Introduction

When designing a cell culture process for a new product, one uses a laboratory-scale model to predict the process performance in manufacturing reactors. In some cases, this may involve designing new reactors. More often, it involves taking the new process to an existing manufacturing plant or to a reactor that is designed for generic purposes. The staggeringly high cost of constructing a new manufacturing plant, the trend to decentralize the manufacturing facility and divide it among multiple sites, the increasing use of platform process with disposable reactors, and continued improvement of productivity (thus freeing up manufacturing capacity for new products) all contribute to the increased importance of taking a new process to a pre-existing manufacturing reactor. Understanding the principle of reactor scale translation and developing the ability to evaluate key factors that are sensitive to scale in laboratory equipment is important for cell bioprocess technologists (Panel 12.1).

Panel 12.1. Scale Translation

Objective

- Predict process performance in large-scale reactors
- Specify operating conditions in small-scale equipment that simulates conditions at the large scale

Major Effects of Scale

- Oxygen (gas) transfer
- Heat transfer
- Hydrodynamic effects
- CO_2 removal
- May impact the chemical environment and physiological variables

When the scale of a reactor changes by its length dimension, many associated physical parameters (e.g., volume and total surface area) also change. Changes in physical parameters may influence the chemical environment or have a direct

impact on cells' behavior. Chemical changes may cause biological alterations. As will be discussed below, an increase in the scale of a stirred tank reactor is accompanied by a reduction in the agitation rate. As a consequence, liquid pumping, shear stress, mixing time, etc., are all affected. Some changes to the physical parameters have a direct effect on cells. For example, the changes in the mechanical agitation rate may influence the extent of potential mechanical damage to cells. Fundamentally, the various surface areas related to the reactor (e.g., the heat transfer surface area in the cooling water jacket or the cross-sectional area of the reactor) all decrease with increasing scale on a per reactor volume basis. Changes to the ratio of surface area to volume influence the kinetics of temperature shift that are often practiced at the end of the rapid cell growth stage of production. The physical constraints of decreasing the cross-sectional area to reactor volume ratio leads to a less-than-proportional increase in the aeration rate relative to the scale increase. This affects the gas–liquid mass transfer, which in turn causes changes to the accumulation of CO_2, the pH and the addition of a base for controlling the pH, and the consequential osmolality of the culture fluid. Hence, changes to the physical parameters when scaling up inevitably lead to changes in the chemical environment that may affect cell behavior.

In this chapter, we will discuss the scaling up of a bioreactor based on geometrical similarity. We will keep constant the ratio of the geometrical lengths in the reactor when comparing different scales. In other words, when scaling up all different lengths will scale proportionally. For example, in scaling 10 liters to 10,000 liters, the reactor height, reactor and impeller diameter, and relative size of the internal parts (e.g., impeller, flow diverter) will all be increased by a factor of 10. By scaling all length dimensions of the reactor proportionally, all surface areas will increase with the length dimension to the second power, while the volume will increase to the third power. As a result, the scale-related surface area per unit volume of equipment will decrease. In microbial fermentation, the decrease in the surface area to volume ratio creates an impediment for the removal of heat generated by the metabolism and mechanical agitation. In mammalian cell processing, the generation of metabolic heat is less of a concern. However, the process may still be sensitive to other variables related to scale change.

As the scale of equipment changes, the physical and mechanical parameters may not remain constant. As will be discussed, it is not possible to keep all key operating parameters constant between different scales. Instead of striving to keep scale-related parameters constant, one ought to define the operating range of scale-sensitive physical and mechanical parameters, so that the cellular physiological state and productivity can be maintained within an acceptable range.

Mechanical Agitation

The stirred tank bioreactor is the standard vessel for growing mammalian cells in industrial processes. Figure 12.1 shows an example of a typical industrial cell culture bioreactor. One or more impellers are mounted on a shaft that is driven by a motor mounted at the top of the tank. The impeller diameter is usually around 35–45% of the tank diameter and includes three or four blades. On the inner tank wall, four or six baffle plates are mounted to break the circulation flow pattern around the tank and generate better mixing of the fluid. The sparger is typically mounted some distance from the bottom impeller. Mechanical agitation provides the lifting force that keeps cells in suspension (Panel 12.2). This agitation sustains the fluid flow that mixes the chemical components and creates a more homogeneous chemical environment. In many cases, it also creates a flow pattern that helps reduce the size of gas bubbles and increase their retention time in the culture fluid to enhance oxygen transfer. For cells grown as aggregates, agitation also helps reduce the formation of oversized particles. In many fermentations of mycelial mold or actinomycete, extensive agitation is used to overcome the high viscosity of culture fluid and to reduce the mycelial pellet size to enhance oxygen transfer.

Among all the reasons for providing mechanical agitation, supplying oxygen is the most important. In microbial fermentation, oxygen demand is high (often exceeding 120 mmol /L-h). Mechanical agitation

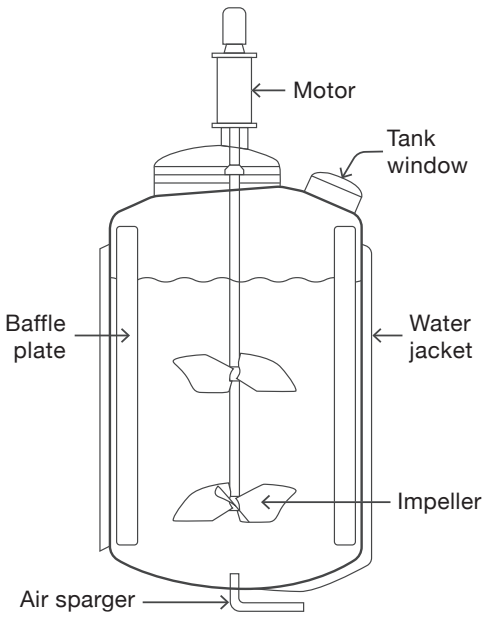


Figure 12.1. Schematic of a typical stirred tank bioreactor for cell culture process.

Panel 12.2. Purposes of Agitation

- Gas–liquid mass transfer
  - The higher shear field near the impeller tip produces small bubbles, thereby increasing the gas–liquid interfacial area
- Suspension of solids (e.g., microcarriers, cell aggregates) or dispersion of liquid
- Liquid–liquid, liquid–solid mass transfer (e.g., quick mixing of pH-neutralizing base)
- Minimization of pellets or aggregates
  - Pellets are cell aggregates of mycelial microorganisms (streptomyces, molds)
- Mixing, especially for viscous fluid (e.g., xanthan gum)

breaks up air bubbles to achieve the level of oxygen transfer capacity needed for a high process productivity. In cell culture processes, the oxygen demand is at least one order of magnitude lower than that in microbial fermentation. Even then, the most important role of mechanical agitation is supplying oxygen.

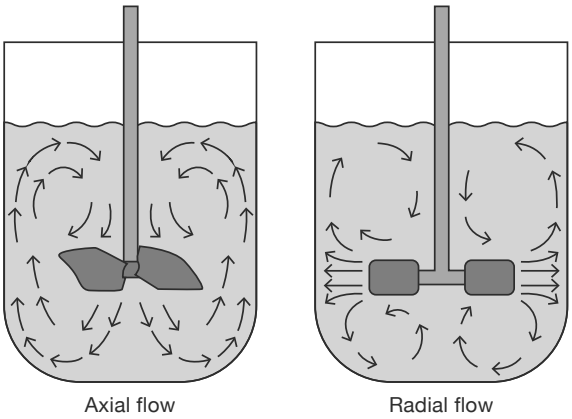


Figure 12.2. Two different kinds of fluid flow patterns in a stirred tank reactor: axial and radial flow.

Mechanisms of Agitation

Impellers can generally be classified into two types according to the flow pattern they generate: axial flow and radial flow (Figure 12.2). An axial flow impeller primarily generates upward or downward flows due to its pumping action. With a radial flow impeller, the liquid moves primarily outward, toward the walls of the vessel. In cell culture processing, impellers that generate axial flow are used because the shear fields generated by axial flow patterns are lower than those generated by radial flow patterns (Figure 12.3a and b).

Rushton disk turbines, which are often used with multiple installations in large reactors, are the predominant type used in microbial fermentation (Figure 12.3c). Rushton turbines are usually used with an air sparger placed directly underneath it. Gas bubbles from the sparger rise, hit the disk, and are directed outward. The blades, which rotate at a fast speed, then break the bubbles up.[1] Very high-energy dissipation in the area immediately surrounding the blades causes a high degree of turbulence in the fluid, further contributing to bubble break-up. The agitation also creates a high shear zone surrounding the impeller that can potentially damage cells. Despite this, it has been shown that mammalian cells grown in suspension can proliferate well with a Rushton turbine-type impeller rotating at a moderate agitation rate. Many cells are perhaps more tolerant to fluid flow stress than commonly thought.

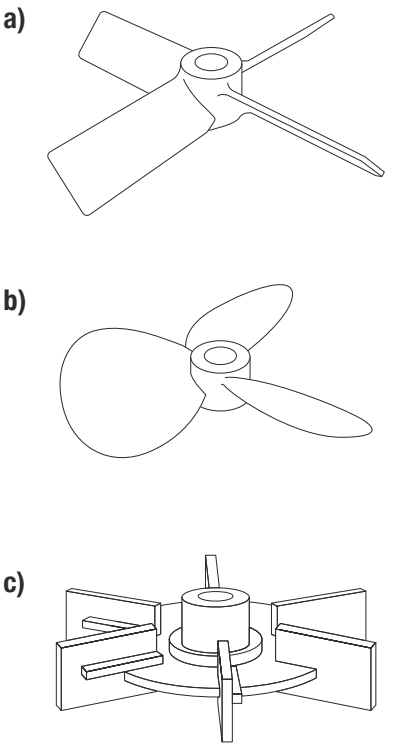


Figure 12.3. Three types of impellers commonly seen in stirred tank reactors: (a) pitched-blade, (b) propeller, and (c) Rushton turbine.

Propeller or pitch blade-type impellers focus on moving the liquid to create lift for mixing and suspending solids. Various designs of axial flow impellers are used in cell culture. As in microbial fermentation, in large bioreactors two to three axial flow impellers are often installed to provide adequate lifting force and to retain air bubbles for oxygen transfer.

The agitation needed to suspend microcarriers for cell growth is somewhat more delicate than for suspension cells. Microcarriers are generally more than ten times larger in diameter than suspension cells. They have a much higher settling velocity (>100 times) and are subjected to higher fluid shear stress. The impeller of a stirred tank for microcarrier culture tends to be larger relative to the tank diameter, but rotates at a slower rate. While the "propeller three blades" is used extensively in microbial fermentation to enhance oxygen transfer, the "axial flow three blades" provides less shear stress and a more uniform velocity in the entire discharged area. Some have attempted to use more specialized impellers, such as elephant ear or "sail" impellers. The latter, which rotates at a very slow rate of only a few rotations per minute, has been used at a pilot plant scale for cell aggregates and microcarriers.[2]

Power Consumption for Mixing Tanks

Power Consumption of Impeller-Agitated Bioreactors

In designing equipment and processes involving fluid flow, heat transfer, and mass transfer, engineers often employ a relationship established using dimensionless parameters. These dimensionless parameters are combinations of descriptors of physical properties such as force, time, and mass. The combination of those physical parameters cancels out the unit of time, mass, and length. Such parameters are therefore called dimensionless numbers. Fundamentally, these dimensionless numbers represent the ratio of a physical quantity in a system, for example the ratio of inertial forces to viscous forces exerted on an object. An important dimensionless quantity that is used widely to describe the dynamics of fluid flow in pipes is the Reynolds number. The number takes the ratio of inertial force $((\rho D^3)(v/t))$ to viscous force (μvD). It was used to establish a unified relationship of the dimensionless energy loss of fluid flow in different viscosities, through different scales and flow conditions. The dimensionless number has since been called the Reynolds number (Re) (Panel 12.3). The quantity that describes the energy loss of fluid flow in a pipe is called the friction factor (f). The relationship between the Reynolds number and the friction factor has distinct regions: in the laminar flow (low Re) region, f decreases linearly with increasing Re; after a critical region, f becomes less dependent on Re; and in the turbulent (high Re) region, it becomes relatively constant. In the turbulent flow region,

Panel 12.3. Dimensionless Quantities Used to Describe Energy Loss in Fluid Flow in Pipes

Reynolds Number

$$\mathrm{Re} = \frac{\text{inertial force}}{\text{viscous force}}$$

$$= \frac{(\rho D^3 v)/t}{\mu v D} [=] \frac{(\mathrm{kg \cdot m^{-3}})(\mathrm{m^3})(\mathrm{m \cdot s^{-1}}) \cdot \mathrm{s^{-1}}}{(\mathrm{kg \cdot m^{-1} \cdot s^{-1}})(\mathrm{m \cdot s^{-1}})(\mathrm{m})} \rightarrow \text{dimensionless}$$

Fanning Friction Factor

$$f = \frac{\text{sheer stress}}{\text{kinetic energy density}} = \frac{\text{force/area}}{\text{kinetic energy/volume}}$$

$$= \frac{(\mathrm{kg \cdot m \cdot s^{-2}}) \cdot \mathrm{m^{-2}}}{(\mathrm{kg \cdot m^{-3}})(\mathrm{m \cdot s^{-1}})^2} = \frac{\mathrm{kg \cdot m^{-1} \cdot s^{-2}}}{\mathrm{kg \cdot m^{-1} \cdot s^{-2}}} \rightarrow \text{dimensionless}$$

pipes of different surface roughness give somewhat different relationships. These are typically presented as multiple lines representing the different surface roughnesses in high Re regions in a f vs. Re plot. Correlations between the Reynolds number (Re) and various dimensionless numbers (in addition to the friction factor) were developed and used in the design of equipment and processes involving fluid flow, mass transfer, heat transfer, etc.

During the development of design principles for stirred tank reactors, the concept of a fundamental relationship among dimensionless numbers was also adopted. A correlation was established between power consumption and the Reynolds number in a stirred tank reactor. In this case, the Reynolds number is denoted as Re_I (Impeller Reynolds number) to indicate that the characteristic length is the diameter of the impeller (Panel 12.4). The dimensionless number for power consumption by impeller is the power number, N_p.

The plot of N_p vs. Re_I compiled from experimental data resembles that of the friction factor vs. Re. At

Panel 12.4. The Impeller Reynolds Number and Power Number

Impeller Reynolds Number (Re_I): $\dfrac{ND_I^2\rho}{\mu}$

Power Number (N_p): $\dfrac{P_o}{N^3 D_I^5 \rho}$

- With an aqueous medium, a bioreactor is always operated in the turbulent region
- In turbulent regions, N_p is constant, independent of Re_I:

$$\frac{P_o}{N^3 D_I^5 \rho} = K \qquad \textbf{(Eq. 12-1)}$$

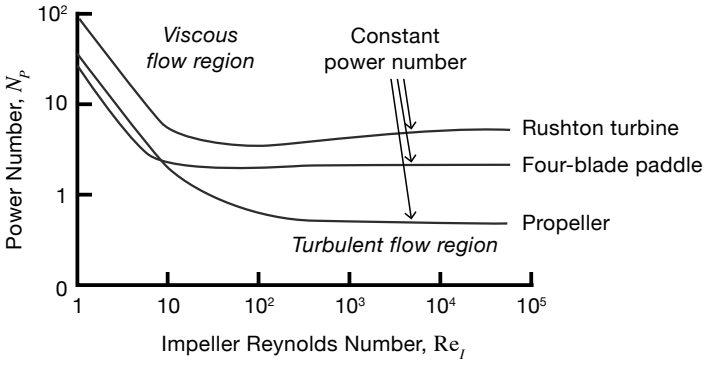


Figure 12.4. Relationship between impeller power number and impeller Reynolds number for different types of impellers.

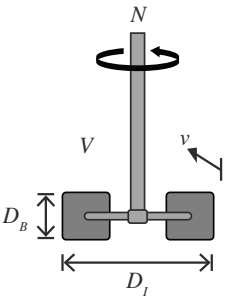
low Re, N_p decreases linearly until the Reynolds number is ~2000, where a short break is seen, and then continues at a relatively constant value in the high Re region (Figure 12.4). The first region is the laminar (or viscous) flow region, and the constant value region is the turbulent flow region. In the turbulent region, different types of impellers give different correlations. They all exhibit a similar behavior of having a relatively constant N_p over a wide range of Re_I.

In a stirred tank, laminar flow is only seen when the viscosity of the fluid is very high, such as when glycerol is used. The flow in a cell culture bioreactor is always in the turbulent regime. Since NP is constant in the turbulent region for a given type of impeller, we consider it to be constant in the scaling up of cell culture bioreactors. From the definition of N_p, one can see that the impeller power (P) divided by $N_3 D_I^5$ (N is the agitation rate, D_I is the impeller diameter) is constant.

Other Scale-Sensitive Variables for Stirred Tank Reactors

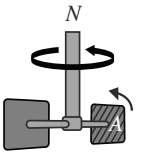
In addition to power consumption, a number of other properties of a stirred tank may profoundly affect cell growth and productivity. For example, the liquid velocity and its associated shear rate and shear stress influence the size of cell aggregates and exert mechanical stress on the cells. The volumetric flow rate (or pumping rate) affects the time that the content of the reactor is mixed, called the mixing time (Figure 12.5). For our discussion, we will choose a representative quantity in the reactor and call them the characteristic liquid velocity, pumping rate, and mixing time, and use them to discuss the effects of scaling up.

The maximum liquid velocity that occurs at the tip of the impeller can be taken as the characteristic liquid velocity in a stirred tank. It can be represented by the multiplicative product of the rotation rate of the impeller and its diameter, ND_I (Eq. 12-2, Figure 12.5). (Note: we will



Maximum velocity

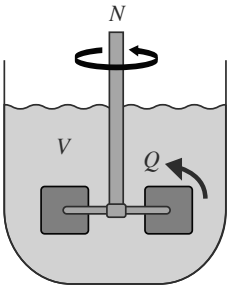
$$v = ND_I \qquad \textbf{(Eq. 12-2)}$$



Pumping

$$Q = \text{pumping area} \cdot \text{pumping velocity} = A \cdot v$$

$$= D_B^2 \cdot ND_I \propto ND_I^3 \qquad \textbf{(Eq. 12-3)}$$



Mixing time

$$\theta = \frac{V}{Q} \propto \frac{D_I^3}{ND_I^3} = \frac{1}{N} \qquad \textbf{(Eq. 12-4)}$$

Figure 12.5. The characteristic liquid velocity (impeller tip velocity), characteristic pumping rate, and characteristic mixing time in a stirred tank bioreactor.

ignore π in our discussion of the perimeter, area of circle, etc. The constant value π is cancelled out when comparing different scales.) ND_I is also the impeller tip speed.

The amount of fluid that the impeller moves with its rotational motion is directly dependent on its rotating velocity and the area of the impeller blades. Since we are considering scale translation under the conditions of geometrical similarity, we may use any length dimension of the reactor for comparison of reactors of different scales. We will use the length of the impeller, instead of the length of the impeller blade, to represent the length scale for our discussion. The pumping, then, is the projected area (D_I^2) of the impeller multiplied by the velocity of its rotation (ND_I), which gives ND_I^3 (Eq. 12-3, Figure 12.5).

How quickly a solute can be mixed after it is added to the fluid in a stirred tank is dependent on the volume of the fluid in the tank (D_T^3) and the amount of liquid the impeller can pump (ND_I^3). Since we are considering the case of geometrical similarity, the ratio of the length dimensions

can be represented by D_I. The changes in mixing time in scaling up can be estimated by dividing the reactor volume by the pumping. The characteristic mixing time in a stirred tank is the inverse of its rotation speed ($1/N$) (Eq. 12-4, Figure 12.5).

As the scale changes, the characteristic fluid velocity (v), liquid volumetric flow rate (Q), and mixing time (θ) also change. By comparing changes in these characteristic quantities across different scales, we can gain much insight into the effects scaling up has on many process-related physical effects.

Effects of Scale on Physical Behavior in Bioreactors

We will use the correlations between the power number and the impeller Reynolds number and the three characteristic quantities described above to explore the effects of a changing scale. We will assume that the equipment between the different scales will remain geometrically similar (Figure 12.6). The effects of different reactor sizes can then be compared using characteristic length D (the tank diameter). If the tank diameter increases by 10-fold, all the other reactor parts (tank height, impeller diameter, etc.) will increase by the same proportion of 10-fold. Hence, in the subsequent discussion we will use only one length symbol, D, without specifying whether it is tank diameter or impeller diameter. As noted above, the constant π will be dropped from our equations.

When scaling up different processes, one ought to keep the most important variable(s) constant or within an acceptable range. Common

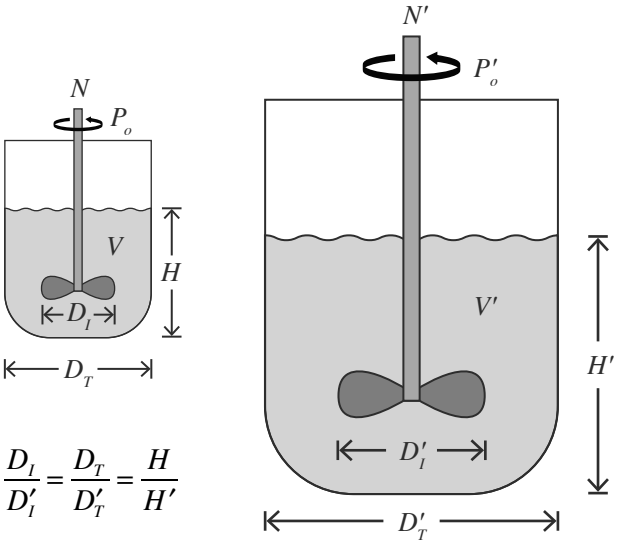


Figure 12.6. Notation of an impeller-based mixing reactor and geometrical similarity in scaling up. H: liquid height, V: liquid volume, N: impeller rotation rate, P_O: agitation power, D_I: impeller diameter, D_T: tank diameter.

Panel 12.5. Scale Translation Approaches

- Constant $K_L a$
- Constant impeller tip speed (ND_I)
- Constant power per unit volume (P_o/V)
- Constant mixing time ($1/N$)

design objectives for scaling up are: (1) keep $K_L a$ constant, so that mass transfer can be maintained; (2) keep impeller tip speed constant, thus maintaining a critical value of high shear velocity to avoid mechanical damage to cells, or to break up agglomerating pellets of mycelial cells in fungal fermentation; (3) keep power input per volume constant, usually for less power-intensive processes such as crystallization and blending; and (4) keep mixing time constant (Panel 12.5).

Consider the case of scaling up by keeping the power input per reactor volume constant. Recall that the power number in a turbulent region is constant (Eq. 12-1, Panel 12.4), so the power input (P) is proportional to N^3D^5 (Eq. 12-5, Panel 12.6). The reactor volume is described by πHD^2. Because of geometrical similarity, we represent H by D and ignore the constant π. The reactor volume (V) is thus represented by D^3 (Eq. 12-6,

Panel 12.6. Scaling Up Geometrically Similarly by Keeping Power Per Unit Volume Constant

Constant parameters, such as π, can be dropped in the discussion of the effect of scale (D_I). One can use a characteristic length D to represent the length dimension.

$\dfrac{P_o}{N^3D^5\rho}$ is constant in a turbulent region.

The density of water, ρ, is constant. Thus,

$$P_o = KN^3D^5 \qquad \textbf{(Eq. 12-5)}$$

The volume of the reactor can be expressed as the characteristic length raised to the third power,

$$V = \pi HD^2 = cD^3 \qquad \textbf{(Eq. 12-6)}$$

The power per unit volume is described as

$$\frac{P_o}{V} = \frac{KN^3D^5}{cD^3} = K'N^3D^2 \qquad \textbf{(Eq. 12-7)}$$

This leads to the conclusion that, when power per unit volume is kept constant, $N^3D_I^2$ is also constant.

$$N^3D^2 = \text{constant} \qquad \textbf{(Eq. 12-8)}$$

$$N = \frac{\text{constant}}{D^{2/3}}$$

Panel 12.7. Effect on Agitation Rate

Comparing scale 1 and scale 2:

$$N_1^3 D_1^2 = N_2^3 D_2^2 \qquad \textbf{(Eq. 12-9)}$$

$$\frac{N_1}{N_2} = \left(\frac{D_2}{D_1}\right)^{2/3} \qquad \textbf{(Eq. 12-10)}$$

The agitation rate N decreases with increasing scale. When the diameter increases eight times, the agitation rate is reduced to 1/4 in the larger scale.

Panel 12.8. Effect on Impeller Tip Speed

Tip speed is described by N multiplied by D, from Eq. 12-6. By keeping P/V constant,

$$N_1^3 D_1^2 = N_2^3 D_2^2$$

$$\frac{N_1^3 D_1^3}{D_1} = \frac{N_2^3 D_2^3}{D_2}$$

$$\frac{N_1^3 D_1^3}{N_2^3 D_2^3} = \frac{D_1}{D_2}$$

$$\frac{N_1 D_1}{N_2 D_2} = \left(\frac{D_1}{D_2}\right)^{1/3} \qquad \textbf{(Eq. 12-11)}$$

Tip speed increases with increasing scale, but only at 1/3 power of the length of scale.

Panel 12.6). By keeping P_o/V constant, N^3D^2 is also constant across different scales (Eq. 12-7). As D increases, the rotation speed must decrease by $1/D^{2/3}$ (Eq. 12-8). The relationship can be written even more explicitly by comparing N across different scales (Eq. 12-9, Panel 12.7). By keeping power per volume constant in scaling up, it is inevitable that larger reactors will need to be operated at lower rotation speeds.

By similar algebraic manipulation, one can also see that scaling up by keeping power input per reactor volume (P/N^3D^2) constant will lead to changes in impeller tip speed (ND) (Panel 12.8), total pumping (ND^3) (Panel 12.9), and mixing time (Panel 12.10). The impeller tip speed increases with the length scale factor to 1/3 power (Eq. 12-11). The liquid pumping also increases with the length scale factor to 7/3 power (Eq. 12-12, Panel 12.9). However, pumping per volume will decrease as the scale increases (Eqs. 12-13 and 12-14, Panel 12.9). Since mixing time can be represented by reactor volume divided by total pumping, mixing time increases with scale (Eq. 12-15, Panel 12.10).

Table 12.1 explores the effects of scaling up a geometrically similar bioreactor by a factor of 10 using three different criteria: constant power per volume (P/D^3), constant agitation rate (N), constant pumping rate, and constant tip speed. The calculation is based on the power number remaining constant when scaling up. By keeping the power input per volume constant, the agitation rate is reduced to $10^{-2/3}$ ($\approx$0.21), but the impeller tip speed is increased by 2.15-fold. The total pumping is increased, but pumping per volume and mixing time increase. If the impeller tip speed is kept constant when scaling up, the decrease in the agitation rate will become much steeper with respect to the scale. Instead of decreasing with $D^{-2/3}$ as in constant power per volume, it decreases proportionally to the scale. Consequently, the power input and the liquid pumping per volume are

Panel 12.9. Effect on Liquid Pumping

The capacity of liquid pumping can be described by the impeller tip speed, ND_I, and by the area that it moves against the liquid, D_{I2}.

At constant power per volume,

$$N_1^3 D_1^2 = N_2^3 D_2^2$$

Multiply both the numerator and the denominator by the diameter to the seventh power.

$$\frac{N_1^3 D_1^9}{D_1^7} = \frac{N_2^3 D_2^9}{D_2^7} \qquad \textbf{(Eq. 12-12)}$$

$$\frac{N_1 D_1^3}{N_2 D_2^3} = \left(\frac{D_1}{D_2}\right)^{7/3}$$

Liquid pumping capacity increases with scale. By dividing both sides by the characteristic length raised to the third power, we can obtain the pumping capacity on a per volume basis.

$$\frac{ND^3}{D^3} = \text{pumping per volume} = \frac{Q_p}{V} \qquad \textbf{(Eq. 12-13)}$$

$$\frac{Q_{p_1}}{V_1} \Big/ \frac{Q_{p_2}}{V_2} = \left(\frac{D_1}{D_2}\right)^{-2/3} = \left(\frac{D_2}{D_1}\right)^{2/3} \qquad \textbf{(Eq. 12-14)}$$

The pumping capacity per volume decreases with increasing scale.

much reduced. This is accompanied by an equal magnitude of increase in mixing time. When the agitation rate is kept constant, the pumping rate and mixing time will also be constant. However, a tremendous increase in power consumption would be needed to keep the agitation rate constant, and as such cannot be implemented in practice.

Panel 12.10. Effect on Mixing Time

Pumping per volume decreases with scale. Thus, mixing time increases when scale increases.

From Eq. 12-4 and Eq. 12-10:

$$\frac{\theta_1}{\theta_2} = \frac{N_2}{N_1} = \left(\frac{D_2}{D_1}\right)^{2/3} \qquad \textbf{(Eq. 12-15)}$$

This example illustrates a very important point in the discussion of scaling up: all physical properties cannot be kept constant at different scales. If one property is chosen to be kept constant, many other properties must change.

Mixing Time

The purpose of agitation is to provide a homogenous chemical environment. Spatial heterogeneity of nutrients may subject cells to an oscillating environment as they traverse the reactor, leading to inferior growth or production performance. Since nutrients that are present from the beginning of the culture eventually become homogeneous in a stirred tank, mixing is potentially a concern only for the components that are added continuously or intermittently during the cultivation. In most reactors, the feeding stream is added in a fixed position(s). It takes a finite amount of time for the added materials to become uniformly distributed throughout the reactor. When mixing is inadequate, cells may encounter regions of excessively high concentrations of added solute(s), potentially causing physiological changes. When areas with especially low concentration are encountered, cells may experience nutrient starvation.

Nutrient Starvation Time

When cells are grown in a closed, nutrient-rich environment without any external supply, oxygen is always the first nutrient to be depleted. Because of its low solubility in medium, oxygen concentration in the medium is orders of magnitude lower than glucose. The ratio of the molar specific consumption rate of oxygen to glucose ranges from about 1.0 (when most glucose is converted to lactate) to close to 6.0 (when most glucose is converted to CO_2). With a cell concentration commonly seen

Table 12.1. Effects of Scaling Up Based on Different Criteria. N_p (P/N^3D^5) is constant.

		Small scale: $D=1$, $V=1$	*Large scale:* $D=10$, $V=1000$		
			Constant power per volume	Constant agitation rate	Constant impeller tip speed
Power	P	1	1000	10^5	100
Power per volume	P/V	1	1	100	0.1
Rotation rate	N	1	0.21	1	0.1
Tip speed	ND	1	2.15	10	1
Pumping	ND^3	1	215	1000	100
Pumping per volume	Q_p/V	1	0.215	1	0.1
Mixing time	θ	1	4.76	1	10

Table 12.2. Comparison of Oxygen and Glucose Saturation Times in a Typical Culture (for 10^{10} cells/L)

	Oxygen	*Glucose*
Concentration	0.1 mM (50% saturation with air space)	1 g / L (5.6 mM)
Specific consumption rate	1 x 10^{-10} mmol / cell-h	0.15–1.0 x 10^{-10} mmol / cell-h
Volumetric consumption	1 mmol / L-h	0.15–1 mmol / L-h
Time to depletion	0.1 h (6 min)	12 h

in industrial processes and no external supply, oxygen is depleted within minutes, whereas the depletion time for glucose is orders of magnitude longer (Table 12.2). To avoid adverse physiological consequences or even apoptosis, nutrients must be replenished before they are reduced to below their critical levels.

Mixing Time

Panel 12.11. Mixing Time Measurement

Measurement

- At $t = 0$, add tracer to the reactor and measure the fluctuation of tracer concentration over time
- Measure terminal mixing time, defined as the point when an arbitrarily chosen uniformity (e.g., 90% or 95%) is reached

The mixing time of a reactor can be quantified using a tracer solute (Panel 12.11). First, a solution of dye is injected into the reactor and allowed to be carried by the fluid and dispersed. A sensor placed in a fixed position in the reactor then records the change of dye concentration over time (Figure 12.7). The concentration will fluctuate, initially over a wide range, and then gradually ebb to a steady value. The time needed for the concentration to reach within a bounded range of steady value is considered the "mixing time." For example, if two ranges are +/-10% and +/-5% of the final steady value, then the dye concentration begins to enter the range at 90% and 95% mixing time, respectively.

Let us denote the final, well-mixed steady dye concentration as c_s, the concentration at any time point as c, and the concentration deviation from its final steady value as Δc. The semilogarithmic plot of the time profile of $\ln(\Delta c)$ vs. t can often be approximated by a constant slope, suggesting that the kinetic behavior is first order. The slope, which is a kinetic constant of mixing, can be taken as the pumping rate (Q_p) divided by the volume of the reactor. The slope is a rate constant and its inverse is a characteristic time (Eq. 12-16, Figure 12.7). After one characteristic time, the tracer concentration will be $0.37\Delta c$ from the final c_s, and after three characteristic times it will be $0.02\Delta c$ from c_s. In other words, the content of the reactor can be considered well-mixed after three characteristic mixing times.

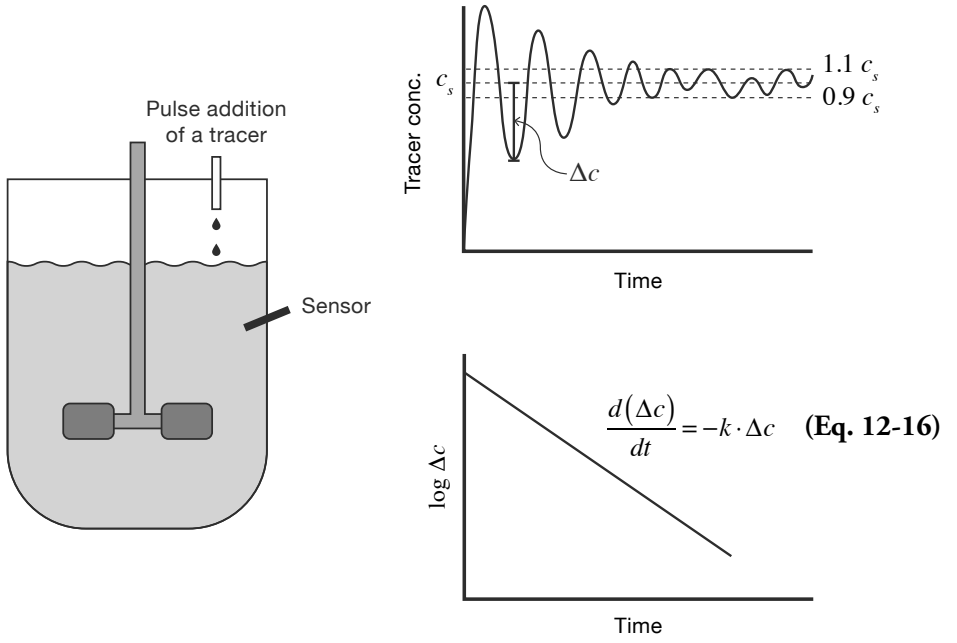


Figure 12.7. Measurement of averaged mixing time in a stirred tank.

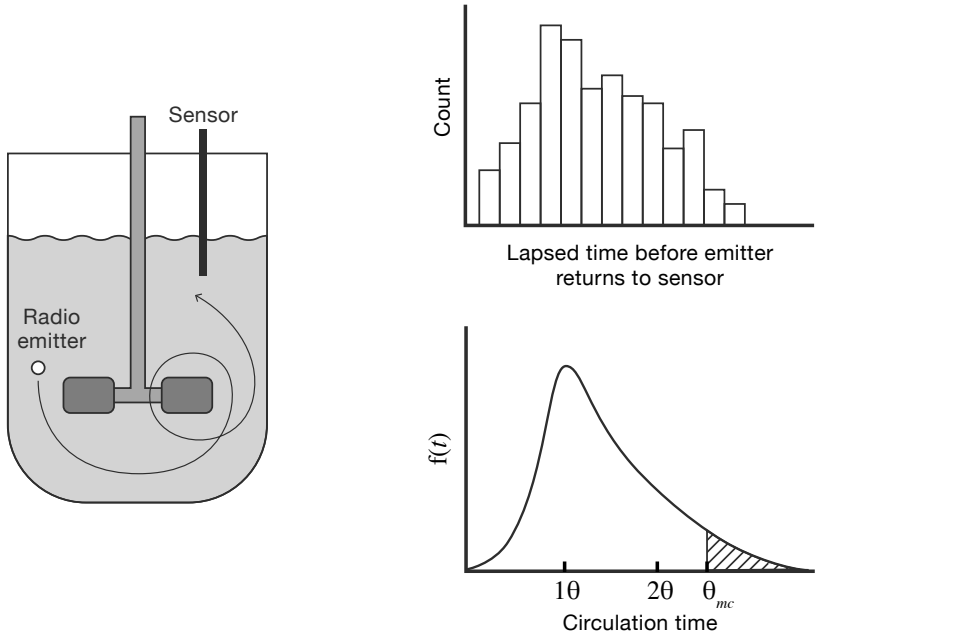


Figure 12.8. Circulation time distribution in a stirred tank and its measurement.

In scale-up, Q_p/V decreases as the scale increases, meaning that the time to reach complete mixing lengthens. To avoid nutrient deprivation, one may keep the set point nutrient concentration (c_s) higher so that the level will not drop too low before the next nutrient addition time. One may also feed more frequently. For very large reactors or if the nutrient level cannot be raised (possibly because of toxicity), multiple nutrient addition points in the reactor are sometimes implemented.

Mixing Time Distribution

The mixing time described above is an averaged value measured at a fixed position in the reactor. Consider the case that a nutrient solution is added at a fixed position in a reactor. A fluid element with a certain volume and cell concentration passes by the position and carries an amount of the added nutrient as it traverses the reactor via the fluid flow created by the mixing mechanism. At some point, the fluid element will return to the feeding position, acquire more nutrient, move away to circulate around the reactor again, and finally returns to the feeding position again. The time elapsed between the two consecutive returns to the feeding position, or the circulation time/medium re-enrichment time, is not uniform. Instead, it is distributed over a range; sometimes the time is short, other times it is long (Panel 12.12). As discussed above, the amount of nutrient that the fluid acquires at the nutrient feeding zone must be sufficient to sustain the metabolic needs of the cells before it returns to the feeding zone. In other words, the nutrient re-enrichment time must be shorter than the nutrient depletion time. The nutrient depletion time is dependent on cell concentration and the consumption rate. If the nutrient re-enrichment time is longer than the nutrient depletion time, a time period of nutrient depletion will appear during the circulation of the fluid element.

Panel 12.12. Medium Circulation Time Distribution

- The circulation in a tank is not uniform. If the average circulation time is 6 minutes, many fluid elements will have a circulation time shorter than 6 minutes, and others will have longer times.
- The average circulation time should be shorter than the critical circulation time

To measure the distribution of the circulation time (defined as the time elapsed between two consecutive feedings), imagine that a signal-emitting ball with an identical density to the fluid is being carried freely by fluid motion (Figure 12.8, Panel 12.13). A sensor at the position where the nutrient is added to the reactor picks up the signal when the ball is close by and records the time interval between consecutive detections of the signal. The recorded time interval is not uniform, and instead distributes over a range because sometimes the ball returns to the sensor shortly after it moves away, while other times it roams around the reactor for a while before returning to the sensor position. The

Panel 12.13. Mixing Time Distribution Measurement

- Add a radio emitter to the reactor. A sensor picks up the signal when the emitter, in the course of circulating around the reactor, passes by.
- Measure the circulation time for each encounter and plot the frequency distribution of the circulation time
- Determine the mean and median circulation time and the standard deviation σ
- The distribution of the circulation time can be plotted as a population density function. The portion of the distribution with a circulation time lying between t and $t + \Delta t$ is the area under the curve between t and $t + \Delta t$.

distribution function thus describes the frequency of the circulation time or the nutrient feeding interval.

In general, the distribution follows a logarithmic normal distribution. The mean or median of the distribution function is a descriptor of the mixing characteristics of the reactor, but it does not present the entire picture of mixing. Two reactors that have the same median or mean nutrient feeding interval may still have a very different frequency distribution. Cells that circulate with the fluid element receive nutrients only when the fluid returns to that feeding position. If the circulation time is longer than the nutrient depletion time, then the nutrient level will fall below the critical value. If the duration of nutrient depletion exceeds a critical time period, apoptosis or other metabolic anomalies may occur. A wide distribution of the circulation time can be a concern. Even low occurrences of long circulation times may cause nutrient starvation once cell concentration becomes high. When very high cell concentrations are used—as is often the case during the search for ever-higher target productivities—the oxygen depletion time can be only a few seconds long. The effect of circulation time distribution should therefore be taken into consideration when scaling up.

Scaling Up and Mechanical Forces on Cells

Cells in a stirred bioreactor may encounter shear stress caused by fluid flow, or be subjected to direct impact by moving mechanical parts such as the impeller. Since cells are neutrally buoyant particles, they tend to follow the motion of relatively large eddies. In general, direct impact of cells on the impeller is therefore minimal, since cells are usually carried away by fluid before direct mechanical impact. Damage to cells due to mechanical agitation in a stirred tank is more likely to arise from shear stress caused by large fluid velocity gradients, or from the transference of kinetic energy from swirling pockets of fluid (called eddies) in a turbulent flow than from mechanical impacts (Figure 12.9).

The maximum fluid velocity in a stirred tank occurs at the tip of the impeller (Panel 12.14). As the scale increases, the impeller tip speed increases. The shear rate (i.e., the steepness of the fluid velocity change over a unit distance), increases with the agitation rate. Because of this, shear damage was a considerable concern when cell culture was undergoing the transition from a research platform to a major biomanufacturing technology in the 1980s and early 1990s. Nevertheless, large stirred tanks of up to tens of cubic meters in volume have been commonly used for cell culture-based manufacturing since the mid-twentieth century. Agitation conditions well below those causing significant cell damage can be identified and employed in industrial processes for the vast majority of cell lines of industrial interest. In general, the conditions of mechanical agitation that are commonly employed in cell culture processes are much less energy-intensive and impart much less mechanical stress than those in microbial fermentation. It is advantageous to employ a higher agitation rate when possible, as it provides more efficient oxygen transfer and carbon dioxide removal.

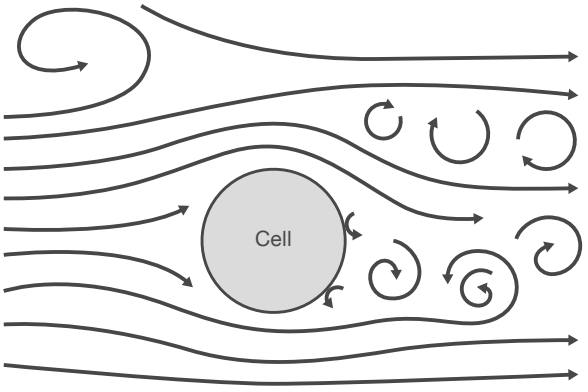


Figure 12.9. Fluid flow and eddies surrounding a cell. Eddy size increases with scale if the power per unit volume decreases with scale.

Panel 12.14. Effects of Mechanical Agitation on Cells

- The most velocity and energy released in a stirred tank occurs at the impeller tip
- An increasing agitation rate increases the impeller tip speed and the shear rate
- Most suspension cells are sensitive to mechanical stress. Satisfactory, non-cell damaging conditions are identified and used in large-scale cell culture.
- More intensive agitation may be advantageous in enhancing oxygen and CO_2 transfer in high density cell culture process
- Most studies employ cell viability and growth as criterion for evaluating cell damage. Evaluating the non-lethal effects on cells' physiology is also important.

It has long been reported that cells are not as fragile as they were thought to be at the dawn of cell culture bioprocessing. Even Rushton turbines were shown to support normal growth in laboratory bioreactors, and elephant ear impellers with high agitation rates have been successfully used in small reactors. Many of the early studies on the effects of mechanical agitation focused on cell death—an irreparable event—as the outcome variable. Before cell death is caused, however, mechanical stress may elicit changes in the cell metabolism, stress response, and other physiological processes. Many studies have been undertaken on the physiological differences among endothelial cells that have been exposed to different flow conditions. Cells grown

under different hydrodynamic conditions show gene expression changes in some functional classes, including the stress response. But, in general, our knowledge of non-lethal cellular changes in industrial cell lines under different mechanical agitation conditions is still relatively scarce.

Cells grown on microcarriers are more susceptible to damage caused by forces exerted by fluid flow. With their much larger particle diameters, microcarriers have a higher velocity relative to the fluid compared to suspension cells. By using the energy dissipation rate to compare the agitation conditions that cause cell damage, a study reported that the energy dissipation rate that causes cell damage in microcarrier cultures is at least two orders of magnitude lower than that for suspension cells.[3] It should be noted that different cell lines have different sensitivities to mechanical agitation. Even for suspension cell lines, the maximal shear-stress tolerance range spans over two orders of magnitude, suggesting that the sensitivity to fluidic dynamic damage is highly dependent on the cell line, reactor, and many other factors. Nevertheless, the low tolerance of microcarrier cultures to high mechanical energy dissipation is generally reflected in their higher sensitivity to mechanical agitation when scaling up.

A mechanistic understanding of cell damage caused by fluid flow in a stirred tank is still lacking, despite attempts to link Kolmogorov micro-eddies to damage on cells grown on microcarriers. Further complicating the matter is the nature of cell damage. Cells in a reactor roam around different locales that have vastly different energy dissipation rates. It has been reported that a vast amount of energy is often released in a very small region. The energy released in the bulk can differ from the high energy release zone by several orders of magnitude. Cells may be damaged by a constant exposure to moderate energy dissipation or by a short exposure to a very high level of mechanical stress. Analyses based on the average energy dissipation consideration may not reflect all possible causes that can lead to cell damage in scale-up. The potential of cell damage or other biological changes caused by hydrodynamics that affect productivity in scaling up is still a concern.

Oxygen and Carbon Dioxide Transfer

The design objective in scaling up a process is to produce product in quantities proportional to the scale. To meet that goal, all nutrients are supplied proportionally to the scale. For liquid nutrients, this is easily met. However, for oxygen supply and CO_2 removal, scaling up presents a challenge due to the constraints of physical factors.

Scale-Up and Oxygen Transfer

Oxygen balance on liquid phase

In Chapter 11, we established that the oxygen transfer rate (OTR) into the culture medium is dependent on the overall mass transfer coefficient (K_La) and the driving force ($c^*–c$). The dissolved oxygen content in the reactor is informed by the balance between the transfer rate through the gas–liquid interface and the oxygen uptake rate (OUR) (Eqs. 12-17 and 12-18, Panel 12.15). OUR is the specific oxygen consumption rate multiplied by the cell concentration. In a reactor, the oxygen content (i.e., the oxygen partial pressure) in the incoming air is different from that in the air exiting the reactor. Which c^*, then, is the one to use for the driving force calculation? For small reactors, we can assume that culture content in the reactor is well-mixed. In cases like these, the concentration at the outlet is the same as the concentration in the reactor. The c^* for calculating the magnitude of the driving force should thus be that at the air outlet (Eq. 12-19, Panel 12.15). For a large reactor, it is assumed that

Panel 12.15. Liquid Phase Oxygen Balance

The dynamics of the dissolved oxygen concentration are described by the balance between OTR and OUR at a quasi-steady state.

$$V\frac{dc}{dt} = K_La\left(c^*-c\right)\cdot V - \mathrm{OUR}\cdot V = \mathrm{OTR}\cdot V - \mathrm{OUR}\cdot V \qquad \textbf{(Eq. 12-17)}$$

At a steady state, OTR = OUR

$$K_La\left(c^*-c\right) = \mathrm{OUR} = q_{O_2}x \qquad \textbf{(Eq. 12-18)}$$

For small-scale bioreactors, one can assume both the liquid phase and the gas phase are well-mixed. The gas phase in the reactor is thus the same as that in the exit gas stream, and c^* is related to the oxygen concentration at the exhaust gas by Henry's law constant:

$$c^* = \frac{P_{O_2,\mathrm{out}}}{H_{O_2}} = \frac{Py_{O_2,\mathrm{out}}}{H_{O_2}} \qquad \textbf{(Eq. 12-19)}$$

For large-scale bioreactors, the inlet and outlet oxygen concentrations may be very different. One uses the logarithmic mean driving force described below:

$$K_La\left(c^*-c\right)_{LM} = K_La\frac{\left(c^*_{\mathrm{in}}-c\right)-\left(c^*_{\mathrm{out}}-c\right)}{\ln\dfrac{c^*_{\mathrm{in}}-c}{c^*_{\mathrm{out}}-c}} \approx K_La\left(\frac{c^*_{\mathrm{in}}+c^*_{\mathrm{out}}}{2}-c\right) \qquad \textbf{(Eq. 12-20)}$$

the gas phase behaves like in a tubular reactor (plug flow), and a logarithmic mean of the driving force is used (Eq. 12-20, Panel 12.15). In most cell culture processes, though, the difference between the logarithmic mean or a simple average is rather small.

As the reactor volume increases, the magnitude of OTR ought to be maintained in order to supply sufficient oxygen for growth. The increased liquid height in a large-scale reactor increases the hydrostatic pressure, and thus also the value of c^*. Recall that 10 m of water height gives an additional 1 atm of hydrostatic pressure. The effect of hydrostatic pressure on c^* should be accounted for in the calculation of OTR, although we do not express it explicitly in the equation.

Oxygen balance on gas phase

In an aerated bioreactor, air bubbles travel through the culture fluid, dispersing oxygen as they go (Figure 12.10). When air exits the reactor, the amount of oxygen it carries is less than that at the gas inlet at the bottom of the reactor. While oxygen is transferred into the liquid phase, water vapor and CO_2 produced by cells in the medium are stripped out of the culture broth, potentially causing the volume flow rate at the outlet to differ from that at the inlet. However, on a dry air basis, the molar (or volumetric) flow rate at the inlet and outlet can be considered to be the same because the RQ (respiratory quotient, or CO_2 produced/oxygen consumed) is very close to 1.0. The oxygen transferred into the liquid is simply the difference between the rate of oxygen entering the reactor at the inlet and exiting at the outlet (Eq. 12-21, Panel 12.16). Assuming ideal gas behavior ($PV = nRT$), the molar flow rate of the oxygen at the inlet and the outlet is the total air flow rate (PQ/RT) multiplied by the molar fractions of oxygen at the inlet and the outlet ($y_{O2,\,in}$ and $y_{O2,\,out}$), respectively. The difference between the inlet and the outlet is the amount retained in the reactor. It is also the amount that is transferred into the liquid. Therefore, the OTRs determined from the gas and liquid phase transfers are equal (Eq. 12-22, Panel 12.17).

In a cell culture process, the operating conditions are such that the dissolved oxygen level does not change rapidly because of the balance of OTR and OUR. The dissolved oxygen level can be taken as being at a quasi-steady state (i.e., the change in dissolved oxygen (dc/dt) is ~0). The OUR can be approximated from the OTR calculated from the gas phase balance or liquid phase balance.

Aeration Rate and Superficial Velocity

Consider again scaling up a reactor by geometrical similarity. All length dimensions (diameter, height, etc.) increase by the same proportion factor. The reactor volume, V, thus increases proportionally with the tank diameter to the third power, while the cross-sectional area of the

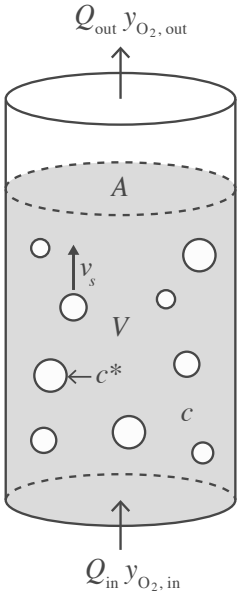


Figure 12.10. Gas sparging for aeration in a stirred tank bioreactor. Oxygen content in the gas at the inlet and outlet is different.

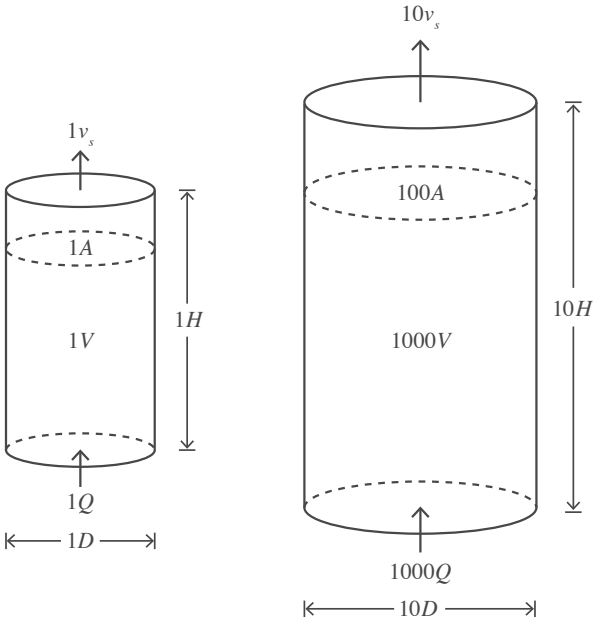


Figure 12.11. Depiction of scaling up 1000 times with geometrical similarity. Gas flow is increased 1000 times to meet the demand of 1000 times the culture volume.

Panel 12.16. Gas Phase Oxygen Balance

On the gas side, the oxygen transferred from the gas side to the liquid side is reflected in the difference of oxygen concentration between the gas inlet and outlet.

$$\text{OTR} \cdot V = \left(Q_{\text{in}} N_{O_2,\text{in}} - Q_{\text{out}} N_{O_2,\text{out}}\right) \qquad \textbf{(Eq. 12-21)}$$

$$= \frac{P}{RT}\left(Q_{\text{in}} y_{O_2,\text{in}} - Q_{\text{out}} y_{O_2,\text{out}}\right)$$

Panel 12.17. Steady State Gas Phase and Liquid Phase Balance

On the liquid side, OTR and OUR can be assumed to be at a steady state since the rate of change of dissolved oxygen is small. In cell culture process, the air flow rate can be considered to be the same at the inlet and outlet. Overall, the relationship is described as:

$$\text{OUR} = K_L a\left(c^* - c\right) = \frac{PQ}{RT}\left(y_{O_2,\text{in}} - y_{O_2,\text{out}}\right) \qquad \textbf{(Eq. 12-22)}$$

Note: P/RT converts volume flow rate Q to a molar flow rate using the ideal gas law.

Panel 12.18. Gas Superficial Velocity in Scale-Up

Superficial gas velocity = gas flow rate/ reactor cross-sectional area

$$v_s = \frac{Q}{A} = \frac{Q}{\pi D^2} \qquad \textbf{(Eq. 12-23)}$$

The reactor volume increases with the length scale raised to the third power, while the cross-sectional area increases only by the second power.

$$\frac{V_1}{V_2} = \frac{D_1^3}{D_2^3} \qquad \textbf{(Eq. 12-24)}$$

$$\frac{A_1}{A_2} = \frac{D_1^2}{D_2^2} \qquad \textbf{(Eq. 12-25)}$$

When scaling up, one may choose to increase the air flow rate proportionally to the increasing reactor volume:

$$\frac{Q_1}{Q_2} = \frac{V_1}{V_2}$$

One can see that

$$\frac{v_{s_1}}{v_{s_2}} = \frac{Q_1/A_1}{Q_2/A_2} = \frac{V_1/A_1}{V_2/A_2} = \frac{D_1}{D_2} \qquad \textbf{(Eq. 12-26)}$$

Superficial gas velocity will increase linearly with increasing scale.

tank, A, increases with the diameter to the second power (see Figure 12.11 for an example of scaling the reactor volume by 1000-fold). To supply a sufficient amount of oxygen, we provide an airflow rate (Q) in a proportional fashion to the reactor volume ratio. The velocity of the air blowing through the liquid surface in the reactor (i.e., the air flow rate divided by the cross-sectional area, referred to as the superficial velocity) increases with the same factor as the tank diameter (Panel 12.18). For a case of scaling up 1000 times in volume (i.e., 10 times in diameter), the superficial air velocity will be 10 times higher in the large scale.

A very high superficial velocity can cause flooding of the impeller, a situation in which the moving impellers encounter more air rather than liquid. Additionally, as superficial velocity increases, foaming becomes a more serious concern. To avoid these problems, airflow per reactor volume generally decreases with increasing scale.

Given the same OUR, if a lower air flow rate per reactor volume is used in scaling up, then the exiting gas will have a lower oxygen content. This, in turn, will reduce OTR through a lower c^*_{out} and a lower driving force, as shown in Eqs. 12-19 and 12-20. The decreased driving force must be compensated for by increasing $K_L a$ in order to maintain the same oxygen supply rate, or by enriching the air with oxygen (Panel 12.19, Table 12.3). The decreased air flow rate per reactor volume (typically expressed as vvm, or volume air per volume liquid per minute) is somewhat compensated for by the increased holding time and hydrostatic pressure in the large-scale reactor. The greater height of the large-scale reactor increases the travel time for air bubbles reaching the top of the reactor, thus increasing the gas hold-up and the interfacial area for transfer with a given gas flow rate.

Panel 12.19. Effect of Air Flow Rate in Scaling Up on Oxygen Transfer Driving Force

Considering gas phase balance in the scale up from scale 1 to scale 2:

$$\frac{Q_1}{V_1}\left(y_{O_2,\,in,\,1} - y_{O_2,\,out,\,1}\right) = \frac{Q_2}{V_2}\left(y_{O_2,\,in,\,2} - y_{O_2,\,out,\,2}\right)$$

$y_{O_2,\,in}$ (oxygen concentration in the inlet air) is the same in both cases. If Q_2/V_2 is smaller, $y_{O_2,\,out,\,2}$ and c_2^* will be smaller to keep $OUR_1 = OUR_2$.

Considering the liquid phase, OTR needs to be maintained at the same level in the two scales:

$$(K_La)_1\left(c_1^* - c_1\right) = (K_La)_2\left(c_2^* - c_2\right)$$

This can be accomplished by:

- With a smaller c_2^*, $(K_La)_2$ must increase to keep $c_2 = c_1$.
- If $(K_La)_2$ is to be kept the same as $(K_La)_1$, the concentration driving force of oxygen must be increased (i.e., $(c_2^* - c_2) > (c_1^* - c_1)$) by either
  - Allowing c_2 to decrease
  - Increasing c_2^* by using enriched oxygen

Table 12.3. Effects of Scale on Oxygen Transfer

	Reference scale	*Constant air flow*	*Constant superficial velocity*
Scale (volume)	1	1,000	1,000
Cross-sectional area	1	100	100
Air flow rate	1	1,000	100
Superficial air velocity	1	10	1
O_2 consumption	1	1,000	1,000
CO_2 production	1	1,000	1,000
$Q(y_{in} - y_{out})$	1	1	$(y_{in} - y_{out})$ ~10 times larger (y_{out} is very low)
Comments		May reach flooding, excessive foaming	Need to increase K_La or power input; overall driving force for O_2 transfer is low

Effects of Scaling Up on CO_2 Removal

The respiratory quotient for cells using glucose and glutamine as their main sources of energy is very close to 1.0. Every mole of oxygen consumed by the cell generates one mole of CO_2. A highly active culture with a high cell concentration may produce nearly 100 mmol/L of CO_2

per day at the peak of its metabolic activities. In comparison, a cell culture medium initially has about 20–40 mM of sodium bicarbonate. The CO_2 produced by cells will therefore exceed the amount that is included in the medium initially.

Many cells, such as hepatocytes, are rather tolerant to CO_2, but others are more sensitive. The growth of most cells may begin to be affected once the CO_2 concentration exceeds 15% (~65 mM HCO_3^-). The CO_2 produced needs to be stripped off from the culture medium to prevent it from accumulating to high levels that can affect growth or productivity.

The rate of CO_2 stripping is dependent on the concentration difference of CO_2 in the liquid and gas phases. The direction of the driving force for CO_2 is in the opposite direction than that of oxygen transfer (Figure 12.12, Panel 12.20). A higher concentration of CO_2 in the gas phase reduces the driving force of CO_2 and thus the stripping efficiency. When scaling up, the airflow rate per reactor volume often decreases somewhat with increasing scale. As a consequence, less air per culture volume is used to strip CO_2 off the medium. The CO_2 level in the gas stream exiting the reactor will thus be higher in the large-scale reactor, causing the CO_2 in the liquid phase to also be higher.

To compensate for the reduced O_2 driving force when scaling up, one can use O_2-enriched air. However, such a measure cannot compensate for the diminished efficiency of CO_2 stripping. Therefore, CO_2 accumulation patterns in large-scale bioreactors can differ significantly from small-scale reactors.

The overall mass transfer coefficient (K_La) for CO_2 is about 15%–30% lower than that for oxygen (depending on the temperature and solution composition) due to its larger molecular size. The difference,

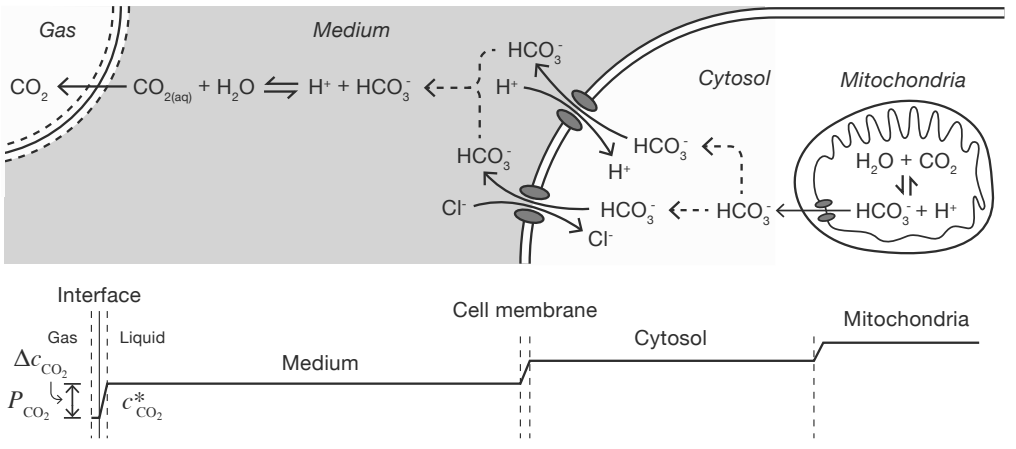


Figure 12.12. Schematic of the removal of carbon dioxide produced by cells.

Panel 12.20. CO_2 Removal from Culture Medium

- The mass transfer coefficients for oxygen and carbon dioxide are about the same
- R.Q. (moles of CO_2 produced / moles O_2 consumed) for mammalian cells is very close to 1.0. Thus, the oxygen uptake rate (OUR) and carbon dioxide evolution rate (CER) are about equal.
- The inhibitory level of CO_2 is around 15–20% (110–150 mm Hg)

Gas phase CO_2 balance:

$$\mathrm{CER} = \frac{PQ}{RT}\left(y_{\mathrm{CO_2,out}} - y_{\mathrm{CO_2,in}}\right) = \frac{QH_{\mathrm{CO_2}}}{RT}\left(c^*_{\mathrm{CO_2,out}} - c^*_{\mathrm{CO_2,in}}\right) \qquad \textbf{(Eq. 12-27)}$$

Liquid side balance:

$$\mathrm{CER} = K_{L,\mathrm{CO_2}}a\left(c_{\mathrm{CO_2}} - c^*_{\mathrm{CO_2,out}}\right) \qquad \textbf{(Eq. 12-28)}$$

- A high level of CO_2 in the exit air can be caused by a high metabolic rate / high cell concentration, or by a low air flow rate. It diminishes the driving force for CO_2 stripping and leads to the accumulation of CO_2/HCO_3^- in the medium.
- The driving force for CO_2 removal decreases with increasing scale

approximately the square root of their molar weight ratio, is significant but not huge.

Unlike O_2, the solubility of CO_2 in an aqueous solution is very high. At the gas bubble interface, O_2 in the liquid phase can be assumed to be in equilibrium with the gas phase, so (c^*-c) is a good estimate of the driving force. The same assumption is not valid for CO_2. CO_2 in the medium exists as CO_2, HCO_3^-, and CO_3^{2-}, with HCO_3^- being the dominant form at a neutral pH. At the interface, CO_2 crosses the film and is transferred out of solution, but HCO_3^- does not diffuse into the gas phase. HCO_3^- must dissociate to CO_2 before being transferred to the gas phase. The kinetics of the dissociation of HCO_3^- to $CO_{2(aq)}$ is slow. Because of this, the actual driving force is smaller than it would be were the kinetics faster. An enzyme carbonic anhydrase is expressed in the mitochondria and in many tissues to facilitate the hydration of CO_2. A similar mechanism facilitating the kinetics of $HCO_3^-/CO_{2(aq)}$ conversion is not available in a reactor.

As the scale increases, the hydrostatic pressure exerted by the liquid height increases the solubility of CO_2. All factors combined, the stripping of CO_2 from the culture medium becomes less efficient as the scale increases.

Variation in Chemical Environment in Scale Translation

As discussed earlier in this chapter, when scaling up many physical parameters cannot be kept constant. Changes in some of those parameters, such as maximal fluid velocity in the reactor and the frequency and nature of bubbles bursting through the liquid surface, can directly impart physical stress on cells and cause metabolic or other physiological changes. In other cases, a change in scale necessitates the alteration of operating conditions that in turn elicit changes in the culture's chemical environment and then in cellular physiology. As a notable example, the efficiency of CO_2 removal and the time profile of CO_2 concentration in culture changes with the scale of the reactor. CO_2 concentration is not controlled explicitly, but is affected by the aeration rate that is used to control the dissolved oxygen level. Over time, the CO_2 concentration in a culture distributes over a wider range than dissolved oxygen does.

CO_2 is a major contributor to the pH buffer in culture and the intracellular environment. The vast majority of CO_2 production in the cell takes place in the TCA cycle in the mitochondria. The CO_2 produced by cells is exported from the mitochondria and excreted from the cell. The excretion of CO_2 is necessary to maintain a physiological range of intracellular pH.

Carbon dioxide is transported through the plasma membranes as CO_2 and HCO_3^-. CO_2 can diffuse through cellular membranes, while HCO_3^- transport is mediated by transporters. A symporter co-transports HCO_3^- and H^+, while another antiporter co-transports Cl^- and HCO_3^- in the opposite direction. In some tissues, Na^+/HCO_3^- symporters play a major role in intracellular pH regulation.

When scaling up, the CO_2 level in culture is likely to increase in the late culture stage. This has an effect on the intracellular CO_2/HCO_3^- level and possibly the intracellular pH. As CO_2 builds up in the culture medium, extracellular HCO_3^- levels increase, thus reducing the concentration difference between the intracellular and extracellular levels of HCO_3^- (or the driving force of HCO_3^- excretion). To compensate, a higher Cl^- gradient is needed to achieve the necessary driving force for excreting HCO_3^- out of the cell. However, experimental evaluation of the effects of scale on cellular levels of CO_2 and intracellular pH is still lacking.

The increased accumulation of CO_2 in the medium also increases the need for a base addition to neutralize the pH and the associated change in osmolality. This further changes the chemical environment and possibly influences cell physiology.

Process Scale-Up and Scale-Down Models

The discussion above focuses on the reactor aspects of scale translation. Besides the reactor, many operations and pieces of auxiliary equipment are also affected by the process scale. For example, the fluid flow pattern and time duration involved in liquid transfer, the kinetics of the dissolution of media components, and the variability of raw materials all differ with the process scale. In cell recycle, the amount of time needed for cells to settle in the cell separator, and the frequency of passing through the device per day (thus, the frequency of cells' subjugation to shear stress incurred by pumping and temperature fluctuation), are likely to vary with the scale of operation. With the increasing practice of perfusion process, the impact of cell retention devices in large-scale operations should be examined. The throughput of the cell retention device should be kept in proportion to the process volume. In principle, this is accomplished by keeping the cell settling surface area in a settling device, or the transmembrane flow area in a membrane filtration device, proportional to the culture volume. This constraint can be met in small-scale reactors, but is difficult to implement at larger scales. In a high-throughput perfusion operation, multiple units of hollow fiber cell retention devices are sometimes needed in order to provide a sufficient surface for filtration. Another approach is to devise a more balanced nutrient feed that minimizes the accumulation of inhibitory metabolites and reduces the volumetric flow rate in large-scale operations.

A rational approach to scale translation is to establish a scale-down model that predicts the process kinetics and performance in large-scale operations or that reproduces large-scale process conditions (Panel 12.21). To establish a scale-down model, it is critical to identify the major physical and chemical factors that affect process performance. The physical factors that may affect cell growth, productivity, metabolism, and overall process performance include: the mechanical stress of agitation, the mechanical stress of aeration, the heat transfer feature (temperature gradient, speed of temperature change), and the mixing time. The chemical parameters include: the CO_2 level, the osmolality, the bulk salt profile (e.g., sodium), and the antifoam concentration. It is not possible to keep all physical parameters constant. A better understanding of the sensitivity or insensitivity of the cell and process to these parameters will allow for the selection of certain pivotal variables to be examined in a scale-down model. For example, one can vary the value of a pivotal variable over a given range in both small- and large-scale reactors while allowing less important variables to vary over a wider range. For chemical parameters, one can reproduce in the scale-down model a profile that was seen at a larger scale by the manipulation of the medium or through dynamic feeding.

Panel 12.21. Establishing a Scale-Down Model of a Production Process

- Identify the sensitivity/insensitivity of a cell line to major physical (e.g., impeller tip speed, energy input) and chemical (e.g., CO_2, lactate, hydroxybutyrate) factors in terms of the effects on growth, productivity, and metabolism, in both the early and late growth stages
- Evaluate the effects of major physical and chemical factors on quality attributes if the mode of action of the product calls for its evaluation
- For creating a scale-down model of an established production process:
  - Obtain the time profile and its range of variability of the physical and chemical factors with a high sensitivity
  - Reproduce the time profile of those factors, individually or in combination, in a scale-down model bioreactor while keeping the insensitive factors at conditions typically used in small scales
  - Evaluate the similarity of the growth and metabolic characteristics between the large-scale process and the scale-down model
  - Once a scale-down model has been established, it can be used to explore new process conditions
- For predicting the performance of a new process in a large-scale bioreactor:
  - Use the time profiles of the aeration rate, gas composition, dissolved oxygen and CO_2 levels, base addition, and osmolality in the large reactor to estimate the mass transfer characteristics of the large reactor
  - Obtain the metabolic profile of the cell line in a small bioreactor, including its metabolic rate of glucose, lactate, etc., oxygen uptake, and CO_2 production
  - Compute the time profile of the base addition and osmolality, CO_2, and key metabolites in a large-scale process
  - Perform experiments in the scale-down model to reproduce the computed time profile of the chemical environment
  - Perform experiments in the scale-down model to reproduce the profile of the physical factors with a high sensitivity
  - Use the data to define the range of operation conditions in the large-scale reactor

In the long term, a rational approach to scale translation will be to combine *in silico* process simulation with experimentation in a scale-down model (Figure 12.13). The process model will likely be a multiscale model that integrates intracellular metabolic metabolism with an empirical cell growth model and a reactor operation model. The metabolic model predicts how cells change their chemical environment through the consumption of nutrients and the production of metabolites. It also describes how cell metabolism adjusts to changing chemical environments. It is thus the heart of the model. The growth model charts out the trajectory of the process. The reactor model describes how external controls of oxygen, CO_2, base additions, and nutrient levels affect the chemical environment. The integrated multiscale model can combine experimentally determined metabolic, growth, and production behavior with the knowledge of large-scale reactor data on mass transfer characteristics to prescribe the operating conditions in a scale-down model that would optimize large-scale operations, or to predict their outcome.

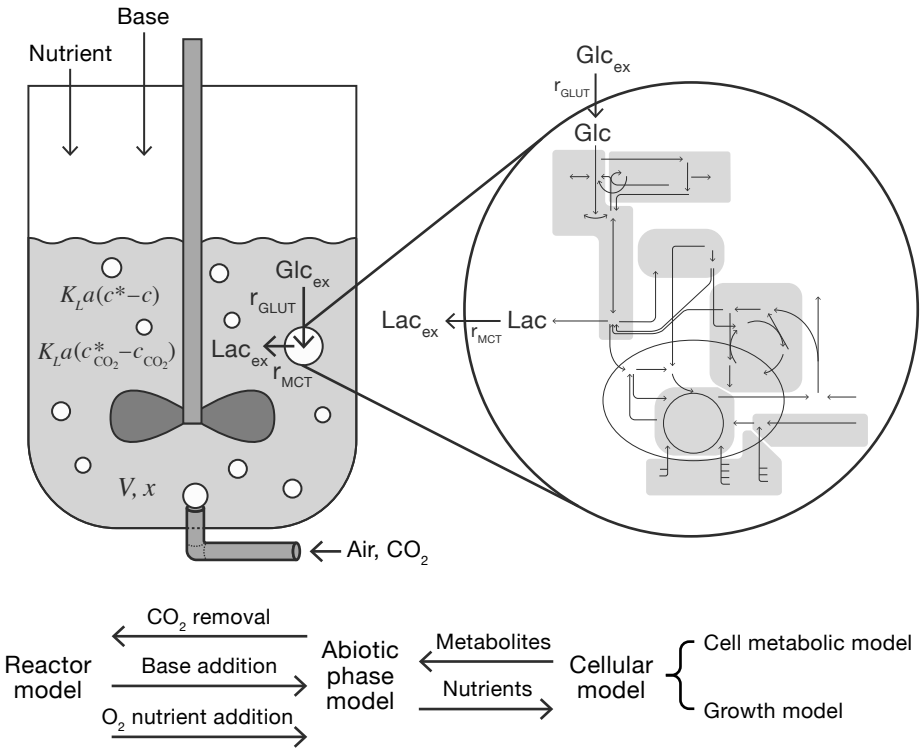


Figure 12.13. Depiction of a mathematical model for a cell culture process.

Concluding Remarks

In scale translation, the relationship of geometry-related physical parameters cannot all be kept constant. One must identify the relevant parameters that affect their cell's growth, physiology, and productivity, and focus on keeping those parameters in the acceptable range. In most cases, one chooses not to scale-up with complete geometrical similarity. Most large-scale reactors have a larger height to diameter ratio than smaller-scale ones. Nevertheless, the physical constraints on scaling up are the same regardless of whether one scales up geometrically similarly or not. In scaling up, the gas flow rate is likely to change in its proportion to the reactor volume. This causes the mass transfer characteristics to be different for different scales. While the dissolved oxygen can be controlled at the same level, the CO_2 concentration profiles are likely to differ for reactors of different scales. Differences in CO_2 concentration in the reactor cause pH control actions (including base and CO_2 addition and stripping) to vary at different scales. Differences in pH control actions may further change the chemical environment of the culture. Given that the physical and chemical parameters related to scaling up cannot easily be manipulated or controlled, one may resort to adapting cells