Introduction

Oxygen is the main energy driver for all mammals. It drives the oxidation of organic nutrients from food and extracts energy from them for cellular functions and growth. The requirement of oxygen is universal in cells derived from mammals. Blood circulation brings oxygen to cells in different tissues to sustain their viability and functions. The solubility of oxygen in water is very low. In Chapter 8 we briefly discussed the tendency of oxygen to deplete rapidly in a plug flow reactor due to its low solubility. The artery is essentially a plug flow system. Our body overcomes the limitations of plug flow by using red blood cells to increase the oxygen carrying capacity of blood. Even then, blood must be frequently recirculated back to the lungs to be re-oxygenated. Ultimately, in a system containing living cells that consume oxygen, there must be a source of oxygen in a gas phase to continuously replenish oxygen in the liquid phase in which cells reside. For cell culture process, gas bubbles in the culture medium constitute the gas phase.

When an air bubble is placed in a cell suspension, oxygen will diffuse from the bubble into the liquid (Figure 11.1). To cross the gas–liquid boundary, oxygen crosses a relatively stagnant film of gas on the gas side and another liquid film on the liquid side. The convection inside the bubble makes the oxygen concentration in the bulk (outside the gas film) relatively uniform. Similarly, within the bulk liquid and outside the liquid film the concentration of oxygen can be considered uniform. However, across the film the oxygen concentration decreases, and oxygen diffuses from the gas side to the liquid side. Once in the liquid phase, oxygen travels to reach cells. If cells are growing in a stagnant medium,

an appreciable concentration difference between the bubble's surface and the cell may be seen, and oxygen molecules may diffuse from the bubble to the cell. In a stirred tank, the bulk oxygen level can be considered to be uniform. At the cell's surface, oxygen diffuses through the cell membrane and is mostly consumed in the mitochondria. However, in the case that cells form aggregates, the diffusion of oxygen through the aggregates may be slow, causing oxygen starvation in the interior of the aggregate. For the vast majority of industrial cell culture, the rate-limiting step of oxygen transfer in the bioreactor is in the diffusion from the gas to the liquid. In this chapter, we will focus on oxygen transfer across the gas phase and liquid phase interface.

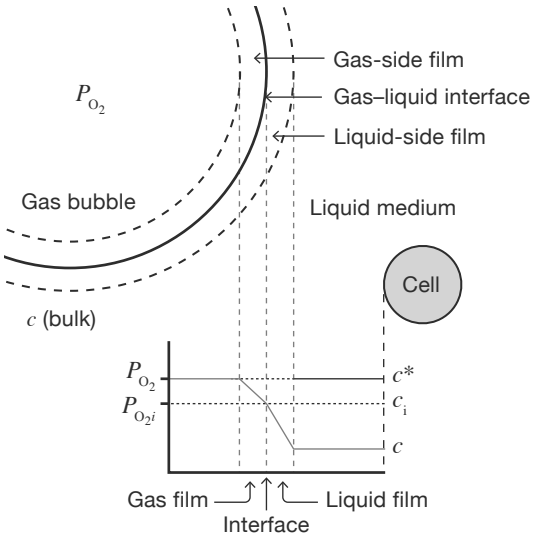

Figure 11.1. Transfer of oxygen across an air bubble in culture medium.

Oxygen Concentration across the Gas–Liquid Interface

Equilibrium and Oxygen Transfer

If a gas phase and a liquid phase are brought in contact and a component in the gas phase is soluble in the liquid phase, given enough time the component gas will reach equilibrium between the two phases. Before equilibrium is reached, molecules of the soluble species are exchanged between the liquid phase and the gas phase; once equilibrium is reached, the liquid phase becomes "saturated" with the component gas and there is no longer any net transfer in either direction.

As discussed above in the interfacial transfer, oxygen is hypothesized to cross a gas film and a liquid film (Figure 11.1). In between the two films is a boundary where oxygen is in equilibrium between gas and liquid. The oxygen level on the gas-side surface of the gas film (P_{O2}) is higher than that at the interface (P_i), so oxygen diffuses from the bulk gas phase towards the interface. On the liquid side of the film, the oxygen concentration is lower than that in the interface, and oxygen diffuses toward the bulk liquid. However, the thickness of the film and the concentration at the interface are difficult to measure. In the subsequent discussion, we will treat the interfacial boundary (i.e., the gas film and the liquid film) as one homogeneous interface.

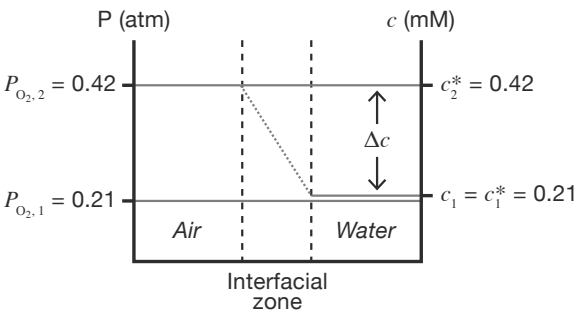

Figure 11.2. Oxygen concentration profile across the air–liquid interface.

Consider the case that a cup of water is brought in contact with air consisting of 79% N_2 and 21% O_2 at 1 atm initially. A concentration profile of oxygen across its air–water interface is depicted in Figure 11.2. Let us assume that the volume of the air is very large, so that losing a small quantity of N_2 and O_2 from their diffusion into water will not change the composition of the air. Given a long enough time, the water will become saturated with oxygen, or the oxygen level in the air and water will reach an equilibrium. Let us denote the concentration of oxygen in the bulk liquid (i.e., the liquid at some distance away from the interface) as c, and that of the saturation as c^*. In the bulk gas phase, the oxygen partial pressure ($P_{O2,1}$) is 0.21 atm. After the two phases reach an equilibrium, c_1 is the same as c_1^*. The nitrogen solubility in water is very low, about 20 mg/L. At such a low level, the dissolution of nitrogen into water does not interfere with the transfer of oxygen. If the oxygen content in the gas phase is increased to 42%, then the partial pressure of oxygen becomes 0.42 atm ($P_{O2,2}$) and the oxygen in the liquid phase is no longer saturated. An oxygen concentration difference across the interface will arise and is sketched qualitatively. Oxygen will then begin to diffuse from the gas into the liquid phase and the dissolved oxygen concentration in the water will gradually increase until the water reaches a new equilibrium (c_2^*) with the new air containing 42% oxygen. Conversely, if the oxygen level in the gas phase is decreased to 10%, then oxygen will escape from the liquid into the gas phase until a new equilibrium is reached. In the above discussion, the composition of the gas phase is changed and the total pressure remains at 1 atm. One can achieve the same change of oxygen partial pressure by changing the total pressure while keeping the gas phase composition static.

Description of Oxygen Concentration and Its Solubility

Oxygen level in a gas phase is usually described as a mole fraction (y_{O2}) or as partial pressure (P_{O2}), which is the mole fraction of oxygen multiplied by total pressure. If the ambient pressure is 1 atm (or 760 mm Hg) and the oxygen mole fraction is 0.21 (y_{O2} = 0.21), then the partial pressure of oxygen in the air is 0.21 atm (or 159.6 mm Hg). The oxygen concentration in a liquid phase (or dissolved oxygen concentration) is often expressed as mmol/L (mM). Since 0.21 mmol/L in water is the equilibrium value with an oxygen partial pressure of 159.6 mm Hg, some describe the concentration in water using the equivalent of gas phase and

express it as 159.6 mm Hg, especially in the healthcare profession (Panel 11.1). For example, water which is at 50% of saturation with air at 1 atm and 37 °C has a dissolved oxygen concentration of 0.105 mmol/L, or 79.8 mm Hg. The difference between concentration in the liquid phase, c, and the saturation concentration, c^*, is the driving force for oxygen transfer. The driving force can also be described in terms of liquid phase concentration (i.e., mM), or gas phase concentration (mm Hg, atm).

For sparingly soluble species, the solubility of the gas solute in the liquid phase is described by Henry's law (Panel 11.2). Henry's law states that, at equilibrium, the concentration of a gas in a liquid solvent is proportional to the partial pressure of that gas (Eq. 11-1, Panel 11.2 and example in Panel 11.3). The concentration in each phase can be described in different ways. Commonly used units are partial pressure and the molar or mass concentration (mmol/L, mg/L). The value of Henry's law constant is, thus, dependent on the units used to describe the concentration.

Henry's law describes oxygen concentration in an ideal solution. Despite all of its components, cell culture medium is close to an ideal solution. The saturation concentration calculated using an aqueous solution applies to cell culture media. However, the gas phase in a cell culture bioreactor also contains carbon dioxide that is intentionally added to provide the pH buffer (see Chapter 7), thus reducing the oxygen level from the 21% in ambient air. Additionally, in a bioreactor the interior pressure

Panel 11.1. Dissolved Oxygen Concentration in Water at 1 Atm of Air

- Solubility in water at 37 °C with air of 1 atm, 21% O_2
  - 0.21 mmol / L
  - 6.7 mg / L
- Since air in equilibrium contains 159.6 mm Hg or 0.21 atm, sometimes solubility is expressed also in mm Hg, especially for blood oxygen level

Panel 11.2. Henry's Law

$$x_A = \frac{P_A}{H} \qquad \textbf{(Eq. 11-1)}$$

- Oxygen solubility is not affected by other dissolved species in the medium. Its solubility is virtually identical to PBS and water.

Panel 11.3. Henry's Law Example

- At 37 °C, in 1 atm air (P_{O2} = 0.21 atm), O_2 concentration in H_2O is:

$$c^* = \frac{0.21\ \text{atm}}{\left[5.18 \times 10^4 \frac{\text{atm} \cdot \text{mole } H_2O}{\text{mole } O_2}\right]} \cdot 55.5 \frac{\text{mole } H_2O}{\text{L } H_2O}$$

$$= 0.21 \frac{\text{mmol } O_2}{\text{L } H_2O}$$

- In the bracket is Henry's law constant for water at 37 °C
- In ambient air (oxygen partial pressure = 0.21 atm), c^* ~0.21 mmole / L in H_2O at 37 °C = 160 mmHg = 6.7 mg / L (6.7 ppm)

is usually greater than 1 atm. These effects on oxygen solubility need to be accounted for.

The solubility of carbon dioxide can also be calculated using Henry's law. However, in an aqueous solution carbon dioxide associates with water molecules and then dissociates into HCO_3^- and H^+. The value calculated using Henry's law is the concentration of $CO_{2(aq)}$. In addition to $CO_{2(aq)}$, water also contains HCO_3^- and CO_3^{2-}, and the amount varies with pH. The total content of CO_2 and related species (HCO_3^- and CO_3^{2-}) is affected by the pH of the solution.

The height of a large microbial bioreactor (~300 m^3 in volume) can reach tens of meters. Large cell culture bioreactors, about 20 m^3, reach a few meters vertically. At the bottom of the bioreactor, the hydrostatic pressure incurred by the liquid mass is substantially higher than that at the top. At the bottom of the bioreactor, the saturation concentration (c^*) of oxygen and carbon dioxide is higher. The hydrostatic pressure enhances the driving force for oxygen transfer but diminishes that for carbon dioxide removal.

Panel 11.4. Effects of Hydrostatic Pressure

- 10 m liquid height gives a 1 atm hydrostatic pressure at the bottom of the tank
- Up to almost 9 atm has no adverse effects on hybridoma cell growth and specific glucose consumption

Cells circulating in the bioreactor will travel to different regions and periodically reach an area where the hydrostatic pressure is high (Panel 11.4). Experimental data has shown that cells grow normally at up to nine atmospheric pressure and exhibit little discernible metabolic differences compared to normal conditions. It thus appears that in the range of hydrostatic pressures practiced in bioprocess, cell growth is not visibly affected.

Oxygen Transfer at the Gas–Liquid Interface

The boundary between a gas phase and a liquid phase can be modeled as an interface separating a gas film and a liquid film that connect to the bulk liquid phase and gas phase as shown in Figure 11.1. The distance between the liquid phase concentration and equilibrium is c^*-c. The larger the deviation is from equilibrium, the faster the transfer. The magnitude of the driving force for oxygen transfer across the interface can thus be described as c^*-c.

In addition to the concentration difference, the rate of oxygen transfer is affected by the area of the interface that is available for oxygen to diffuse across (Panel 11.5). Just like the evaporation of water, the same amount of water evaporates faster when it is spread out on a large plate than when it is contained in a tall cup with a small top surface. The

interfacial area (a) is typically expressed as interfacial area per volume of liquid. It therefore has a unit of inverse of length (like cm^{-1}).

The mass transfer coefficient is a descriptor that quantifies the mass transfer rate at the interface. It reflects the combined effects of the liquid film and the gas film at the interface and is often referred to as the overall mass transfer coefficient. It is affected by the physical and chemical features of the molecule that is crossing the interface and the liquid. The overall mass transfer coefficient (K_L) has the same units as velocity (like cm per second). Under vigorous mixing, the resistance for mass transfer is lower and the value of K_L is higher. Again, consider the evaporation of water placed in a plate, where stirring the liquid increases the mass transfer coefficient and therefore speeds up evaporation.

Note that the "L" in K_L stands for liquid, as opposed to "G" for gas. The resistance of oxygen transfer across the interface is mostly contributed by the liquid phase. Hence, L is used in the notation by convention.

The transfer rate of oxygen (OTR) or other gas species with a low solubility is described as multiplicative product of three factors: the driving force, the mass transfer coefficient, and the interfacial area (Eq. 11-2, Panel 11.5). All three are leveraged in a bioreactor to modulate the supply rate of oxygen (Panel 11.6). The driving force can be increased by augmenting the oxygen content in the air or by increasing the head pressure in the reactor. In a bioreactor where the dissolved oxygen level is controlled at a set point, the set point can be set at a lower level (e.g., at 30% of saturation as opposed to 60% of saturation) to increase (c^*-c). The interfacial area can be increased by supplying air as small bubbles, and K_L can be enhanced by increasing the turbulence at the gas–liquid interface.

Panel 11.5. Oxygen Transfer Rate (OTR)

Three Factors Affecting OTR

- Mass transfer coefficient (K_L)
- Specific transfer area (i.e., the interfacial area)
- Driving force (i.e., the gradient across the interface)

$$\mathrm{OTR} = K_L a \cdot (c^* - c) = K_L a \Delta c \qquad \textbf{(Eq. 11-2)}$$

$$[\mathrm{mmol/L \cdot hr}] = [\mathrm{cm/hr}] \cdot [1/\mathrm{cm}] \cdot [\mathrm{mmol/L}]$$

- To transfer oxygen or CO_2 between the gas and liquid phases through the interface, the two phases must not be in equilibrium

Panel 11.6. Means to Enhance Oxygen Transfer

- Increase K_L (make the interface more "turbulent")
- Increase a (use smaller bubbles for sparging, or use silicone tubing)
- Increase Δc (use oxygen-enriched air or maintain dissolved oxygen (c) at a low/optimal level)

Methods of Supplying Oxygen

Surface aeration

Due to its low solubility, oxygen must be supplied to the culture medium continuously from a gas phase. In a T-flask or small spinner flask, the open space above the culture medium supplies oxygen for cell growth.

Panel 11.7. Methods of Supplying Oxygen

- Surface aeration
  - Used in laboratory culture wares
  - Effectiveness diminishes as the culture volume increases
  - Contribution to oxygen transfer is significant in small reactors, but insignificant in large reactors
- Highly oxygen-permeable membrane tubing
  - Provides bubble-free aeration
  - Only used in small reactors with a low oxygen demand
- Sparging
  - Gas holdup in the reactor provides interfacial areas for oxygen transfer
  - Less effective in very small-scale laboratory reactors due to the short hold time of air bubbles in the liquid phase
  - The magnitude of the interfacial area can be estimated roughly by the following process:
    - Estimate the gas holdup, V_g (difference of total reactor volume before and after aeration starts)
    - Estimate the bubble size, d_p; surface area per bubble volume is $6/d_p$.

$$a = \left(\frac{V_g}{V}\right)\left(\frac{6}{d_p}\right) \qquad \textbf{(Eq. 11-3)}$$

The top surface area relative to the total culture volume deceases as culture volume increases. Surface aeration alone is insufficient to support cell growth at a high cell concentration even in a laboratory bioreactor. In most process-scale reactors, the contribution of surface aeration to overall mass transfer is insignificant and the direct sparging of air into the culture medium is the most effective means of supplying oxygen (Panel 11.7).

Indirect contact with gas phase

Oxygen transfer by air sparging in small bioreactors is inefficient due to the short air bubble holding time in the liquid. Hence, a high aeration rate in terms of volume of air per volume of liquid per minute (vvm) is necessary to supply oxygen. A high air flow rate may lead to excessive foaming or even cause cell damage. Excessive sparging is also avoided in microcarrier cultures. Microcarriers covered with cells on their external surface have a propensity to stick to air bubbles, possibly due to the somewhat hydrophobic nature of the cells' surfaces. As the bubbles rise to the liquid surface, microcarriers sticking to the bubble are carried along. The fluid shear caused by bubble rising can cause cell damage.

To avoid direct air sparging, oxygen can be supplied through silicone or Teflon tubings. Air is passed through the tubing, which is submerged in the medium in the reactor. Oxygen diffuses through the tubing membrane and dissolves into the medium. The method is effective in the small scale, but the long length of tubing required makes it impractical beyond small laboratory reactors.

Sparging

In an aerated stirred tank reactor, air is typically introduced through a sparger located near the bottom of the reactor and released as swarms of bubbles into the culture medium. As the bubbles rise, the rotating

impeller further breaks them up and increases the total bubble surface area, thus enhancing oxygen transfer.

The efficiency of oxygen delivery, or the amount of oxygen that is transferred into the liquid phase per unit volume of air supplied, is affected by the total amount of interfacial area provided by the amount of gas entrapped in the liquid phase. It takes a finite amount of time for air bubbles to rise from the bottom to the top of the culture broth. In a laboratory-scale cell culture bioreactor, where air bubbles rise quickly to exit from the liquid's surface, the holding time in the culture broth is short, providing only a small amount of interfacial area. Conversely, as the scale increases, the bubble holding time and the efficiency of oxygen utilization per volume of air supplied also increases. The total reactor content thus includes both the liquid and the air bubbles that are retained in the liquid. As the aeration rate increases, the liquid level rises because more air is trapped, and the interfacial area increases.

Bubble size, interfacial area, and the mass transfer coefficient

The air bubbles traveling through the bioreactor are the main contributor to the interfacial area for oxygen transfer. As the height of the bioreactor increases, the bubble traveling time increases. Given the same gas provision rate (typically denoted by volume gas/volume culture broth/min, or vvm), the gas-hold up also increases. In a microbial fermenter, a gas holdup of 25% (meaning every liter of medium contains 250 mL of gas bubbles) is not unusual, although for cell culture bioreactors the gas-hold is typically only in the range of a few percent.

The interfacial area can be estimated from the gas holdup volume divided by the specific surface area of the bubble (Panel 11.7). The specific surface area of the bubble is inversely proportional to bubble diameter ($6/d_p$). Smaller bubbles give larger surface area for a given volume of gas. Small bubbles also rise slower in a stagnant liquid. Small bioreactors tend to employ microspargers that generate very fine bubbles.

Under an idealized condition of a single bubble being slowly released from a submerged orifice in a stagnant fluid, the bubble leaves the orifice when the buoyant force just overcomes the surface tension exerted on the bubble. The force balance between buoyancy and surface tension indicates a modest decrease of bubble diameter, d_p, with a smaller orifice diameter, d_o (Panel 11.8). Using orifice size to control bubbles is thus not very effective. As the scale increases and multi-orifice spargers and higher flow rates are used, inertial effects of local liquid velocity, medium properties, and the momentum of gas flow become dominant, making the initial bubble size

Panel 11.8. Effect of Orifice Diameter on Bubble Size

- A bubble bursting into a stagnant liquid from an orifice:

$$d_p = \left(\frac{6 d_o \sigma}{\Delta \rho} \right)^{1/3}$$

- Orifice diameter has a weak effect on bubble diameter
- In a stirred tank, the fluid dynamics have a stronger effect on bubble diameter

almost independent of the orifice size. Bubbles agglomerate as they rise to the liquid surface and coalesce into larger bubbles. They are also broken up by the impeller. Thus, in large-scale reactors, using fine bubbles to increase the interfacial area for oxygen transfer is not effective.

Bubble size also affects bubble rising velocity and the mass transfer coefficient. In principle, larger bubbles have a higher terminal rising velocity and a higher K_L if bubbles are considered to be rigid spheres. However, as bubble diameter increases, the resistance from the liquid increases and the bubble tends to deform and become flatter on the top, thus decreasing its rise velocity. Experimentally, it has been observed that both rising velocity and K_L only increase with bubble diameter when the bubble size is small (<3 mm). Beyond that, they are relatively invariant. Hence the effect of bubble size on K_L is relatively moderate.

Oxygen Consumption and CO_2 Production

Oxygen, like other nutrients, needs to be maintained at a certain level in the medium to allow cells to grow and function normally. For most mammalian cells, an oxygen level of ~30% saturation with ambient air, or ~0.06 mmol/L, is sufficient for optimal growth (Panel 11.9). The oxygen demand of cells is described as the specific oxygen consumption rate (q_{O2}). Its magnitude is affected by metabolic activities, the growth status (proliferating or quiescent), the temperature of cultivation, cell type and size, etc. Most cells have a q_{O2} in the order of 1–5 x 10^{-10} mmol/cell-h (Panel 11.9). The cell concentration that can be reached in a reactor before oxygen supply becomes limiting can be calculated from the oxygen transfer capacity and specific oxygen consumption rate (Panel 11.10).

Panel 11.9. Optimal Dissolved Oxygen Level and Specific Oxygen Consumption Rate

Optimal Oxygen Concentration

- Most cells grow well at a dissolved oxygen level of 30% saturation with ambient air (1 atm)

Oxygen Demand by Cells

- On the average, $q_{O2} \approx$ 1.0 x 10^{-10} mmole/cell-h

Most oxygen taken up by cells is used to oxidize carbon sources. The most important carbon sources for mammalian cells in culture are glucose and glutamine. The oxidation reaction of both compounds yields carbon dioxide at a stoichiometric ratio (CO_2:O_2) of 1.0 and 1.1, respectively. The mole ratio of CO_2 produced to that of O_2 consumed is called the respiratory quotient (RQ). Since the carbon source consumed in the largest quantity by cells is glucose and the next is glutamine at about 1/5 of that of glucose, the RQ of cells in culture is very close to 1.0 (Panel 11.11). The CO_2 produced by cells needs to be removed from the culture to avoid excessive accumulation, which would cause a drop in pH or an inhibition of growth. A key role of aeration in cell culture is thus the removal of carbon dioxide, in addition to the supplying of oxygen. The removal of carbon dioxide generated by cell metabolism requires the presence of a gas phase just like the supply of oxygen, except that the transfer through the gas–liquid interface is in the opposite direction (Panel 11.12).

Panel 11.10. Oxygen Transfer Rate and Achievable Cell Concentration

- K_L is about 3–6 cm/h for surface aeration in a cell culture vessel

What cell concentrations can be reached in a 400 mL spinner flask with a diameter of 8 cm?

Estimate K_L = 5 cm/h.

Surface area is:

$$A = (4\text{cm})^2 \cdot \pi = 50.2\text{cm}^2$$

$$A = 50.2\ \text{cm}^2 / 500\ \text{cm}^3 = 0.1\ \text{cm}^{-1}$$

Assume D.O. is almost zero at maximum cell concentration (maximum driving force)

$$\Delta c = 0.18\ \text{mmol/L}$$

$$q_{O_2} = 1.0 \text{ x } 10^{-10}\ \text{mmol/cell} \cdot \text{h}$$

$$q_{O_2} \cdot x = \text{OTR} = \frac{5\ \text{cm}}{\text{h}} \cdot \frac{0.1}{\text{cm}} \cdot \frac{0.18\ \text{mmol}}{\text{L}} = \frac{0.09\ \text{mmol}}{\text{L} \cdot \text{h}}$$

$$x = \frac{0.09\ \text{mmol}}{\text{L} \cdot \text{h}} \div \frac{1 \text{ x } 10^{-10}\ \text{mmol}}{\text{cell} \cdot \text{h}} = \frac{9 \text{ x } 10^8}{\text{L}} = \frac{9 \text{ x } 10^5 \text{cell}}{\text{mL}}$$

Panel 11.11. CO_2 Production by Cells

- Respiratory quotient of cells is about 1.0, i.e., 1 mole CO_2 is produced for every mole of O_2 consumed, or $q_{CO2} \approx 1.0 \text{ x } 10^{-10}$ mmol / cell-h
- A high CO_2 concentration is inhibitory
- The inhibitory level usually starts at 15% to 20% CO_2

Panel 11.12. Stripping of CO_2 by Aeration

- Aeration supplies oxygen as well as stripping CO_2 from culture medium
- CO_2 is required for cell growth (e.g., in fatty acid synthesis) in addition to providing a pH buffer *in vivo*
- Cells can grow well even in sodium-bicarbonate-free medium, especially in a stationary culture, because CO_2 produced by cells accumulates in the medium to condition their growth
- When cell concentration is low in a sodium-bicarbonate-free medium, excessive aeration may strip off CO_2 to inhibit cell growth, unless some CO_2 (~0.5–1%) is in the gas

The accumulation of carbon dioxide to a high level causes growth inhibition. A medium containing 44 mM of $NaHCO_3$ as a pH buffer, like Dulbecco's modified Eagle's medium (DMEM) is typically used in conjunction with air containing 10% CO_2 to maintain a neutral pH. Thus, 10% CO_2 is within the optimal range for cell growth. The level of carbon dioxide that begins to exert growth-inhibitory effect is usually higher than 0.15 atm of P_{CO2} (15% CO_2). Carbon dioxide is also required for growth because of its participation as a substrate in a number of biosynthetic reactions, notably fatty acid synthesis. If carbon dioxide is completely stripped from the medium, cell growth may be inhibited.

Balancing Oxygen Transfer and Consumption

The oxygen content in cell culture fluid is the balance between oxygen supply and cell consumption. The rate of change in the dissolved oxygen level is the difference between the rate of transfer into the culture broth from the gas phase and the rate of oxygen uptake by the cells (Eq. 11-4, Panel 11.13). The oxygen uptake rate (OUR) is the specific oxygen consumption rate multiplied by cell concentration (Eq. 11-5), and the oxygen transfer rate (OTR) is shown in Eq. 11-2. Both OUR and OTR are expressed on a per culture volume basis. The total of oxygen consumption and oxygen transfer in the reactor is thus those terms multiplied by the culture volume (Eq. 11-5). It is convenient to express the balance of oxygen on a per unit culture volume basis (Eq. 11-6).

Panel 11.13. Balance of Oxygen in a Reactor

$$V\frac{dc}{dt} = \mathrm{OTR}\cdot V - \mathrm{OUR}\cdot V \qquad \textbf{(Eq. 11-4)}$$

$$V\frac{dc}{dt} = K_L aV\left(c^* - c_2\right) - q_{O_2} xV \qquad \textbf{(Eq. 11-5)}$$

$$\frac{dc}{dt} = K_L a\left(c^* - c\right) - q_{O_2} x \qquad \textbf{(Eq. 11-6)}$$

Panel 11.14. Oxygen Transfer and Uptake in a Stirred Tank Bioreactor

- A reactor can be considered to be in a quasi-steady state due to the slow change of the oxygen level
- OTR ≈ OUR

$$K_L a\left(c^* - c\right) \approx q_{O_2} x \qquad \textbf{(Eq. 11-7)}$$

In a cell culture bioreactor, the dissolved oxygen level changes relatively slowly once an appropriate air flow rate and agitation condition are set. As cells grow, the dissolved oxygen level decreases over time, but the rate of change is rather slow because the cell doubling time is long. In contrast, if the air supply is shut off, the dissolved oxygen level will decrease rapidly due to consumption by cells. Similarly, if cells are rendered incapable of oxygen consumption suddenly, the dissolved oxygen level will rise quickly once the culture is deprived of oxygen consumption but continues to receive the supply. At a given moment, the dissolved oxygen level in a culture can be considered to be at a quasi-steady state because the time constant of OUR and OTR is in minutes, while that for change in the reactor is in hours. In general, OUR and OTR in a cell culture bioreactor can be considered equal in magnitude (Eq. 11-7, Panel 11.14). With the steady state assumption, OTR and OUR are equal, as can be seen in Eq. 11-4.

Measurement of $K_L a$

The capacity of oxygen transfer in a bioreactor is often the limiting factor, or at least one of the limiting factors, that dictates the maximum cell concentration or productivity achievable in a bioreactor. Knowing the oxygen transfer capacity of a bioreactor is important in process design and scale translation. The magnitude of K_L and a is highly dependent on

the properties of the liquid and gas, the flow conditions, power input, etc. Except under idealized conditions, such as a single bubble rising in stagnant fluid, it is very difficult to theoretically predict the value of K_L and a. Experimentally, K_L and a are extremely difficult to measure independently. As a result, mass K_L and a are lumped together as "K_La" in experimental measurements and reported as a single value. K_La is sometimes called the volumetric mass transfer. The K_La of a reactor varies under different operating conditions, such as different aeration rate, media composition (e.g., protein content, surfactant concentration), and agitation rate. Thus, ideally the oxygen transfer capacity should be measured under the cultivation conditions for process evaluation. However, few process engineers have the opportunity to perform K_La measurements under manufacturing conditions.

The K_La of a reactor is usually measured in the absence of cells using water, culture medium, or a buffer solution. First, the liquid is aerated to reach an initial dissolved oxygen level. Then the gas phase is switched to nitrogen under the same aeration and other operating conditions to strip oxygen off the liquid. The time profile of decreasing dissolved oxygen level is recorded and plotted in a semilogarithmic graph of $\ln(c^*-c)$ or $\ln(c)$ vs. time (Panel 11.15). The slope of the plot gives $-K_La$ (Figure 11.3). One can also perform the experiment by first stripping oxygen from the medium and then starting the measurement at a low level of c. By sparging air into the liquid, the dissolved oxygen level begins to rise. The time profile of the dissolved oxygen level is then plotted on a semilogarithmic graph. The slope in the semilogarithmic plot is then K_La. Multiple measurements of K_La under different operating conditions can be carried out to develop correlations between K_La and process conditions.

Panel 11.15. Measurements of K_La

- In the absence of cells

$$\frac{dc}{dt} = K_La(c^*-c) \qquad \textbf{(Eq. 11-8)}$$

Initial condition: $t = 0$, $c = c_O$

- After integration: $\ln\frac{c}{c_O} = -K_Lat$
- Plot $\ln(c^*-c)$ vs. t, the slope is $-K_La$

It should be noted that the measured K_La is the combined contribution of bubble aeration and surface aeration from the top surface of the liquid phase (Panel 11.16) where the gas composition may be the same or different from that in the reactor. In small-scale reactors, the contribution of the top surface is significant.

Measurement of Specific Oxygen Consumption Rate and OUR

OUR and the specific oxygen consumption rate can be easily measured off-line using culture broth sampled from the bioreactor (Figure 11.4). By depriving the cell suspension of any gas phase to eliminate any oxygen supply, the dissolved oxygen level will decrease over time

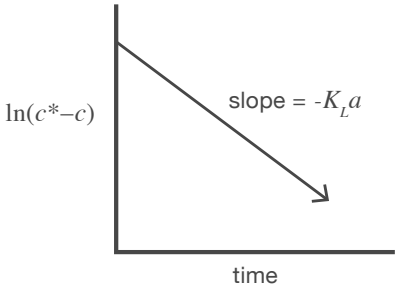

Figure 11.3. Measurement of $K_L a$ by degassing, or stripping of oxygen from the liquid phase.

Panel 11.16. OTR Contribution from Gas Sparging and Surface Aeration

$$OTR = K_{L_s} a_s \left(c_s^* - c\right) + K_L a\left(c^* - c\right)$$

$$= K_{L_s} \frac{A}{V}\left(c_s^* - c\right) + K_L a \Delta c \qquad \textbf{(Eq. 11-9)}$$

$$a_s = \frac{A}{V} \qquad \textbf{(Eq. 11-10)}$$

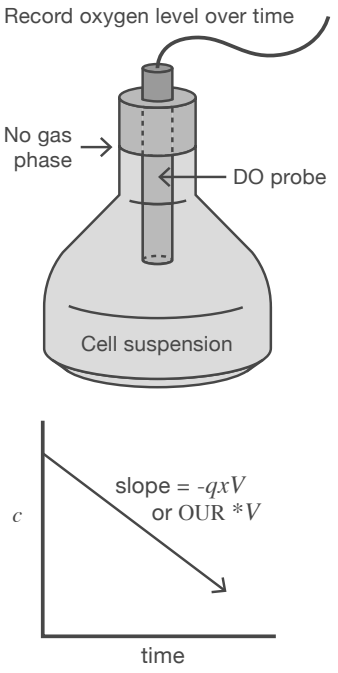

Figure 11.4. Measurement of oxygen uptake rate off-line.

due to consumption by cells. This can be done by filling up a container with a cell suspension without leaving room for a gas phase. The change in the dissolved oxygen level will be relatively linear over time. The slope of the linearly decreasing dissolved oxygen level gives the oxygen uptake rate (OUR) of the culture (Figure 11.4). The specific oxygen consumption rate can be obtained by dividing OUR by the cell concentration in the cell suspension.

OUR measurement can be practiced in real time using in-line dissolved oxygen measurement. A commonly used method is referred to as dynamic OUR measurement because it relies on changing gas composition or allowing aeration to be temporarily turned off to allow the dissolved oxygen level to change. It can be easily practiced in large-scale manufacturing bioreactors, as it requires no additional instrumentation. In a large reactor, the surface area of the liquid top is relatively small compared to the interfacial area generated by gas sparging. Thus, if the gas sparging is turned off, the oxygen transfer rate into the culture fluid is negligible and the dissolved oxygen concentration will decrease linearly with time. The slope of the decreasing dissolved oxygen level gives the OUR (Figure 11.5). When the method is used in a small bioreactor, the contribution from surface aeration cannot be neglected. In that case, $K_{Ls}a_s$ of surface aeration needs to be determined separately, and the transfer of oxygen into the liquid from surface aeration must be subtracted from the measured slope. Such an on-line measurement of OUR is a very sensitive method of determining changes in total oxygen consumption in a bioreactor, far more sensitive than measurements of the concentration changes of nutrients or metabolites. It is even more sensitive than detecting pH changes caused by proton release from lactic

acid production. The pH buffering effect of the medium renders the pH less sensitive. OUR has been shown to detect the rapid change in cell vitality during virus production, faster than detecting the cell viability change caused by virus replication.[1–2] Its high sensitivity also allows for continuous feeding of glucose to control its concentration at rather low levels, thus eliciting metabolic shift and guiding continuous culture to steady states with low flux metabolic states.[3]

When the method is used in a small bioreactor, the contribution from the surface aeration cannot be neglected. In that case, $K_{Ls}a_s$ of the surface aeration needs to be determined separately and the transfer of oxygen into the liquid from surface aeration must be subtracted from the measured slope.

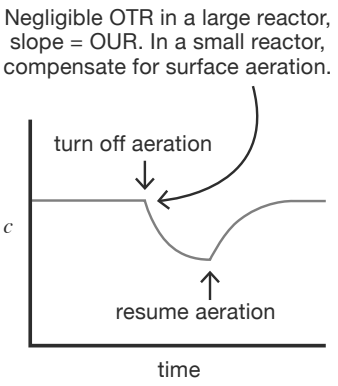

Figure 11.5. Dynamic in-line measurement of oxygen uptake rate in a bioreactor.

Damage to Cells by Gas Sparging

In an aerated mixing vessel, the many different types of force exerted on cells may cause cellular structural reorganization, deformation, or biochemical alterations that lead to cell damage or death. Cells may be squeezed by forces normal to their surface from opposite sides (Figure 11.6). They may impinge on moving mechanical objects, or be subjected to a fluid shear field with fluid moving faster on one side of the cell than the other (Figure 11.7). In a short time scale, the stress may cause deformation in cell shape. For example, squeezing cells from opposite sides changes their relatively spherical shape to an ovoid. At long time scales, cells may reorganize their cytoskeleton and redistribute the lipid bilayer membrane. For example, exposing endothelial cells to fluid flow may induce them to align in the flow direction. The effects of mechanical force on cell behavior can thus be physical or biological. If the rate of

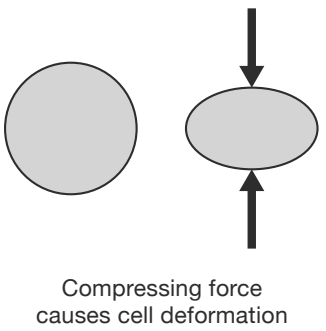

Figure 11.6. Deformation of a cell by a compressing force.

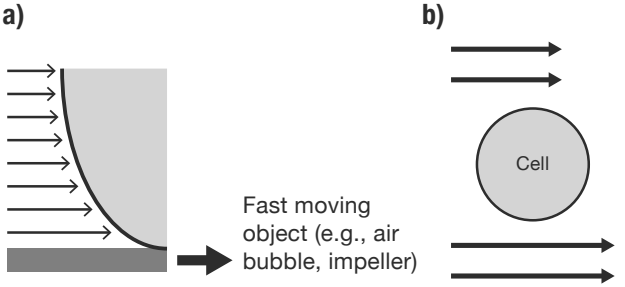

Figure 11.7. A difference in the liquid velocity on opposite sides of a cell causes shear stress. (a) The liquid velocity profile near the surface of a moving plate. (b) The velocity gradient around a cell in a high shear zone.

deformation is fast and the extent is extensive, the cytoskeletal network and the membrane integrity can be compromised, leading to stress response or even cell death. In bioreactors, the concern over mechanical forces has been mostly on the physical effects, especially cell damage. In most studies, the damage was measured in cell death. One should keep in mind that preceding cell death, cell damage may be incurred on other physiological events, such as metabolism, gene expression, or productivity. However, the damage in those respects has been less well documented.

Vigorous sparging of air bubbles through the culture causes cell damage. The mechanism most likely to cause cell damage is a fluid shear field caused by bubbles. Several possible events may dissipate a high-intensity burst of mechanical energy during the bubbles' time traversing the suspended cells in the bioreactor (Figure 11.8).[4] As the bubble escapes from the orifice, the volume originally occupied by the orifice-attached bubble suddenly becomes void, causing the surrounding, cell-containing fluid to rush in to fill the space. A large velocity gradient and high shear field that may cause cell damage inevitably occurs. As the bubble rises, a velocity gradient develops around the bubble: very high in proximity to the bubble's surface, but decreasing rapidly with distance. Similarly, when bubbles coalesce or break up, a liquid velocity gradient develops. Bubbles bursting from the liquid surface cause the volume originally occupied by gas to be rapidly replaced by liquid, thereby creating a strong shear field around the region of shear bursting.

Under typical operating conditions, the event that generates the greatest shear stress is the bursting of gas bubbles from the liquid surface (Panel 11.17). As the bubbles burst off the liquid surface, the high liquid velocity at the air–liquid interface decreases to the velocity in the bulk

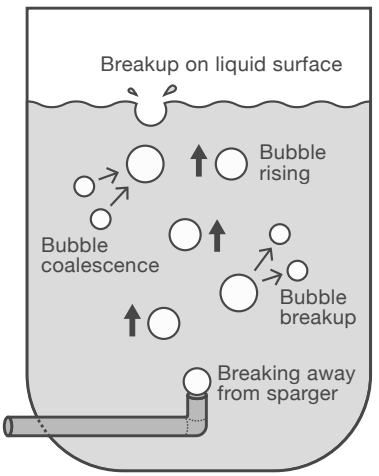

Figure 11.8. Various points during bubble rising in the reactor that may impart damaging shear force on cells.

Panel 11.17. Damage to Cells by Gas Sparging

- It is generally thought that bubble breakup at the liquid surface dissipates the most energy and is most damaging to cells
- Cells and cell-laden microcarriers adhere to rising gas bubbles
- Energy dissipation in gas sparging causes cell damage
- Protective agents are added to the medium via the sparger

liquid over a very small distance. The momentum of the liquid movement caused by the bursting bubbles causes the liquid to rise above the liquid level surface (Figure 11.9). As a result, the liquid moving downward to fill the empty space left by the bubble reverses its direction to rise upward. In the upward-moving liquid cone, the velocity is highest in the center and lowest on the surface of the rising cone. When the rising liquid reaches its maximum height, it quickly reverses direction and drops down, causing the velocity gradient to change direction. The

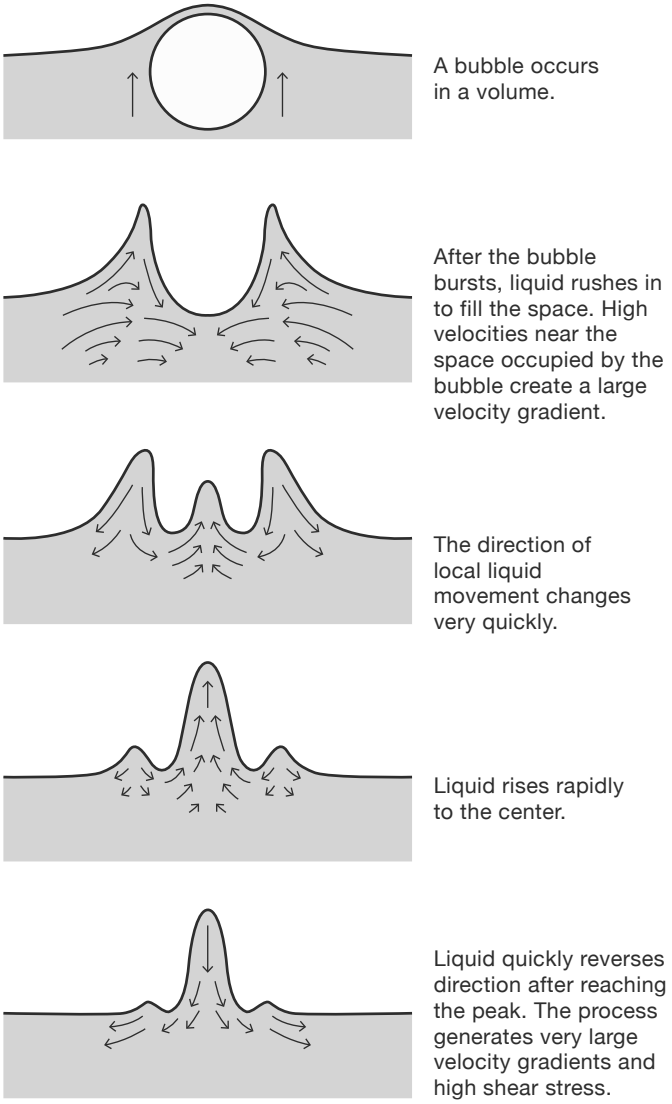

Figure 11.9. Conceptual depiction of fluid movement surrounding the point at which a bubble emerges from the liquid surface.

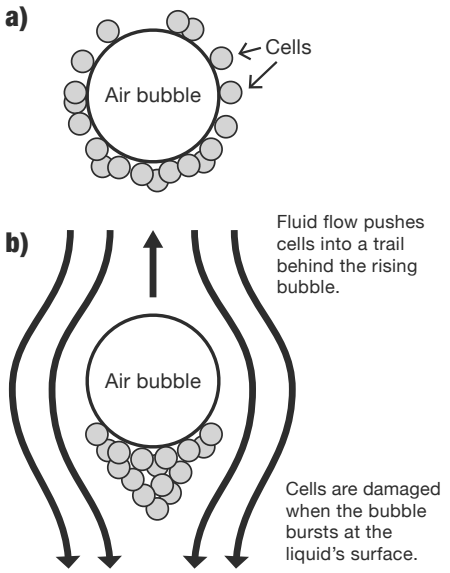

Figure 11.10. Accumulation of cells to a bubble's surface due to hydrophobic interactions and their accumulation to the tail end while the bubble rises.

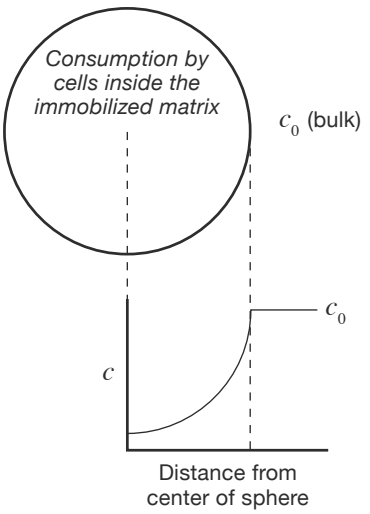

Figure 11.11. Concentration gradient of oxygen in a cell aggregate or an immobilized cell matrix particle.

energy released is very high and its damaging effects on cells entrapped in the bubble-bursting zone can be tremendous.

A bubble submerged in a cell suspension is often surrounded by a layer of cells under microscopic observation, possibly due to hydrophobic interactions between the cells and the bubble's surface (Figure 11.10). Once the bubble begins to rise, cells can be seen trailing the bubble in its tail, or the wake. As the bubble bursts from the liquid surface, cells can be severely damaged, if not outright killed. The block copolymer Pluronic F-68, which is commonly used in culture medium in bioprocessing, reduces the number of cells adhering to rising bubbles through its surface tension modulation effect.[5–6] This reduction of cell attachment to bubbles likely contributes to the "protective" effect of Pluronic F-68 (Poloxamer 188 (P188)) on cell damage from sparging.

Oxygen Transfer in Aggregates and Membrane Bioreactors

Cell Aggregates

Many cell lines and differentiated cells grow as aggregates or organized three-dimensional structures. For example, embryonic stem cells can grow as embryoid bodies and liver cells can grow as spheroids. In some cases, adherent cells are grown inside macroporous microcarriers. In these cases, oxygen must be supplied from a gas phase to the culture medium, travel to the aggregate, and then diffuse from the surface of the particle to the cells in the interior. As oxygen diffuses into the interior, it is consumed by cells. The concentration of oxygen thus decreases toward the interior from the particle surface (Figure 11.11). The steepness of the decrease is dependent on how fast the cells consume oxygen, and on the geometry and size of the particle. Further into the interior, oxygen may become depleted or become too low to

support optimal growth. Oxygen deprivation causes cell death. The use of viability stain shows that dead cells often accumulate in the interior of large cell aggregates. To minimize oxygen limitations, one might reduce the size of particles or maintain a higher oxygen concentration in the culture medium.

Plug-Flow-Like Membrane Bioreactors

Some bioreactors for cell cultivation are comprised of multiple compartments separated by nutrient- and oxygen-permeable membranes. The most commonly seen of such devices is the hollow fiber bioreactor (Figure 11.12, also Figure 8.19). Cells reside in the cell chamber as adherent cells or as suspension cells so that they are not carried out of the reactor by fluid flow in the medium chamber. A medium stream flows through the medium chamber (the intraluminal space of the fiber) to allow nutrients and oxygen to diffuse across the membrane and reach cells in the extracapillary space (i.e., the shell side). In the lumen of the fiber, medium flows through in a plug-flow fashion. The oxygen is supplied by the medium entering from one end and exiting at the other end of the reactor. After exiting the bioreactor, the medium is re-oxygenated in an external oxygenator, usually also a hollow fiber membrane device with oxygen-enriched air in the shell side, to replenish oxygen in the medium before it is recirculated back to the bioreactor (Figure 11.13). The amount of oxygen carried in by fluid flow must be sufficient to supply for cell consumption (Panel 11.18).

It can be easily shown that, at a cell concentration of 2 x 10^{11}/L, a very high circulation rate is needed to supply enough oxygen. Once started up, a hollow fiber bioreactor can be continuously operated over months to produce a product. It is a convenient device for producing antibodies for diagnostic use.

Figure 11.12. Photographs of a hollow fiber bioreactor. Large-diameter fibers are shown for illustration.

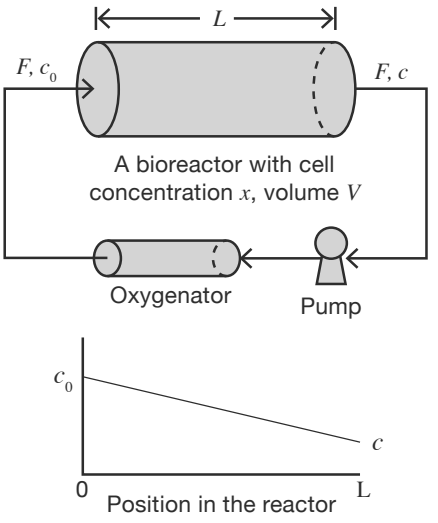

Figure 11.13. Medium recirculation and oxygen concentration gradient in the axial direction of fluid flow in a plug flow reactor.

Panel 11.18. Oxygen Supply by Medium Recirculation

Balance of oxygen concentration:

$$V\frac{dc}{dt} = F\left(c_{\text{in}} - c\right) - q_{O_2} xV \qquad \textbf{(Eq. 11-11)}$$

Example: The medium flow rate required to supply oxygen in a hollow fiber bioreactor with 0.2 L of cell chamber at 2 x 10^{11} cell/L is:

$$q_{O_2} \cdot x \cdot V = \frac{5 \text{ x } 10^{-10} \text{ mmol}}{\text{cell} \cdot \text{h}} \cdot \frac{2 \text{ x } 10^{11} \text{ cell}}{\text{L}} \cdot 0.2 \text{ L}$$

$$= 20 \frac{\text{mmol}}{\text{L} \cdot \text{h}}$$

Assume $c_{\text{in}} = 0.4 \text{ mmol/L}$ (saturated by air)

$$F = \frac{20}{0.4} = 50 \frac{\text{L}}{\text{h}}$$

$$F/V = 250 \text{ h}^{-1}$$

The recirculation rate is 250 times per hour.

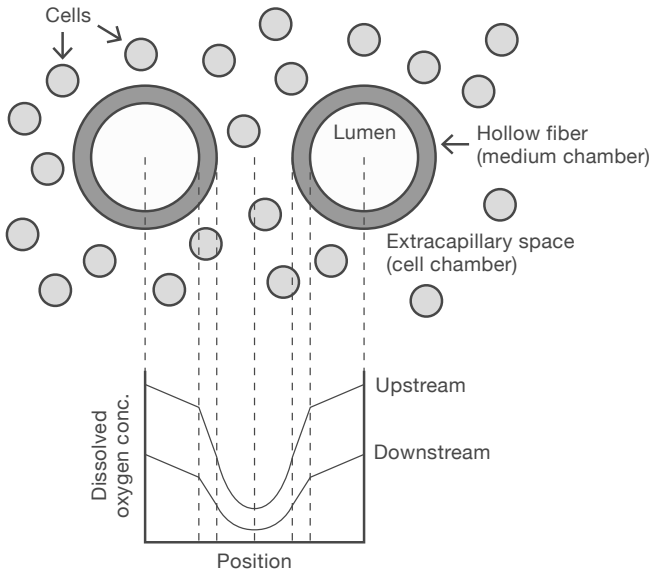

Figure 11.14. Oxygen gradient in the radial direction of a fiber in a hollow fiber bioreactor.

In a hollow fiber bioreactor, the oxygen concentration gradient exists in both axial and radial directions. It was realized very early on, when various reactors were being explored for cell culture bioprocessing, that oxygen transfer limitations would restrict the widespread application of hollow fiber bioreactors. Hence, oxygen transfer in hollow fiber bioreactors was extensively studied.[7] As the medium flows downstream in the lumen, oxygen is diffused through the membrane into the cell compartment. Oxygen concentration therefore decreases along the axial direction of the medium flow (Figure 11.13). In the radial direction, oxygen first diffuses from the bulk liquid in the fiber lumen to the wall of the fiber, and then through the fiber wall into the cell chamber. In the cell chamber, oxygen travels through cell clusters where it is consumed by cells before reaching the cells distal to the fiber. At a high cell concentration, the drop in oxygen concentration is steep in both the axial and radial directions (Panel 11.19). Figure 11.14 is a qualitative description of oxygen concentration in a single fiber, in the direction of medium flow. Under some conditions, an oxygen concentration difference can be seen between the center point of the fiber lumen and the inner wall. The fiber wall often presents a large resistance, causing oxygen concentration to decrease across significantly. In the cell chamber, oxygen decreases rapidly due to a high consumption rate. While the dissolved oxygen concentration in a stirred tank bioreactor is often maintained at 30% of the saturation, that in the exit stream of a hollow fiber bioreactor is typically kept at a much higher level to avoid cells in the downstream suffering from oxygen starvation. To improve oxygen transfer, one may increase the flow rate of medium recirculation or reverse the flow direction periodically to alternate the upstream and downstream ends. Even with those practices, the longitudinal length of a hollow fiber bioreactor is limited due to oxygen transfer limitations. A hollow fiber bioreactor with two sets of hollow fibers, each supplying medium and oxygen respectively, can alleviate the problem of oxygen transfer limitation. The demand for such bioreactors comes during scale-up.

Panel 11.19. Oxygen Transfer in a Hollow Fiber Bioreactor

- Oxygen transfer limitation occurs in both axial and radial directions
- Periodic reversal of the direction of flow helps

Concluding Remarks

The solubility of oxygen is very low in cell culture medium. It must be supplied continuously from a gas phase to sustain cellular demand. Since the respiratory quotient for cells in culture is close to 1.0, almost equal molar of carbon dioxide to that of oxygen consumption should be removed from the culture as cells grow. The most direct and effective method of supplying oxygen and removing carbon dioxide is by

direct sparging of air into the culture broth in the bioreactor. The transfer rate of oxygen through the liquid–gas interface is affected by the mass transfer coefficient, the interfacial area, and the driving force, or the extent of deviation from equilibrium. All three factors can be manipulated to enhance oxygen transfer.

In some cases, such as when a medium containing a high concentration of serum is used, direct sparging is to be avoided because of excessive foaming or other adverse effects. Oxygen can then be supplied from an air stream that is separated from the medium by means of tubing or a membrane with high oxygen permeability, such as silicone or Teflon membranes. In a hollow fiber bioreactor, a recirculating medium passes through an oxygenator before re-entering the reactor to supply oxygen and other nutrients. The oxygenator is often a membrane device such as a hollow fiber oxygenator. The culture medium that exits from the bioreactor is re-oxygenated in the oxygenator and then recirculated back into the bioreactor.

References

1. Galvez, J., et al., *Optimization of HEK-293S cell cultures for the production of adenoviral vectors in bioreactors using on-line OUR measurements*. J. Biotechnol., 2012. **157**(1): p. 214–22.
2. Kussow, C.M., et al., *Monitoring of mammalian cell growth and virus production process using on-line oxygen uptake rate measurement.* Enzyme & Microbial Technology, 1995. **17**: p. 779–83.
3. Europa, A.F., et al., *Multiple steady states with distinct cellular metabolism in continuous culture of mammalian cells*. Biotechnology Bioengineering, 2000. **67**: p. 25–34.
4. Papoutsakis, E.T., *Fluid-mechanical damage of animal cells in bioreactor*. Trends in Biotechnology, 1991. **9**: p. 427–37.
5. Ma, N., et al., *Quantitative studies of cell-bubble interactions and cell damage at different Pluronic F-68 and cell concentrations*. Biotechnol. Progress, 2004. **20**: p. 1183–91.
6. Michael, J.D., et al., *Analysis of cell-to-bubble attachment in sparged bioreactors in the presence of cell-protecting additives*. Biotechnology Bioengineering, 1995. **47**: p. 407–19.
7. Piret, J.M. and C.L. Cooney, *Model of oxygen transport limitations in hollow fiber bioreactors*. Biotechnology Bioengineering, 1991. **37**: p. 80–92.