Bioreactor Design

6.1 INTRODUCTION

To design a bioreactor, some objectives have to be defined. The decisions made in the design of the bioreactor might have a significant impact on overall process performance. Knowledge of reaction kinetics is essential for understanding how a biological reactor works. Other areas of bioprocess engineering such as mass and energy balances, mixing, mass transfer and heat transfer are also required.

The bioreactor is the heart of any biochemical process in which enzymes, microbial, mammalian or plant cell systems are used for manufacture of a wide range of useful biological products. The performance of any bioreactor depends on many functions, such as those listed below:

- Biomass concentration
- Sterile conditions
- Effective agitations
- Heat removal
- Correct shear conditions

- Nutrient supply
- Product removal
- Product inhibition
- Aeration
- Metabolisms/microbial activities

There are three groups of bioreactor currently in use for industrial production:

1. Non-stirred, non-aerated system: about 70% of bioreactors are in this category.
2. Non-stirred, aerated system: about 10% of bioreactors.
3. Stirred and aerated systems: about 20% of the bioreactors in industrial operation.

Non-stirred, aerated vessels are used in the process for traditional products such as wine, beer and cheese production. Most of the newly found bioprocesses require microbial growth in an aerated and agitated system. The percentage distribution of aerated and stirred vessels for bioreactor applications is shown in Table 6.1. The performances of various bioreactor systems are compared in Table 6.2. Since these processes are kinetically controlled, transport phenomena are of minor importance.

Non-stirred, non-aerated vessels are used for traditional products such as wine, beer and cheese. Most of the new products require growth of microorganisms in aerated, agitated vessels.

TABLE 6.1. *Percentage of distribution aerated and stirred vessel in bioreactor application*

Non-stirred, non-aerated	76%
Non-stirred, aerated	11%
Stirred, aerated	13%
Total	100%

TABLE 6.2. *Performances of bioreactors*

Concentration	Productive bioreactors		Wastewater treatment	
	10–50		Aerobic	Anaerobic
(kg/m^3)			5	50
	Moulds	Bacteria/yeast		Mixed Culture
Viscosity	High	Low	Low	Low
Oxygen consumption	High	High	Low	Absent
Mass transfer	Low	High	Low	Absent
Heat production metabolic (kW/m^3)	3–15	3–15	0.03–0.14	Negligible
Power consumption, hp	3–15	fewer than 5	0.02–05	Negligible

6.2   BACKGROUND TO BIOREACTORS

The main function of a properly designed bioreactor is to provide a controlled environment to achieve optimal growth and/or product formation in the particular cell system employed. Frequently the term "fermenter" is used in the literature to mean "bioreactor".[1-3] The performance of any bioreactor depends on many functions including:

• Biomass concentration must remain high enough to show high yield.
• Sterile conditions must be maintained for pure culture system.
• Effective agitation is required for uniform distribution of substrate and microbes in the working volume of the bioreactor.
• Heat transfer is needed to operate the bioreactor at constant temperature, as the desired optimal microbial growth temperature.
• Creation of the correct shear conditions. High shear rate may be harmful to the organism and disrupt the cell wall; low shear may also be undesirable because of unwanted flocculation and aggregation of the cells, or even growth of bacteria on the reactor wall and stirrer.

6.3   TYPE OF BIOREACTOR

Aerobic bioreactors are classified into four categories, depending on how the gas is distributed.

- Stirred tank reactor: the most common type of bioreactor used in industry. A draught is fitted which provides a defined circulation pattern.
- Airlift pressure cycle bioreactor: the gas is circulated by means of pressurised air.
- Loop bioreactor: a modified type of airlift system in which a pump transports the air and liquid through the vessel.
- Immobilized system: the air circulates over a film of microorganisms that grows on a solid surface. In an immobilized bioreactor, particulate biocatalysts for enzyme production and conversion of penicillin to 6-aminopenicillanic acid are used.
- Fluidized bed: when packed beds are operated in upflow mode, the bed expands at high flow rates; channelling and clogging of the bed are avoided. Normal application is waste-water treatment and the production of vinegar.
- Trickle bed: another variation of the packed bed, fluid is sprayed onto the top of the packing and trickles down through the bed. Air is introduced at the base, because liquid is not continuous throughout the column, so air moves easily around the packing. This type of bioreactor is widely used for aerobic wastewater treatment.
- Fed-batch mixed reactor: starting with a relatively dilute solution of substrate this provides control over the substrate concentration. High rates are avoided. Fed batch is used for baker's yeast to overcome catabolite repression and to control oxygen demand. It is also used routinely for production of Penicillin.
- Batch mixed reactor: There are three principal modes of bioreactor operation: (a) batch; (b) fed batch; (c) continuous.

Industrial bioreactors can withstand up to 3 atmospheres positive pressure. Large fermenters are equipped with a lit vertical sight glass for inspecting the contents of the reactor. Side parts for pH, temperature and dissolved oxygen sensors are a minimum requirement. A steam sterilisation sample port is provided. Mechanical agitators are installed on the top or bottom of the tank for adequate mixing.

Choice of operating strategy has a significant effect on substrate conversion, product susceptibility to contamination and process reliability.

Mass balance: $$\frac{dm}{dt} = m_i - m_o + r_p - r_s \qquad (6.3.1)$$

where r_p is the rate of product formation and $-r_s$ is the rate of substrate consumption.

The design emphasis of this section will be on stirred tank bioreactors, which are the most common type used commercially in many bioprocess industries.

6.3.1   Airlift Bioreactors

In an airlift fermenter, mixing is accomplished without any mechanical agitation. Airlift bioreactors are used for tissue culture because the tissues are shear sensitive and normal mixing is not possible. There are many forms of airlift bioreactor. In the usual form, air is fed into the bottom of a central draught tube through a sparger ring, so reducing the apparent density of the liquid in the tube relative to the annular space within the bioreactor. The flow passes

up through the draught tube to the head space of the bioreactor, where the excess air and the by-product, CO_2, disengage. The degassed liquid then flows down the annular space outside the draft to the bottom of the bioreactor. Cooling can be provided by either making the draught tube an internal heat exchanger or with a heat exchanger in an external recirculation loop.

The advantages of airlift bioreactor are:

1. In low shear, there is low mixing which means the bioreactor can be used for growing plant and animal cells.
2. Since there is no agitation, sterility is easily maintained.
3. In a large vessel, the height of liquid can be as high as 60 m, the pressure at the bottom of the vessel will increase the oxygen solubility, and the value of $K_L a$ will increase.
4. Extremely large vessels can be constructed. In one single cell protein plant, the reactor had a total volume of $2300\,m^3$ (a column of 7 m diameter and 60 m height with a reactor working volume of $1560\,m^3$). Further, in this reactor the microorganisms were grown on methanol for SCP, the biochemical reaction resulting in an extremely large heat release. It was not possible to remove such a high exothermic heat of reaction with a conventional stirred-tank design.

In applications of airlift bioreactor there are various types of fermenter. The most common airlift bioreactors are pressure cycle, internal and external loop bioreactors.

6.3.2   Airlift Pressure Cycle Bioreactors

The gas is circulated by means of pressurised air. In airlift bioreactors, circulation is caused by the motion of injected gas through a central tube, with fluid recirculation through the annulus between the tube and the tower or vice versa. Figure 6.1 shows an airlift bioreactor with an internal loop cycle of fluid flow.

6.3.3   Loop Bioreactor

A modified type of airlift system with gas and liquid flow patterns in which a pump transports the air and liquid through the vessel. Here, an external loop is used, with a mechanical pump to remove the liquid. Gas and circulated liquid are injected into the tower through a nozzle. Figure 6.2 shows an airlift bioreactor that operates with an external recirculation pump.

6.4   STIRRED TANK BIOREACTORS

The most important bioreactor for industrial application is the conventional mixing vessel, which has the dual advantages of low capital and low operating costs. Figure 6.3 is a schematic diagram for such a reactor. Vessels for laboratory experiments of volume up to 20 litres are made of glass. For larger volumes, construction is made of stainless steel. The height:diameter ratio of the vessel can vary between 2:1 and 6:1, depending largely on the amount of the heat to be removed, and the stirrer may be top- or bottom driven. All tanks are fitted with baffles, which prevent a large central vortex being formed as well as improve
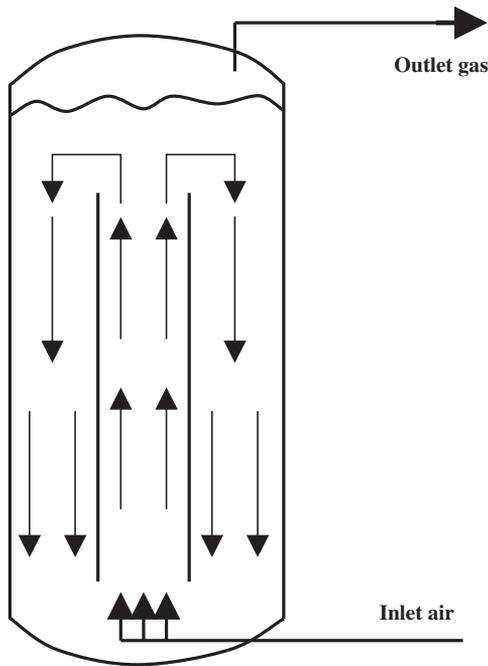
FIG. 6.1. Gas and liquid flow pattern with internal loop cycle.
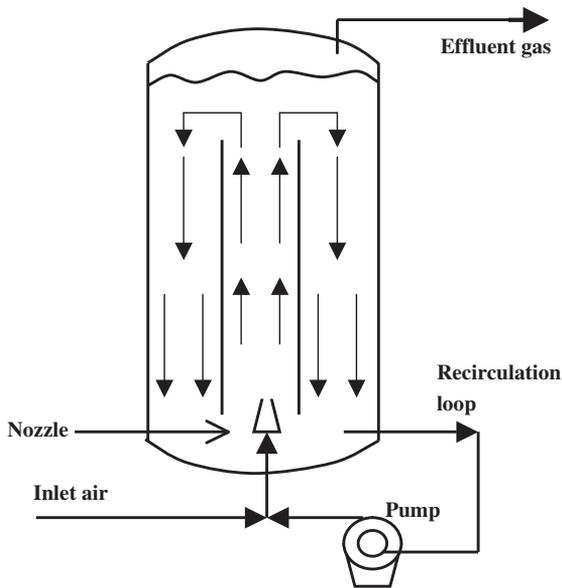
FIG. 6.2. Airlift bioreactor with external recirculation pump.
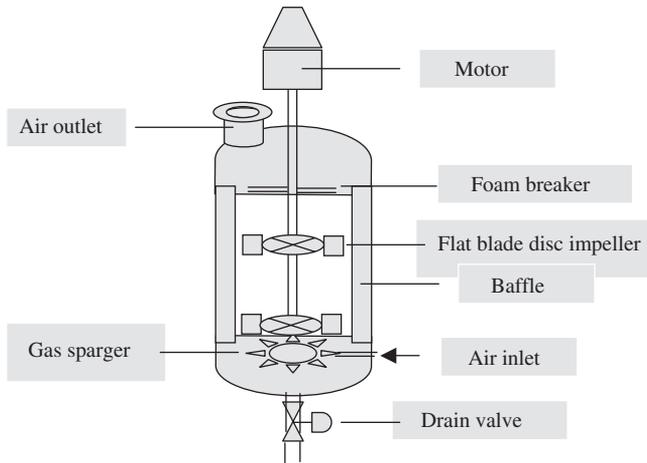
F<small>IG</small>. 6.3. Stirred tank bioreactor.

mixing. Four baffles are used for vessels less than 3 metres in diameter, and six to eight baffles are used in larger vessels. The width of the baffle is usually between $T/10$ and $T/12$, in which T is the tank diameter.[4,5]

Height of vessel to diameter:

$$\frac{H}{D_t} = 2{:}1 \quad \text{and} \quad 6{:}1 \tag{6.4.1}$$

Diameter of vessel to baffle:

$$10 < \frac{D_t}{D_b} < 12 \tag{6.4.2}$$

The diameter of the tank, D_{tank} is less than 3 m, four baffles of 6–8 inches may prevent a central vortex. Typically, 75% of the designed volume is used as working volume, in a fermentation vessel about 75% of the total CSTR volume is filled with liquid, the remaining 25% is used for gas space. If foaming takes place, there is no chance of immediate contamination. If the vessel height is equal to the diameter ($H = D$), one agitator is sufficient. If the vessel height is twice the diameter ($H = 2D$) or more, additional sets of agitators should be mounted on the shaft, separated by a distance φ. Installation of multiple sets of impellers improves mixing and mass transfer. Spargers should always be located near the bottom of the vessel with a distance $D_i/2$ below the agitator, where D_i is the diameter of the impellers. Power input per unit volume of fermentation vessel for a normal fermenter should be greater than 100 W/m^3, and the impeller tip speed ($\pi N D_i$) should be greater than

1.5 m/s. Let us define a dimensionless number that is known as the Froude number, *Fr*; the value of the stated dimensionless number has to be greater than 0.1:

$$Fr = \frac{N^2 D_i}{g} > 0.1 \tag{6.4.3}$$

High agitation and aeration cause major problems such as foaming, which may lead the fermentation vessel to unknown contamination. Antifoam cannot be always added for the reduction of foam: it may have inhibitory effects on the growth of microorganisms, so the simplest devices have rakes mounted on the stirrer shaft and located on the surface of the fluid.

If heat removal is a problem, as it can be in large bioreactors greater than 100 m^3, up to 12 baffles can be used, through which coolant passes.

Careful consideration has to be given to agitator design within a bioreactor because it controls the operation of the bioreactor.

The most common type of agitator used is the four-bladed disk turbine. However, research on the hydrodynamics of the system has shown that other disk turbine agitators with 12, 18 or concave blades have advantages.

Considerable research has been undertaken in gas/liquid systems with no solids present and where shear is not a problem. In systems that are shear-sensitive and where solids are present, there are advantages in using an inclined bladed turbine. The number of agitators mounted on the shaft will be dependent on the height of liquid in the vessel. For specification of the correct number of agitators on the shaft, the height of liquid in the vessel should be equal to the tank diameter, one agitator is required; if the height of liquid is two or three times of the tank diameter ($H = 2T$ or $3T$), additional agitators should be mounted on the shaft, separated by a distance φ; then $\varphi = T$, where T represents tank diameter. Installation of multi-sets of impellers improves mixing and enhances mass transfer.

High turbulence is required for efficient mixing; this is created by the vortex field which forms behind the blades. For all the gas to flow through this region it must enter the vessel close to and preferably underneath the disk; hence it is recommended that spargers should always be nearer, about a distance of $D_i/2$ below the agitator, where D_i is the impeller diameter.

The centrifugal force will draw the gas into the system, which ensures that sufficient turbulence is created. For this, a power input greater than 100 W/m^3 is required from the agitator.[6] Alternatively, a tip speed ($\pi N D_i$) greater than 1.5 m/s or a Froude number ($N^2 D_i/g$) greater than 0.1 are often used, where N is the agitator speed in Hz, and g is gravitational acceleration in m/s^2.

The design of the gas inlet device is of only secondary importance for the capture and dispersal of the gas by the agitator. For efficient mass transfer, a multiple-orifice ring sparger is generally used with a gas outflow diameter of $3D_i/4$. However, it is only slightly better than a single open-pipe sparging located centrally beneath the disk.

Foaming is often a problem in large-scale aerated systems. Antifoam cannot always be added for the reduction of foam because it may inhibit the growth of the microorganisms. However, there are several mechanical methods by which the foam can be broken up.

The simplest devices have rakes mounted on the stirrer shaft located on the surface of the liquid. A more sophisticated device is the 'Funda-foam system', in which the foam is destroyed by centrifugal forces. The nutrient solution held in the foam flows back into the bioreactor, and the air released from the foam leaves the vessel.

There should be a minimum number of openings in the bioreactor so that sterility can be maintained. Small openings must be made leak-proof with an O-ring, and larger openings fitted with gaskets. One of the most difficult areas to seal effectively is the point where the agitator shaft passes into the vessel; here a double mechanical shaft seal should be fitted. If possible the joints of all the parts connected within the sterile vessel as well as all of the pipes both inside and outside the bioreactor should be welded. There should not be any direct connection between the non-sterile and sterile area; that is, sampling devices and injection ports must be accommodated in steam-sterilisation closures.

6.5 BUBBLE COLUMN FERMENTER

For the production of baker's yeast, beer and vinegar, bubble column fermenters are used. They are also often used for sufficient aeration and treatment of wastewater. In designing such a bioreactor, the height of liquid to tank diameter ($H:D$) is about 2:1, a common ratio of $H:D$ is also about 3:1; in bakers' yeast production the ratio of $H:D$ is 6:1. In bubble columns the hydrodynamics and mass transfer depend on the size of the bubbles and how they are released from the sparger. The upward liquid velocity at the centre of the column, for the column diameter range 10 cm to 7.5 m ($0.1 < D < 7.5$ m) and the superficial gas velocity is in the range of $0 < u_{gas} < 0.4$ m/s.[7] The liquid velocity is correlated in the following equation:

$$u_{liquid} = 0.9 \, [gDu_{gas}]^{0.33} \qquad (6.5.1)$$

The gas superficial velocity is defined as the ratio of gas flow rate to column cross sectional area:

$$U_{gas} = \frac{Q_{gas}}{A} \qquad (6.5.2)$$

The mixing time is calculated by:

$$t_{mixing} = 11 \left(\frac{H}{D} \right) \left(\frac{gu_g}{D^2} \right)^{-0.33} \qquad (6.5.3)$$

where H is the height of bubble column and D is the column diameter. Figure 6.4 shows a simple column with an air sparger installed at the bottom of the column which allows sufficient air to pass through the liquid.

Exhaust gas
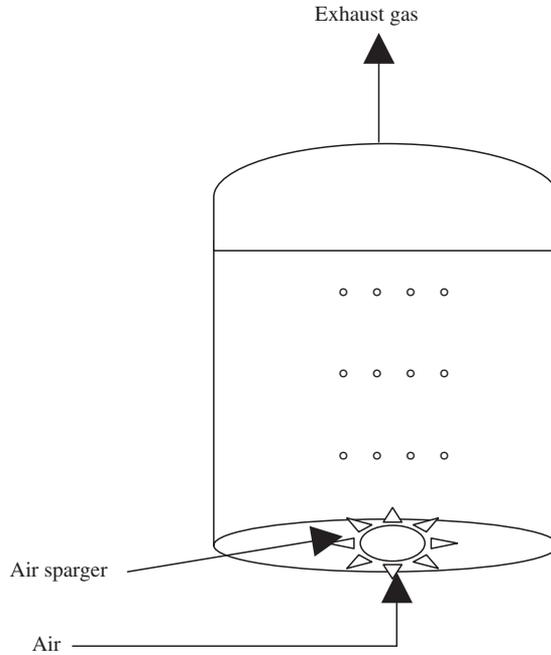
Fig. 6.4. Bubble column bioreactor.

6.6   AIRLIFT BIOREACTORS

In an airlift fermenter, mixing is accomplished without any mechanical agitation. An airlift fermenter is used for tissue culture, because the tissues are shear sensitive and normal mixing is not possible. With the airlift, because the shear levels are significantly lower than in stirred vessels, it is suitable for tissue culture. The gas is sparged only up to the part of the vessel cross section called the riser. Gas is held up, fluid density decreases causing liquid in the riser to move upwards and the bubble-free liquid to circulate through the down-comer. The liquid circulates in airlift reactors as a result of the density difference between riser and down-comer.

There are many forms of airlift bioreactor. In the usual form, air is fed into the bottom of a central draught tube through a sparger ring, so reducing the apparent density of the liquid in the tube relative to the annular space within the bioreactor. The flow passes up through the draught tube to the head space of the bioreactor, where the excess air and the by-product, CO_2, disengage. The degassed liquid then flows down the annular space outside the draft to the bottom of the bioreactor. In general, airlift bioreactors have the following features:

• Internal-loop vessels
• Draft tubes
• External loop or outer-loop

The cooling duty can be provided by either making the draught tube an internal heat exchanger or with a heat exchanger in an external circulation loop. The mass transfer coefficient for external loop airlift Fermenter is estimated as:[8]

$$K_L a < 0.32 u_g^{0.7} \qquad (6.6.1)$$

The height of airlift reactors is typically about 10 times the diameter of the column ($H = 10D$). For deep-shaft systems the ratio of H:D is about 100. For large fermenters ($500\,m^3$), a bubble column is an attractive choice, because it is simple and cheap to operate.

The main disadvantages of airlift reactors are:

1. High capital cost with large-scale vessels.
2. High energy costs. Although an agitator is not required, a greater air throughput is necessary, and the air has to be at a higher pressure, particularly on a large scale. Also, the efficiency of gas compression is low.
3. As the microorganisms circulate through the bioreactor, the conditions change, and it is impossible to maintain consistent levels of carbon source, nutrients and oxygen throughout the vessel.
4. The separation of gas from the liquid is not very efficient when foam is present. In the design of an airlift bioreactor, these disadvantages have to be minimised. If the feed comes in at only one location, the organism would experience continuous cycles of high growth, followed by starvation. This would result in the production of undesirable by-products, low yields and high death rates. Therefore, particularly on a large scale, multiple feed points should be used. Similarly, air should be admitted at various points up the column. However, the air must mainly enter from the bottom to circulate the fluid through the reactor.

6.7   HEAT TRANSFER

The temperature in a vessel can be controlled by removing heat by means of water circulating through a jacket on the outside of the vessel and/or by passing the water through hollow baffles situated in the vessel. With an airlift bioreactor the heat can be removed through the hollow draught tube. The rate at which heat is transferred is given by:

$$Q = UA\Delta T \qquad (6.7.1)$$

where Q is heat transferred in W, U is the overall heat transfer coefficient in $W/m^2{\cdot}K$, A is the surface area for heat transfer in m^2, and ΔT is the temperature difference between media and cooling water in K. The coefficient U represents the conductivity of the system, which depends on the system geometry, fluid properties, flow velocity, wall material and thickness. The overall resistance to heat transfer is the reciprocal of the overall heat transfer coefficient.

It is defined as the sum of the individual resistances to heat transfer as heat passes from one fluid to another, and can be written as:

$$\frac{1}{U} = \frac{1}{h_o} + \frac{1}{h_i} + \frac{1}{h_{of}} + \frac{1}{h_{if}} + \frac{1}{h_w}$$

(6.7.2)

where, h_o is the outside film coefficient, h_i is the inside film coefficient, h_{of} is the outside fouling film coefficient, h_{if} is the inside fouling film coefficient, h_w is the wall heat transfer coefficient (which is k/x), k is the thermal conductivity of the wall, and x is the wall thickness in m. The units for all film coefficients are W/m^2·K. This equation is applicable for all cases except a thick-walled tube where a correction factor has to be used. The outside and inside film coefficients can be evaluated from semi-empirical correlations of the following form:

$$Nu = k(Re)^a (Pr)^b$$

(6.7.3)

where Nu is the Nusselt number, the ratio of convective to conductive heat transfer coefficients. The terms k, a, and b are constants. Re is the Reynolds number, which is the ratio of inertial over viscous forces, and Pr is the Prandtl number, which is the ratio of kinematic viscosity over the thermal diffusivity:

$$Nu = \frac{hD_t}{k}$$

(6.7.4)

$$Re = \frac{D_i V \rho}{\mu} \quad \text{or} \quad \frac{D_i^2 N_\rho}{\mu}$$

(6.7.5)

$$Pr = \frac{C_p \mu}{k} = \frac{v}{\alpha}$$

(6.7.6)

where D_t is the vessel diameter, D_i is the impeller diameter, all in m; ρ is the density in kg·m^{-3}, μ is the viscosity in kg/m·s, ν the kinematic viscosity in m^2/s, k is the thermal conductivity in W·m^{-1}·K^{-1}, h is the convective heat transfer coefficient in W·m^{-2}·K^{-1}, C_p is the specific heat in J·kg^{-1}·K^{-1}, α is the thermal diffusivity in m^2·s^{-1}, V is the velocity in m·s^{-1} and N is the impeller speed in Hz. The above equation applies to turbulent conditions for Newtonian fluids. In stirred-tank bioreactors, normally turbulent conditions are attained. However, non-Newtonian behaviour can occur, especially if polysaccharides pass into the broth. An extensive literature survey of heat transfer correlations for both Newtonian and non-Newtonian single-phase systems has been done by many researchers. They have shown that for hold-ups of less than 15%, the rates of heat transfer with gas addition are very close to the values obtained without gas addition.[8,9] Gas hold-up is defined as the volume of gas in the vessel per vessel volume, and can be calculated from the equation

$$\varepsilon = K\left(\frac{P_g}{V_L}\right)^{0.48}(v_s)^{0.4} \qquad (6.7.7)$$

where P_g is power consumed by gassed liquid in W, V_L is liquid volume without gassing, v_s is the superficial gas velocity in m/s and K is a constant. Other correlations for gas hold-up are defined in the literature.[10,11]

The calculation of heat transfer film coefficients in an air-lift bioreactor is more complex, as small reactors may operate under laminar flow conditions whereas large-scale vessels operate under turbulent flow conditions. It has been found that under laminar flow conditions, the fermentation broths show non-Newtonian behaviour, so the heat transfer coefficient can be evaluated with a modified form of the equation known as the Graetz–Leveque equation:[9]

$$Nu = 1.75\delta^{0.33}Gz^{0.33} \qquad (6.7.8)$$

where δ is correction for non-Newtonian behaviour equal to $(3n + 1)/4n$, where n is the flow behaviour index of power-law fluid. Gz is the Graetz number, a dimensionless number related to mass flow rate, heat capacity and conductive heat transfer coefficient.

$$Gz = \frac{\dot{m}C_p}{kL} \qquad (6.7.9)$$

where $\dot{m}$ is the mass flow rate of fluid through the tube in kg/s, and C_p is specific heat in J/kg K, k is thermal conductivity in W/m·K, and L is the length along the tube in m. This equation is most accurately applied in the initial stages of the bioreactor. In later stages growing *Xanthmonas campestris*, the value of the film coefficients were up to 45% lower than predicted by the Graetz–Leveque equation, because of fouling of the heat transfer surface. However, with *Aspergillus niger*, values of up to four times those predicted by the non-Newtonian form of the Graetz–Leveque equation were observed. The enhancement was found to be dependent on cell concentration and morphology of the microorganisms, and was probably due to the increased turbulence of the boundary layer caused by the mycelial aggregates.

The overall heat transfer coefficient is dependent on the agitation rate in the vessel, throughput of the liquid and gas in an airlift bioreactor and the rate of circulation of cooling water in the jacket. The expected value of the overall heat transfer coefficient including all resistance for a non-fouling system should be in the range 500–1500 $W\cdot m^{-2}\cdot K^{-1}$. In case of any problems, for instance animal and plant cells, which are shear-sensitive the vessel side turbulence has to be reduced; consequently the heat transfer coefficient will be lowered. In such cases, the heat transfer will increase only by providing more heat transfer area. The additional effective surface area can be obtained by having a vessel with a large height:diameter ratio, because the volume of a vessel is proportional to the height multiplied by the cross-sectional area, whereas the surface area is the external area of the vessel that is αHD, where

α is the proportionality factor. Where the total heat transferred has to be calculated, the power of the agitator should be included, because a considerable amount of energy is converted to heat in the vessel.

Small temperature differences, ΔT, in a bioreactor are usually easily stabilised, unless refrigerated cooling water is used, which means that the product of overall heat transfer coefficient and the heat transfer area, 'UA', has to be large. Therefore the heat transfer area can be maximised by having cooling water in the baffles as well as in the jacket of the bioreactor.

6.8   DESIGN EQUATIONS FOR CSTR FERMENTER

In designing a bioreactor, material balance is used for all the streams associated with the fermentation vessel. The biomass at inlet, outlet and the generated biomass must be balanced while the fermentation proceeds. The cell balance without any cell accumulation is shown in the following equation:

$$F(X_0 - X) + Vr_x = 0 \tag{6.8.1}$$

where X is viable cell in the effluent stream and X_0 is viable cell in the feed stream, F is the volumetric flow rate, V is the reactor working volume, and r_x is the rate of cell formation per unit volume. The rate equation is explained in detail by a Monod rate model. The Monod rate equation is well known in microbial growth kinetics:

$$\mu = \frac{\mu_{\max}S}{K_s + S} \tag{6.8.2}$$

where μ is the specific growth rate, $\mu_{\max}$ is the maximum specific growth rate, and K_s is the Monod constant.

6.8.1   Monod Model for a Chemostat

A Monod rate model is used to demonstrate the rate of biomass generation. We neglect the cell death rate. Let us denote the ratio of biomass rate of generation to biomass concentration, r_x/X, that is the specific growth rate; μ also denotes the dilution rate; D is defined as number of tank volumes passed through per unit time, F/V. After substitution of D and μ into (6.8.1), the following equation is obtained:

$$DX_o = (D - \mu)X \tag{6.8.1.1}$$

Substituting specific growth rate based on the Monod rate equation into (6.8.2), the rearranging results in:

$$\left(\frac{\mu_{max}S}{K_s + S} - D\right)X + DX_0 = 0 \qquad (6.8.1.2)$$

For sterile media with suitable nutrients in absence of any organisms,

$$X_O = 0, \quad 0 = (D - \mu)X$$

Biomass generated is considered as $X \neq 0$, therefore $D - \mu = 0$

$$D = \mu \qquad (6.8.1.3)$$

At steady state, substrate utilisation is balanced with a rate equation:

$$F(S_i - S) = \left(\frac{\mu_{max}S}{K_m + S}\right)V \qquad (6.8.1.4)$$

When the volume of the vessel is divided by the flow rate, retention time and dilution rate are defined in the following equation:

$$\frac{V}{F} = \tau = \frac{1}{D} \qquad (6.8.1.5)$$

Plug in (6.8.1.5) to (6.8.1.4):

$$D(S_i - S) = \frac{\mu_{max}S}{K_m + S} \qquad (6.8.1.6)$$

Solve (6.8.1.6) for dilution rate or substrate concentration, as follows:

$$\frac{\mu_{max}S}{K_s + S} = D \quad \text{or} \quad S = \frac{DK_s}{\mu_{max} - D} \qquad (6.8.1.7)$$

Material balance in terms of cell density is written as:

$$\frac{d\rho_{cell}}{dt} = \frac{F}{V}(\rho_i - \rho_o) + (\mu - \alpha)\rho_{cell} \qquad (6.8.1.8)$$

At steady state, $d\rho/dt = 0$ for a sterile fermenter, $\rho_i = 0$, (6.8.1.8) is simplified and reduced to dilution rate, which is similar to (6.8.1.3) above.

Substrate balance may also lead to the same results as the following relations:

$$\frac{dS}{dt} = \frac{F}{V}(S_i - S) - \frac{\mu \rho_{cell}}{\text{Yield of cell}} - m\rho_{cell} - \frac{q_p \rho_{cell}}{Y_{p/s}} \qquad (6.8.1.9)$$

At steady-state condition, where $dS/dt = 0$ and $m\rho_{cell} << \mu\rho_{cell}/Y$, (6.8.1.9) can be simplified and leads to substrate balance with growth rate:

$$D(S_O - S) = \frac{\mu \rho_{cell}}{Y} \qquad (6.8.1.10)$$

For the special case when $\mu = D$, the substrate balance equation reduces to yield of substrate to cell biomass:

$$\rho_{cell} = Y(S_i - S) \qquad (6.8.1.11)$$

Let us define yield factor, Y:

$$Y = \frac{\text{mass of cell formed}}{\text{mass of substrate consumed}}$$

By rearrangement of (6.8.1.11) and when yield factor is inserted, it becomes the same equation as in (6.8.1.2):

$$DX_o + \left(\frac{\mu_{max} S}{K_s + S} - D\right)X = 0$$

Substituting into the mass balance yields, the cell mass balance is arranged. At steady-state condition:

$$D(S_o - S) - \frac{\mu_{max} SX}{Y(K_S + S)} = 0 \qquad (6.8.1.12)$$

For sterile conditions X_o is zero, because no microbe is present in the feed stream and the feed is sterile without any contamination.

$$0 = (D - \mu)X \qquad (6.8.1.13)$$

When the cell concentration is appreciable, the dilution rate must reach a specific rate ($X \neq 0$, $D = \mu$). The cell mass concentration is defined in (6.8.1.13) as the dilution rate approaches zero; the cell density is the product of yield and initial substrate concentration:

$$Y = \frac{X}{S_o - S} \tag{6.8.1.14}$$

Substituting (6.8.1.7) into (6.8.1.14), the biomass concentration is defined:

$$X = Y(S_o - S) = Y\left(S_o - \frac{DK_S}{\mu_{max} - D}\right) \tag{6.8.1.15}$$

As the dilution rate increases, the concentration level of final substrate will linearly increase with D, and D approaches μ_{max}. The result of a high dilution rate would cause the cell density to drop. When $D = \mu_{max}$, $X = 0$. This phenomenon is known as wash out.

$$D_{max} = \frac{\mu_{max} S_o}{K_s + S_o} = \mu_{max}\left(1 - \sqrt{\frac{K_s}{K_s + S_o}}\right) \tag{6.8.1.16}$$

Near the wash out, the reactor is very sensitive to variations of dilution rate D. A small change in D gives a relatively large shift in X and S. The rate of cell production per unit volume of reactor is DX. These quantities are shown in Figure 6.5, where there is a sharp maximum in the curve of DX. We can compute maximal cell rate by taking the derivative of DX with respect to D, then solving the equation. The derivative of DX with respect to D is defined as:

$$\frac{d(DX)}{dD} = 0 \tag{6.8.1.17}$$

$$\frac{d(XD)}{dD} = \frac{d}{dD}\left[YD\left(S_o - \frac{DK_s}{\mu_{max} - D}\right)\right] = 0 \tag{6.8.1.18}$$

After differentiation, the result is simplified for initial substrate concentration with respect to dilution rate:

$$S_o - \frac{DK_s}{\mu_{max} - D} - \frac{DK_s \mu_{max}}{(\mu_{max} - D)^2} = 0 \tag{6.8.1.19}$$
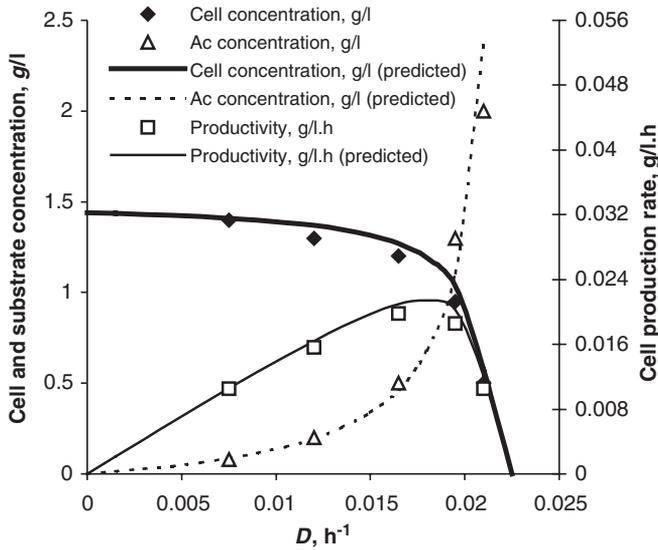
FIG. 6.5. Effect of dilution rate on cell density, substrate concentration and cell production rate.

Rearranging (6.8.1.19) gives a second-order equation with respect to D:

$$\left(\frac{D}{\mu_{\max}}\right)^2 (S_o + K_s) + S_o - 2\frac{D}{\mu_{\max}}(S_o + K_s) = 0 \qquad (6.8.1.20)$$

Solving the quadratic equation will lead to (6.8.1.21):

$$\frac{D}{\mu_{\max}} = \frac{(S_o + K_s) \pm \sqrt{(S_o + K_s)^2 - 4S_o(S_o + K_s)}}{2(S_o + K_s)} = 1 \pm \sqrt{1 - \frac{S_o}{S_o + K_s}}$$

$$\frac{D}{\mu_{\max}} = 1 - \sqrt{\frac{K_s}{K_s + S_o}} \qquad (6.8.1.21)$$

6.9   TEMPERATURE EFFECT ON RATE CONSTANT

Generally, in an equation of a chemical reaction rate, the rate constant often does not change with temperature. There are many biochemical reactions that may be influenced by temperature and the rate constant depends on temperature as well. The effect of temperature on

reaction rate constant may follow Arrhenius' law. The differential form of rate constant is shown as follows:

$$\frac{d \ln k}{dT} = \frac{E}{RT^2} \qquad (6.9.1)$$

Integration may lead to a relation for rate constant with temperature dependency in the form of Arrhenius' law:

$$k = Ae^{-E/RT} \qquad (6.9.2)$$

For a plug flow reactor, differential volume moves along the length. The following equation may express the material balance for a plug flow reactor:

In = Out
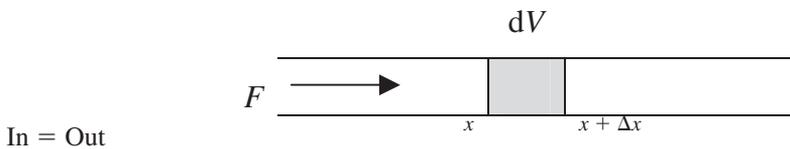
$$FX + dV \left(\frac{dx}{dt} \right)_{growth} = F(x + dx) \qquad (6.9.3)$$

The integration may simply express the residence time for PFR:

$$t_p = \int_{x_1}^{x_2} \frac{dx}{\left(\dfrac{dx}{dt} \right)_{growth}} = \int_{0}^{x_A} \frac{dx_A}{-r_A}$$

$$\tau = \frac{V}{F} \qquad (6.9.4)$$

The differentiation of (6.9.4) results in the following ratio:

$$dt_p = \frac{dV}{F} = \frac{dx}{\left(\dfrac{dx}{dt} \right)_{growth}} \qquad (6.9.5)$$

The result in (6.9.5) shows a discrete time, which is numerically used for a PFR bioreactor.

6.10   SCALE-UP OF STIRRED-TANK BIOREACTOR

A general rule, which is often applied in scale-up, is that of geometric similarity between the small and large vessels. However, as shown in Table 6.3, the relevant parameters that

TABLE 6.3. *Various parameters on scale-up using geometric similarities*

Property	Pilot scale (100 Litres)	Constant P/volume	Plant-scale constant ND_i^2	(125,000 litres) constant ND_i	Constant N_{Re}
Power, P (hp)	1.0	15.63	7800	6.25	0.005
P/Volume	1.0	1.00	6.25	0.005	4×10^{-6}
N, mixing time^{-1}	1.0	0.48	1.00	0.005	2×10^{-3}
D_i, m	1.0	2.50	2.50	2.50	2.50
Agitator flow Discharge, $Q\alpha ND_i^2$	1.0	7.50	15.63	6.25	2.50
ND_i	1.0	1.20	2.50	1.00	0.40
Reynolds number $\rho ND_i^2/\mu$	1.0	3.00	6.25	2.50	1.00
Froude number $N^2 D_i/g$	1.0	0.60	2.50	1.00	0.0003

ND_i^2, impeller speed (Hz); $N^2 D_i^3 \alpha$, power; $ND_i \alpha$, average shear rate; $N\alpha$ 1/mixing time.

affect mixing can vary widely between the two scales. The pilot scale is a base line; the parameters in the second column are given a numerical value of 1. Several strategies were used to observe the effect of design parameters on scale-up process. The third column considers the situation with geometric similarity and where constant power per unit volume was implemented in the design calculation. The new volume is 1250 times the old volume, and the linear dimension scale-up is 5:1.

The important parameters that affect mixing and growth of a microorganism are summarised as follows:

- Oxygen transfer rate (mass transfer coefficient).
- Power per unit volume, agitation and mixing.
- Volumetric flow rate of gas per unit volume of reactor.
- Maximum shear rate, average shear rate and mixing time, impeller tip velocity, ND_i.
- Pumping rate per unit volume, N.
- Heat transfer, Reynolds number and surface area of the vessel.

Referring to Table 6.3, it can be seen that with geometric similarities in self controls there is no mixing variable. In practice, we would select the important criterion that needs to be controlled and then size the vessel accordingly.

Let us summarise the results of Table 6.3. In column 2, constant power per unit volume is maintained, giving larger mixing times and maximum shear rates than in the pilot-scale vessel, but with a lower average shear rate. In column 3, a constant impeller speed and mixing time are maintained, which gives an increase in the power/unit volume of 6.25 times. This is not on scale-up as the maximum shear is also considerably increased. If constant tip velocity is maintained, as shown in column 4, the power per unit volume is drastically decreased and consequently the mass transfer rate of oxygen to microorganisms. In all of

these scale-up calculations, the Reynolds number is increased. In column 5, an attempt was made to maintain a constant Reynolds number, which resulted in a dramatic fall in the power requirement and an increase in mixing time. The extremely low Reynolds number caused very low agitation and low power input. This is usually not a practical situation, and generally the Reynolds number always increases in the scale-up process. The special criteria chosen for the scale-up process are based on three concepts:

• Constant power/unit volume.
• Constant gas flow rate/unit volume.
• Geometric similarity of the vessel.

These criteria have been found to give comparable growth and product rates compared with the pilot-scale operation. If we need to control maximum shear, the value of ND_i should be the same in both the pilot- and large-scale vessels.

Example 1

A bacterial fermentation was carried out in a reactor containing broth with average density $\bar{\rho} = 1200\,\text{kg/m}^3$ and viscosity $0.02\,\text{N·s/m}^2$. The broth was agitated at 90 rpm and air was introduced through the sparger at a flow rate of 0.4 vvm. The fermenter was equipped with two sets of flat blade turbine impellers and four baffles. The dimensions of vessel, impellers and baffle width were:

   tank diameter, $D_t = 4\,\text{m}$;
   impeller diameter, $D_i = 2\,\text{m}$;
   baffle width, $W_b = 0.4\,\text{m}$;
   also the liquid depth was $H = 6.5\,\text{m}$.

Determine: (a) ungassed power, P; (b) gassed power, P_g; (c) K_La; (d) gas hold-up.

Solution

Let us define the ratio of tank diameter to impeller diameter:

$$\frac{D_t}{D_i} = \frac{4}{2} = 2 \tag{E.1.1}$$

Also, the ratio of the height of the liquid level to impeller diameter is:

$$\frac{H_L}{D_i} = \frac{6.5}{2} = 3.25 \tag{E.1.2}$$

$$n = \frac{90\,\text{rpm}}{60} = 1.5\,\text{rps} \tag{E.1.3}$$
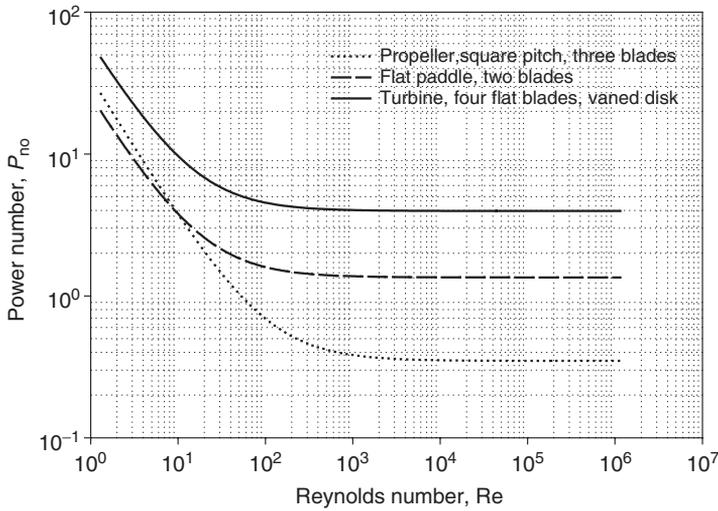
F<small>IG</small>. 6.6. Power number versus Reynolds number for various impellers (flat blades, turbine, vaned disk and marine propeller).

Now define the Reynolds number:

$$N_{Re} = \frac{ND_i^2 \rho}{\mu} = \frac{(1.5)\,(2)^2(1200)}{0.02} = 3.6 \times 10^5 \qquad (E.1.4)$$

Since the Reynolds number is greater than 10^4, therefore the flow is turbulent. Based on a power number defined in the turbulent regime, the power number is defined as about 6 (from Figure 6.6).

$$N_P = 6 = \frac{Pg_c}{\rho N^3 D_i^5} = \frac{P \times 9.81}{(1200)(1.5)^3(2)^5} \qquad (E.1.5)$$

Power is calculated as

$$P = \frac{(6)\,(1.5)^3(2)^5(1200)}{9.81} = 79266 \; \frac{\text{kg m}}{\text{s}} = 106 \text{ hp} \qquad (E.1.6)$$

Correction factors are used to define actual power

$$f_c = \sqrt{\frac{\left(\dfrac{D_t}{D_i}\right)^*\left(\dfrac{H_L}{D_i}\right)^*}{\left(\dfrac{D_t}{D_i}\right)\left(\dfrac{H_L}{D_i}\right)}} = \sqrt{\frac{3 \times 3.25}{3 \times 3}} = 0.85 \qquad (E.1.7)$$

For two sets of impellers with application of a correction factor, ungassed power is

$$P = (2)(0.85)(106) = 180 \text{ hp} \tag{E.1.8}$$

Dimensionless aeration rate is defined as:

$$N_a = \frac{F_g}{N_i D_i^3} \tag{E.1.9}$$

$$F_g = 0.4 \ (\text{volume}) = 0.4 \, (4) 2 \left(\frac{\pi}{4} \right) (6.5) = 32.67 \, \frac{\text{m}^3}{\text{min}} = 0.5445 \frac{\text{m}^3}{\text{s}} \tag{E.1.10}$$

$$N_a = \frac{0.5445}{(1.5)(2)^3} = 4.5 \times 10^{-2} \tag{E.1.11}$$

Using the plot of P_g/P versus N_a (Figure 6.7), the ratio of gassed power to ungassed power is defined.

$$\frac{P_g}{P} = 0.74 \tag{E.1.12}$$

The gassed power is:

$$P_g = 0.74(180) = 133 \text{ hp} \tag{E.1.13}$$
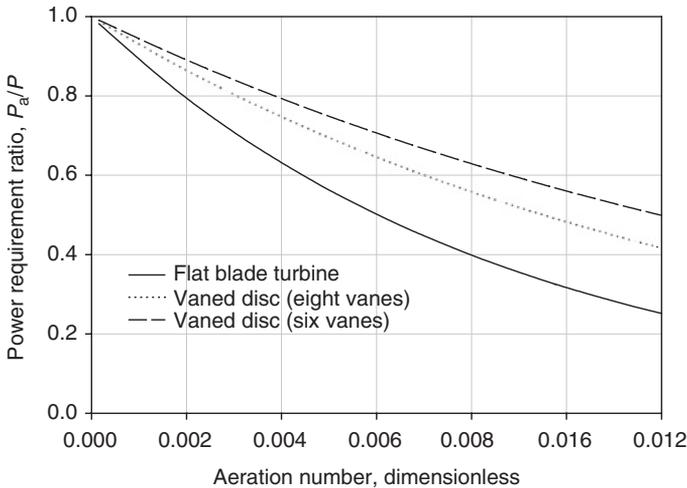
FIG. 6.7. Ratio of power requirement for aerated versus non-aerated systems.

The gas superficial velocity is:

$$V_s = \frac{32.67}{(4)^2\left(\dfrac{\pi}{4}\right)} = 2.6\,\frac{m}{min} \tag{E.1.14}$$

The mass transfer coefficient is defined as turbulent:

$$K_L a = 2\times10^{-3}\left(\frac{P_g}{V}\right)^{0.6} V_S^{0.667} = 10.97\,s^{-1} \tag{E.1.15}$$

$$\text{Gas hold-up,} \quad H_o = \frac{\text{Bubble volume}}{\text{Reactor volume}}$$

Gas hold-up is defined as volume of gas per unit volume of reactor. For air in water, Richard's data are by:

$$\left(\frac{P}{V}\right)^{0.4} (V_s)^{0.5} = 7.63H + 2.37 \tag{E.1.16}$$

$$\left(\frac{180}{81.68}\right)^{0.4}\left(2.6\times60\,\frac{min}{h}\right)^{0.5} = 7.63H + 2.37 \tag{E.1.17}$$

$H = 1.94\,m$ for aeration

$$H_o = \frac{V_g}{V_g + V_L} = \frac{1.94}{1.94 + 6.5} = 0.23 \tag{E.1.18}$$

Gas hold-up = 23%.

Example 2

The Monod rate model is valid for a CSTR bioreactor with maximum specific growth rate of $0.5\,h^{-1}$ and K_s $2\,g\cdot l^{-1}$. What would be a suitable dilution rate at steady-state condition, where there is no cell death if initial substrate concentration is $50\,g\cdot l^{-1}$ and yield of biomass on substrate is 100%.

Solution

The Monod rate is

$$\mu = \frac{\mu_{\max} S}{K_S + S} \rightarrow D = \frac{\mu_{\max} S}{K_S + S}$$

(E.2.1)

$$S_{\mathrm{out}} = \frac{K_S D}{\mu_{\max} - D}$$

$$Y_{x/s} = 1$$

$$S_o = 50 \text{ g.l}^{-1}$$

Substrate balance:

$$-\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{F}{V}(S_{\mathrm{in}} - S_{\mathrm{out}}) - \left(\frac{\mu}{Y_{x/s}}\right)X - \left(\frac{q_p}{Y_{p/x}}\right)X$$

Assume no death rate,

$$\text{steady state} -\frac{\mathrm{d}S}{\mathrm{d}t} = 0, \quad \mu = D, \quad \frac{F}{V} = D$$

Use (6.34) and rearrange to get DX:

$$q_p = 0, \qquad \overline{X} = Y_{x/s}(S_{\mathrm{in}} - S_{\mathrm{out}})$$

$$D(S_{\mathrm{in}} - S_{\mathrm{out}}) = \left(\frac{\mu_{\max} S}{K_S + S}\right)\left(\frac{1}{Y_{x/s}}\right)X$$

$$\rightarrow D\overline{X} = Y_{x/s}\left(S_0 - \frac{K_S D}{\mu_m - D}\right)$$

Take derivative $\mathrm{d}(D\overline{X})/\mathrm{d}D = 0$ to obtain a value for maximum dilution rate:

$$D_{\max} = \mu_{\max}\left(1 - \sqrt{\frac{K_S}{K_S + S}}\right) = 0.5\left(1 - \sqrt{\frac{2}{52}}\right) = 0.4 \text{ h}^{-1}$$

$$\frac{\mathrm{d}(D\overline{X})}{\mathrm{d}D} = Y_{x/s}\left(S_o - \frac{K_S D}{\mu_m - D}\right) - \left[\frac{K_S(\mu_m - D) - K_S D}{(\mu_m - D)^2}\right]D$$

Get
$$D_{max} = \mu_{max}\left(1 - \sqrt{\frac{K_S}{K_S + S}}\right)$$

Then calculate the substrate concentration at leaving stream:

$$S_{out} = \frac{K_S D}{\mu_{max} - D} = \frac{2(0.4)}{0.5 - 0.4} = 8 \text{ g/l}$$

Example 3

A 20 m³ working volume of a bioreactor is used for producting penicillin. What would be the sugar concentration (S_o) you choose if oxygen transfer rate is not the limiting reactant? Given data:

Impeller speed = 1.5 rps (90 rpm)
Number of blades = 8; flat, turbine types of blade
μ = 1 mPa·s
ρ = 1.2 × 10³ kg/m³
Aeration rate = 1 vvm
Ratio of gassed to ungassed power, P_g/P = 0.4
Driving force for OTR = 6 × 10⁻³ kg/m³
Specific O₂ uptake = 0.65 mmol O₂/kg cell
Also, the kinetic data are given as v_{max} = 0.5 h⁻¹
Specific sugar consumption rate of cells = 1.0 kg/kg cell·h

Solution

Given data:

D_{tank} = 2.4 m

Data:

D_{tank} = 2.4 m

$D_i = D_{tank}/3$ = 0.8 m; for three sets of impellers

Impeller speed 150 rpm; assume broth viscosity is 1 cp and the specific gravity of the broth is 1.2; aeration rate is 1 vvm; given ratio of gassed power to un-gassed system is 0.4; specific oxygen uptake is 0.65 mmol O₂/kg cell; OTR = 6 × 10⁻³ kg/m³.

D_{tank}/D_i = 3

D_i = 0.8 m, three sets of impellers are used.

μ = 1 mpas

ρ = 1200 kg/m³

P_g/P = 0.4, v_{max} = 0.5 h⁻¹

Specific sugar consumption rate of cells = 1.0 kg (kg cell)⁻¹·h⁻¹.

Mass transfer is calculated by the empirical correlation defined for non-Newtonian filamentous fermentation:

$$K_L a = 2 \times 10^{-3} \left(\frac{P_g}{V} \right)^{0.6} V_S^{0.667}$$

$$K_L a = S^{-1}$$

$$\frac{P_g}{V} = \frac{\text{Gassed power}}{\text{volume}} = \frac{\text{hp}}{\text{m}^3}$$

$$V_s = \text{gas superficial velocity}, \frac{\text{cm}}{\text{min}}$$

Read power number versus Reynolds number in turbulent region is based on geometry of the impellers. The lowest power number is less than 1, for marine propellers. For flat bladed turbines in a turbulent region, the power number is equal to 6. The power graph is illustrated in Figure 6.6.

$$N_p = 6 = \frac{P g_c}{N^3 D_i^5 \rho}$$

$$\text{Ungassed power, } P = \frac{6 P N^3 D_i^5}{g_c} = \frac{6 \times 1200 \times (2.5)^3 (0.8)^5}{9.81} = 3758 \ \frac{\text{kg} \bullet \text{m}}{\text{s}}$$

$$P = \frac{3758}{745.7} = 5 \, \text{hp}$$

for three sets of impelers, 15 hp

$$P_g = 0.4(15) = 6 \text{ hp}$$

The Correction factor for non-geometrical similarity is:

$$f_c = \sqrt{\frac{\left(\frac{D_t}{D_i} \right)^* \left(\frac{H_L}{D_i} \right)^*}{\left(\frac{D_t}{D_i} \right) \left(\frac{H_L}{D_i} \right)}} = \sqrt{\frac{3 \times 4.42}{3 \times 3}} = 1.25$$

$$P = (\text{three sets})(1.25)(5.04) = 19 \text{ hp}$$

For the agitated and aerated vessel, the ratio of power requirements for aerated versus non-aerated systems is expressed by a dimensionless number known as the aeration rate; the value is obtained from Figure 6.7.

$$N_a = \frac{F_g}{N_i D_i^3}$$

$$N_a = \frac{0.333}{(2.5)(0.8)^3} = 0.26$$

$$F_g = 20\frac{m^3}{min} = 0.333\frac{m^3}{s}$$

$$P_g = 0.4(19) = 7.6 \text{ hp}$$

$$V_S = \frac{20\dfrac{m^3}{min}}{\dfrac{\pi}{4}(2.4)^2 m^2} = 4.42\frac{m}{min} = 7.4\times10^{-2}\frac{m}{s}$$

$$K_L a = 2\times10^{-3}\left(\frac{7.6}{20}\right)^{0.6}(4.42)^{0.667} = 6.7\times10^{-2}\frac{m}{s}$$

$$OTR = K_L a\ (C^* - C)$$

$$OTR = x q_{O_2} = (6.7\times10^{-2})(6\times10^{-3}) = 4.03\times10^{-4}\frac{kg}{m^3 s}$$

Maximum cell concentration, $OTR = x\ qO_2$

$$q_{O_2} = (0.65\times10^{-3})(32\times10^{-3}) = 2.08\times10^{-3}\frac{kg\ O_2}{kg\ cell\ s}$$

$$x_s = x = \frac{4.03\times10^{-4}}{2.08\times10^{-3}} = 19.375\frac{kg}{m^3}$$

$$x_s = x_o + \frac{\mu_m}{q_s}C_s = 0 + \frac{0.5}{1.0}C_s$$

$$C_s = \frac{19.375}{0.5} = 38.75\frac{kg}{m^3}$$

6.11   NOMENCLATURE

r_p Rate of product formation, $g\cdot l^{-1}\cdot h^{-1}$
$-r_s$ Rate of substrate consumption, $g\cdot l^{-1}\cdot h^{-1}$
Fr Froude number, dimensionless
g gravity, m/s^2
N rotational speed, Hz

D_i Impeller diameter, m
N_P Power number, dimensionless
V_S Gas superficial velocity, cm·min^{-1}
N_a Dimensionless aeration rate

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