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**Biopharmaceutics**

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MCPHS
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# Bioprocess Development Upstream and Downstream Technologies

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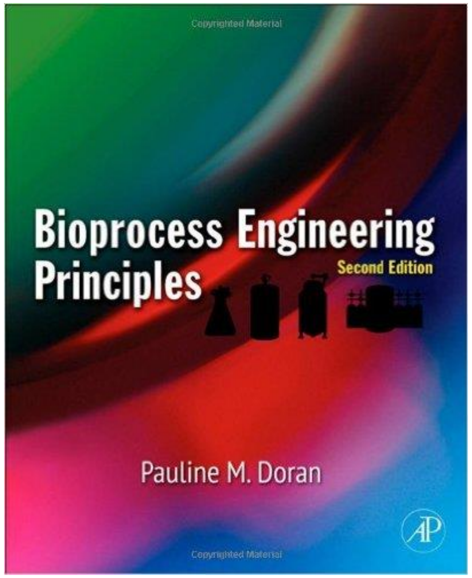
# References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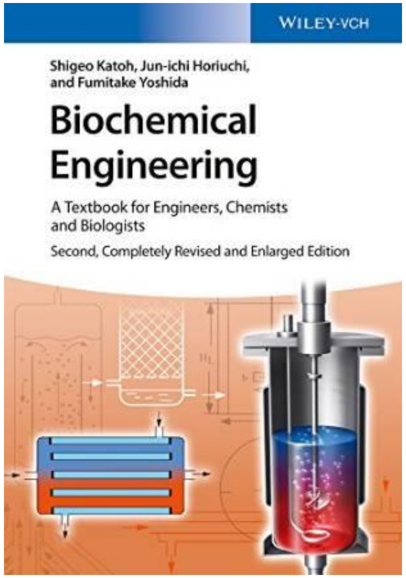
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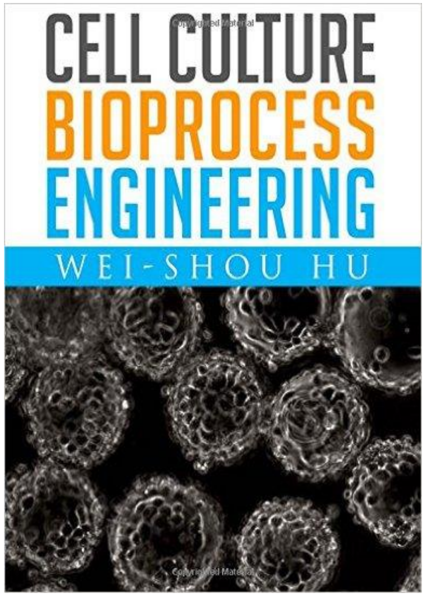
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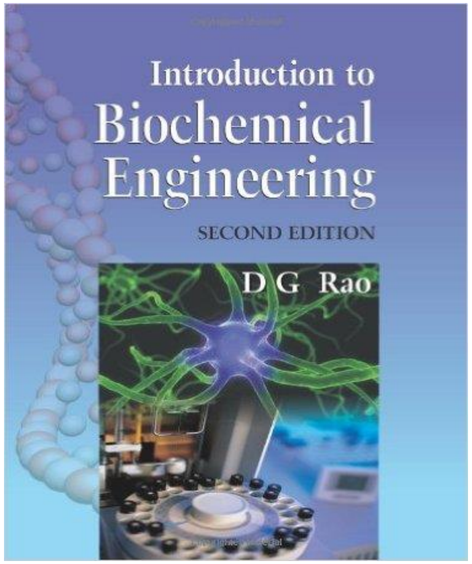
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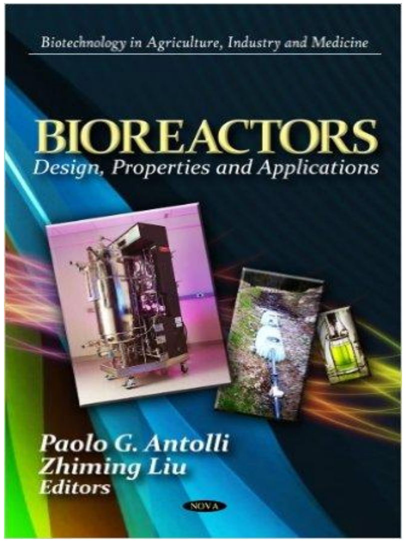
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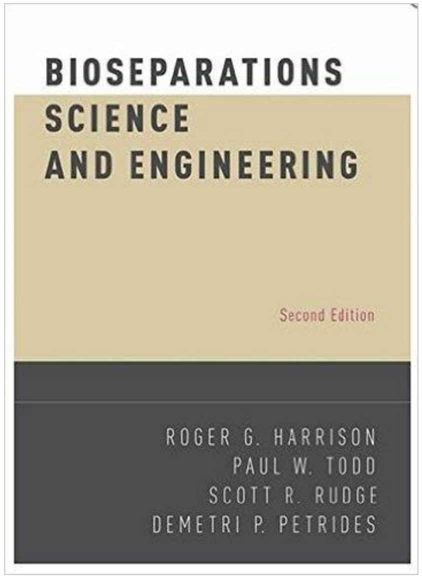
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# Solid State Fermentation

- **Microorganisms grow on a moistened solid surface without free flowing water, but have free access to air.**
- **Low capital investment, water utilization, waste water, and energy requirement (no agitation)**
- **No foam formation**
- **Simple fermentation media**
- **Less space**
- **Less control techniques**
- **Ease in controlling bacterial contamination**
- **Ease in induction and suppression of spores**
- **Low downstream processing**

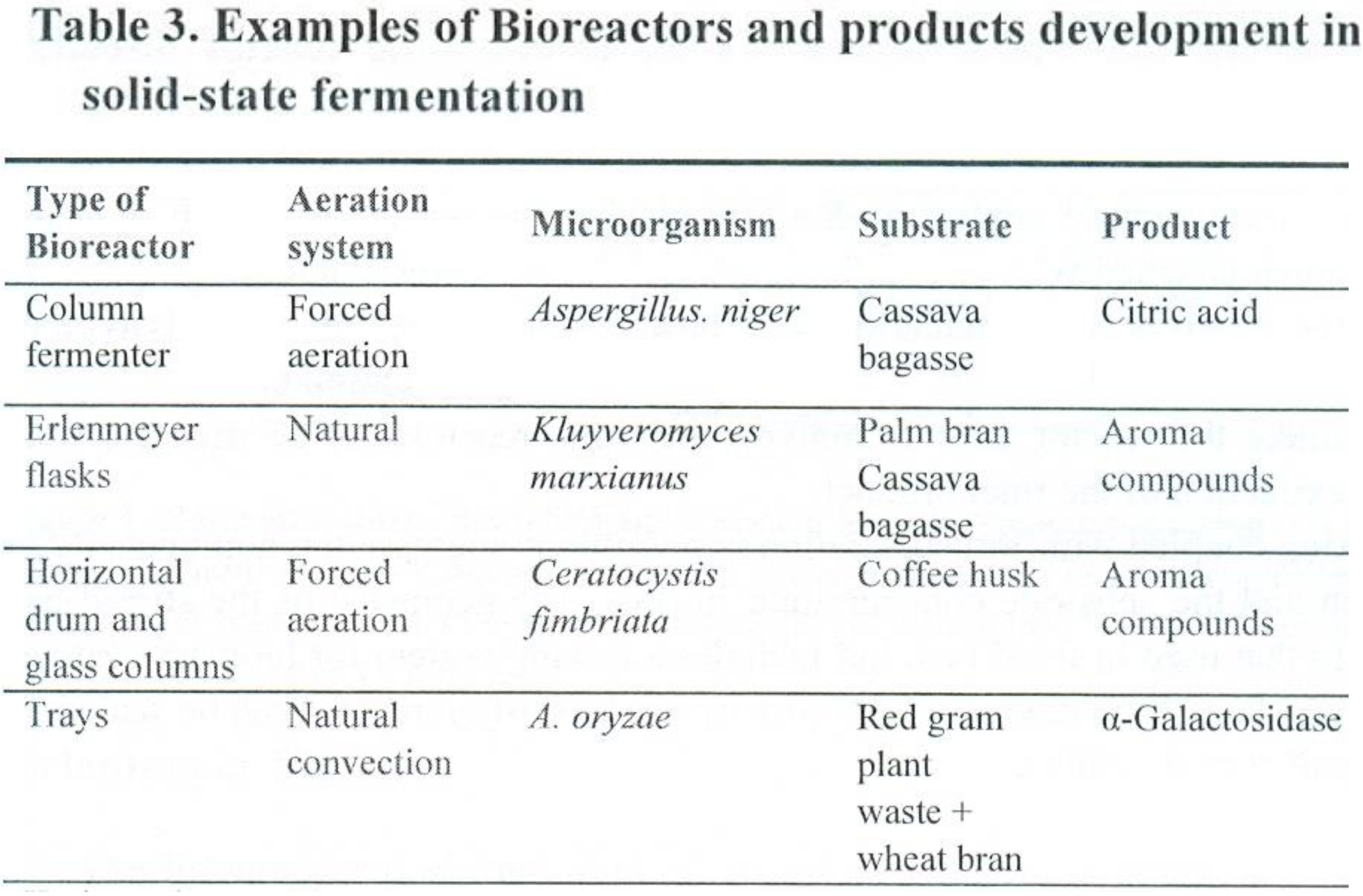
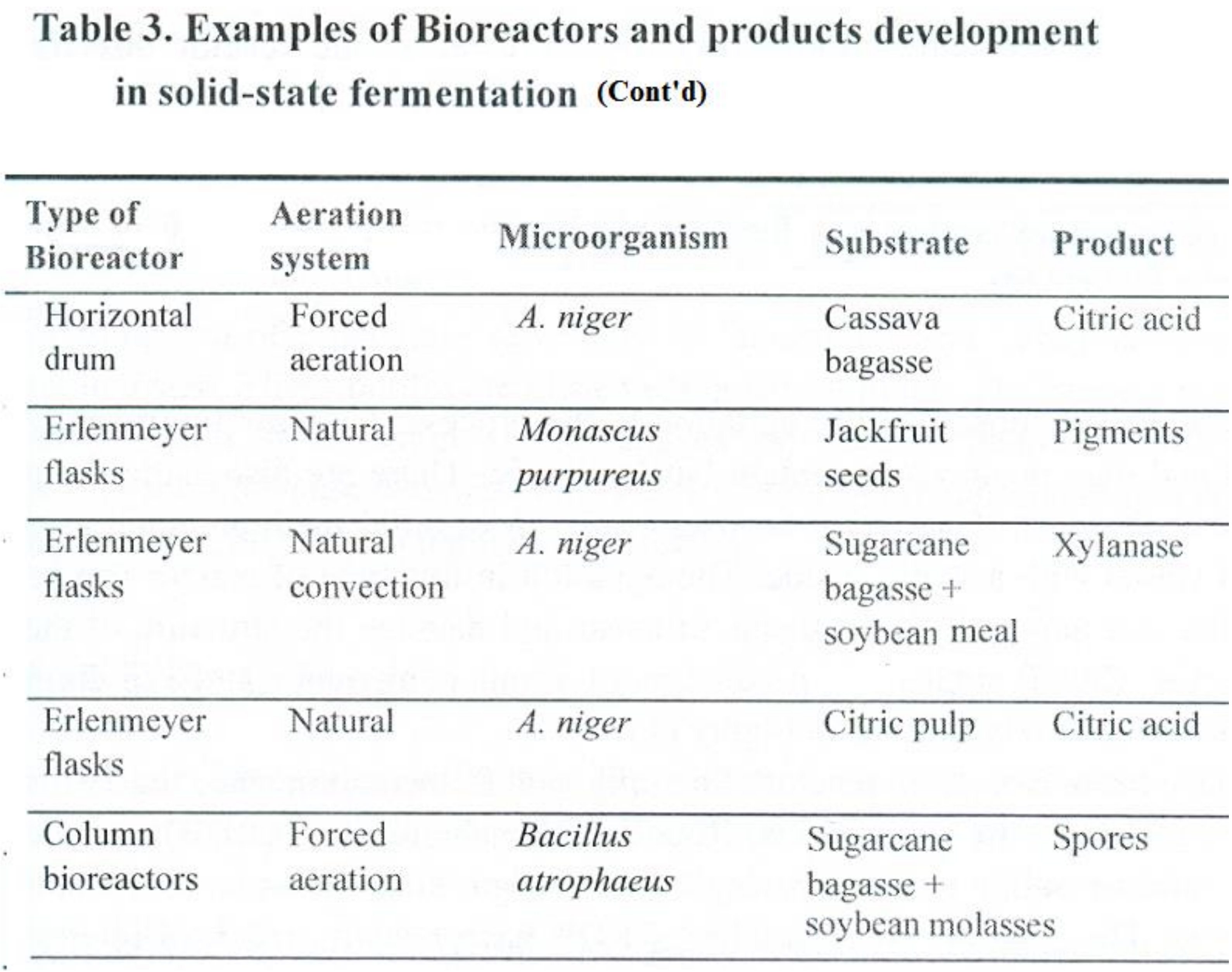


**Table 3. Examples of Bioreactors and products development in solid-state fermentation**

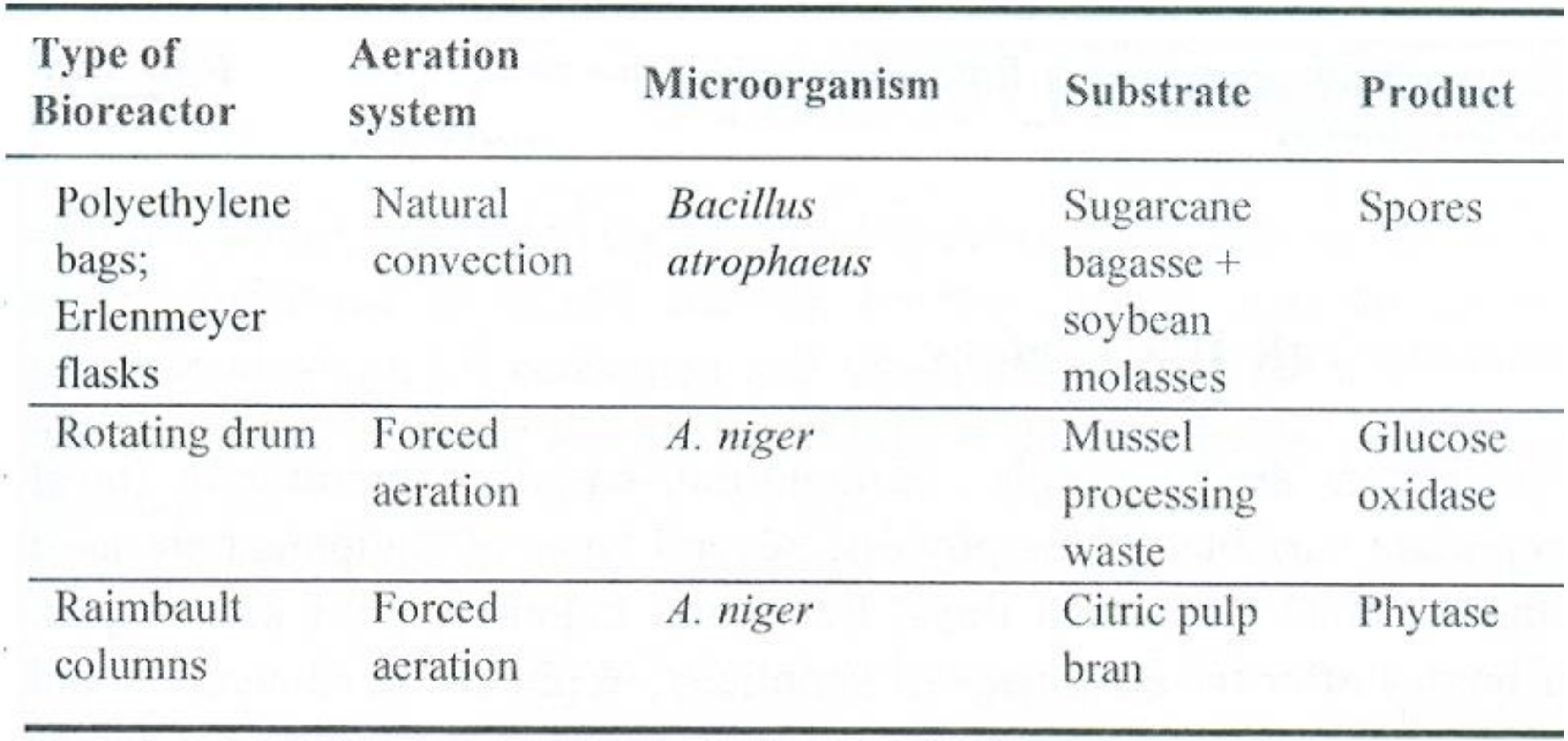
| Type of Bioreactor | Aeration system | Microorganism | Substrate | Product |
|---|---|---|---|---|
| Column fermenter | Forced aeration | *Aspergillus. niger* | Cassava bagasse | Citric acid |
| Erlenmeyer flasks | Natural | *Kluyveromyces marxianus* | Palm bran<br>Cassava bagasse | Aroma compounds |
| Horizontal drum and glass columns | Forced aeration | *Ceratocystis fimbriata* | Coffee husk | Aroma compounds |
| Trays | Natural convection | *A. oryzae* | Red gram plant waste + wheat bran | α-Galactosidase |



## Table 3. Examples of Bioreactors and products development in solid-state fermentation (Cont'd)

| Type of Bioreactor | Aeration system | Microorganism | Substrate | Product |
|---|---|---|---|---|
| Horizontal drum | Forced aeration | *A. niger* | Cassava bagasse | Citric acid |
| Erlenmeyer flasks | Natural | *Monascus purpureus* | Jackfruit seeds | Pigments |
| Erlenmeyer flasks | Natural convection | *A. niger* | Sugarcane bagasse + soybean meal | Xylanase |
| Erlenmeyer flasks | Natural | *A. niger* | Citric pulp | Citric acid |
| Column bioreactors | Forced aeration | *Bacillus atrophaeus* | Sugarcane bagasse + soybean molasses | Spores |

# Table 3. Examples of Bioreactors and products development in solid-state fermentation (Cont'd)



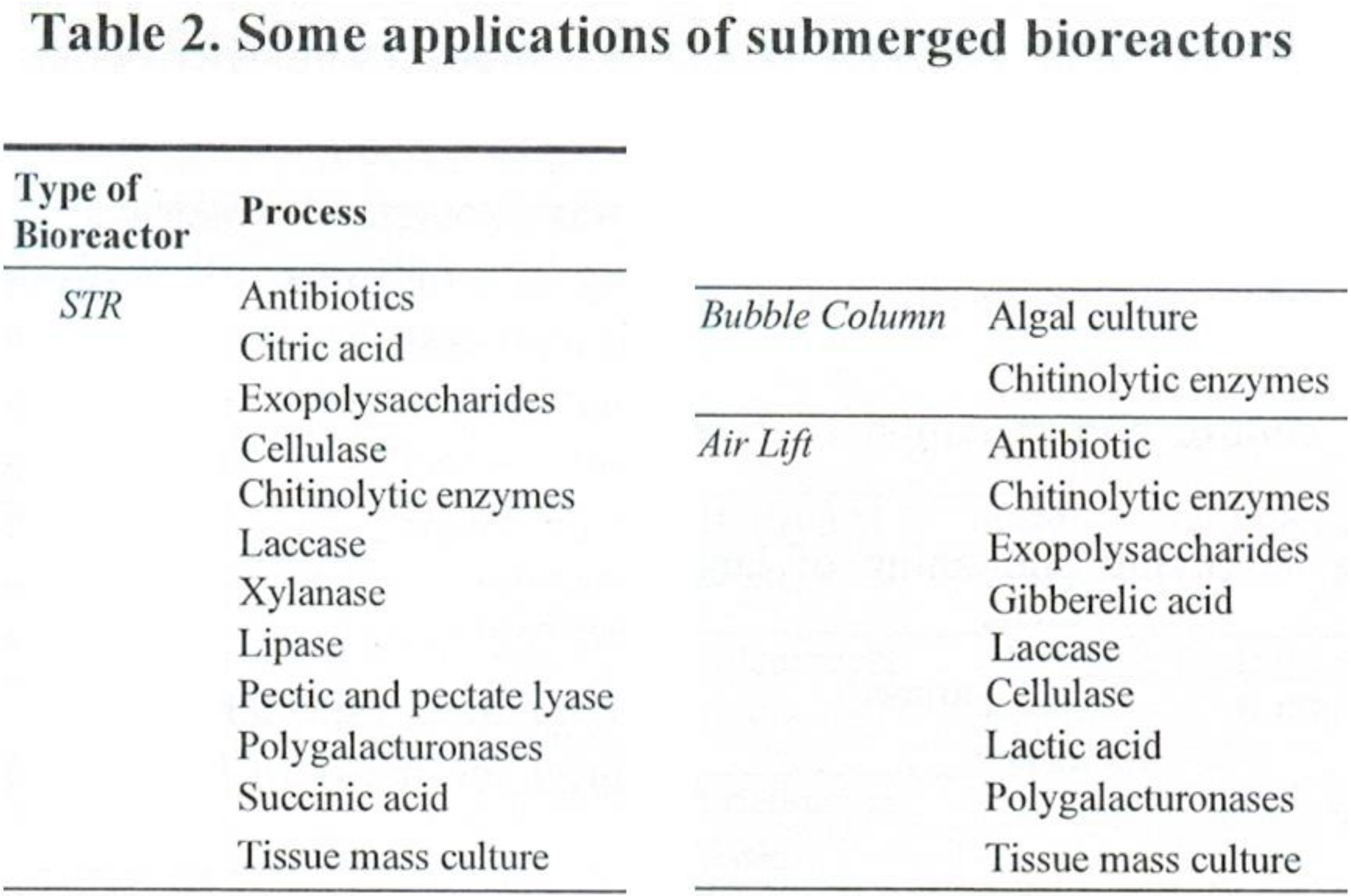
| Type of Bioreactor | Aeration system | Microorganism | Substrate | Product |
|---|---|---|---|---|
| Polyethylene bags; Erlenmeyer flasks | Natural convection | *Bacillus atrophaeus* | Sugarcane bagasse + soybean molasses | Spores |
| Rotating drum | Forced aeration | *A. niger* | Mussel processing waste | Glucose oxidase |
| Raimbault columns | Forced aeration | *A. niger* | Citric pulp bran | Phytase |

# Submerged Fermentation

The microorganisms and the substrate are present in the submerged state in the liquid medium.

- Large solvent
- The heat and mass transfer are more efficient
- Amenable for modeling the process
- Process scale-up is easy.

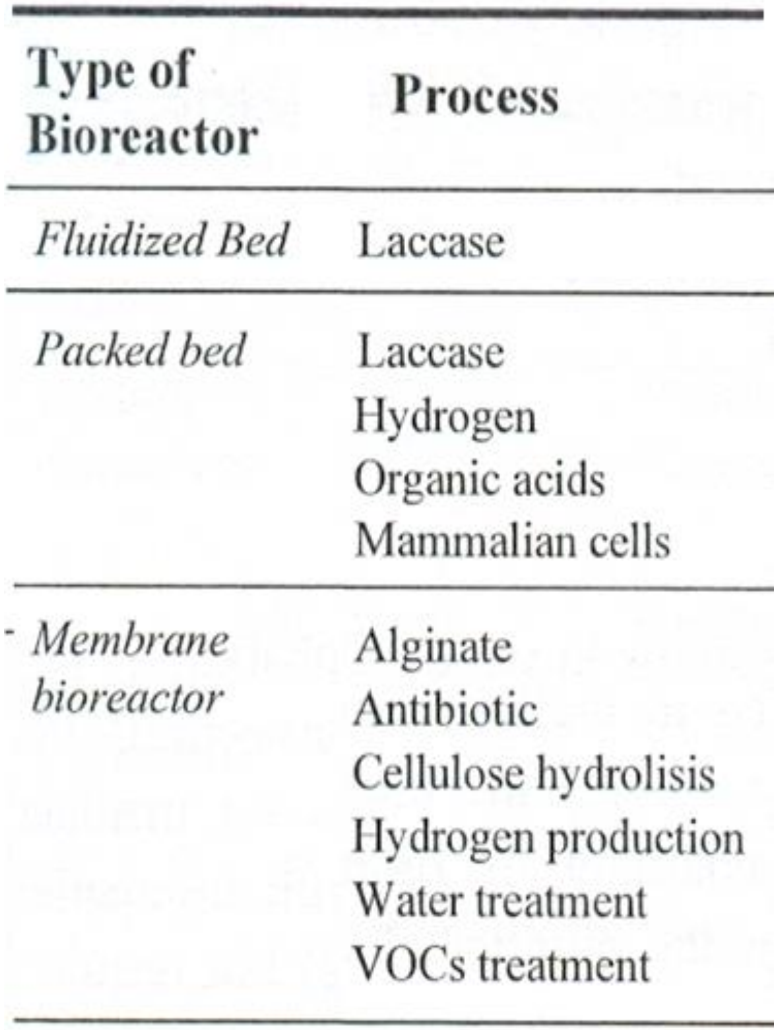
More popular



# Table 2. Some applications of submerged bioreactors

| Type of Bioreactor | Process |
|---|---|
| *STR* | Antibiotics |
| | Citric acid |
| | Exopolysaccharides |
| | Cellulase |
| | Chitinolytic enzymes |
| | Laccase |
| | Xylanase |
| | Lipase |
| | Pectic and pectate lyase |
| | Polygalacturonases |
| | Succinic acid |
| | Tissue mass culture |
| *Bubble Column* | Algal culture |
| | Chitinolytic enzymes |
| *Air Lift* | Antibiotic |
| | Chitinolytic enzymes |
| | Exopolysaccharides |
| | Gibberelic acid |
| | Laccase |
| | Cellulase |
| | Lactic acid |
| | Polygalacturonases |
| | Tissue mass culture |

# Table 2. Submerged bioreactors (Cont'd)



| Type of Bioreactor | Process |
|---|---|
| *Fluidized Bed* | Laccase |
| *Packed bed* | Laccase<br>Hydrogen<br>Organic acids<br>Mammalian cells |
| *Membrane bioreactor* | Alginate<br>Antibiotic<br>Cellulose hydrolisis<br>Hydrogen production<br>Water treatment<br>VOCs treatment |

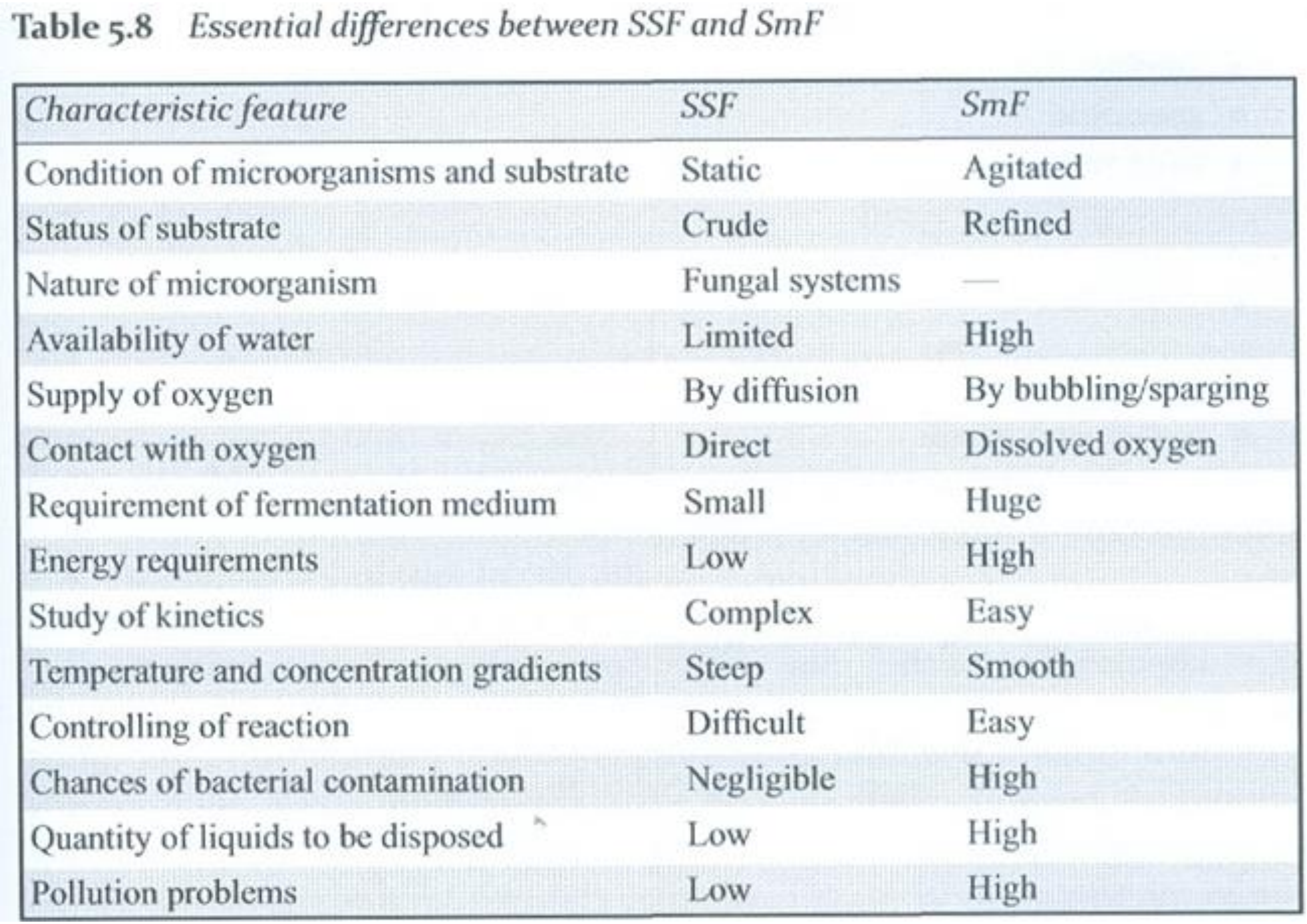


**Table 5.8** *Essential differences between SSF and SmF*

| *Characteristic feature* | *SSF* | *SmF* |
|---|---|---|
| Condition of microorganisms and substrate | Static | Agitated |
| Status of substrate | Crude | Refined |
| Nature of microorganism | Fungal systems | — |
| Availability of water | Limited | High |
| Supply of oxygen | By diffusion | By bubbling/sparging |
| Contact with oxygen | Direct | Dissolved oxygen |
| Requirement of fermentation medium | Small | Huge |
| Energy requirements | Low | High |
| Study of kinetics | Complex | Easy |
| Temperature and concentration gradients | Steep | Smooth |
| Controlling of reaction | Difficult | Easy |
| Chances of bacterial contamination | Negligible | High |
| Quantity of liquids to be disposed | Low | High |
| Pollution problems | Low | High |

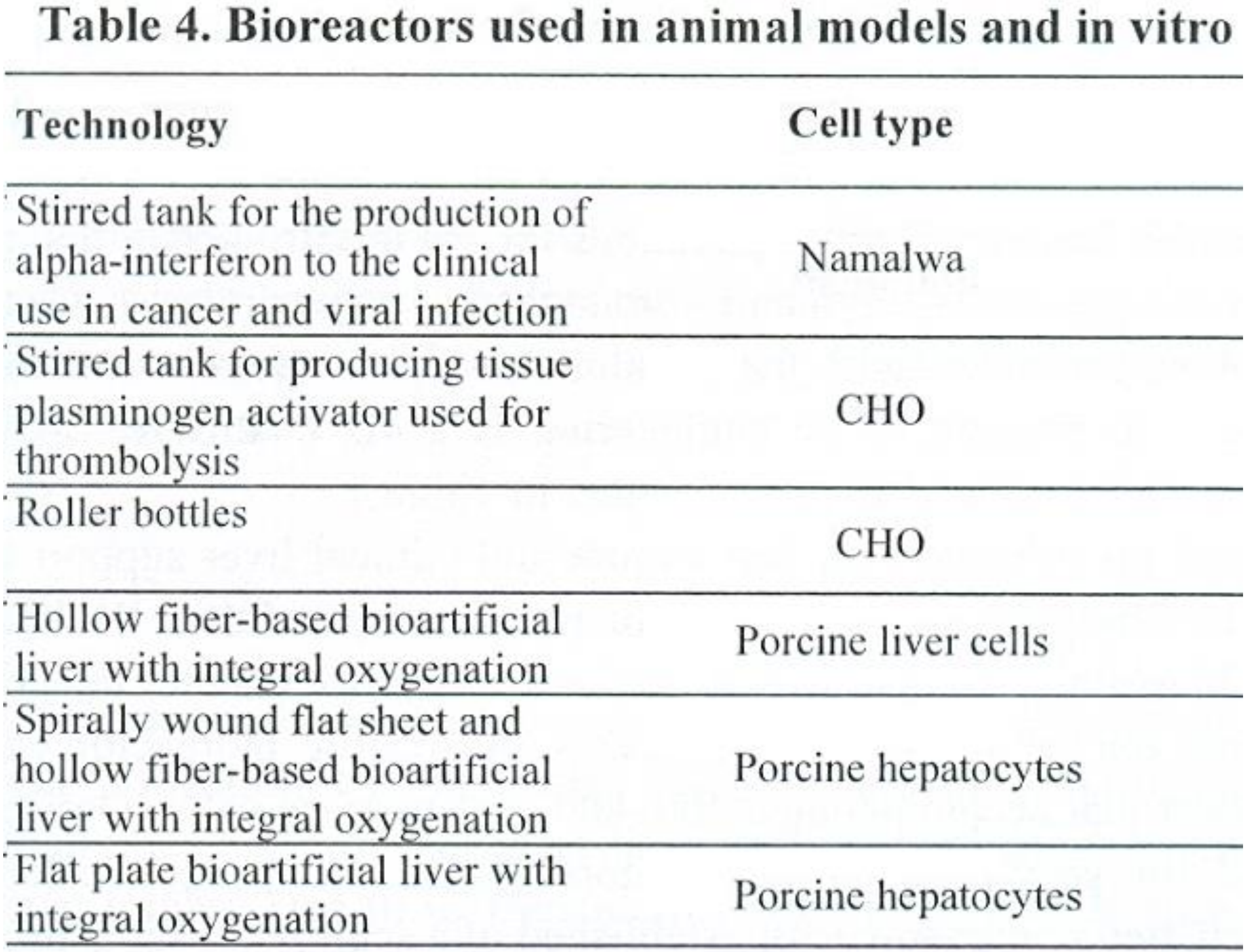
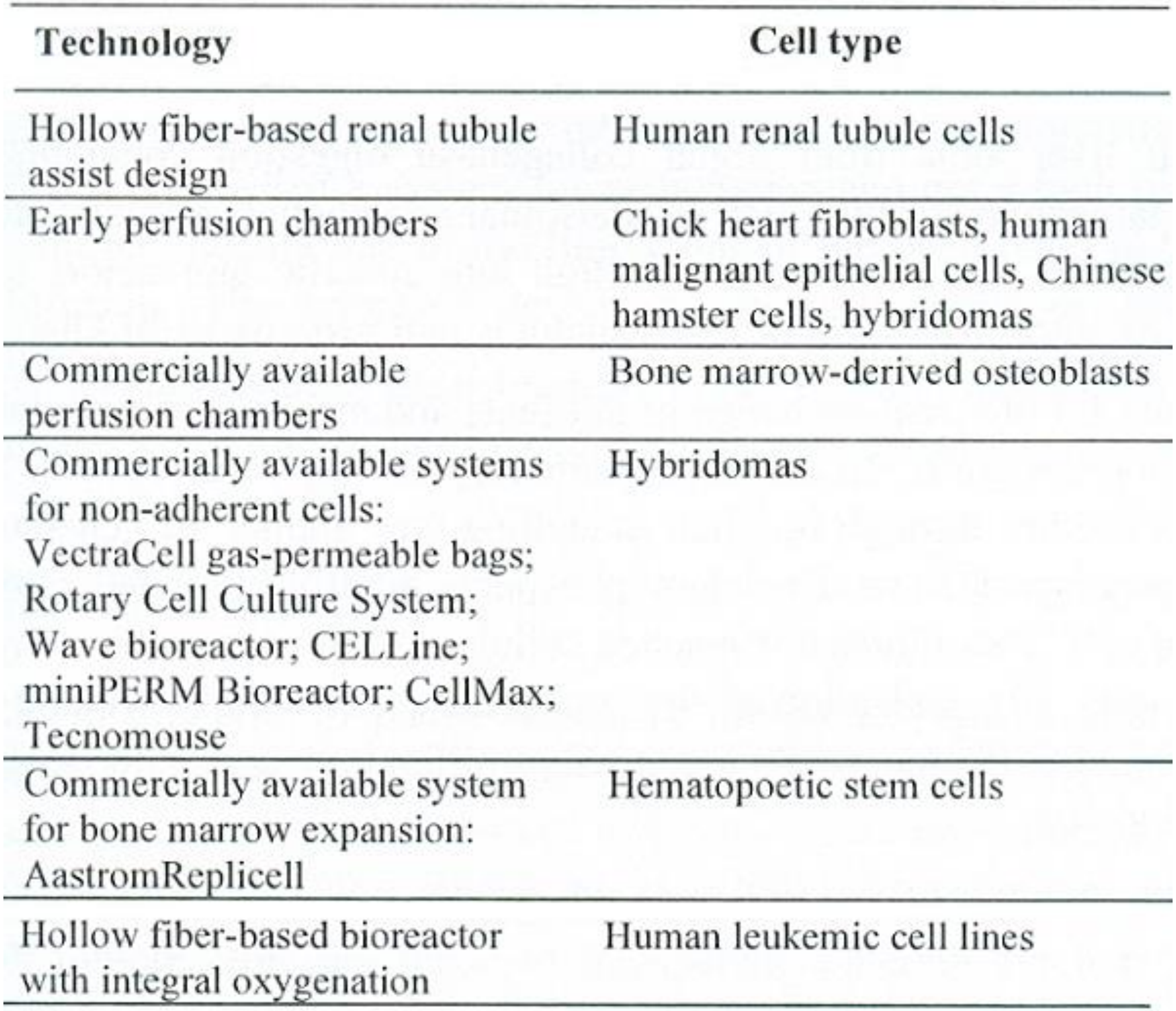


**Table 4. Bioreactors used in animal models and in vitro**

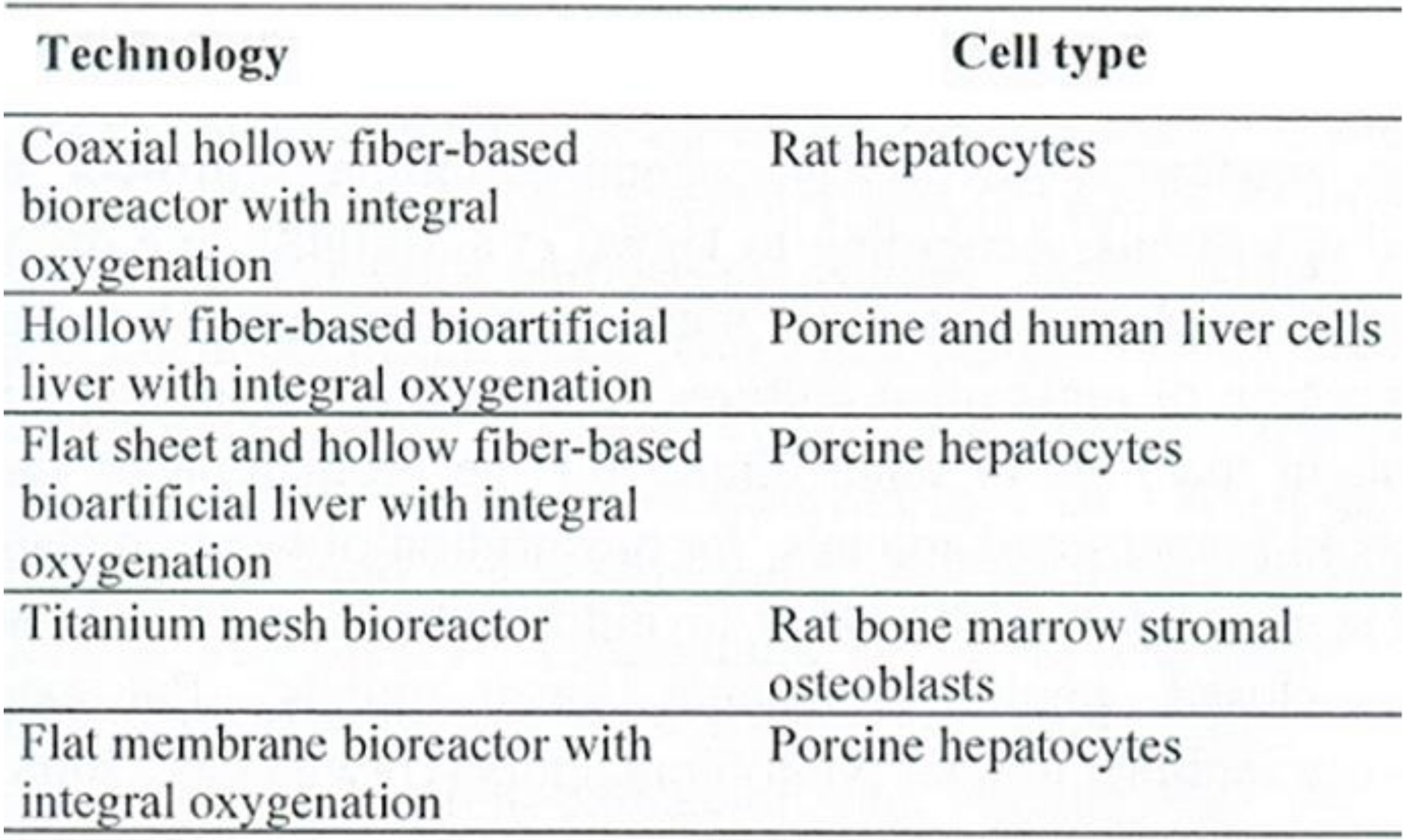
| Technology | Cell type |
|---|---|
| Stirred tank for the production of alpha-interferon to the clinical use in cancer and viral infection | Namalwa |
| Stirred tank for producing tissue plasminogen activator used for thrombolysis | CHO |
| Roller bottles | CHO |
| Hollow fiber-based bioartificial liver with integral oxygenation | Porcine liver cells |
| Spirally wound flat sheet and hollow fiber-based bioartificial liver with integral oxygenation | Porcine hepatocytes |
| Flat plate bioartificial liver with integral oxygenation | Porcine hepatocytes |

**Table 4. Bioreactors used in animal models (Cont'd)**



| Technology | Cell type |
|---|---|
| Hollow fiber-based renal tubule assist design | Human renal tubule cells |
| Early perfusion chambers | Chick heart fibroblasts, human malignant epithelial cells, Chinese hamster cells, hybridomas |
| Commercially available perfusion chambers | Bone marrow-derived osteoblasts |
| Commercially available systems for non-adherent cells:<br>VectraCell gas-permeable bags;<br>Rotary Cell Culture System;<br>Wave bioreactor; CELLine;<br>miniPERM Bioreactor; CellMax;<br>Tecnomouse | Hybridomas |
| Commercially available system for bone marrow expansion:<br>AastromReplicell | Hematopoetic stem cells |
| Hollow fiber-based bioreactor with integral oxygenation | Human leukemic cell lines |

## Table 4. Bioreactors used in animal models (Cont'd)



| Technology | Cell type |
|---|---|
| Coaxial hollow fiber-based bioreactor with integral oxygenation | Rat hepatocytes |
| Hollow fiber-based bioartificial liver with integral oxygenation | Porcine and human liver cells |
| Flat sheet and hollow fiber-based bioartificial liver with integral oxygenation | Porcine hepatocytes |
| Titanium mesh bioreactor | Rat bone marrow stromal osteoblasts |
| Flat membrane bioreactor with integral oxygenation | Porcine hepatocytes |

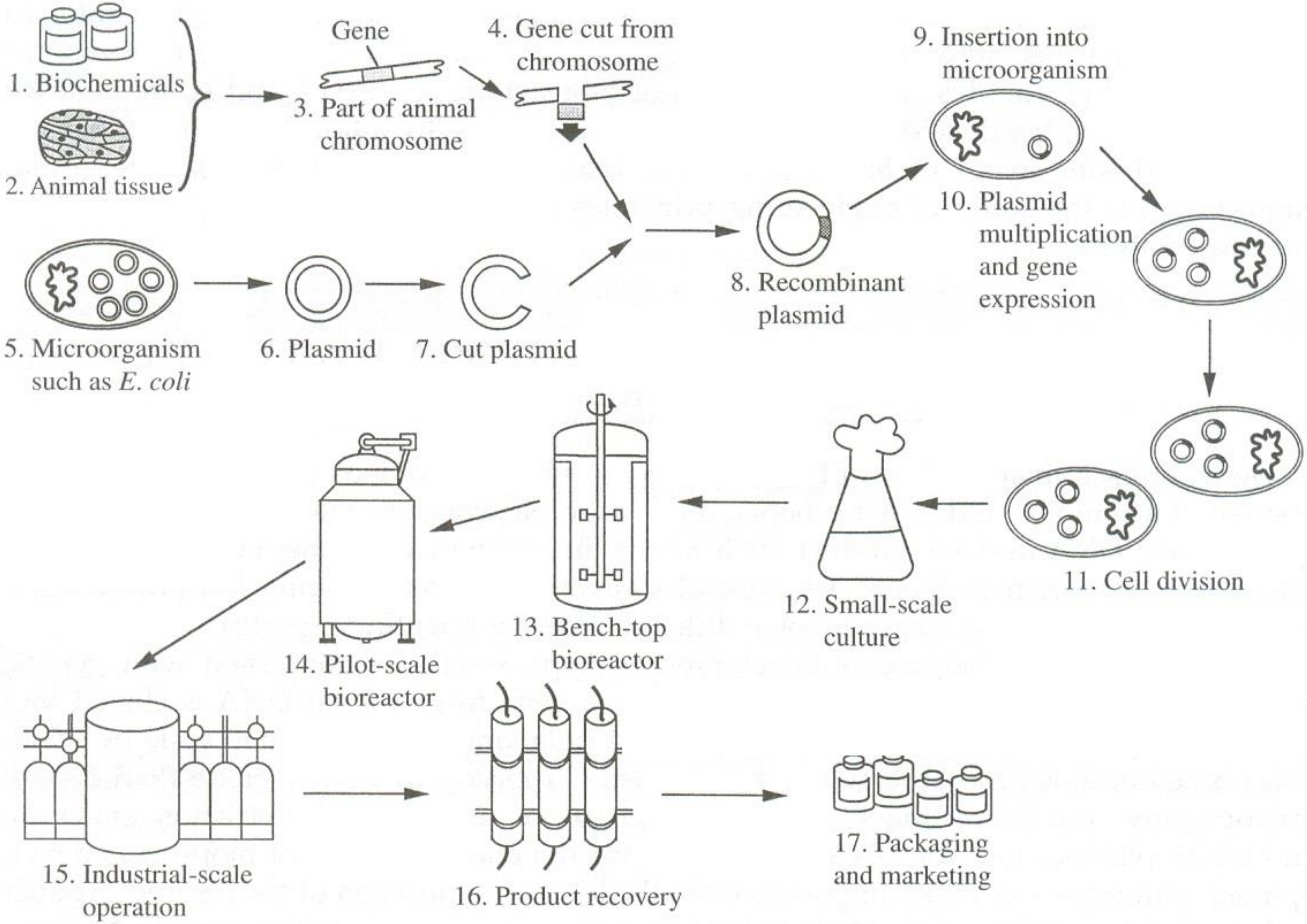


**FIGURE 1.1** Steps involved in the development of a new bioprocess for commercial manufacture of a recombinant DNA-derived product.

## EXAMPLE 12.7 PLASMID INSTABILITY IN BATCH CULTURE

A plasmid-containing strain of *E. coli* is used to produce recombinant protein in a 250-litre fermenter. The probability of plasmid loss per generation is 0.005. The specific growth rate of plasmid-free cells is 1.4 $h^{-1}$; the specific growth rate of plasmid-bearing cells is 1.2 $h^{-1}$. Estimate the fraction of plasmid-bearing cells after 18 h of growth if the inoculum contains only cells with plasmid.

$$F = \frac{1-\alpha-p}{1-\alpha-2^{n(\alpha+p-1)}p} \qquad (12.95)$$

$$F = \frac{x^+}{x^+ + x^-} \qquad (12.94)$$

$$\alpha = \frac{\mu^-}{\mu^+} \qquad n = \frac{\mu^+ t}{\ln 2}$$

$\alpha$ = the ratio of the specific growth rates of plasmid-free ($\mu^-$) and plasmid-carrying cells ($\mu^+$)

*Solution*

The number of generations of plasmid-carrying cells after 18 h is

$$n = \frac{(1.2\ \text{h}^{-1})\ 18\ \text{h}}{\ln 2} = 31$$

$$p = 0.005 \text{ and } \alpha = 1.4\ \text{h}^{-1}/1.2\ \text{h}^{-1} \doteq 1.17:$$

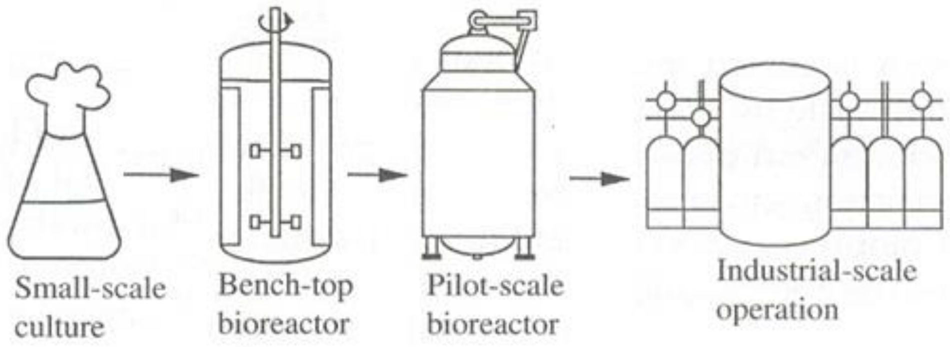
$$F = \frac{1-1.17-0.005}{1-1.17-2^{31(1.17+0.005-1)}\,0.005} = 0.45$$

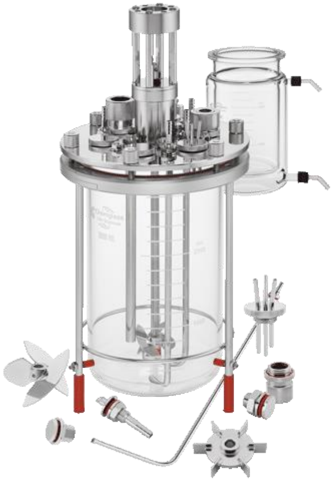
Therefore, after 18 h only 45% of the cells contain plasmid.

*Pall's XRS 20 Bioreactor is a bi-axial agitation bioreactor containing a pre-sterilized single-use biocontainer and a control tower.*

http://vertassets.blob.core.windows.net/image/cb1ce0d0/cb1ce0d0-5ccc-4096-8b57-80ef507bc2e8/wavebioreactorimage.jpg

# Stirred Bioreactors





**15 L**

**1500 L  Bioreactor**

**150 L Bioreactor**

From CRAB Web Master

# Boehringer Ingelheim
# Biotechnology Process Plant

http://www.pharmaceutical-technology.com/projects/biberach/images/bild_2.jpg

# Stirred Tank Reactors

- The least expensive shape is 1:1 ratio (liquid height to tank diameter)

- Aeration (causing air to circulate through) allows longer contact times between the rising bubbles and liquid

- Only 70-80% volume should be filled with liquid.

- Foam breaker is preferred to be installed, because antifoam agents reduce the rate of oxygen transfer.

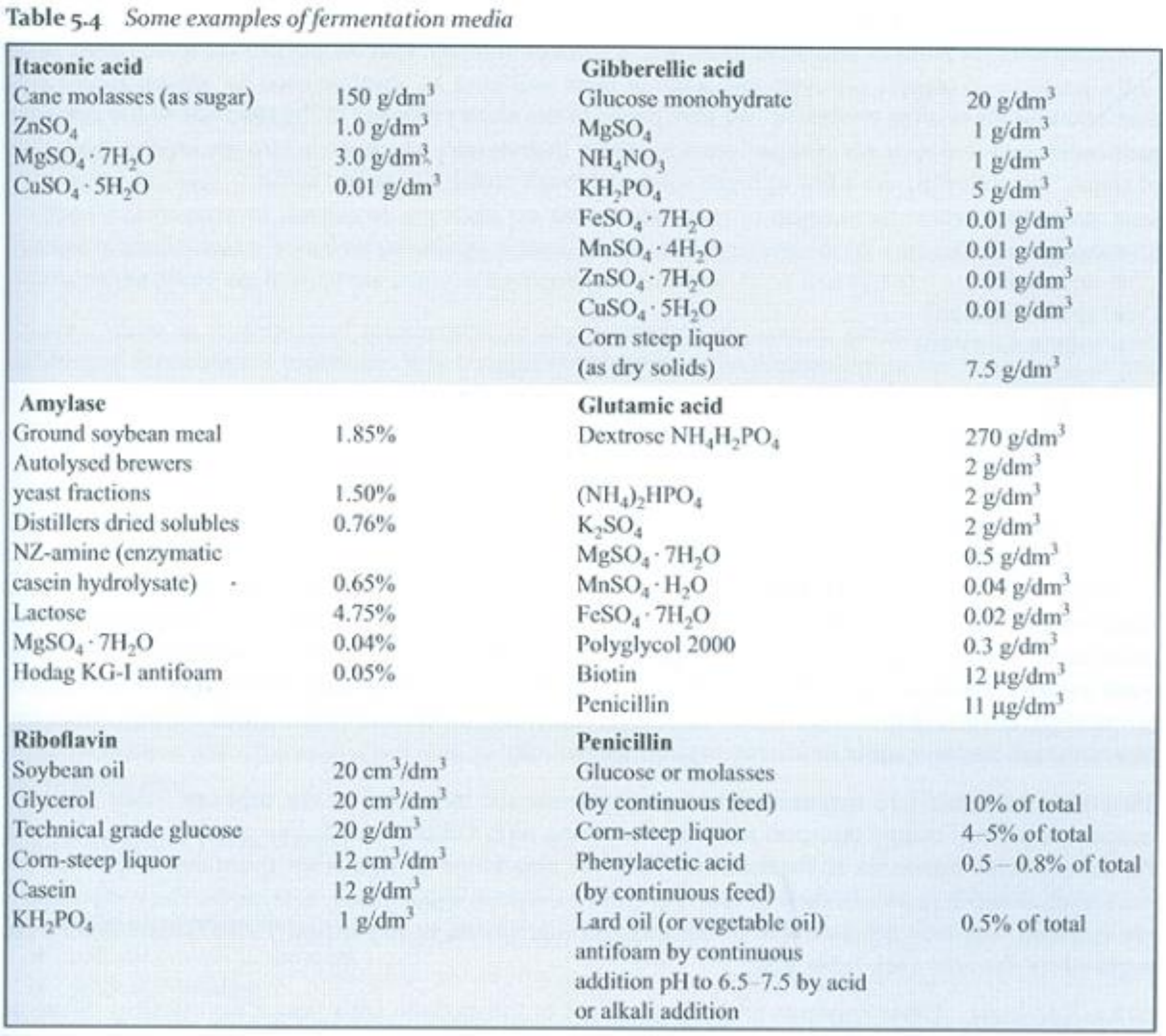
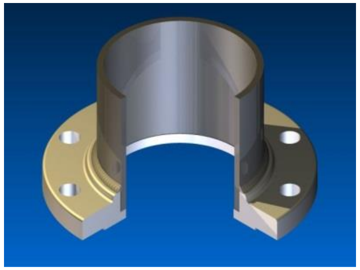


**Table 5.4** *Some examples of fermentation media*

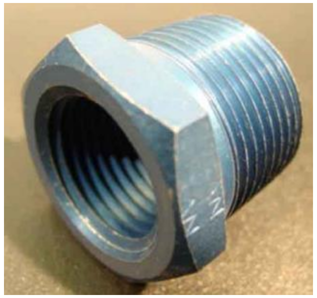
| | | | |
|---|---|---|---|
| **Itaconic acid** | | **Gibberellic acid** | |
| Cane molasses (as sugar) | 150 g/dm$^3$ | Glucose monohydrate | 20 g/dm$^3$ |
| $ZnSO_4$ | 1.0 g/dm$^3$ | $MgSO_4$ | 1 g/dm$^3$ |
| $MgSO_4 \cdot 7H_2O$ | 3.0 g/dm$^3$ | $NH_4NO_3$ | 1 g/dm$^3$ |
| $CuSO_4 \cdot 5H_2O$ | 0.01 g/dm$^3$ | $KH_2PO_4$ | 5 g/dm$^3$ |
| | | $FeSO_4 \cdot 7H_2O$ | 0.01 g/dm$^3$ |
| | | $MnSO_4 \cdot 4H_2O$ | 0.01 g/dm$^3$ |
| | | $ZnSO_4 \cdot 7H_2O$ | 0.01 g/dm$^3$ |
| | | $CuSO_4 \cdot 5H_2O$ | 0.01 g/dm$^3$ |
| | | Corn steep liquor | |
| | | (as dry solids) | 7.5 g/dm$^3$ |
| **Amylase** | | **Glutamic acid** | |
| Ground soybean meal | 1.85% | Dextrose $NH_4H_2PO_4$ | 270 g/dm$^3$ |
| Autolysed brewers | | | 2 g/dm$^3$ |
| yeast fractions | 1.50% | $(NH_4)_2HPO_4$ | 2 g/dm$^3$ |
| Distillers dried solubles | 0.76% | $K_2SO_4$ | 2 g/dm$^3$ |
| NZ-amine (enzymatic | | $MgSO_4 \cdot 7H_2O$ | 0.5 g/dm$^3$ |
| casein hydrolysate) | 0.65% | $MnSO_4 \cdot H_2O$ | 0.04 g/dm$^3$ |
| Lactose | 4.75% | $FeSO_4 \cdot 7H_2O$ | 0.02 g/dm$^3$ |
| $MgSO_4 \cdot 7H_2O$ | 0.04% | Polyglycol 2000 | 0.3 g/dm$^3$ |
| Hodag KG-I antifoam | 0.05% | Biotin | 12 µg/dm$^3$ |
| | | Penicillin | 11 µg/dm$^3$ |
| **Riboflavin** | | **Penicillin** | |
| Soybean oil | 20 cm$^3$/dm$^3$ | Glucose or molasses | |
| Glycerol | 20 cm$^3$/dm$^3$ | (by continuous feed) | 10% of total |
| Technical grade glucose | 20 g/dm$^3$ | Corn-steep liquor | 4–5% of total |
| Corn-steep liquor | 12 cm$^3$/dm$^3$ | Phenylacetic acid | 0.5 – 0.8% of total |
| Casein | 12 g/dm$^3$ | (by continuous feed) | |
| $KH_2PO_4$ | 1 g/dm$^3$ | Lard oil (or vegetable oil) | 0.5% of total |
| | | antifoam by continuous | |
| | | addition pH to 6.5–7.5 by acid | |
| | | or alkali addition | |

# Sterilization

- **Sterilization of the fermenter and medium may be done separately or together.**
- **The transport lines should be maintained aseptic.**
- **Inside the fermenter should have minimum number of joints. The joints are made with welding and the joint points should be made as smooth as possible to avoid potential sources of contamination.**

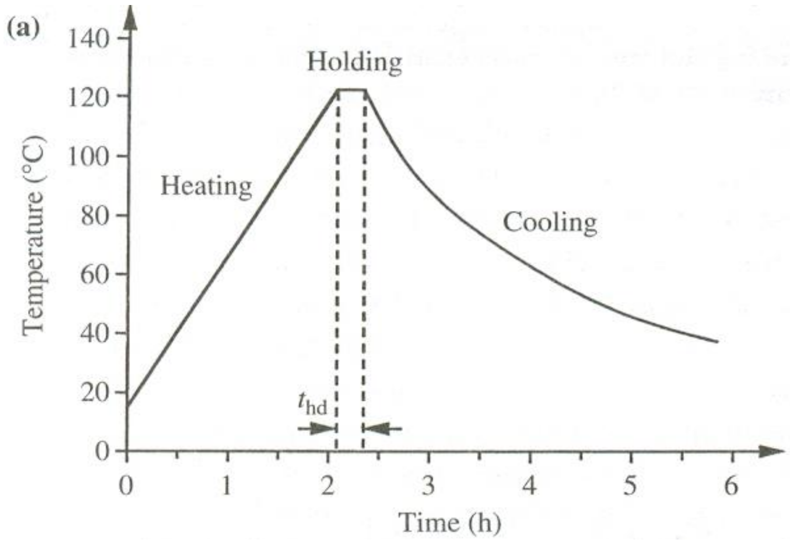


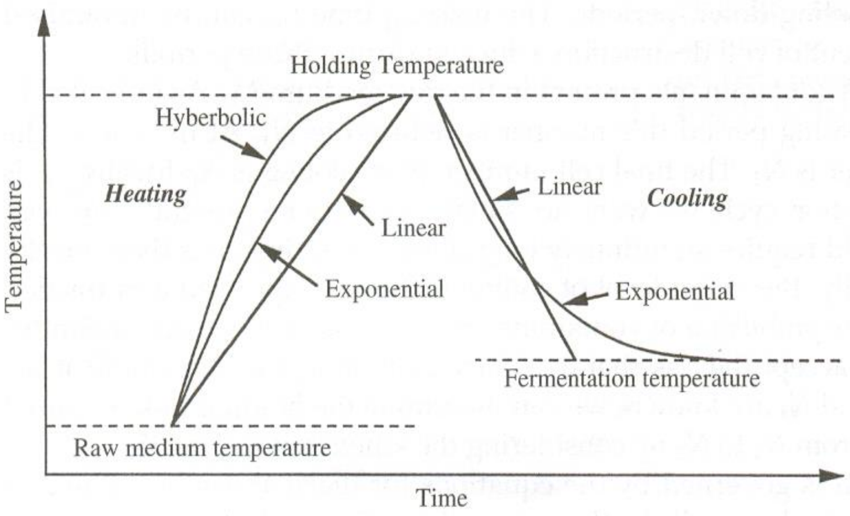
**Threaded Joints**



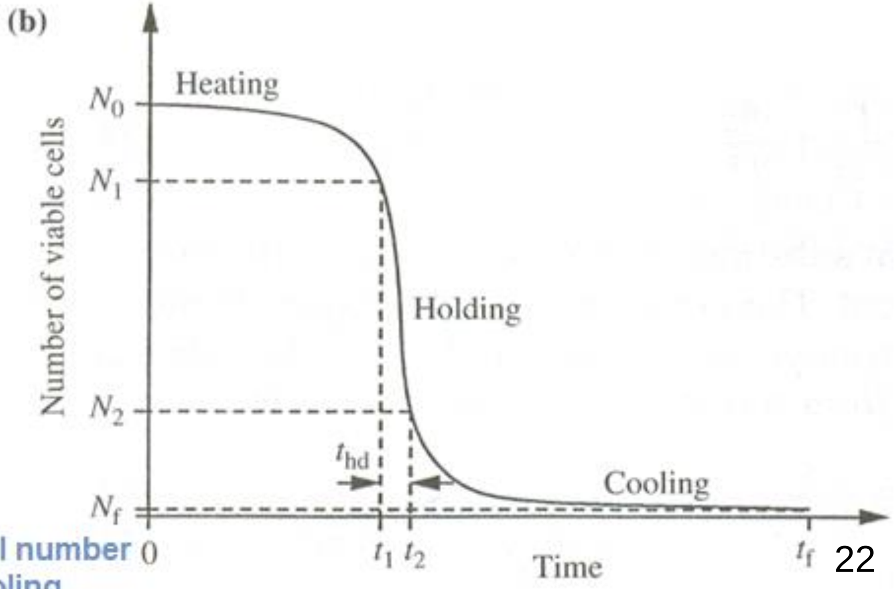
**Flanged Joints**

# Batch Sterilization of Liquid Medium



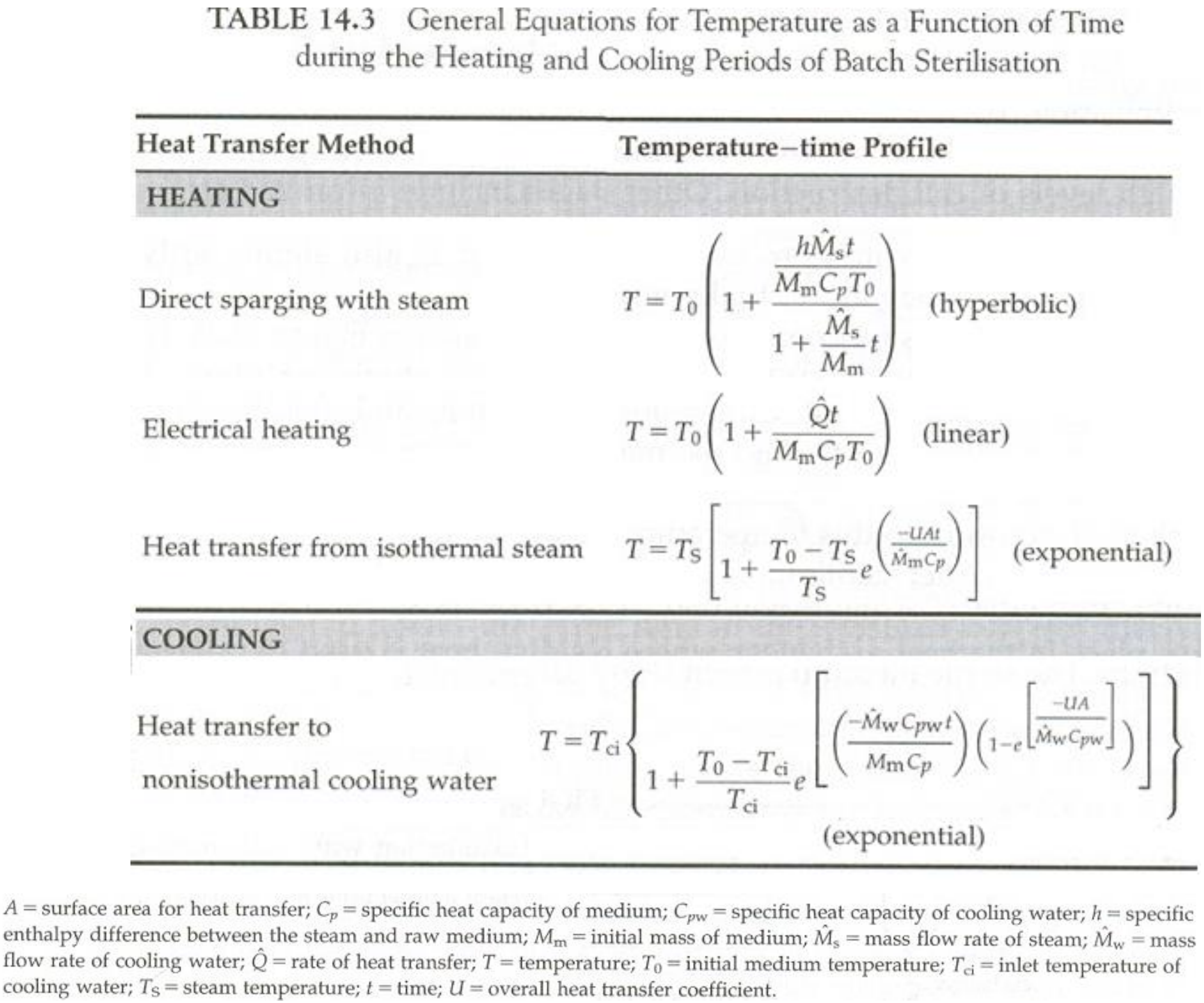


**(a) temperature time plot**



**(b) reduction in the viable cell number with time**

Final cell number after cooling



### TABLE 14.3 General Equations for Temperature as a Function of Time during the Heating and Cooling Periods of Batch Sterilisation

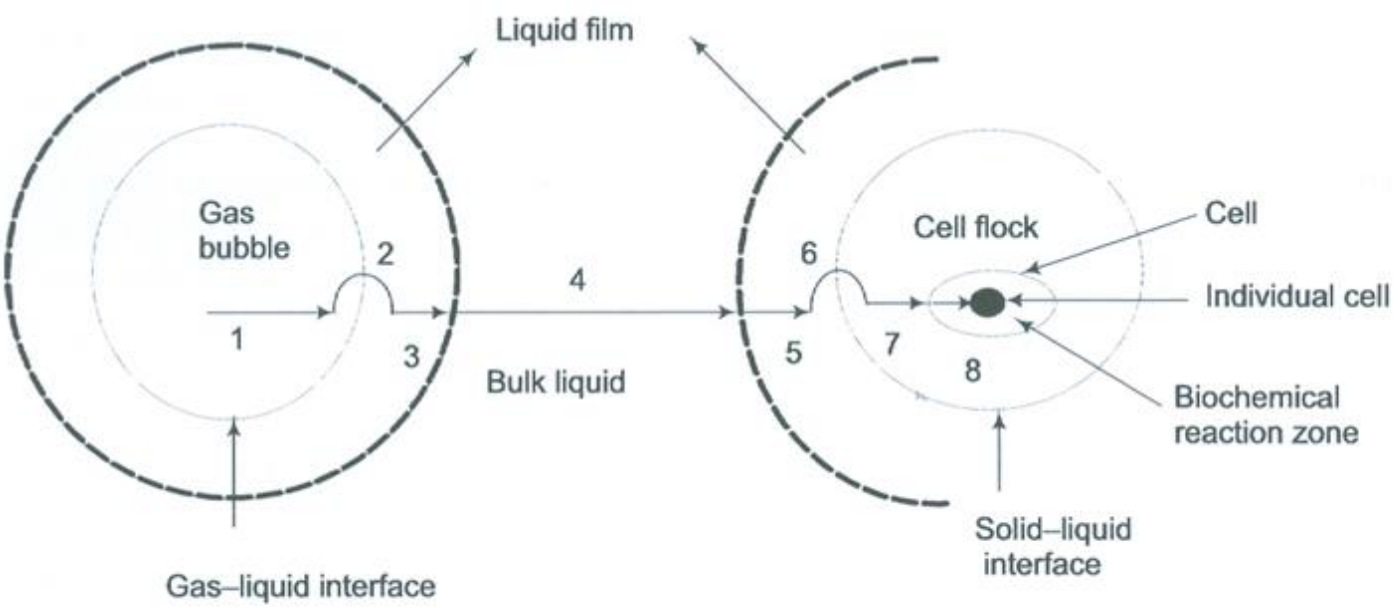
| **Heat Transfer Method** | **Temperature–time Profile** |
|---|---|
| **HEATING** | |
| Direct sparging with steam | $T = T_0\left(1 + \dfrac{\dfrac{h\hat{M}_s t}{M_m C_p T_0}}{1 + \dfrac{\hat{M}_s}{M_m}t}\right)$ (hyperbolic) |
| Electrical heating | $T = T_0\left(1 + \dfrac{\hat{Q}t}{M_m C_p T_0}\right)$ (linear) |
| Heat transfer from isothermal steam | $T = T_S\left[1 + \dfrac{T_0 - T_S}{T_S}e^{\left(\frac{-UAt}{\hat{M}_m C_p}\right)}\right]$ (exponential) |
| **COOLING** | |
| Heat transfer to nonisothermal cooling water | $T = T_{ci}\left\{1 + \dfrac{T_0 - T_{ci}}{T_{ci}}e^{\left[\left(\frac{-\hat{M}_W C_{pw} t}{M_m C_p}\right)\left(1 - e^{\left[\frac{-UA}{\hat{M}_W C_{pw}}\right]}\right)\right]}\right\}$ (exponential) |

$A$ = surface area for heat transfer; $C_p$ = specific heat capacity of medium; $C_{pw}$ = specific heat capacity of cooling water; $h$ = specific enthalpy difference between the steam and raw medium; $M_m$ = initial mass of medium; $\hat{M}_s$ = mass flow rate of steam; $\hat{M}_w$ = mass flow rate of cooling water; $\hat{Q}$ = rate of heat transfer; $T$ = temperature; $T_0$ = initial medium temperature; $T_{ci}$ = inlet temperature of cooling water; $T_S$ = steam temperature; $t$ = time; $U$ = overall heat transfer coefficient.

# EXAMPLE 14.8 HOLDING TEMPERATURE IN A CONTINUOUS STERILISER

Liquid medium at a flow rate of 2 $m^3\ h^{-1}$ is to be sterilised by heat exchange with steam in a continuous steriliser. The medium contains bacterial spores at a concentration of $5 \times 10^{12}\ m^{-3}$. Values of the activation energy and Arrhenius constant for thermal destruction of these contaminants are 283 kJ $gmol^{-1}$ and $5.7 \times 10^{39}\ h^{-1}$, respectively. A contamination risk of one organism surviving every 60 days of operation is considered acceptable. The steriliser pipe has an inner diameter of 0.1 m and the length of the holding section is 24 m. The density of the medium is 1000 kg $m^{-3}$ and the viscosity is 3.6 kg $m^{-1}\ h^{-1}$. What sterilising temperature is required?

# Oxygen Transfer from Gas Bubble to Cell



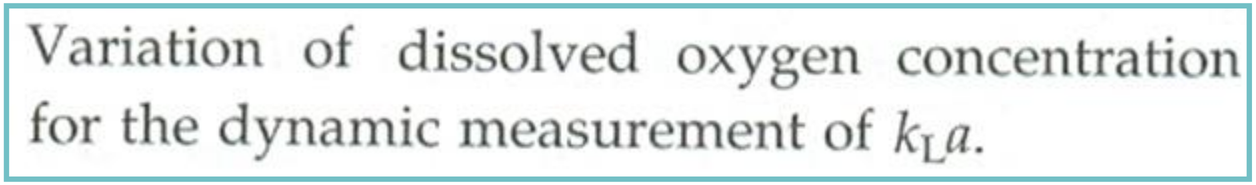
1. Transfer from the interior of the bubble to the gas–liquid interface
2. Movement across the gas–liquid interface
3. Diffusion through the relatively stagnant liquid film surrounding the bubble
4. Transport through the bulk liquid
5. Diffusion through the relatively stagnant liquid film surrounding the cells
6. Movement across the liquid–cell interface

8. Transport through the cytoplasm to the site of reaction

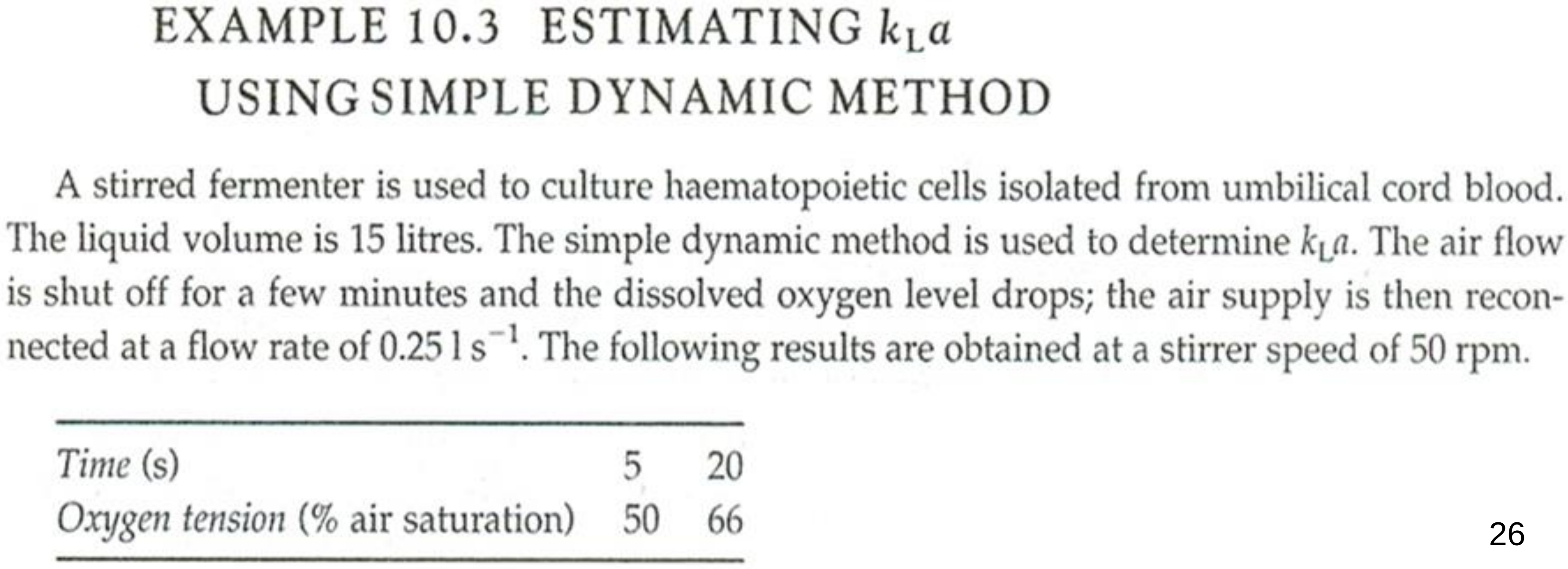
Mass transfer coefficient $k_La$: characterize the oxygen transfer capability of fermenters (oxygen transfer coefficient)

a = liquid gas interfacial area;

$k_L$= liquid phase mass transfer coefficient



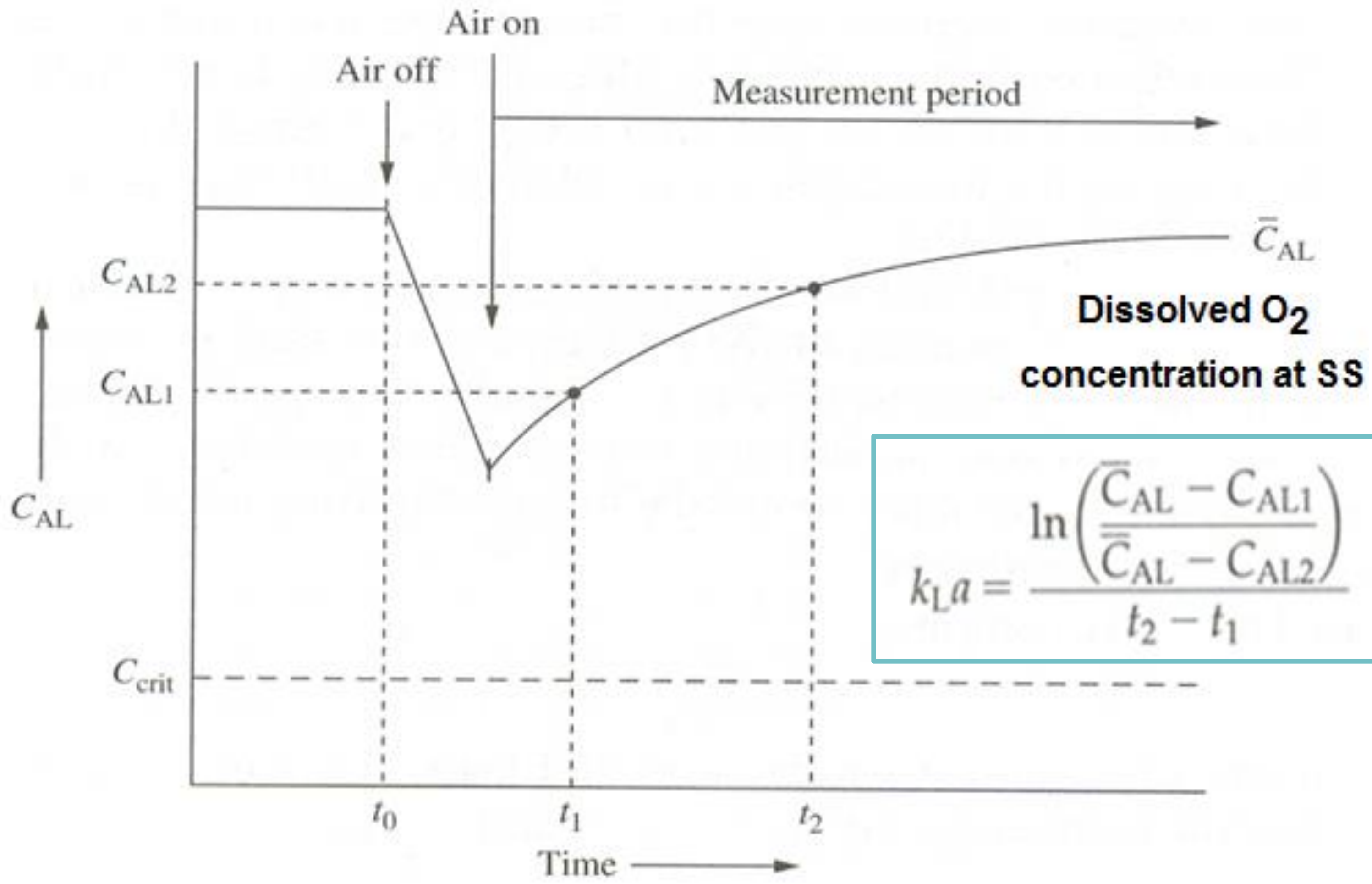
Variation of dissolved oxygen concentration for the dynamic measurement of $k_La$.



EXAMPLE 10.3 ESTIMATING $k_La$ USING SIMPLE DYNAMIC METHOD

A stirred fermenter is used to culture haematopoietic cells isolated from umbilical cord blood. The liquid volume is 15 litres. The simple dynamic method is used to determine $k_La$. The air flow is shut off for a few minutes and the dissolved oxygen level drops; the air supply is then reconnected at a flow rate of 0.25 l $s^{-1}$. The following results are obtained at a stirrer speed of 50 rpm.

| *Time* (s) | 5 | 20 |
|---|---|---|
| *Oxygen tension* (% air saturation) | 50 | 66 |



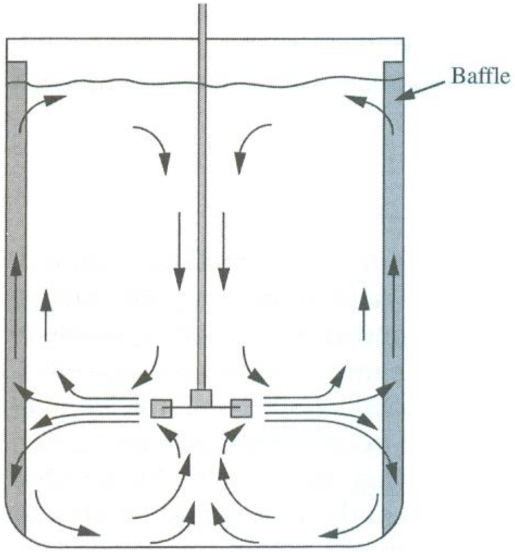
**Dissolved $O_2$ concentration at SS**

$$k_L a = \frac{\ln\left(\dfrac{\bar{C}_{AL} - C_{AL1}}{\bar{C}_{AL} - C_{AL2}}\right)}{t_2 - t_1} \qquad (10.57)$$

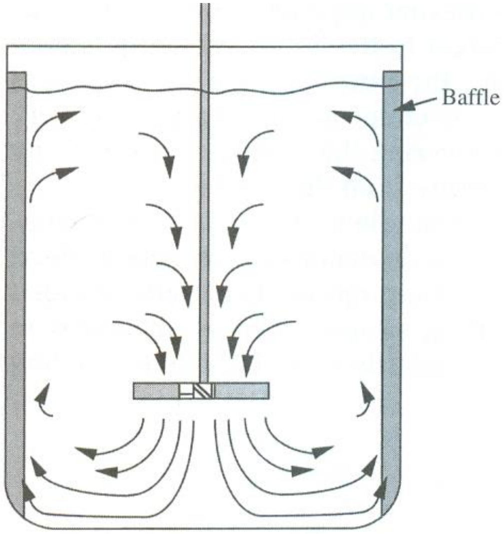
$C_{AL}$: oxygen dissolved in the fermentation broth.
$C_{AL,\,1}$ and $C_{AL,\,2}$: two $[O_2]$ measured during re-oxygenation at $t_1$ and $t_2$ (re-oxgenation is not steady state).

# Stirred Patterns

- **Mixing and bubble dispersion are achieved by mechanical agitation.**
- **Requires high input of energy**
- **Baffles are used to reduce vortexing.**

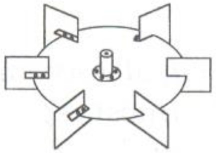


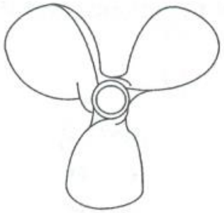
**Radial-flow impeller in a baffled tank**

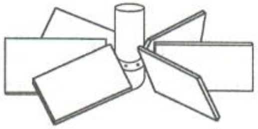


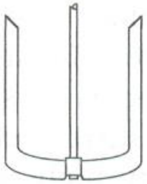
**Axial-flow impeller in a baffled tank**

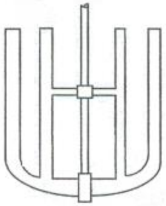
# Impellers


Six-flat-blade disc turbine

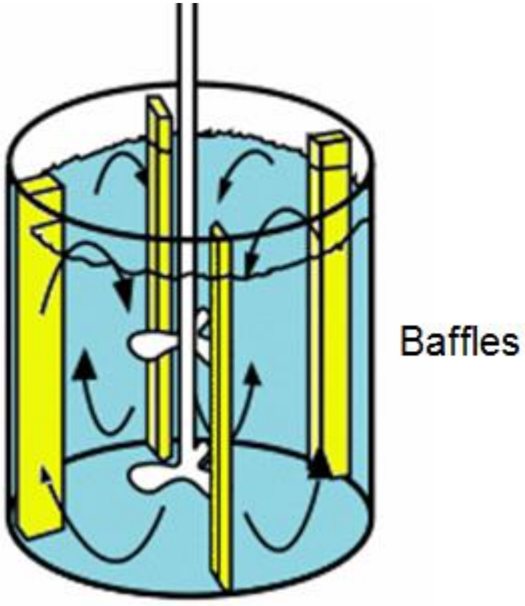

Propeller


Pitched-blade turbine

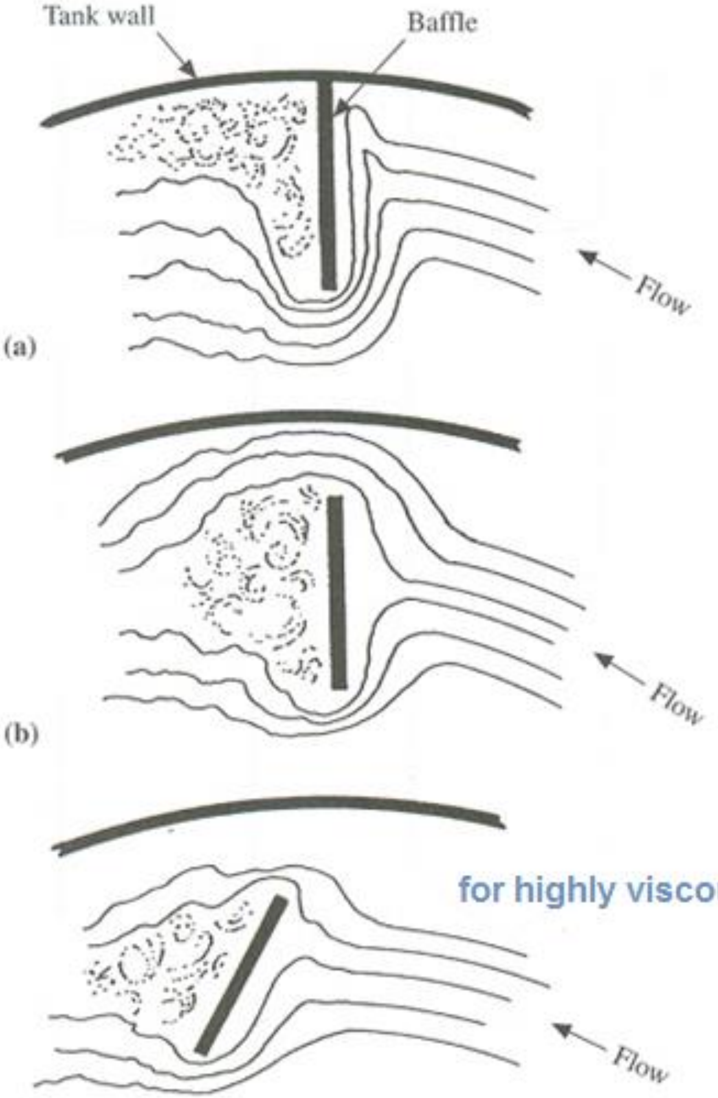

Anchor


Gate anchor


Helical screw

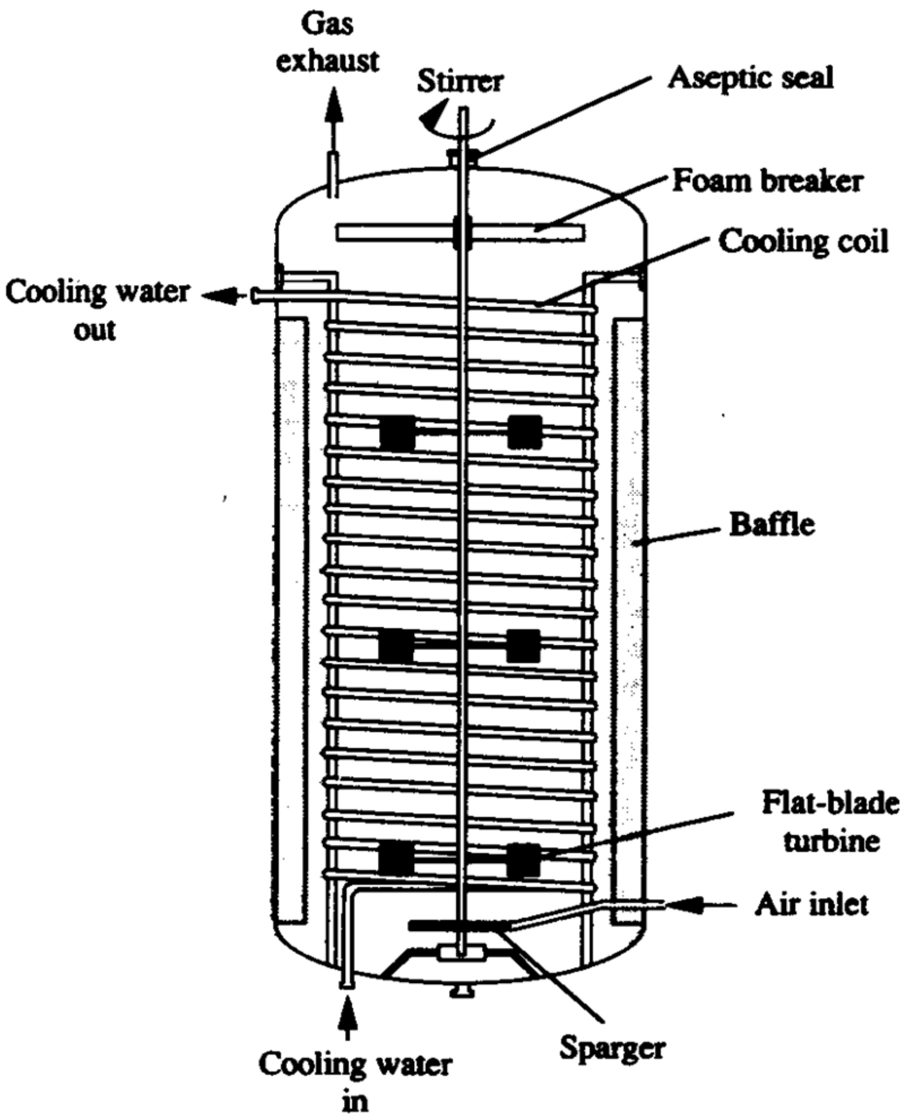


# Stirred Tank With Baffles

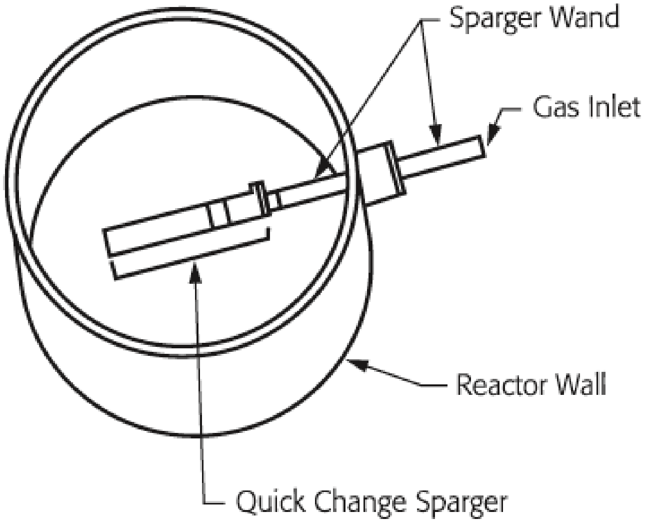


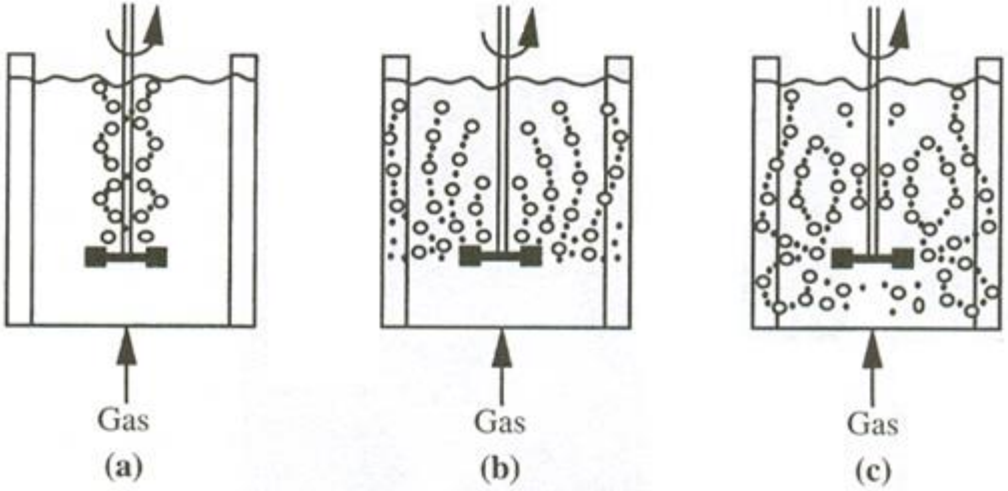
for highly viscous fluid

# Sparger



**Oxygen is measured in its dissolved form as dissolved oxygen (DO)**





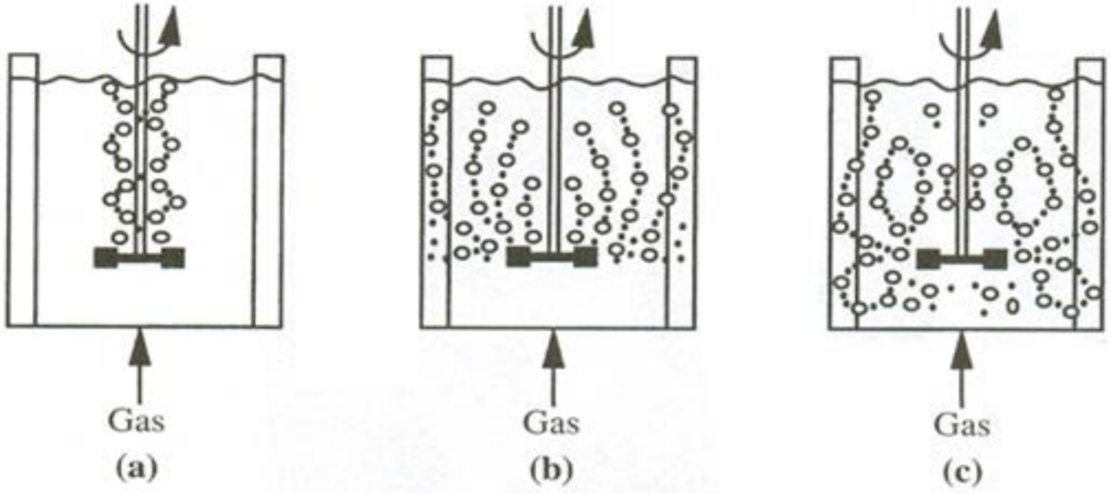
$$Fl_g = \frac{F_g}{N_i D_i^3} \qquad (8.1)$$

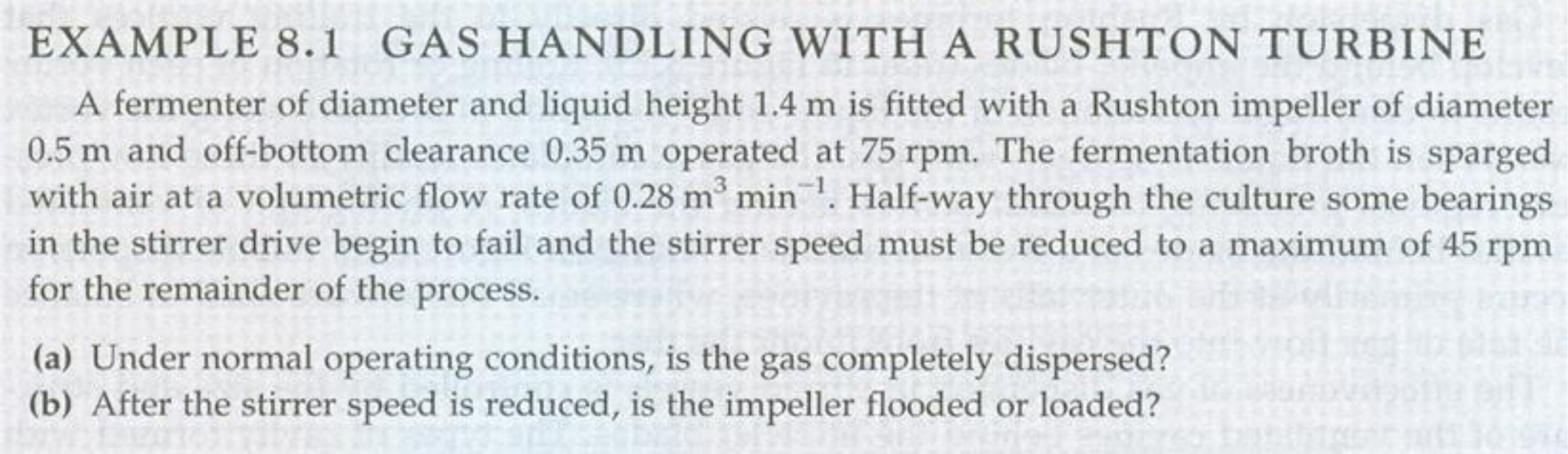
FIGURE 8.18 Patterns of gas distribution in an aerated tank stirred with a Rushton turbine as a function of the impeller speed $N_i$ and gas flow rate $F_g$.

(a) Impeller flooding;
(b) impeller loading;
(c) complete gas dispersion.

- The capacity of the stirrer in handling gas
- The amount of gas introduced.

- Impeller flooding: at high gassing rates or low stirrer speeds, liquid flow up the middle of the vessel.
- Impeller loading: at higher stirrer speeds or lower gas flow rates, the impeller is loaded as gas is dispersed towards the vessel walls.
- Complete gas dispersion.



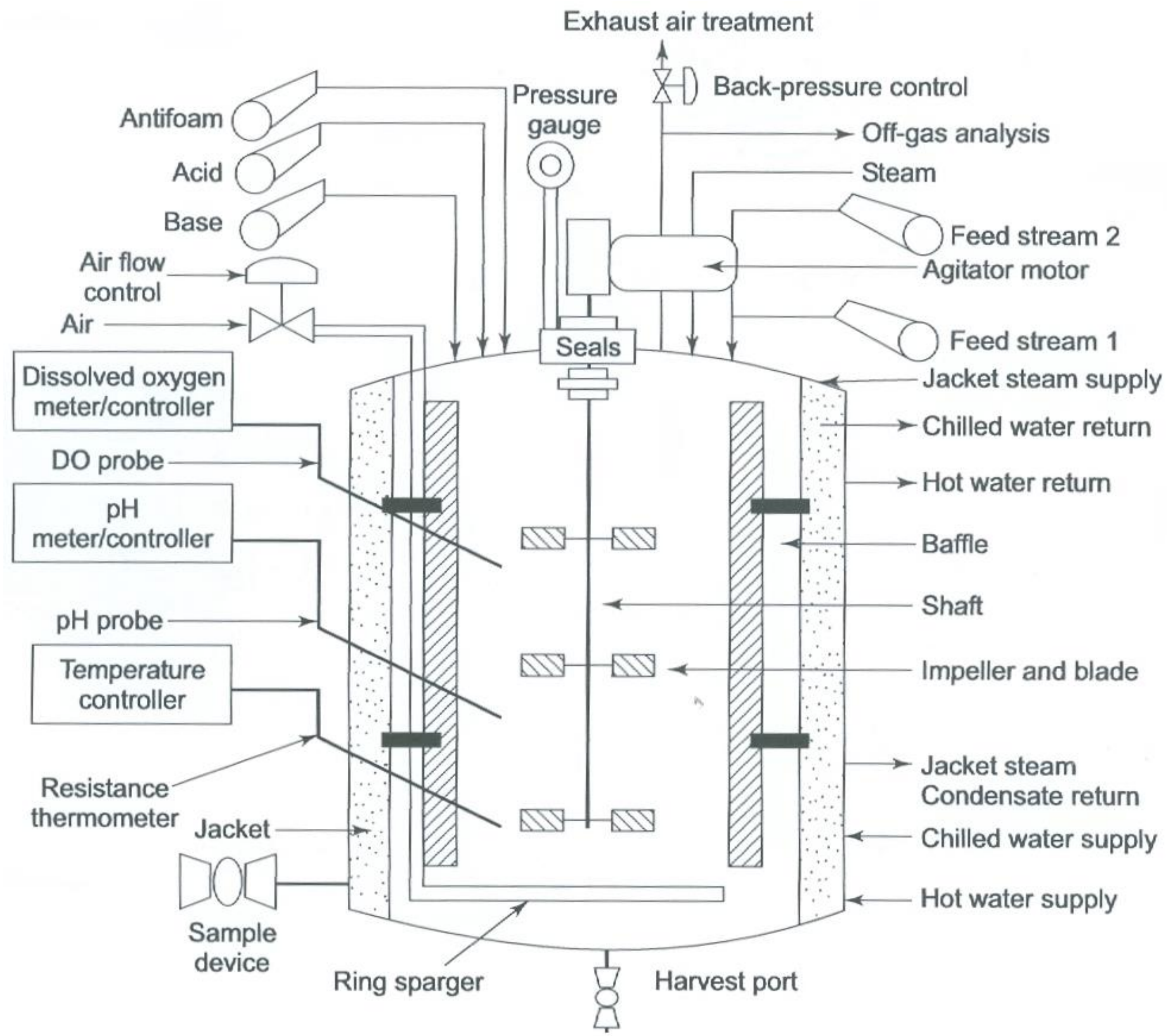


## EXAMPLE 8.1 GAS HANDLING WITH A RUSHTON TURBINE

A fermenter of diameter and liquid height 1.4 m is fitted with a Rushton impeller of diameter 0.5 m and off-bottom clearance 0.35 m operated at 75 rpm. The fermentation broth is sparged with air at a volumetric flow rate of 0.28 $m^3\ min^{-1}$. Half-way through the culture some bearings in the stirrer drive begin to fail and the stirrer speed must be reduced to a maximum of 45 rpm for the remainder of the process.

**(a)** Under normal operating conditions, is the gas completely dispersed?
**(b)** After the stirrer speed is reduced, is the impeller flooded or loaded?

# Process Control

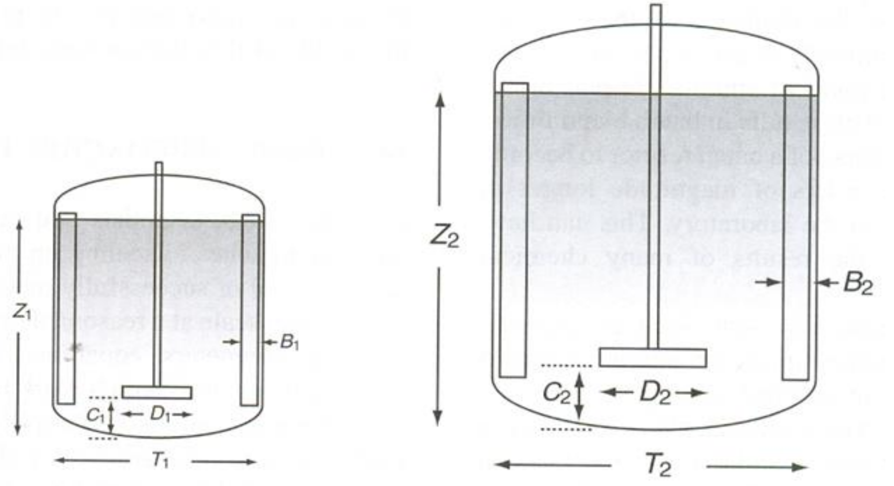


# Scale-Ups vs. Scale-Down

- **Geometric similarity**
- **Kinematic similarity**
- **Dynamic similarity**

## EXAMPLE 14.2

A 2 L laboratory system is being designed to study the mixing characteristics of a commercial vessel. The goal is to operate the model at the same mean rate of energy dissipation ($\varepsilon$) as the commercial vessel. The commercial vessel is a 7500 L working volume (7.5 $m^3$) cylindrical vessel with $T = 2.0$ m, a 0.8 m diameter ($D$) four-blade pitched turbine impeller ($D/T = 0.4$) that turns at a fixed speed of 68 rpm, and two vertical baffles. Assume that the process fluid has the properties of water.



$$\frac{D_1}{T_1} = \frac{D_2}{T_2} \qquad \frac{Z_1}{T_1} = \frac{Z_2}{T_2} \qquad \frac{C_1}{T_1} = \frac{C_2}{T_2} \qquad \frac{B_1}{T_1} = \frac{B_2}{T_2}$$

**Geometric Similarity**

## Kinematic Similarity

Requires geometric similarity and characteristic velocities scale by the same ratio.

## Dynamic Similarity

Requires both geometric and kinematic similarity and adds the characteristic forces scale by the same ratio.

# Dynamic Similarity

- **Maintain the rate of turbulent energy dissipation ($\varepsilon$), or power intensity (power/volume, P/V) constant.**

$$\varepsilon = \frac{P}{\rho V} \quad (14.1)$$

$$N_P = \frac{P}{\rho N^3 D^5} \quad (14.5)$$

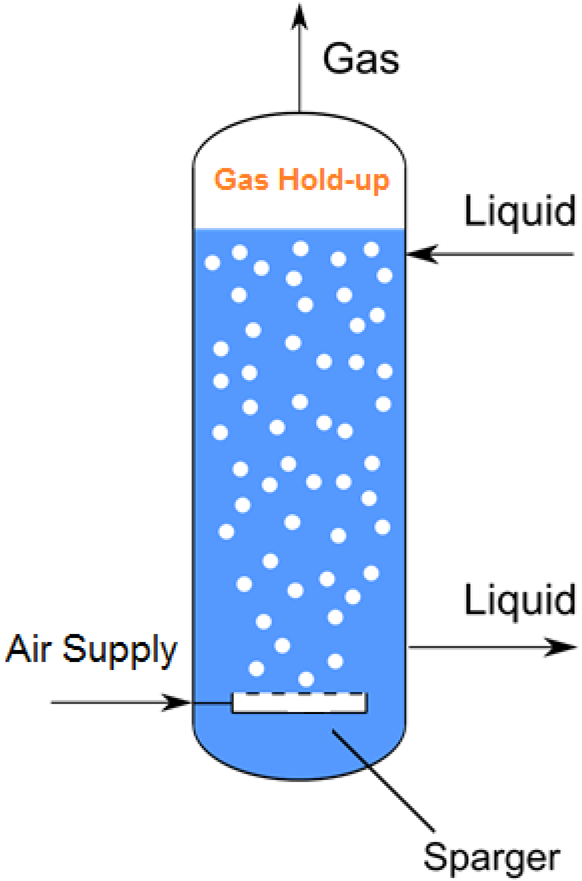
$P$ is power input (W)

$\rho$ is liquid density (kg/m$^3$)

$V$ is liquid volume (m$^3$)

| | |
|---|---|
| $P$ | mixing power |
| $D$ | impeller diameter |
| $N$ | rotational speed |
| $V$ | liquid volume |
| $\rho$ | batch density |
| $N_P$ | power number |

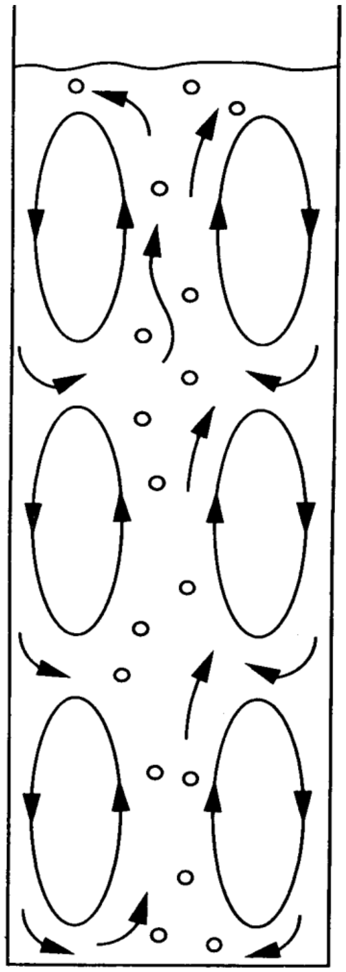
# Bubble Column Bioreactors (BCRs)



# Homogeneous vs. heterogeneous bubble columns

No mechanical agitation.

Aeration and mixing are achieved by gas sparging.



# Bubble Columns (Cont'd)

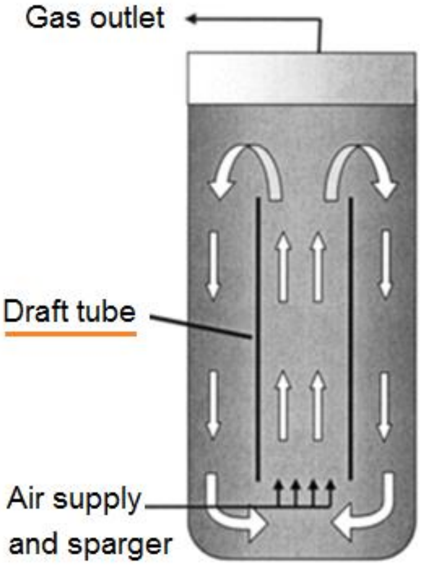
Gas-liquid mass transfer coefficients in reactors depend largely on

- gas flow rate
- bubble diameter
- gas hold-up

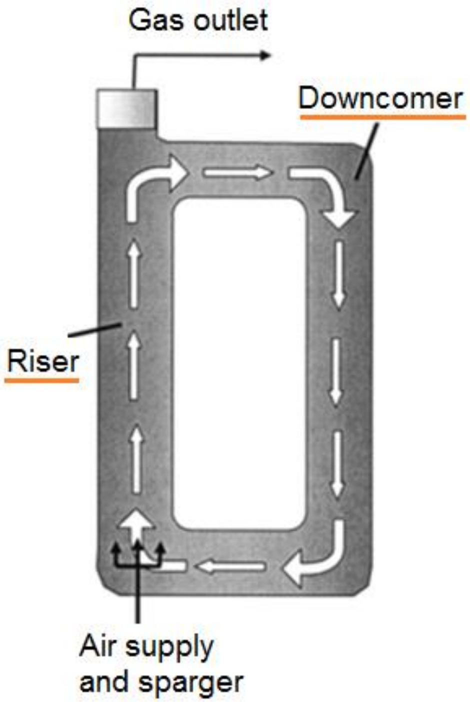
Accurate estimation of the mass-transfer coefficient is difficult (exact bubble sizes and liquid circulation patterns are impossible to predict).

# Air Lift Bioreactors (ARLs)

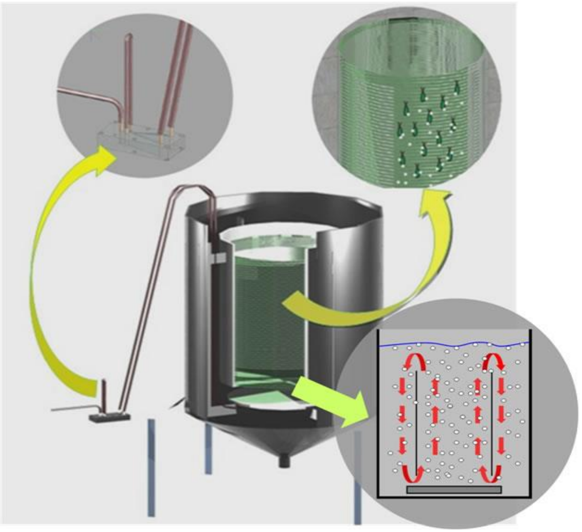
# Airlift Reactors



Internal Loop



External Loop



http://www.babonline.org/bab/045/0001/bab0450001.htm

http://s9.postimg.org/ya85far7j/bab0450001f03.gif

# Airlift Reactors

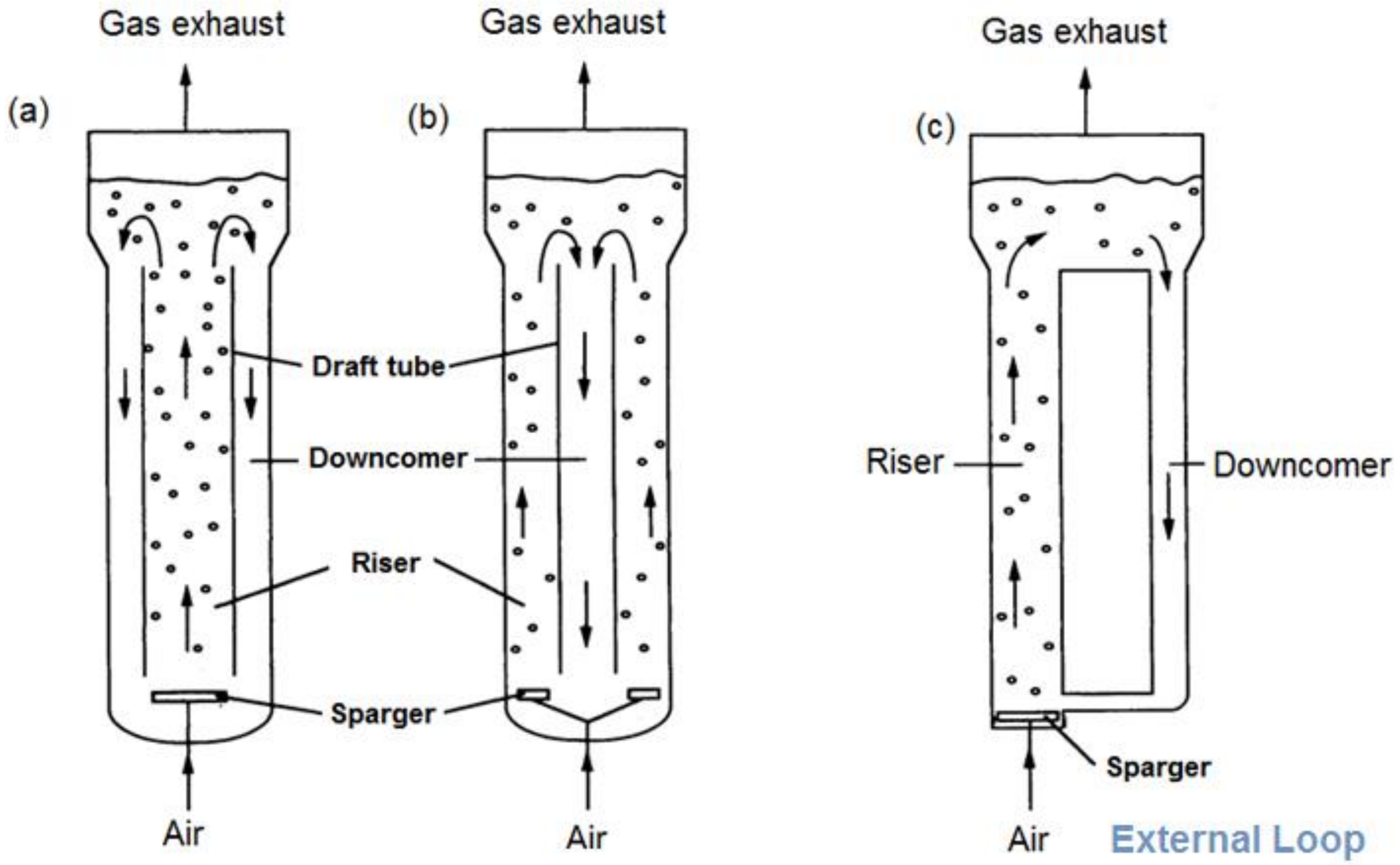
- For plant and animal cell culture, because the shear levels are significantly lower than the stirred vessels.
- The very high hydrostatic pressure at the bottom of these vessels considerably improves gas-liquid mass-transfer.

# Airlift Reactors (Cont'd)

- Gas hold-up and decreased fluid density cause liquid in the riser to move upwards.

- Gas disengages at the top of the vessel leaving heavier bubble-free liquid to recirculate through the downcomer.

- Liquid circulates in airlift reactor as the result of the density different between riser and downcomer.



**Because riser and downcomer are further apart, gas disengagement is more effective.**

**The liquid densities differ in riser and downcomer are greater.**
**The circulation is faster and mixing is better.**

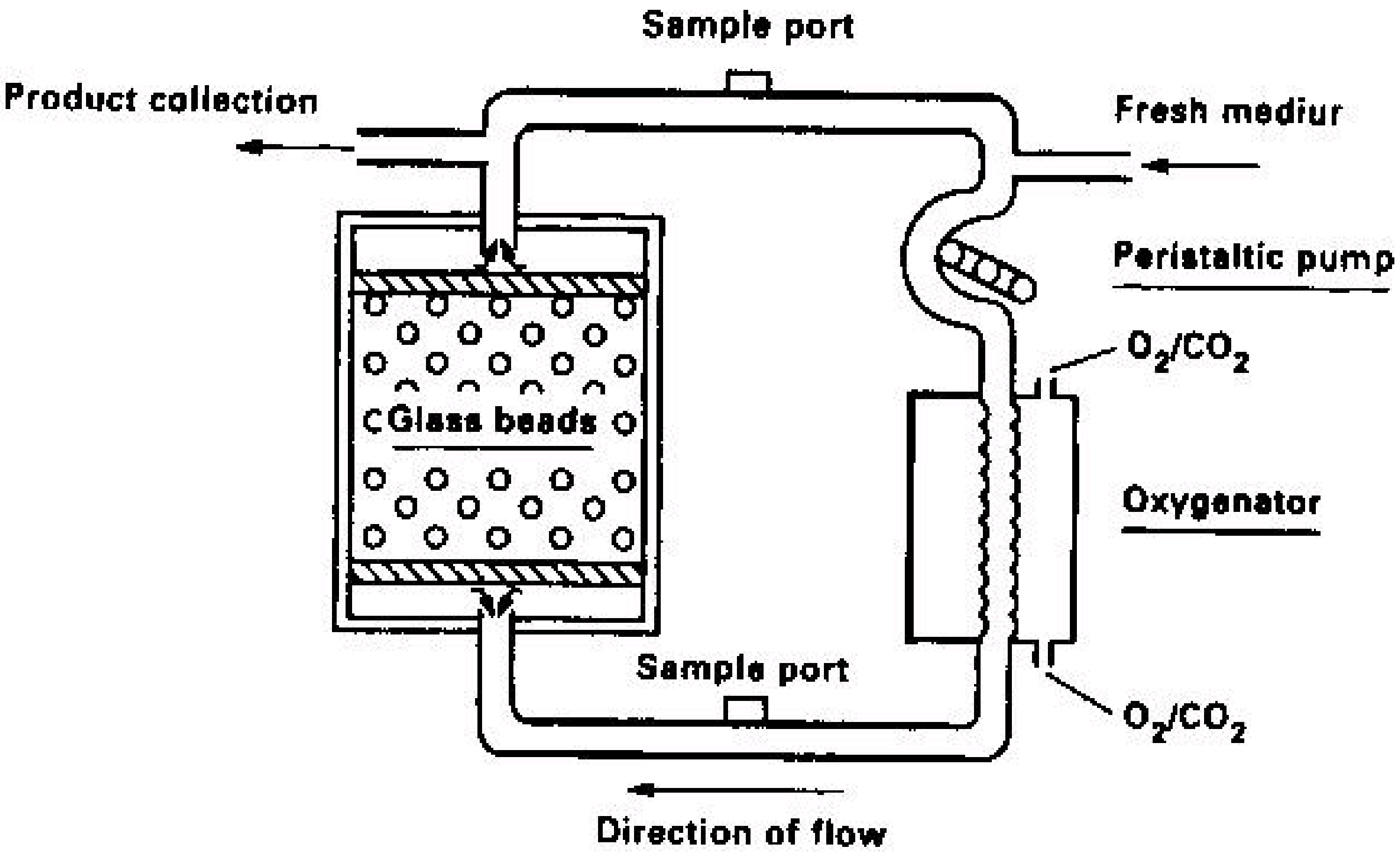
# Airlift Reactors

- Large airlift reactors can have the capacities of thousands of cubic meters.

- The height of airlift reactors is about 10 times the diameter. For deep shaft systems the height-to-diameter ratio may be increased up to 100 (built underground).

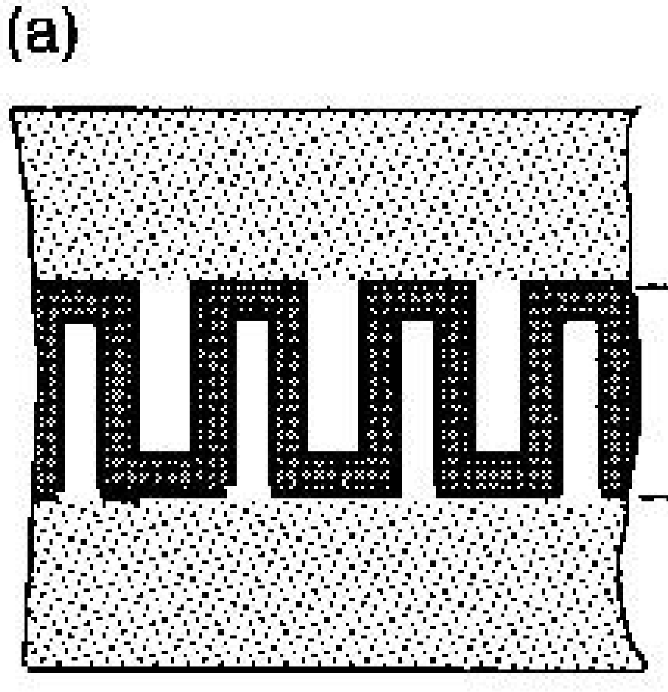
- The very high hydrostatic pressure at the bottom of these vessels considerably improves gas-liquid mass-transfer.

# Glass Bead Reactor

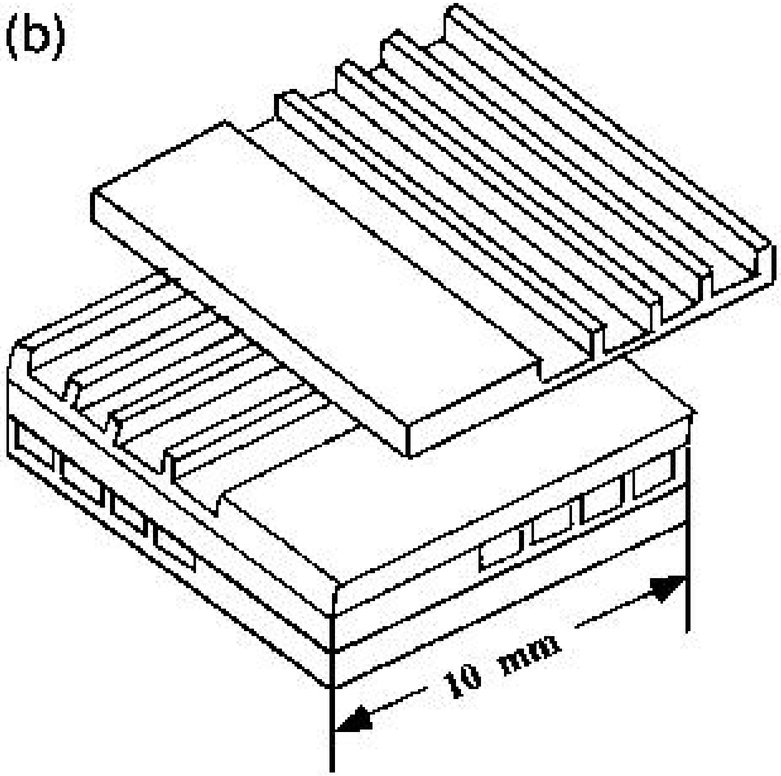


**For long term culture of attached dependent cell lines.**

# Microreactors



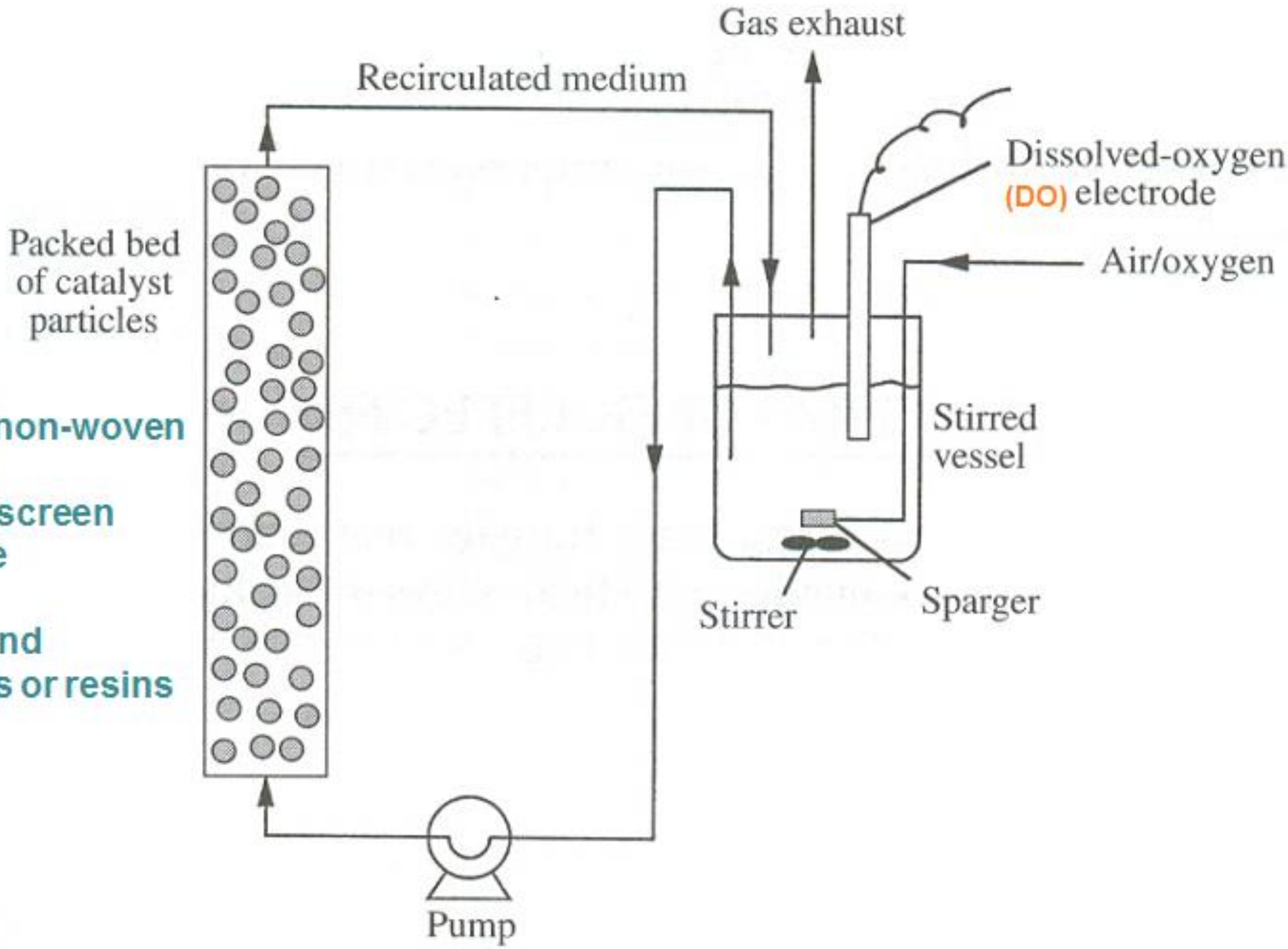
**Parallel Flow**



**Cross Flow**

# Packed Bed Bioreactors (PBRs)

# Packed-Bed Reactor with Medium

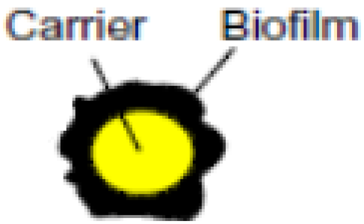


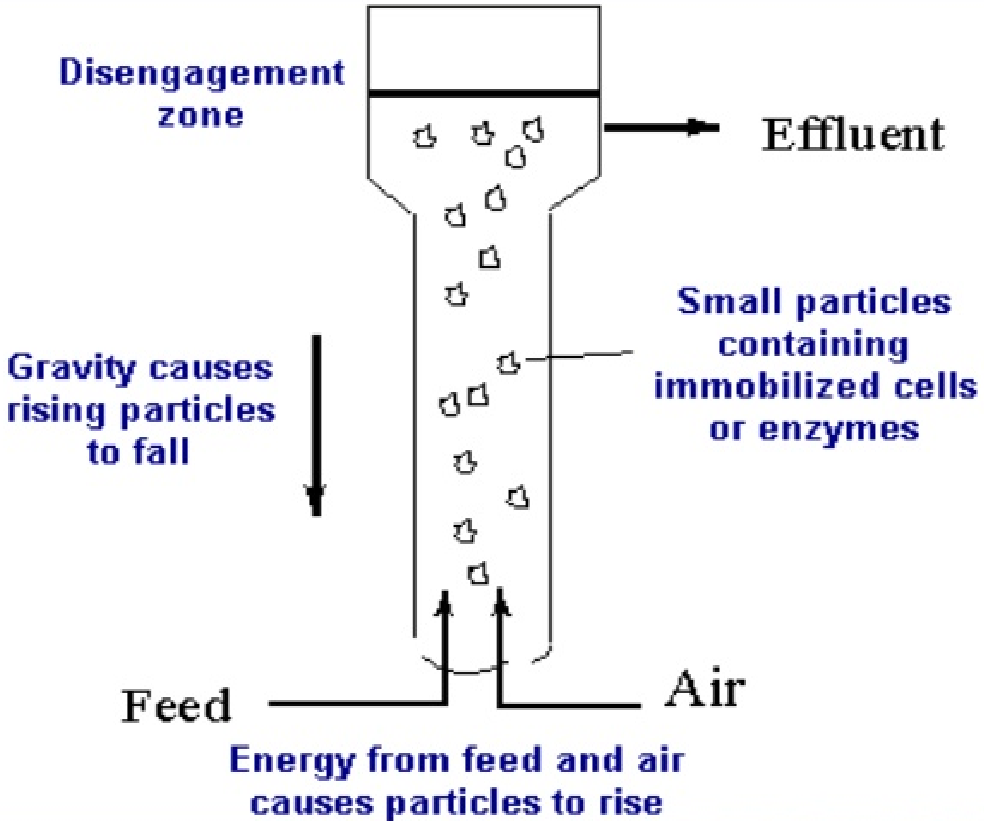
- Disks made of non-woven polyester and polypropylene screen
- ceramic sphere
- glass fibers
- polyurethane and polyvinyl foams or resins

**For perfusion culture of immobilized microorganism cells and mammalian cells.**

# Fluidized Bed Reactors (FBRs)

# Fluidized Bed Reactor





**Fine inert carriers provide large surface area for adherence and growth of microorganism to adhere and grow.**
**The carriers are fluidized to enhance mass transfer and degradation rate of organic pollutants.**

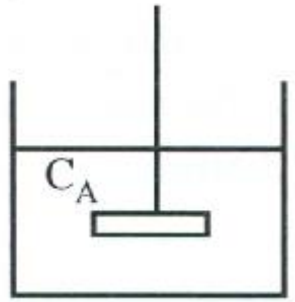
# Four Modes of Operation

- Batch reactor
- Stirred semi-batch
- Continuous stirred

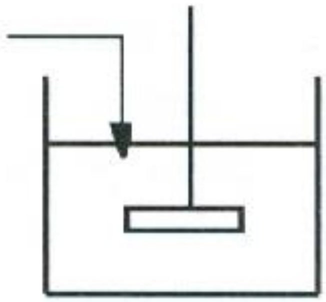
(Above three: Completely stirred and uniform in composition)

- Continuous plug flow reactors

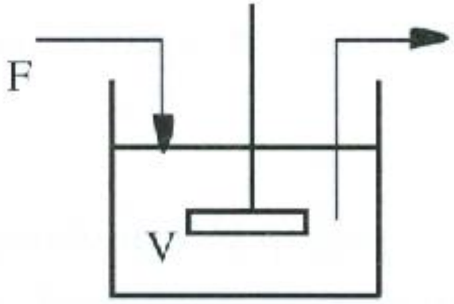
# Modes of Reactor Operations



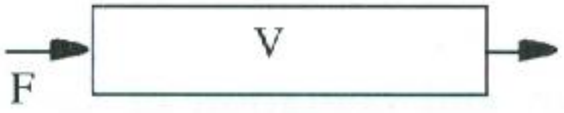
(a) Batch reactor



(b) Semi-batch reactor



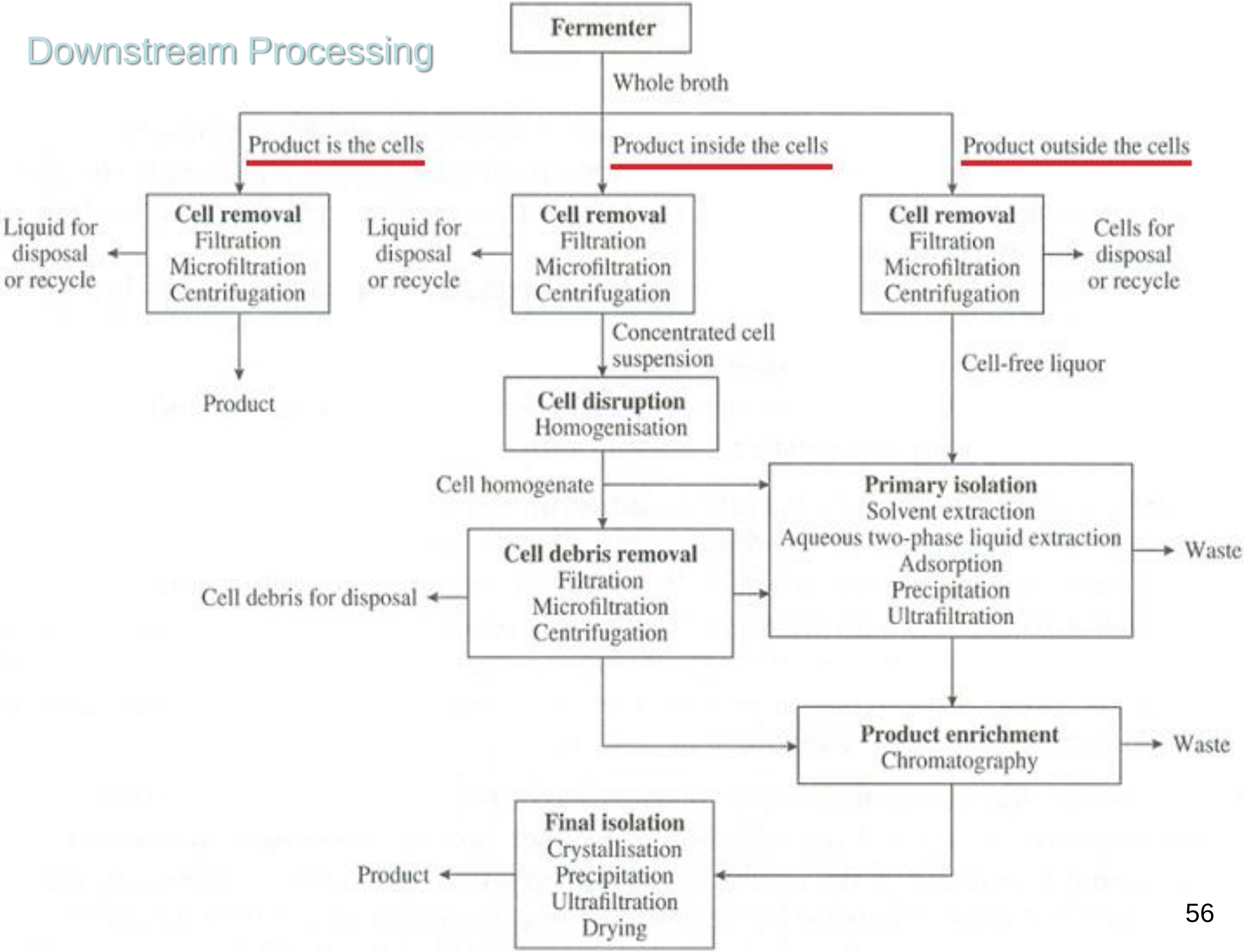
(c) Continuous stirred-tank reactor



(d) Continuous plug-flow reactor

**Plug-flow: The long tube and lack of stirring device in the direction of flow.**

# Downstream Processing



**Fermenter**

Whole broth

Product is the cells

**Cell removal**
Filtration
Microfiltration
Centrifugation

Liquid for disposal or recycle

Product

Product inside the cells

**Cell removal**
Filtration
Microfiltration
Centrifugation

Liquid for disposal or recycle

Concentrated cell suspension

**Cell disruption**
Homogenisation

Cell homogenate

**Cell debris removal**
Filtration
Microfiltration
Centrifugation

Cell debris for disposal

Product outside the cells

**Cell removal**
Filtration
Microfiltration
Centrifugation

Cells for disposal or recycle

Cell-free liquor

**Primary isolation**
Solvent extraction
Aqueous two-phase liquid extraction
Adsorption
Precipitation
Ultrafiltration

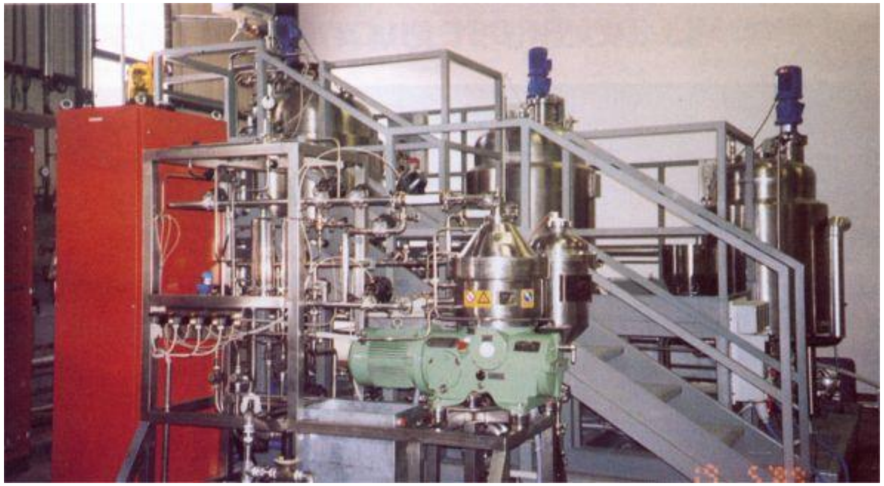
Waste

**Product enrichment**
Chromatography

Waste

**Final isolation**
Crystallisation
Precipitation
Ultrafiltration
Drying

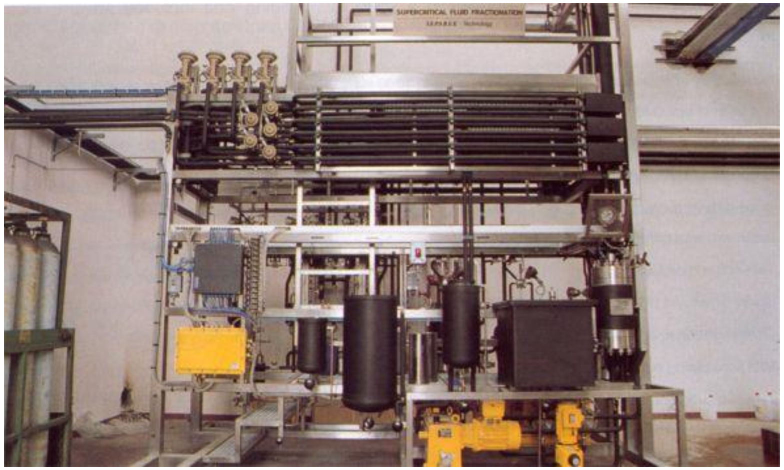
Product



**Spray Dryer Lyophilizer**

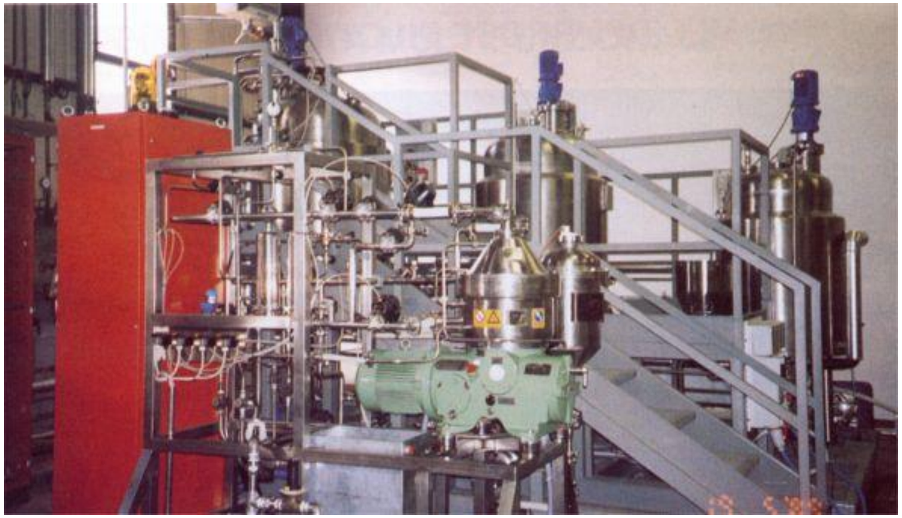
**Nanofiltration (NF)**

**Reverse Osmosis (RO)**



**Supercritical Extraction Plant**

**Distillation Plant**



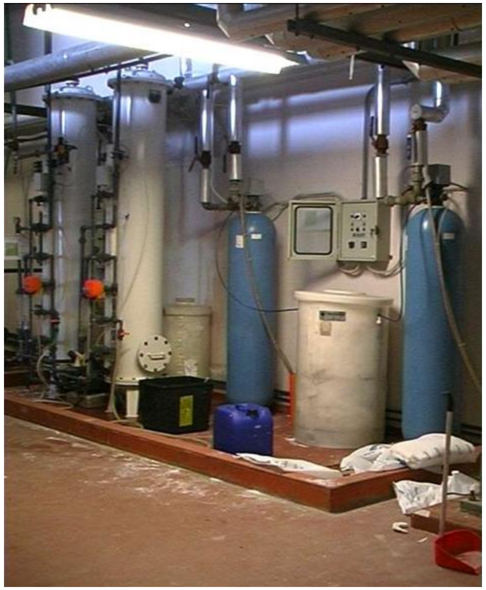
**Centrifugation Plant**

**Homogenization Unit**

**Freezer Rooms**

**Steam Generation Plant**

**Demineralized Water Plant**



Chilled Water Plant

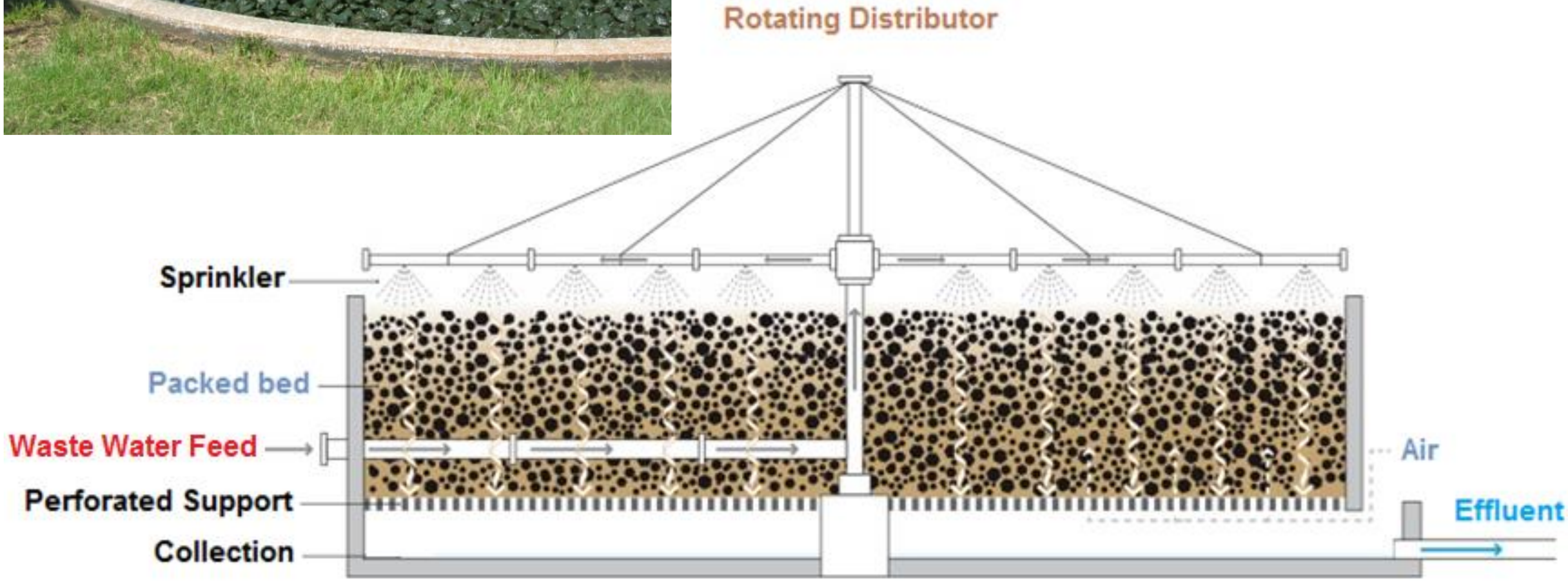
Warm Water Plant

Compressed Air Station

# Effluent Treatments

Incineration

# Trickling Filter (Packed Bed Bioreactors)



**Biochemical oxygen demand (BOD)**
The amount of oxygen consumed by these organisms in breaking down the organic materials in waste water.

**Chemical oxygen demand (COD)**: The amount of oxygen required to chemically oxidize organic compounds in water.

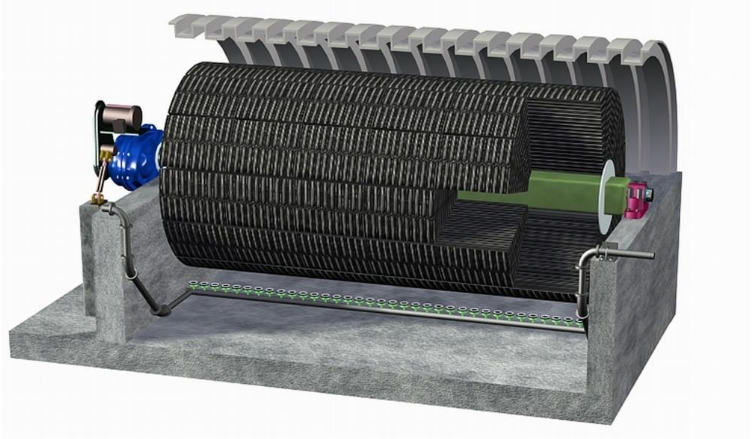
However, COD is less specific, since it measures everything that can be chemically oxidized, rather than just levels of biologically active organic matter.

$S^{-2} + 2\,O_2 \rightarrow SO_4^{-2}$ or $4Fe + 3O_2 \rightarrow 2Fe_2O_3$

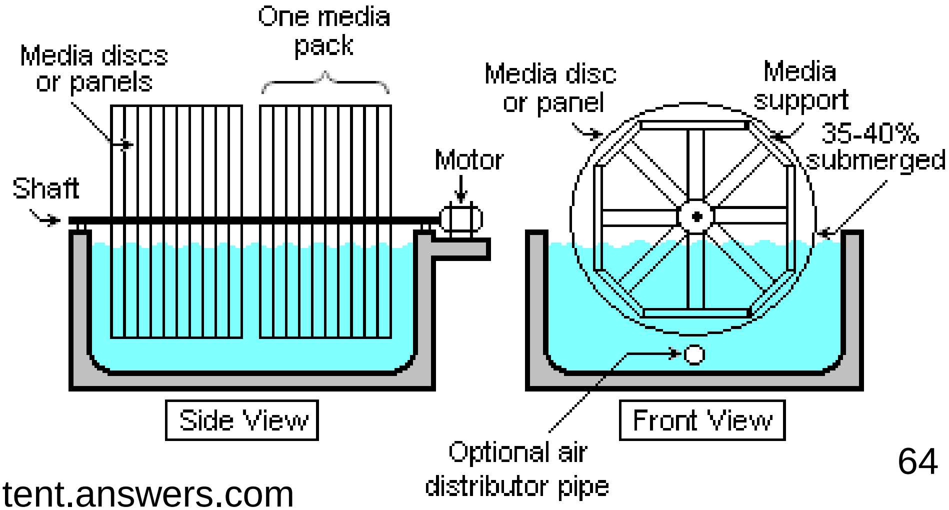
# Rotating Biological Contactors

www.edie.net/products

www.madep-sa.com/english/wwtp.html

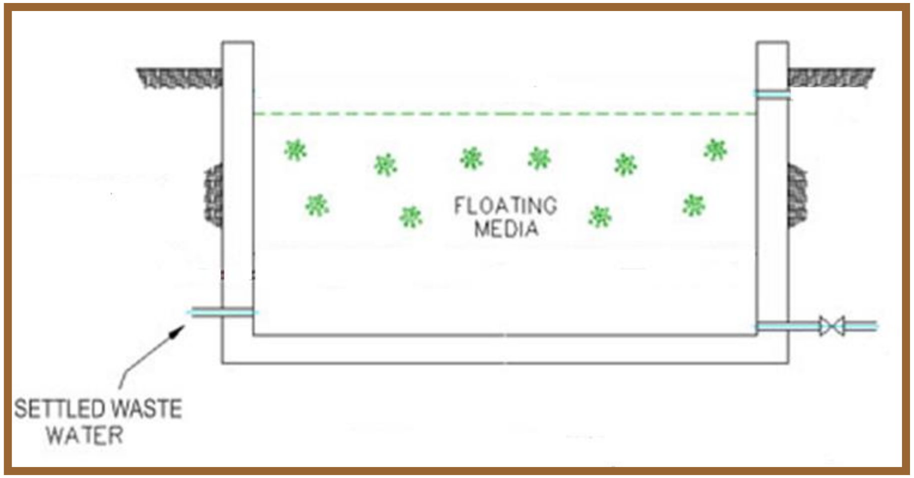


info.industry.siemens.com



wpcontent.answers.com

# Activated Sludge



**Mechanism:**
**Develop biological floc to reduces the organic content of the sewage.**

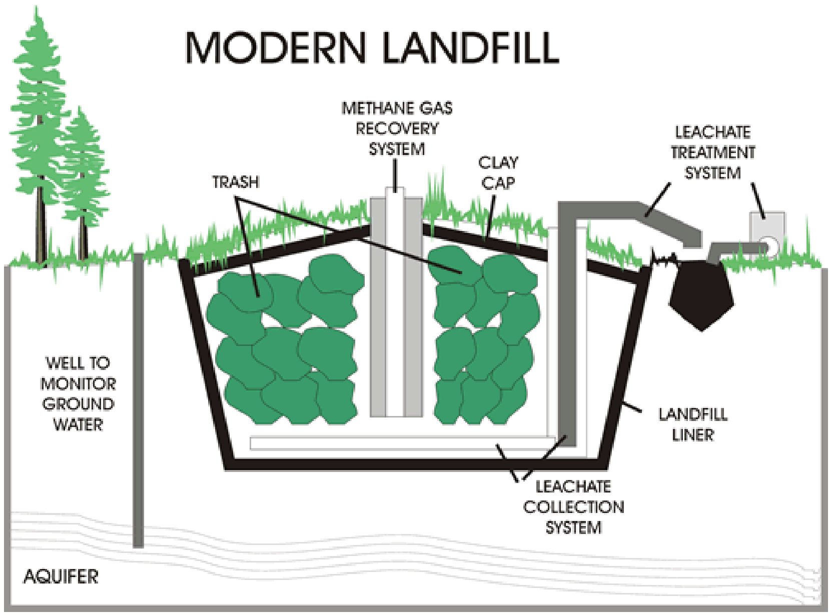
# Anaerobic Digesters

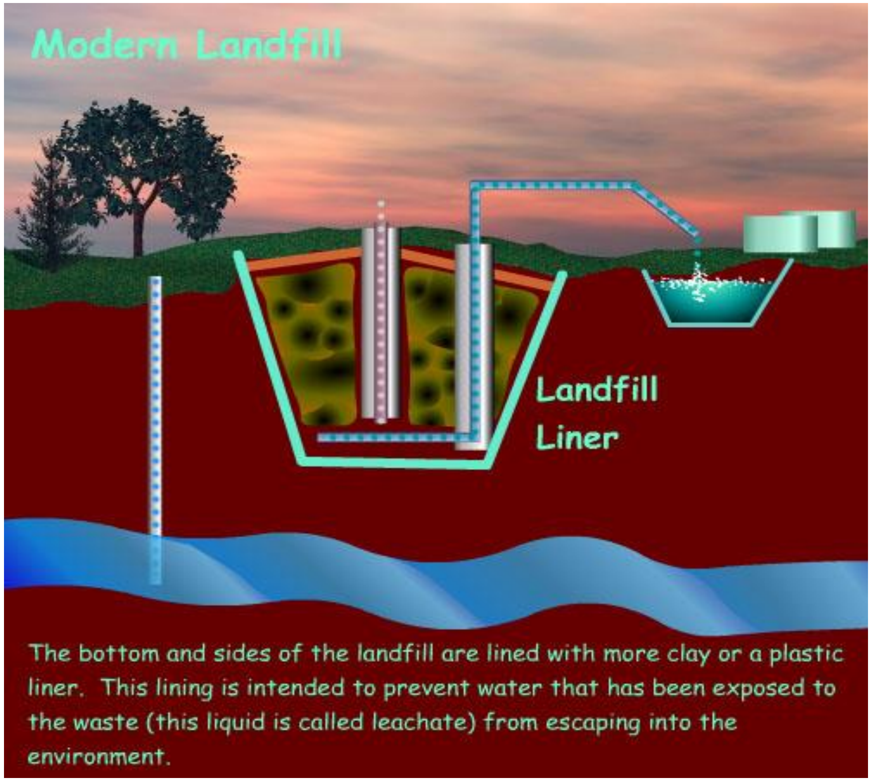
Acid fermenting bacteria degrade the waste to free volatile fatty acids, mainly acetic and propionic acid.

They are then converted to Methane and $CO_2$.

The gas product (biogas) is a useful by-product.

# Landfilling
# (Solid and Liquid Wastes)





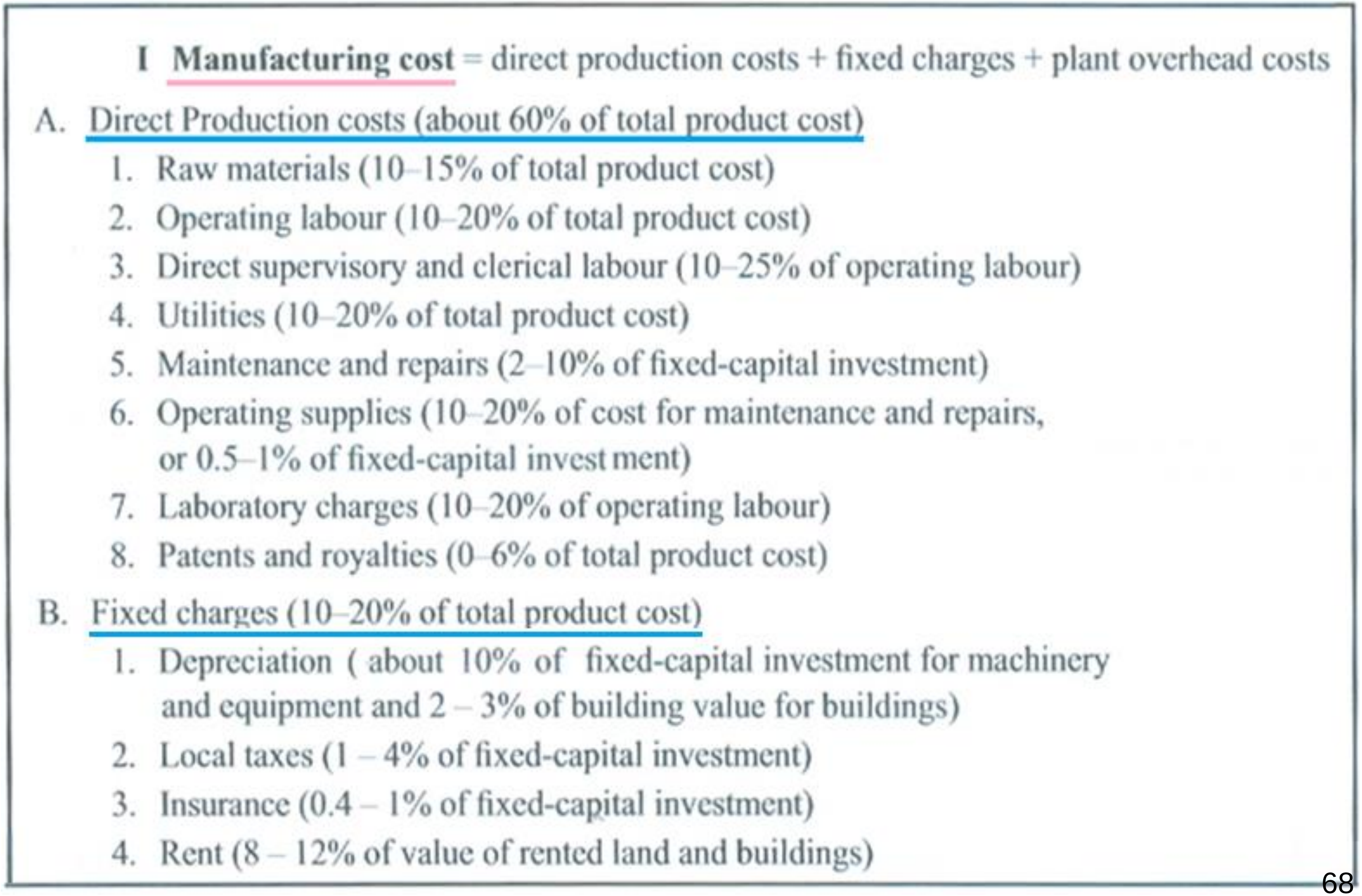
The bottom and sides of the landfill are lined with more clay or a plastic liner. This lining is intended to prevent water that has been exposed to the waste (this liquid is called leachate) from escaping into the environment.

**Landfill gas (50-60% methane) can be collected for energy source.**

www.eia.doe.gov/.../images/landfill.gif

techalive.mtu.edu/.../LandfillCrossSection.jpg

# Bioprocess Economics

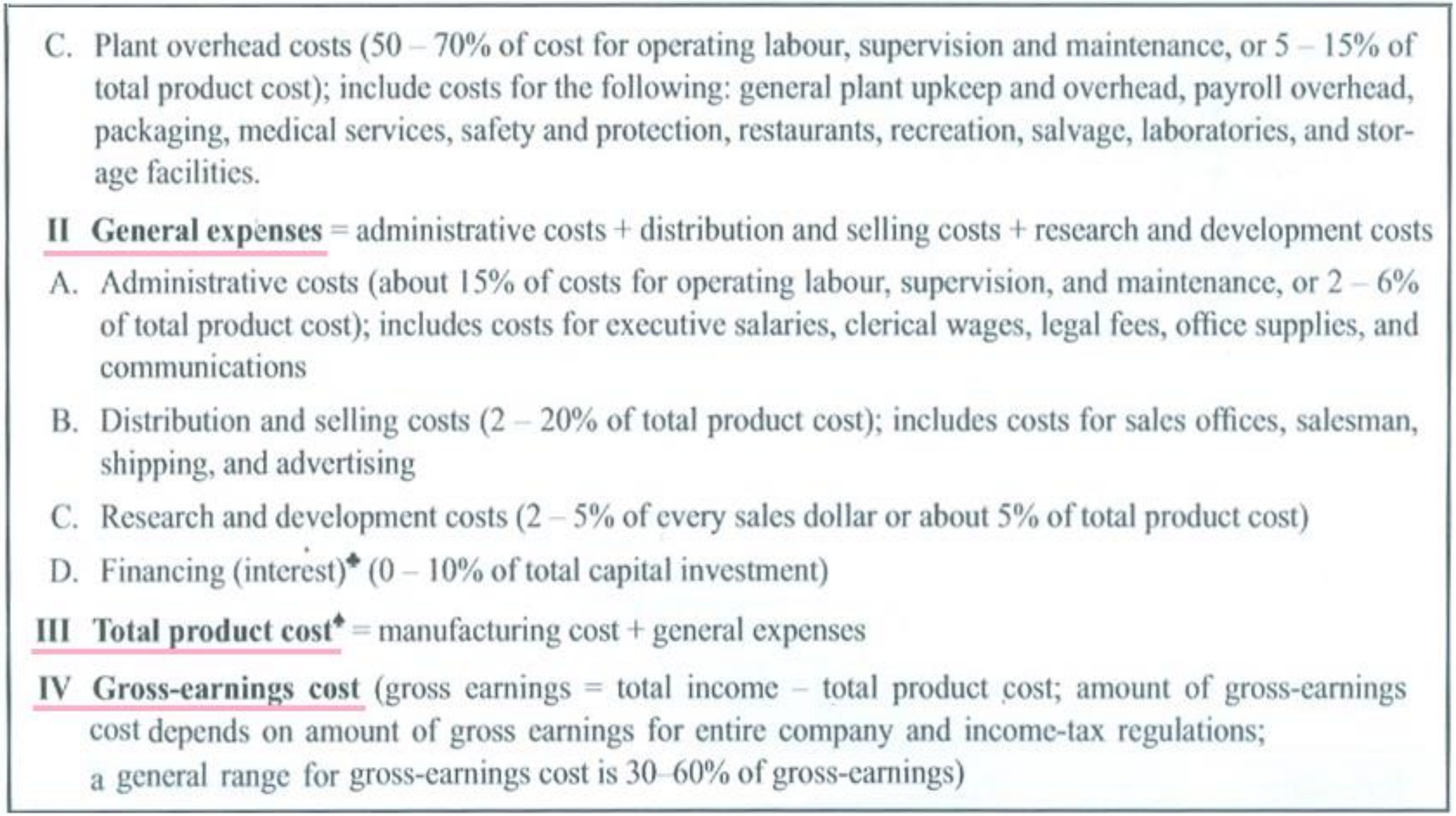


I **Manufacturing cost** = direct production costs + fixed charges + plant overhead costs

A. Direct Production costs (about 60% of total product cost)
  1. Raw materials (10–15% of total product cost)
  2. Operating labour (10–20% of total product cost)
  3. Direct supervisory and clerical labour (10–25% of operating labour)
  4. Utilities (10–20% of total product cost)
  5. Maintenance and repairs (2–10% of fixed-capital investment)
  6. Operating supplies (10–20% of cost for maintenance and repairs, or 0.5–1% of fixed-capital investment)
  7. Laboratory charges (10–20% of operating labour)
  8. Patents and royalties (0–6% of total product cost)

B. Fixed charges (10–20% of total product cost)
  1. Depreciation ( about 10% of fixed-capital investment for machinery and equipment and 2 – 3% of building value for buildings)
  2. Local taxes (1 – 4% of fixed-capital investment)
  3. Insurance (0.4 – 1% of fixed-capital investment)
  4. Rent (8 – 12% of value of rented land and buildings)

# Bioprocess Economics (Cont'd)



C. Plant overhead costs (50 – 70% of cost for operating labour, supervision and maintenance, or 5 – 15% of total product cost); include costs for the following: general plant upkeep and overhead, payroll overhead, packaging, medical services, safety and protection, restaurants, recreation, salvage, laboratories, and storage facilities.

**II General expenses** = administrative costs + distribution and selling costs + research and development costs

A. Administrative costs (about 15% of costs for operating labour, supervision, and maintenance, or 2 – 6% of total product cost); includes costs for executive salaries, clerical wages, legal fees, office supplies, and communications

B. Distribution and selling costs (2 – 20% of total product cost); includes costs for sales offices, salesman, shipping, and advertising

C. Research and development costs (2 – 5% of every sales dollar or about 5% of total product cost)

D. Financing (interest)◆ (0 – 10% of total capital investment)

**III Total product cost◆** = manufacturing cost + general expenses

**IV Gross-earnings cost** (gross earnings = total income – total product cost; amount of gross-earnings cost depends on amount of gross earnings for entire company and income-tax regulations; a general range for gross-earnings cost is 30–60% of gross-earnings)

# Acknowledgements

- **Chemical Engineering, Civil and Environmental Engineering, University of Houston**
- **Chemical Engineering, Oregon State University**
- **School of Pharmacy, Massachusetts College of Pharmacy and Health Sciences (MCPHS) University**

**Thank You!**

**Questions?**

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