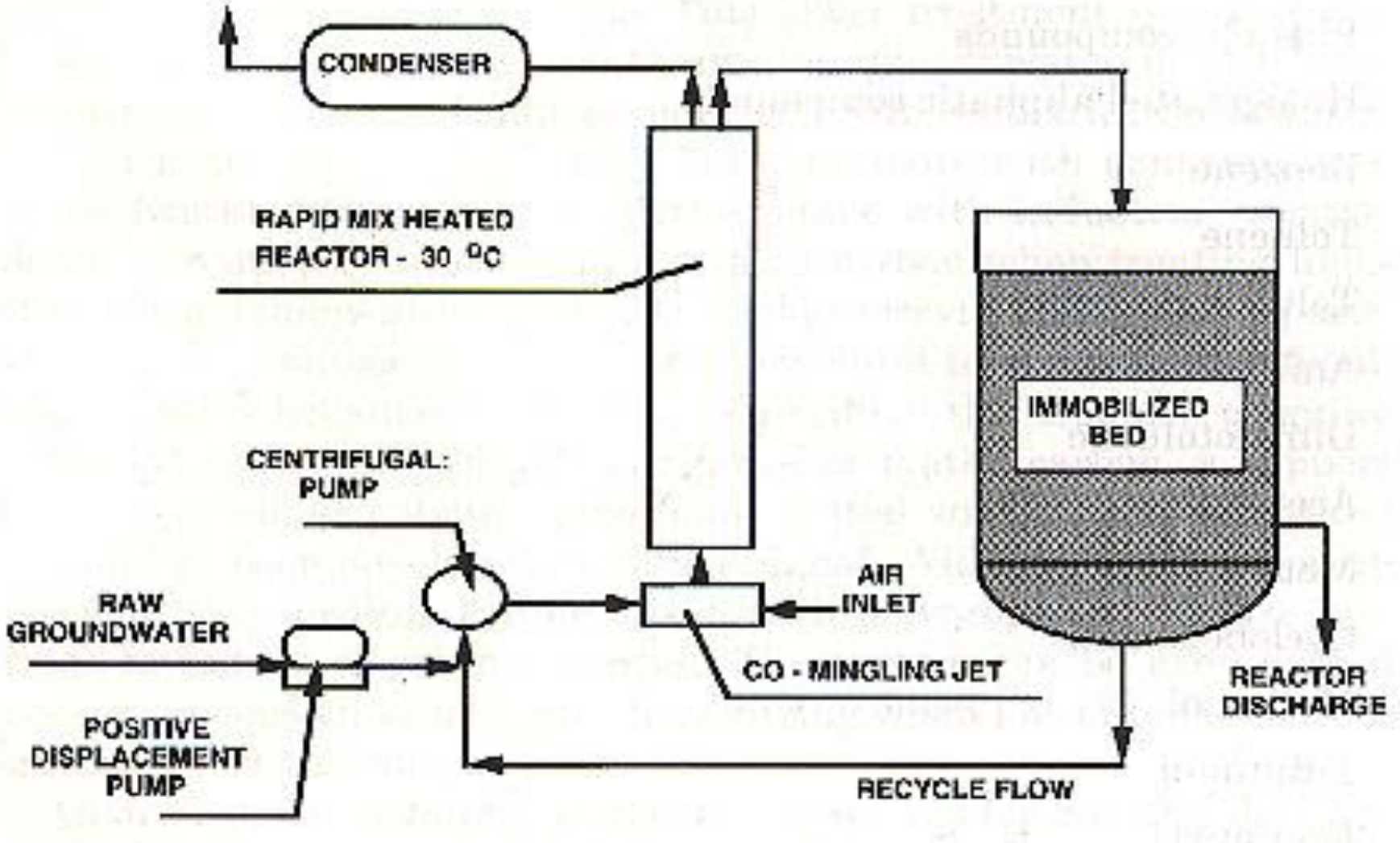
Fluidized bed bioreactor



Fluidized bed bioreactor

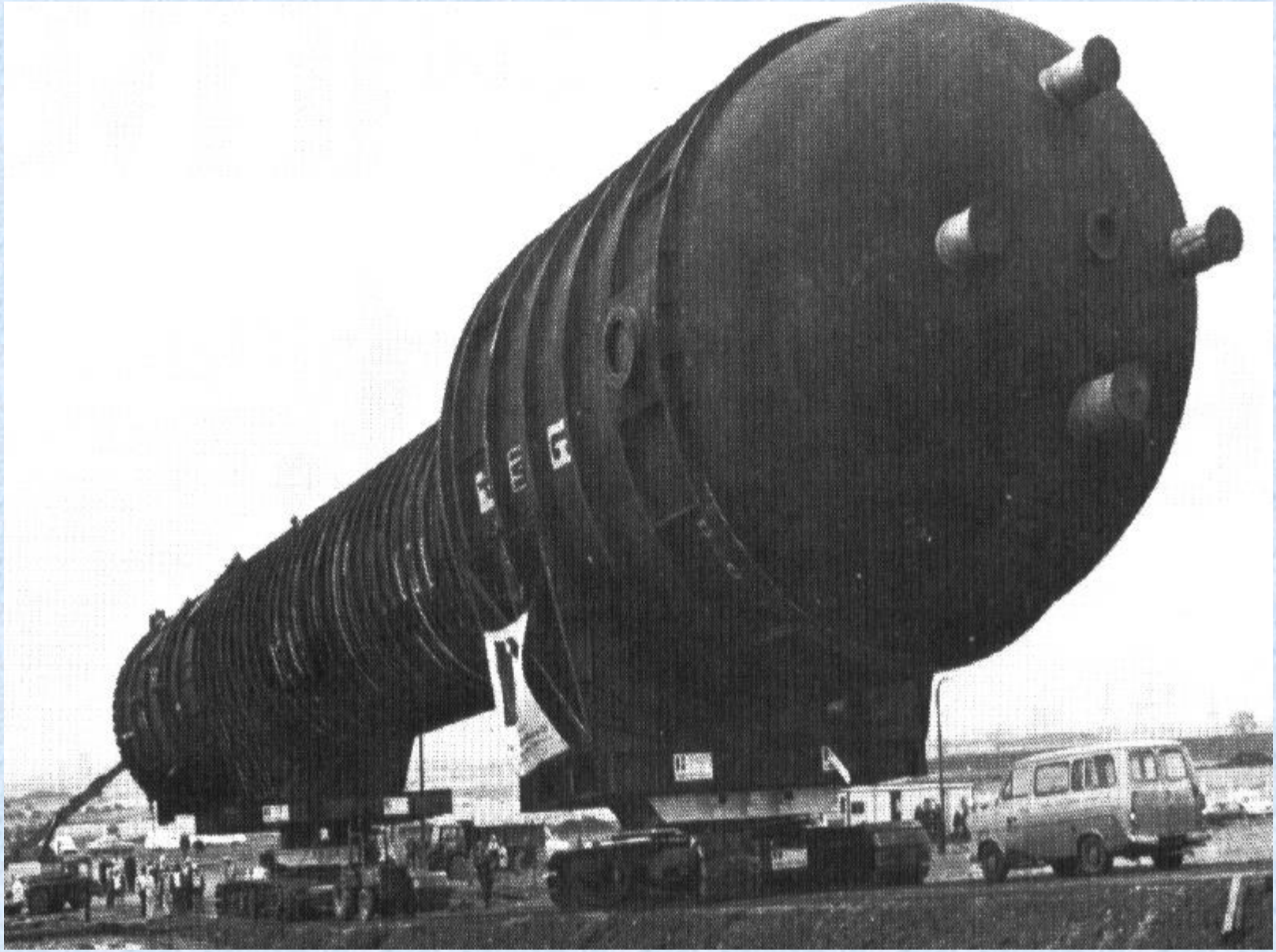


Bed bioreactor

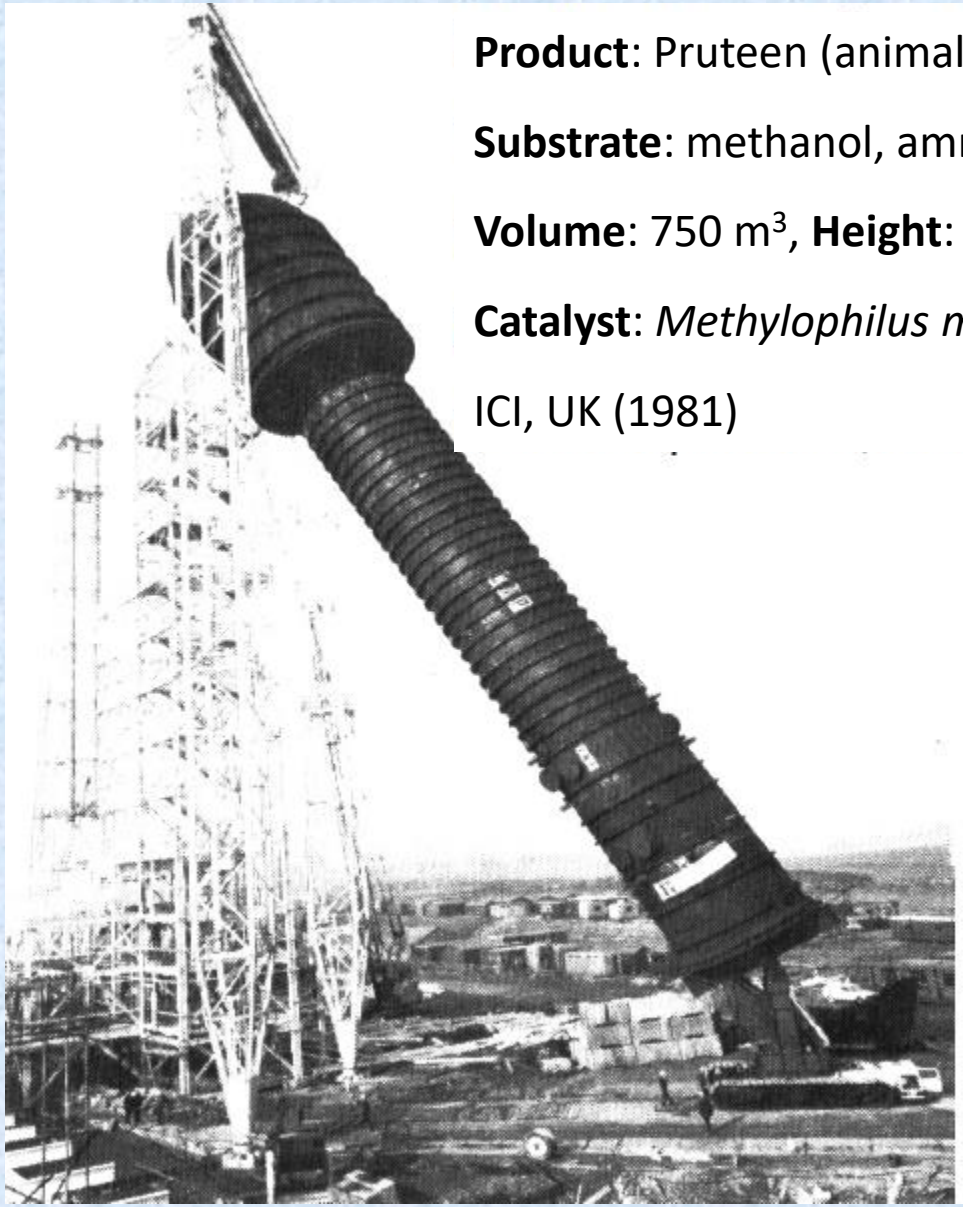



sartorius
BIOSTAT®A plus
sartorius
TOSHIBA
Main
Trend
Calibration
Controller
Maintenance
Remote
Alarm

Complete mixing bioreactor



Complete mixing bioreactor



Product: Pruteen (animal food)

Substrate: methanol, ammonia

Volume: 750 m^3, **Height**: 42 m, **Width**: 11 m

Catalyst: *Methylophilus methylotrophus*

ICI, UK (1981)

Fluidized bed bioreactor

Height: 21 m

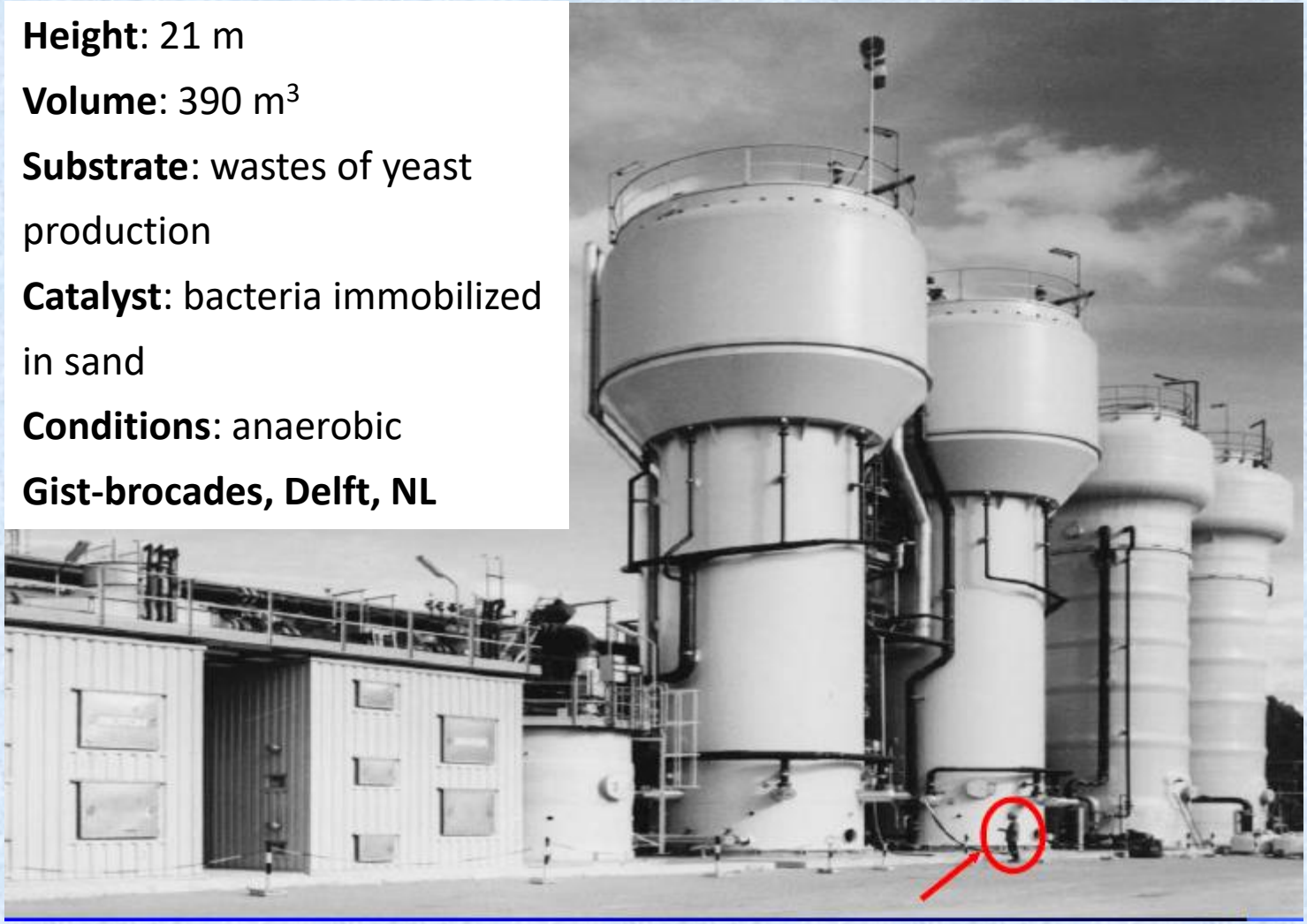
Volume: 390 m^3

Substrate: wastes of yeast production

Catalyst: bacteria immobilized in sand

Conditions: anaerobic

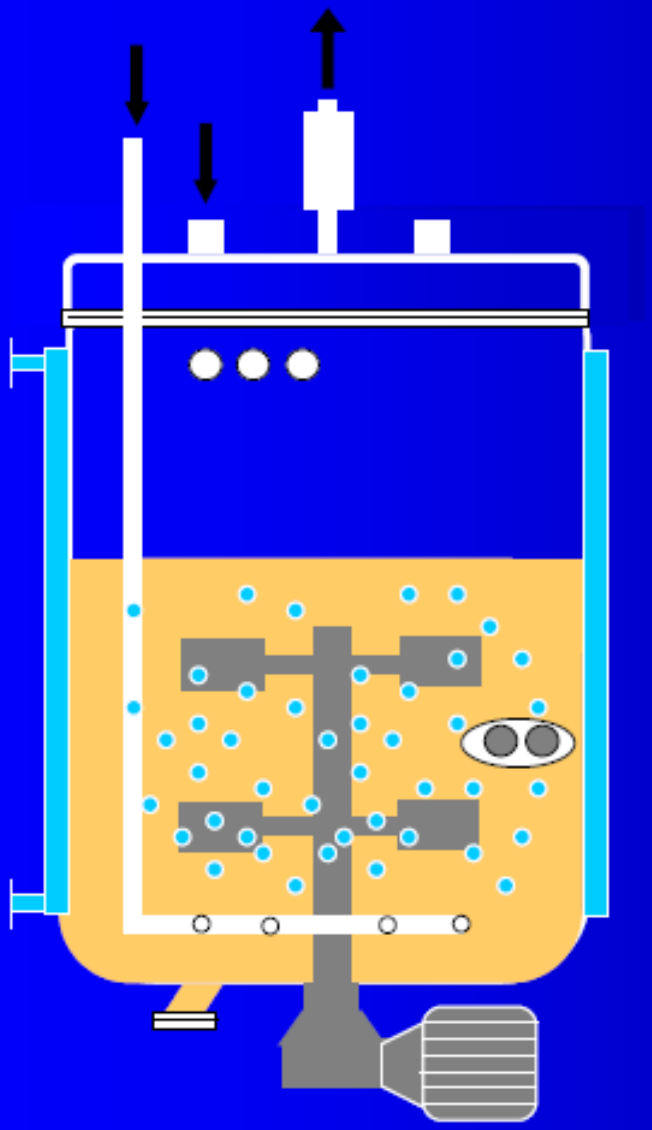
Gist-brocades, Delft, NL



Photovoltaic bioreactor

Type: Bioreactor with 96 polyethylene tubes
(length 120 m, diameter 25 cm, volume 600 m^3)
Catalyst: cyanobacteria *Arthrospira platensis*
Hidrobiologica SA, La Rioja, Spain

Complete mixing bioreactor

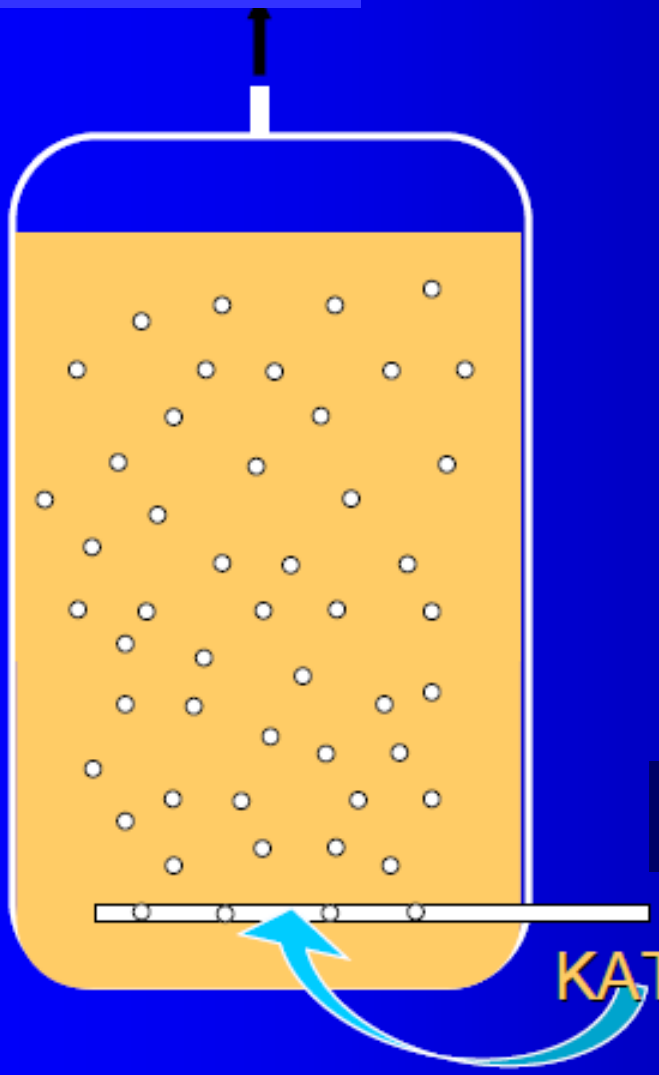


- Optimum control and setting of variables
- A lot of research

✓ Antibiotics
✓ Amino acids
✓ Industrial enzymes

Bubble column bioreactors

AIR OUTLET



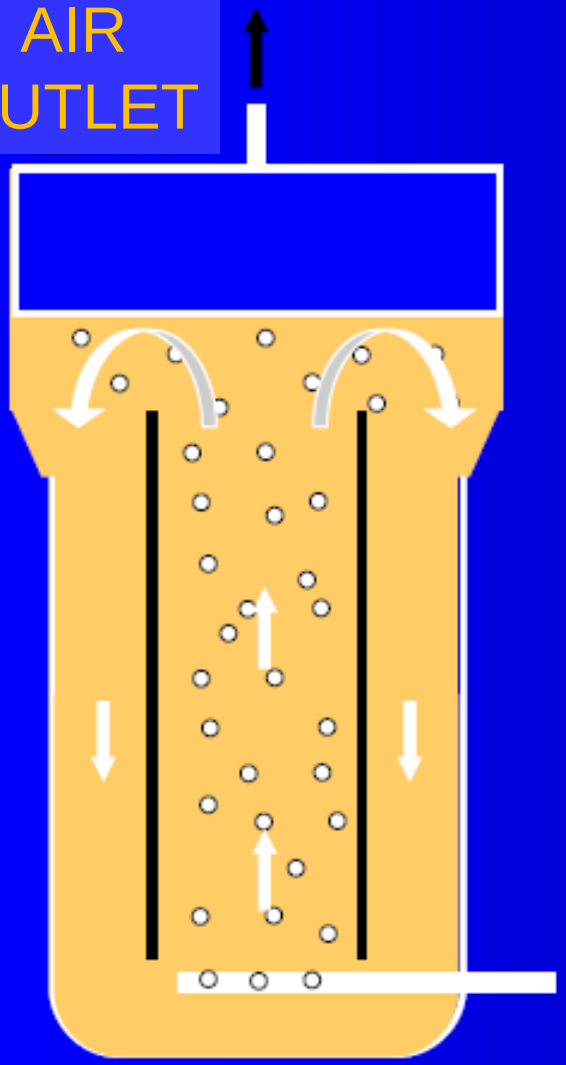
- Low manufacturing cost
- Satisfactory mass and heat transfer

✓ Baker's yeast
✓ Citric acids

AIR INLET

ΚΑΤΑΝΕΜΗΤΗΣ ΑΕΡΑ
(sparger)

Loop bioreactors



- Optimum mixing
- Satisfactory mass transfer
- Sensitive cells protection

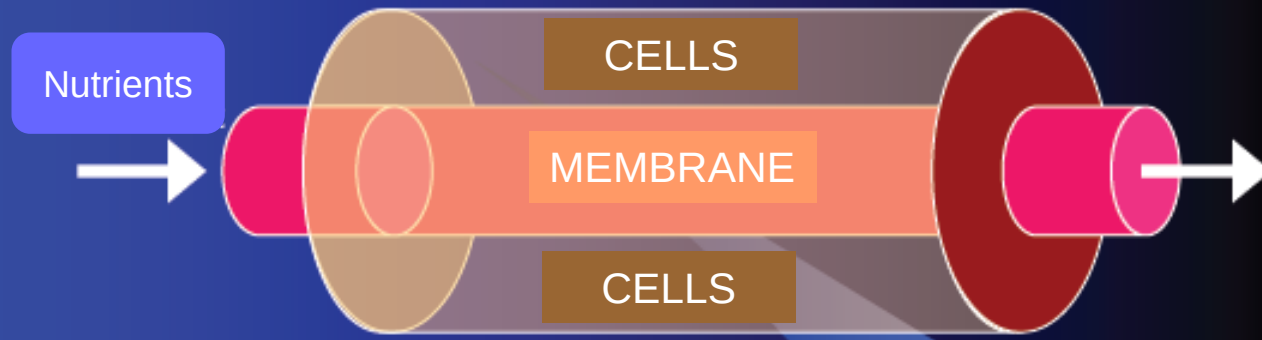
✓ Animal cell culture
✓ Industrial wastes treatment

Bed bioreactors

Σταθερής κλίνης (packed bed)	Ρευστοποιημένης κλίνης (fluidized bed)
Advantages	
• **Ability of continuous operation** • **Increased interface**	
• **High concentration of biocatalyst** • **Handling inhibition from the product**	• **Easy control of pH and T** • **Ability to use colloidal substrates**
✓ Acetic acid (vinegar) production ✓ Biogas production from solid wastes	✓ Liquid wastes treatment ✓ Production of alcohol-free bier ✓ Anti-HIV antibodies production

Membrane bioreactors

Hollow fiber bioreactor



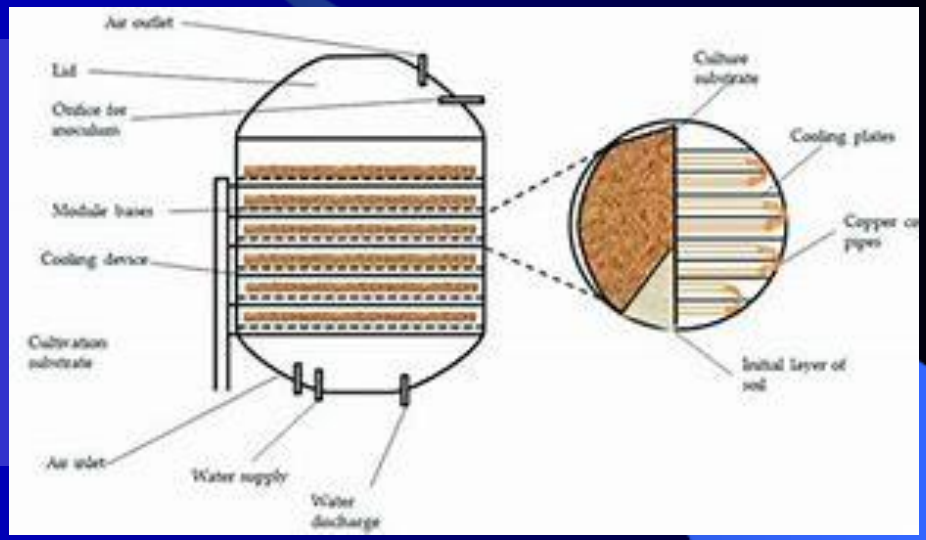
- **Removal of any inhibitor (impurity or product)**
- **Ability of continuous operation**

✓ Enzyme bioreactor
✓ Animal cell cultures
✓ Archaeobacteria cultures

Bioreactors for solid-state fermentation

Solid-state fermentation: microbial growth in solid substrates in the absence of free water

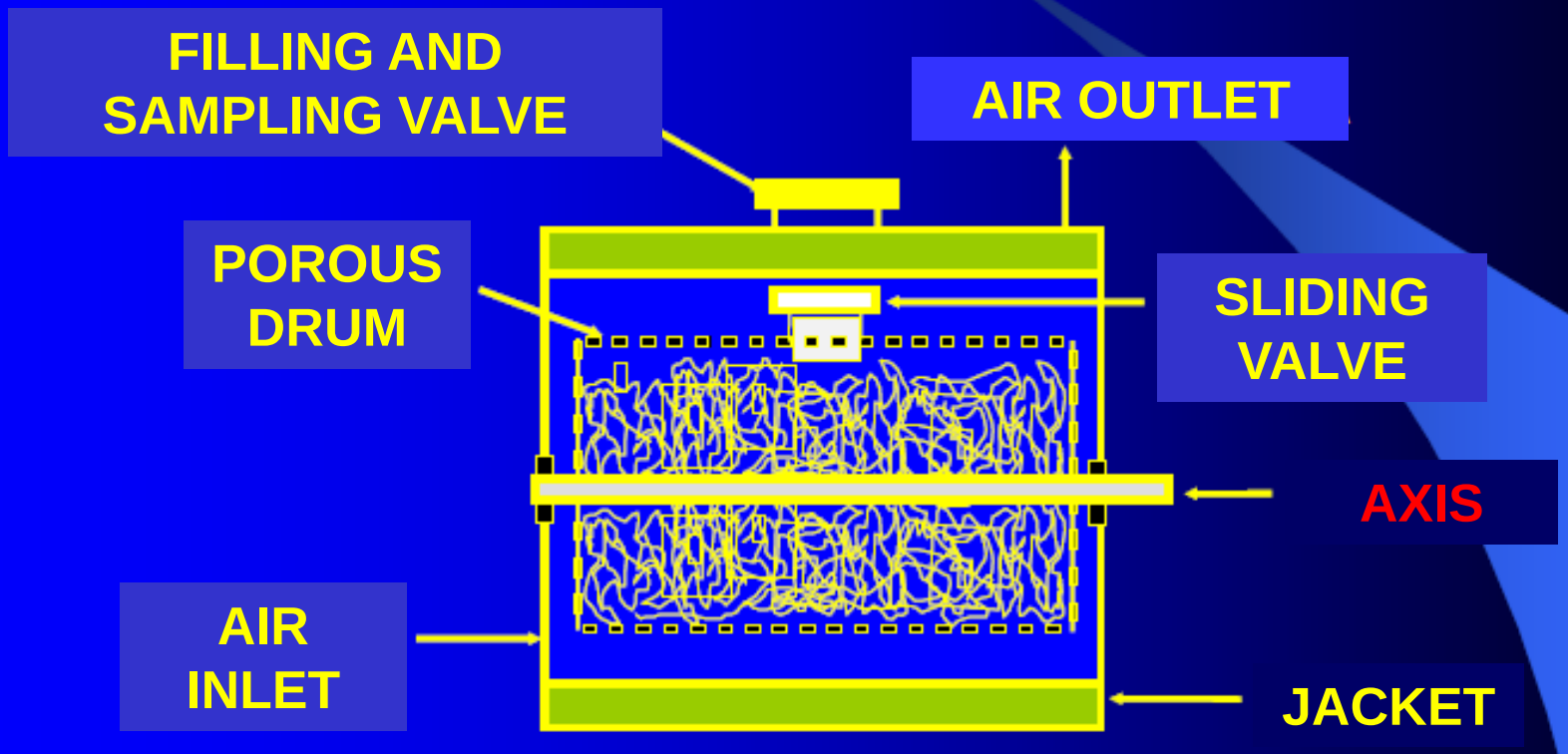
- With disks
- Packed bed
- Rotating drum
- With stirring



- **Low installation and operation costs**
- **Easier product recovery**

- ✓ Amylase
- ✓ Protease
- ✓ Traditional products of East Asia (soy sauce)

Rotating drum fermentor



Selection and design of bioreactor

Criteria

- Microorganism (species, physiology, genetic instability)
- Substrate (type, inhibition)
- Product (relation to the metabolism of microorganism, value)

- **Each case is a different problem and should be solved according to the above restrictions**