Chap 8 Bioreactors

Introduction

- Applications of bioreactors: for production of vaccines, proteins, organic acids, amino acids and antibiotics; enzymatic or microbial biotransformations; bioremediation, etc.
- A production facility usually has a train of bioreactors ranging from 20 L to 250,000 L. The bioreactors are arranged in the series of increasing sizes, starting from small cultures to the final production culture.

I. Bioreactor configurations

Stirred tank reactors

Features:

- Microbial reactors have impellers to provide agitation and generally have 4 baffles from the walls to prevent vortexing of the fluid, the baffle width is 1/10 or 1/12 of the tank diameter.
- The vortex and circular flow result in little mixing between fluids at different heights. At high speeds the vortex may reach down to the impeller so that gas from the surrounding is drawn into the liquid → high mechanical stress in the stirrer shaft, bearings and seal.
- Bioreactors for animal cell cultures usually do not have baffles (especially for small scale reactors) to reduce turbulence.
- The aspect ratio (height-to-diameter ratio) of the vessel is 3-5 for microbial cultures but is normally less than 2 for animal cell culture.
- Sparger: gas is sparged at the bottom using a perforated pipe ring sparger.
- Number of impellers depends on the aspect ratio. The bottom impeller is located at a distance about 1/3 of the tank diameter above the bottom of the tank. Additional impellers are spaced approximately 1 to 2 impeller diameter (d) distances apart.

- The superficial aeration velocity (the volume flow rates of gas divided by the cross-sectional area of the vessel) in stirred vessel must be lower than that can flood the impeller (an impeller is flooded when it receives more gas than it can effectively disperse) otherwise the mixing is poor. Superficial aeration velocities generally do not exceed 0.05 m/s.

- Impellers: choice often depends on the viscosity of the liquid and sensitivity of the cells to mechanical shear.
  - Rushton (6-flat-blade) disc turbine (a) and concave bladed impeller (b): impeller diameter is about 1/3 of the vessel diameter and is often used for bacterial cultures. Rushton turbine is most commonly used in fermentation technology.
  - Hydrofoil impeller (c): diameter is about 0.5 to 0.6 times the tank diameter and is an effective mixer for highly viscous mycelial broths.
  - Marine impeller (d): usually single, large diameter, low shear, used for animal cell culture.
• Impeller speed:
  
  - Usually <120 rpm for animal cell cultures even for vessels >50 liters. Higher stirring speeds can be used for microbial cultures.
  
  - The impeller tip speed \( (3.14 \times \text{impeller diameter} \times \text{speed of rotation}) \) is usually less than 7.6 m/s for filamentous fungi.

◆ Power requirement:

  - critical for large-scale bioreactors
  
  - Smaller for sparged reactors because (1) gas bubble decreases the liquid density; (2) gas-filled cavities develop behind the stirred blades, which reduce the resistance to fluid flow and decrease the drag coefficient of the impeller.

\[
P_0 = N_p \rho_i N_i^3 D_i^5 \quad \text{for ungassed Newtonian fluid}
\]

\( P_0 \): power input (W)
\( \rho_i \): liquid density; \( N_i \): rotational speed (1/s); \( D_i \): diameter of impeller
\( N_p \): power number (related to Reynolds number)

\[
\frac{P_g}{P_0} = 0.10 \cdot \left( \frac{F_g}{\text{NiV}} \right)^{-0.25} \left( \frac{N_i^2 D_i^4}{g \text{W}_i V^{2/3}} \right)^{-0.20} \quad \text{for gassed Newtonian fluid}
\]

\( P_g \): power consumption with sparging; \( F_g \): volumetric gas flow rate; \( V \): liquid volume; \( g \): gravitational acceleration; \( \text{W}_i \): impeller blade width
**Bubble column reactor**

- Usually the height-to-diameter ratio is 4-6.
- Gas is sparged at the base through perforated pipes or plates or metal porous spargers.
- O₂ transfer, mixing and other performance factors are influenced mainly by gas flow rate and rheological properties of the fluid.
- Mixing and mass transfer can be improved by placing perforated plates or vertical baffles in the vessel.

**Airlift bioreactor**

- Separated as two zones: the sparged zone is called the riser, and the zone that receives no gas is the downcomer. The bulk density in the riser region is lower than that in the downcomer region, causing the circulation (so circulation is enhanced if there is little or no gas in the downcomer).
- For optimal mass transfer, the riser to downcomer cross-sectional area ratio should be between 1.8 and 4.3.
- Highly energy efficient and productivities are comparable to those of stirred tank bioreactors.
- The rate of liquid circulation increases with the square root of the height of the airlift device. Consequently, the reactors are designed with high aspect ratios.
- A gas-liquid separator in the head-zone can reduce the gas carry-over to the downcomer and hence increase the liquid circulation.

**Fluidized bed reactor**

- Suitable for reactions involving a fluid-suspended particulate biocatalyst such as immobilized enzyme and cell particles
Similar to the bubble column reactor except that the top section is expanded to reduce the superficial velocity of the fluidizing liquid to a level below that needed to keep the solids in suspension. Consequently, the solids sediment in the expanded zone and drop back, hence the solids are retained in the reactor whereas the liquid flows out.

**Packed bed bioreactor**

- A bed of particles are confined in the reactor. The biocatalyst (or cell) is immobilized on the solids which may be rigid or macroporous particles.
- A fluid containing nutrients flows through the bed to provide the needs of the immobilized biocatalyst. Metabolites and products are released into the fluid and removed in the outflow.
- The flow can be upward or downward. If upward fluid is used, the velocity can not exceed the minimum fluidization velocity.

**II. Bioreactor design features**

- Medium or feed nozzle (19).
- Vertical sight glass (15) and ports for pH, temperature and DO sensors (6).
- Connections for inoculum, acid and alkali (for pH control) and antifoam agents are located above the liquid level in the reactor vessel (16).
- O$_2$ and other gases (CO$_2$ or NH$_3$ for pH control; N$_2$ for O$_2$ control) can be
introduced through a sparger at the bottom (8).

- Foam breaker (22) is used when antifoam is ineffective or the antifoam interferes with downstream processing (antifoam tends to foul the membrane during filtration).

- Can be sterilized in-place using saturated steam (14) at a minimum absolute pressure of 212 kPa. Over-pressure protection is provided by a rupture disc (24) on the top of the reactor, which cracks to relieve the pressure to avoid explosion.

- Maximum allowable working pressure is ≈377-412 kPa (absolute)\(^1\), allowable temperature is usually 150-180\(^\circ\)C (>121\(^\circ\)C for sterilization). The vessel should withstand full vacuum or it could collapse while cooling after sterilization.

BioFlo 6000\(^\circledR\) Sterilizable-In-Place Fermentor-Bioreactor (50 - 130 L) BioFlo 110, 1.3 to 14 liters

- Usually made in Type 316L stainless steel, while the less expensive Type 304 (or 304L) is used for the jacket. The L grades contain less than 0.03% carbon, which reduces chromium carbide formation during welding and lowers the potential corrosion at the welds.

- The vessel should have as few internals as possible and should be free of stagnant areas where pockets of solids or liquids may accumulate.

### III. Design for sterile operation

\(^1\) 1 atm=1.01\times10^5\ Pa=101\ kPa
**Sterilization-in-place**

- A bioreactor must be sterilized before inoculation because contamination is a common cause of process failure.
- For large bioreactors, *in situ* sterilization is common.
- The components should be able to be sterilized independently during fermentation if required.
- The aeration and exhaust groups must also be sterilized. The filters are rated for removing particles down to 0.22 μm or even 0.1 μm. Often the gas streams require two filter cartridges in series, with the first serving to protect the second filter.

**Clean-in-place (CIP) considerations**

- Industrial bioreactors, including all pipings, should be cleaned in-place using automated methods, so as to ensure consistency and reduce down-time.
- To remove solid particles and avoid sedimentation, a flow velocity of 2 m/s is preferred. Also, the piping should be free of dead space as much as possible. For thorough cleaning, the CIP solutions are sprayed through a spray ball. For cleaning with jet spray, pressures of 308 to 377 kPa (absolute) are optimal.
Procedures:
1. Pre-rinse for 5 min with deionized water (sufficient for bacteria, yeast and animal cell cultures).
2. Circulate 1% (w/v) NaOH at 75-80°C through all product contact surfaces for 15-20 min. Discard the solution afterwards.
3. Rinse at 25-35°C with deionized water to remove all alkali.
4. Final wash with hot “water-for-injection” grade water.

For stirred tank bioreactors, it is recommended to fill the vessel and agitate at Reynolds numbers of $10^8$-$10^{8.5}$ during pre-rinse, alkali recirculation and the final rinse for 2-3 min (should be sufficient to dislodge adhering dirt or soil).

Note:
- Disposable bioreactors (e.g. Wave bioreactor, BelloCell) are gaining increasing interest due to smaller capital investment, easier operation and elimination of the CIP process.

BelloCell 500 AP
(Cesco Bioengineering)
Water for Injection (WFI): high quality water subjected to the following treatment:

1. Ion exchanger need to be regenerated regularly by HCl and by NaOH
2. Reverse osmosis removes viruses, microorganisms, pyrogen and virtually all inorganic impurities.
Appendix

IV. Mass transfer steps

- In bioreaction processes, substrates are consumed for the conversion. Typical substrates include carbon sources (e.g. sugar and oil), nitrogen sources (e.g. ammonia and amino acids) and electron acceptors (e.g. O₂).

Effects of transfer limitations

Two effects if one step is slower than the key kinetic reaction step:

1. The overall reaction rate is below the theoretical maximum, and the process output is slower than desired.
   - Reversible effect: for the production of gluconic acid (葡萄糖酸) from glucose by *Gluconobacter oxydans*, the O₂ transfer is limiting. Once O₂ limitation is relieved, there is no irreversible effect on this microorganism.
   - Irreversible effect: for the production of penicillin, O₂ limitation imposes an irreversible damage to the biosynthetic capacity of the cell.

2. The selectivity of the reaction is altered.
   - Ex: O₂ serves as an electron acceptor in the formation of baker’s yeast from glucose. In the absence of O₂ the e⁻ will be directed to pyruvate resulting in the formation of ethanol and CO₂.
Transfer of oxygen involves a chain of mass transfer steps from a gas bubble. The slowest one is the rate-limiting step and determine whether the mass transfer rate would affect the overall process performance. When cells are well dispersed in the liquid and the bulk liquid is well mixed, step (iii) is the limiting step.

**Transfer across the cell envelope**

- Transport across the cell envelope (may include cell wall and cytoplasmic membrane) can be limited.
- Three typical mechanisms:
  1. Free diffusion: passive transport down a concentration gradient
  2. Facilitated diffusion: as above but speeded up by a carrier protein
  3. Active transport: transport by a carrier protein with input of free energy

The diameter of microbial cell itself is small (usually ≈1-5 μm) so diffusion inside the cell is more rapid and not a limiting factor.

The transport barrier imposed by the membranes of intracellular organelles in eucaryotic cells usually does not limit the overall transport rate.

**V. Mass Transfer Equations**

**Fundamentals:**

**Fick’s Eqn.**

\[ J = -D \frac{dC}{dx} \text{ (at steady state)} \]

- **J:** molar mass flux (mol/m²·s)
- **D:** diffusion coefficient (m²/s)
- **C:** concentration of the substance to be transported
- **x:** distance
• Considering transport in solid phase, D is the **effective diffusion coefficient** which lumps the diffusion coefficient, the porosity of the solid, and the shape of the channels.

• For a flat plate with thickness d in a stationary fluid

\[ J = D \Delta C / d \]

Where D/d represents the mass transfer coefficient, while d/D can be interpreted as the resistance against transport.

• For unsteady state:

\[ D \frac{\partial^2 C}{\partial x^2} = \frac{\partial C}{\partial t} \]

• These equations consider the diffusion process only but not convection. In reality, convection is often encountered and flow pattern is not known, thus the mass transport often relies on empirical approach.

**Mass transfer between l-s phase or l-g phase** (two film theory)

For gas film transport:

\[ J_g = k_g (P_i - P) \]  (mass transfer rate, not flux)

Liquid film transport:

\[ J_i = k_i \alpha_i (C_i - C) \]  (the main resistance to oxygen transfer)

\( k_i \): liquid phase mass transfer coefficient (m/h)

\( P_i \neq C_i \) but can be correlated by Henry coefficient H (bar·m³/mol):

\[ P_i = H C_i \]

From here, the volumetric mass transfer rate \( J \) (mg/h/l), can be derived

\[ J = k_i \alpha_i (C^* - C) \]

- \( \alpha_i \) (m²/m³): the gas-liquid interfacial area per unit liquid volume, or area per unit gross vessel volume in the bioreactor.
When dealing with the transfer of O$_2$ from gas to liquid, J is called the OTR (oxygen transfer rate)

- k$_{L,a}$ (1/h): volumetric transfer coefficient, because $a$ and $k_L$ are difficult to evaluate separately, k$_{L,a}$ is often expressed together. Various expressions of k$_{L,a}$ can be found in literature. The value is typically $\approx$0.02~0.25 s$^{-1}$
- C*: saturated DO (in the case of oxygen transfer) concentration (g/l), i.e. the solubility of O$_2$
- C: actual DO concentration in the broth
- Note: mole fraction of O$_2$ in air is 0.2099, so the partial pressure of O$_2$ at 1 atm air pressure is 0.2099 atm. Based on Henry’s law, the solubility of oxygen in water under 1 atm air pressure is 0.2099 times that under 1 atm pure O$_2$.

- Oxygen uptake rate:
  
  $$Q_o = q_o \cdot x \ (g/l-h)$$  
  $q_o$: specific oxygen uptake rate (g/g·s), x: cell concentration (g/l)

- $q_o$ varies with cell species and nutritional environment
- For C> C$_{crit}$ (critical oxygen concentration), $q_o$ is a constant maximum
- For C< C$_{crit}$, $q_o$ (usually 5-10% air saturation) is approximately linearly dependent on C.

At steady state,

$$k_{L,a}(C^* - C) = q_o \cdot x$$

This equation can be used to predict the response of fermenter to changes in mass transfer operating conditions, for example, if k$_{L,a}$ is raised by increasing stirrer speed $\Rightarrow$ C must rise.

**Measurement of k$_{L,a}$**

1. **Oxygen-balance method:** (based on steady state measurement)

   at s.s.

   $$J = \frac{1}{V_L}((F_g C_i) - (F_g C)_o)$$

   where l.h.s: rate of oxygen transfer from gas to liquid
   
   r.h.s.: difference in oxygen flow between inlet (subscript i) and outlet (subscript o).
   
   $V_L$, volume of liquid; $F_g$, volumetric gas flow rate; C, gas phase concentration of O$_2$
Because gas phase concentrations are usually measured as partial pressures, ideal gas law can be incorporated:

\[ J = \frac{1}{RV_L} \left( \left( \frac{F_g p_i}{T} \right) - \left( \frac{F_g p_o}{T} \right) \right) \]

where \( p \) is the partial pressure at the inlet and outlet. The difference between \( p_i \) and \( p_o \) is usually small thus \( p_i \) and \( p_o \) need to be measured very accurately (e.g. by mass spectrometry).

For known \( p, F_g, V_L, R \) and \( T \), \( J \) is obtained, and \( k_La \) can be calculated from

\[ J = k_La(C^* - C) \]

when \( C^* \) and \( C \) are measured.

2. **Dynamic method:** (based on unsteady state measurement)

- At time \( t_0 \), the broth is de-oxygenated by sparging \( N_2 \) or by stopping the air flow if the culture is consuming \( O_2 \) \( \Rightarrow \) \( C \) drops

- Air is then pumped into the broth at a constant flow rate \( \Rightarrow \) \( C \) increases and reaches a s.s. value, \( \bar{C} \) . \( C_1 \) and \( C_2 \) are two oxygen concentrations measured at \( t_1 \) and \( t_2 \).

- During the re-oxygenation step (unsteady state)

\[ \frac{dC}{dt} = k_La(C^* - C) - q_o \cdot x \]

- At s.s., \( k_La(C^* - \bar{C}) = q_o \cdot x \) is substituted into the above equation to yield

\[ \frac{dC}{dt} = k_La(\bar{C} - C) - q_o \cdot x \]

Assume \( k_La \) is a constant, integrating the above equation from \( t_1 \) to \( t_2 \) yields
Gas-Liquid mass transfer in real systems (O₂ transfer)

- Bubble size is critical in determining oxygen transfer, smaller bubbles leads to:
  - higher a
  - slower bubble rise velocity  ➔ higher gas hold-up $\varepsilon$ (volumetric fraction of gas in the liquid).

$$
\varepsilon = \frac{V_G}{V_g + V_L}, \quad V_G: \text{volume of gas bubbles in the reactor, } V_L: \text{volume of liquid}
$$

- Two extreme cases:
  - Coalescing (融合) liquid (a liquid which greatly stimulates bubble coalescence): mass transfer is poorest
  - Non-coalescence fluid: highest mass transfer.

Stirred tank reactor:

- Sparged gas is usually rapidly collected in the gas cavities behind the rotating impeller blades. The cavities flow in a highly turbulent vortex and the gas is dispersed into smaller bubbles. These follow the liquid flow, but will also rise to the surface. They will coalesce in areas that are relatively calm and re-disperse in places where the shear stress is high. A part of the bubbles is re-circulated into the cavities and the rest escapes at the surface.

- Empirical values for gas hold-up

$$
\varepsilon = 0.13 \cdot (P/V)^{0.33} (v_g p_0 / p)^{0.67} \quad \text{(for coalescing fluid)}
$$

where \((P/V)=\text{power input per unit volume, } p=\text{pressure in the system}\)

- Empirical equations of $k_{La}$: (coarse equations with accuracy≈30%, valid for $P/V$=0.5~10 kW/m³).

$$
k_{La} = 0.026 \cdot (P/V)^{0.4} (v_g p_0 / p)^{0.5} \quad \text{for coalescing fluid (e.g. clean air-water system)}
$$
\[ k_L a = 0.002 \cdot (P/V)^{0.7} (v_g p_o / p)^{0.2} \] for non-coalescing fluid (e.g. fermentation broth)

\( v_g \), superficial gas velocity ; \( p_o \), reference pressure of 1 bar.

**Bubble column:**

- When air flow rate is high enough,
  \[ \varepsilon = 0.6(v_g p_o / p)^{0.7} \]
  \[ k_L a = 0.32 \cdot (v_g p_o / p)^{0.7} \]

- In non-coalescing liquids (e.g. some fermentation broths), the bubble rises and does not mix with other bubbles, provided that the size is smaller than the equilibrium average diameter 6 mm.

- In a large bubble column (>50 m³), the bubbles will significantly expand as they rise because of the decreasing hydrostatic pressure, which influence the mass transfer.

- \( k_L \): usually \( \approx 3-4 \times 10^{-4} \) m/s for bubbles>2-3 mm diameter, could be down to \( 1 \times 10^{-4} \) m/s for smaller bubbles, depending on bubble rigidity

**Air-lift reactor:**

- Although the riser resembles a bubble column, the gas-hold up is lower than predicted by the above equation due to the interaction with the liquid flow.

- Correspondingly, \( k_{La} \) will be lower, up to a third of the bubble column. A precise quantification, however, cannot be easily made.