

CHAPTER 3

Centrifugation

Filtration:

Advantages

- * cake formation (dry)
- * less expensive equipment

The first step in most industrial bioseparations is the removal of insoluble solids from the fermentation beer. The concentration and size of these insolubles varies widely. The concentration can be as high as 60% per volume or as low as 0.1%. The size ranges from microorganisms of perhaps $1 \mu\text{m}$ diameter up to insoluble nutrients characteristically 1 mm in diameter.

When these insolubles are dilute, large, and rigid, they can be easily separated by filtration. In the previous chapter, we described filtration, including the use of filter aids. For many beers, these filter aids facilitate filtration. For other beers, filter aids are ineffective.

When the beers are not easily filtered, they sometimes can be separated by centrifugation, which is the subject of this chapter. Centrifugation requires more expensive equipment than filtration. However, it is often effective even when the solid particles are small and, hence, hard to filter.

Centrifugation utilizes the density difference between the solids and the surrounding fluid. When a suspension is allowed to stand, the denser solids slowly settle under the influence of gravity, a process called sedimentation. When this settling is accelerated with a centrifugal field, the process is

Centrifuge : Disadvantages:-

- * expensive
- * particles are separated as paste

called centrifugation. Because sedimentation and centrifugation are similar, both are discussed here.

The solid concentrate produced by centrifugation differs from that produced by filtration. At best centrifugation produces a paste; often it yields only a more concentrated suspension. Filtration produces a relatively dry cake, which is a major advantage. However, many biological feeds which can be centrifuged cannot be effectively filtered, so that centrifugation is often an attractive alternative.

We begin this chapter by describing in Section 3.1 the settling of particles both under gravity and under centrifugal forces. In Section 3.2, we describe the basic types of centrifuges and analyze the performance of two key types. In Section 3.3, we describe scale-up, that is, how the results of laboratory centrifugations can be used to plan large scale centrifugations. We conclude in Section 3.4 with a discussion of the basket centrifuge, which combines centrifugation and filtration.

3.1. SETTLING OF SOLIDS

When a solid particle moves through an infinite continuum, its velocity is affected by two forces. First, the particle is accelerated by the buoyant force resulting from the density difference between the particle and the fluid. Second, the particle is retarded by the drag force caused by this motion. Eventually, the buoyant force and the drag force become equal and the particle moves at a constant velocity.

These ideas are most easily quantified for spheres, which are a good first approximation of many biological particles. The buoyancy force acting on the spheres F_B is given by

$$F_B = \left[\frac{\pi d^3}{6} (\rho_s - \rho) \right] a \quad (3.1-1)$$

where d is the sphere's diameter, ρ_s and ρ are the density of the sphere and the fluid, respectively, and a is the acceleration due to some applied force. Note that the quantity in square brackets is the effective mass of the sphere, so that this equation parallels Newton's definition of force.

The drag force F_D acting on a single sphere in dilute solution is given by Stokes' law:

$$F_D = 3\pi d\mu v \quad (3.1-2)$$

3.1 Settling of Solids

where μ is the viscosity of the continuum and v is the sphere's velocity. This relation is accurate only if the sphere is small. Quantitatively, this means that

$$\frac{dv \rho}{\mu} < 1 \quad (3.1-3)$$

Only if

The dimensionless quantity $(dv \rho / \mu)$, called the Reynolds number, characterizes the flow around the sphere.

Equation (3.1-3) is almost always satisfied for biological solutes, so Eq. (3.1-2) will be basic to the analysis in this chapter. In passing, we note that replacements for Eq. (3.1-2) are available when $(dv \rho / \mu)$ is greater than 1. These replacements have the form:

$$F_D = f \left(\frac{1}{2} \rho v^2 \right) [(\pi/4) d^2] \quad \text{for } NRe > 1 \quad (3.1-4)$$

where f is a friction factor, graphs of which are a fixture of texts on fluid mechanics. We will not need to consider friction factors in this book.

When the spherical particle begins to move in solution, the drag force is small because the particle's velocity is small. As a result, the particle accelerates until the drag and buoyancy forces are equal. Under these conditions, we can combine Eqs. (3.1-1) and (3.1-2) to find

$$3\pi d \mu v = \frac{\pi d^3 (\rho_s - \rho)}{6} a \Rightarrow v = \frac{d^2}{18\mu} (\rho_s - \rho) a \quad (3.1-5)$$

This relation gives the steady state or terminal velocity of the sphere. As an exercise, note how the velocity changes if we double the particle diameter, the fluid's viscosity, or the particle density.

Two limits of Eq. (3.1-5) will be useful in the development to follow. First, for settling, the acceleration is obviously due to gravity g :

$$v_g = \frac{d^2}{18\mu} (\rho_s - \rho) g \rightarrow \text{for sedimentation} \quad (3.1-6)$$

Second, for centrifugation, the acceleration is different:

$$a = g$$

$$v_\omega = \frac{d^2}{18\mu} (\rho_s - \rho) \omega^2 r \quad (3.1-7)$$

$$a = \omega^2 r$$

where ω is the angular rotation in rad/sec, and r is the radial distance from the center of the centrifuge to the sphere. These two equations, which are basic to the rest of this chapter, are illustrated by the following examples.

Ques **Example 3.1-1. Separating Cells Growing on Support.** Many animal cells can be cultivated on the external surface of dextran beads. These cell-laden beads or "microcarriers" have a density of 1.02 g/cm^3 and a diameter of $d = 150 \mu\text{m}$. A 50 liter stirred tank is used to cultivate cells grown on microcarriers to produce a viral vaccine. After growth, the stirring is stopped and the microcarriers are allowed to settle. The microcarrier-free fluid is then withdrawn to isolate the vaccine. The tank has a liquid height to diameter ratio of 1.5; the carrier-free fluid has a density of 1.00 g/cm^3 and a viscosity of 1.1 cP .

- Estimate the settling time by assuming that these beads quickly reach their maximum terminal velocity.
- Estimate the time to reach this velocity.

Solution. (a) Using Eq. (3.1-6), we find that the terminal settling velocity v_g is

$$v_g = \frac{d^2}{18\mu} (\rho_s - \rho) g$$

$$= \frac{(0.015 \text{ cm})^2 (1.02 - 1.00) \text{ g/cm}^3 (980 \text{ cm/sec})^2}{18(0.011 \text{ g/cm sec})}$$

$$= 0.022 \text{ cm/sec}$$

Imp This is true only if Eq. (3.1-3) is correct. To check this, we insert the numerical values:

$$\text{Re} = \frac{dv \rho}{\mu}$$

$$= \frac{(0.015 \text{ cm})(0.022 \text{ cm/sec}) 1 \text{ g/cm}^3}{(0.011 \text{ g/cm sec})}$$

$$= 0.03 < 1.$$

3.1 Settling of Solids

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Thus use of Eq. (3.1-6) is justified. The liquid height can be found from the tank volume

$$\left. \begin{aligned} \frac{\pi}{4} d^2 l &= V \\ \frac{\pi}{4} \left(\frac{l}{1.5} \right)^2 l &= 50 \times 10^3 \text{ cm}^3 \\ l &= 52.3 \text{ cm} \end{aligned} \right\}$$

where l is the tank height and d is the tank diameter in just this one set of equations. The settling time is longest for particles which must move the entire height of the tank, that is,

$$\begin{aligned} t &= \frac{l}{v_g} \\ t &= \frac{52.3 \text{ cm} (\text{min}/60 \text{ sec})}{0.022 \text{ cm/sec}} \\ &= 39.6 \text{ min} \end{aligned}$$

It will take ~ 40 min for the microcarriers to completely settle.

(b) In part (a) we have neglected the time required for the beads to reach terminal velocity. Assuming the velocity of the microcarriers is originally zero, we find the change of settling velocity by a force balance on the particle

$$\frac{\pi d^3}{6} (\rho_s - \rho) \frac{dv}{dt} = F_B - F_D$$

Combining with Eqs. (3.1-1) and (3.1-2), we find for acceleration due to gravity

$$\begin{aligned} \frac{dv_g}{dt} &= g - \frac{3\pi d \mu v}{\pi d^3 / 6 (\rho_s - \rho)} \\ &= g - \frac{18\mu v}{d^2 (\rho_s - \rho)} \end{aligned}$$

This is subject to the initial condition that

$$t = 0, \quad v = 0$$

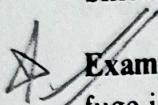
Integrating, we find

$$v_g = \left[\frac{g d^2 (\rho_s - \rho)}{18\mu} \right] [1 - \exp(-18\mu t / [d^2 (\rho_s - \rho)])]$$

which at high time gives Eq. (3.1-6). In our case, we see that for steady state

$$\begin{aligned} t \gg \frac{d^2 (\rho_s - \rho)}{18\mu} &= \frac{(0.015 \text{ cm})^2 (0.02 \text{ g/cm}^3)}{18(0.011 \text{ g/cm sec})} \\ &\gg 2 \times 10^{-5} \text{ sec} \end{aligned}$$

Since our settling time is 40 min, we easily meet this criterion.

 **Example 3.1-2. Centrifugation of Yeast Cells.** A laboratory bottle centrifuge is used to collect yeast cells after fermentation. The centrifuge consists of a number of cylinders rotated perpendicular to the axis of rotation. During centrifugation the distance between the surface of liquid and the axis of rotation is 3 cm, and the distance from the bottom of the cylinder to that axis is 10 cm. The yeast cells can be assumed to be spherical, with a diameter of $8.0 \mu\text{m}$ and a density of 1.05 g/cm^3 . The fluid has physical properties close to those of pure water. The centrifuge is to be operated at 500 r/min. How long does it take to have a complete separation?

Solution. From Eq. (3.1-7), we have that

$$\begin{aligned} \frac{dr}{dt} &= v_\omega \\ &= \frac{d^2}{18\mu} (\rho_s - \rho) \omega^2 r \end{aligned}$$

We are interested in the yeast cell which takes longest to settle, which is that starting near the liquid surface:

$$t = 0, \quad r = 3 \text{ cm}$$

Integrating the initial equation, we find

$$\ln \frac{r}{3 \text{ cm}} = \frac{d^2}{18\mu} (\rho_s - \rho) \omega^2 t$$

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Inserting the values given

$$\ln \frac{10 \text{ cm}}{3 \text{ cm}} = \frac{(8 \times 10^{-4} \text{ cm})^2}{18(0.01 \text{ g/cm sec})} \left(0.05 \frac{\text{g}}{\text{cm}^3} \right) \left(\frac{500 \times 2\pi^2}{60 \text{ sec}} \right),$$

$t = 2500 \text{ sec}$

This separation also takes about 40 min.

3.2. CENTRIFUGES

In this section, we first describe the three most useful types of centrifuges. We then show how the performance of two common types can be analyzed; the third type is deferred to Section 3.4. As in the rest of the book, we are most concerned with simple cases which allow the novice to get started; further details may be found in the specialized references at the end of the chapter.

The three basic types of centrifuges are shown in Figure 3.2-1. One important type is the tubular bowl, which is simple yet can provide a very high centrifugal force. Tubular centrifuges can be cooled, a real advantage

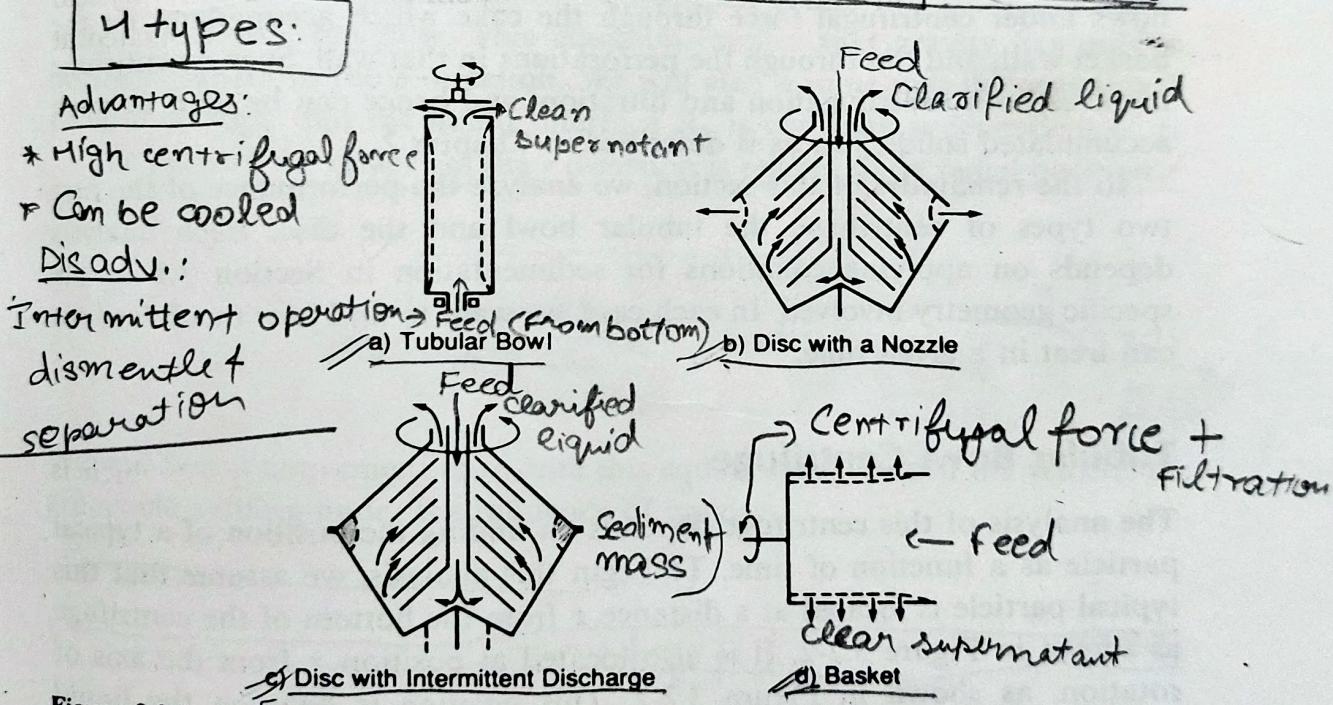


Figure 3.2-1. Four basic types of centrifuge. The bowl and disc types are discussed in this section, and the basket centrifuge is described in the following section. (After H. Hemfort, *Separators*, Westfalia, Oelde, 1984.)

in protein work. In this type, suspension is usually fed through the bottom, and clarified liquid is removed from the top. Solids deposit on the bowl's wall as a thick paste. The suspension can be fed until solid loss in the effluent becomes prohibitive; then the bowl must be dismantled and cleaned. Such intermittent operation may be a significant disadvantage.

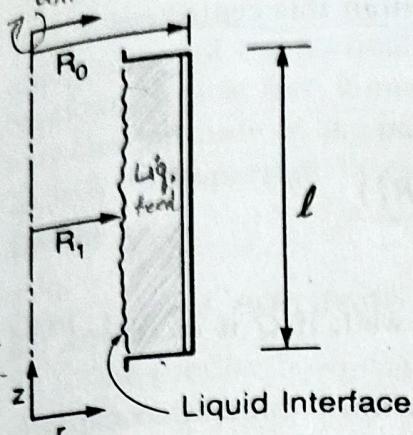
The second basic type of centrifuge is the disc type shown in Figures 3.2-1b and 3.2-1c. These centrifuges, probably the most common type for bioseparations, offer continuous operation at the price of complex equipment. The stacked conical discs allow a large sedimentation area to be contained in a relatively compact volume. Feed usually enters at the top and clarified liquid flows out an annular slit near the feed. The distinguishing characteristic between bowl types is the method of solids discharge. Solids are either removed intermittently, as in the tubular centrifuges, or continuously, out of orifices on the side of the centrifuge. If the solids are removed continuously from the sides, they usually have a higher liquid concentration than in the tubular bowl designs. Thus the nature of the packed solids dictates the type of discharge used.

The third basic type of centrifuge, the basket unit shown in Figure 3.2-1d, is really a combination of a centrifuge and a filter. It consists of a perforated basket which rotates rapidly. Suspension is again fed along the axis of the bowl and solids accumulate on the wall of the basket. Liquid flows under centrifugal force through the cake which accumulates on the basket wall, and out through the perforations in that wall. Such a centrifuge involves both centrifugation and filtration, and hence can be used to wash accumulated solids, just as is described in Chapter 2.

In the remainder of this section, we analyze the performance of the first two types of centrifuge, the tubular bowl and the disc. Each analysis depends on applying equations for sedimentation in Section 3.1 to the specific geometry involved. In each case, we want to find how much feed we can treat in a given time.

Tubular Bowl Centrifuge

The analysis of this centrifuge depends on finding the position of a typical particle as a function of time. To begin this analysis, we assume that this typical particle is located at a distance z from the bottom of the centrifuge as shown in Figure 3.2-2. It is also located at position r from the axis of rotation, as shown in Figure 3.2-2. This position is between the liquid surface R_1 and the bowl radius R_0 .

3.2 Centrifuges
axis of rotation

R_0 : bowl radius
 R_1 : Dist from the axis to b/w surface

Convection $\rightarrow z \uparrow$ $\frac{dz}{dt}$
Centrifuge $\rightarrow r \rightarrow$ $\frac{dr}{dt}$

Figure 3.2-2. Idealization of the tubular bowl centrifuge. Feed slowly flows in the bottom and out the top. Solids in the liquid are thrown outward by centrifugal force, and trapped against the bowl's wall, located at R_0 .

The particle is moving in both the z and r directions. Its movement in the z direction comes from convection of the feed pumped in the bottom of the centrifuge:

$$\frac{dz}{dt} \propto \frac{Q}{\pi(R_0^2 - R_1^2)}$$

$$\boxed{\frac{dz}{dt} = \frac{Q}{\pi(R_0^2 - R_1^2)}} \quad (3.2-1)$$

where Q is the feed flow. This equation implies that gravity has only a negligible effect in the z direction. We will also assume that the centrifugal force is so high that the liquid interface R_1 is constant, independent of z .

The particle movement in the r direction is related to its radial position r by Eq. (3.1-7):

$$\boxed{\frac{dr}{dt} = \frac{d^2}{18\mu} (\rho_s - \rho) r \omega^2} \quad (3.2-2)$$

We will find it convenient to rewrite this equation in terms of the velocity of a particle settling under the influence of gravity:

$$\frac{dr}{dt} = v_g \left(\frac{r \omega^2}{g} \right) \quad (3.2-3)$$

where v_g is the velocity given by Eq. (3.1-6). We now combine Eqs. (3.2-1)

and (3.2-2) to find the trajectory of a particle within this centrifuge:

Particle properties: v_g

centrifugal: $r, \omega, g, R_0, R,$
properties

$$\frac{dr}{dz} = \frac{dr/dt}{dz/dt} = v_g \left(\frac{r\omega^2}{g} \right) \frac{\pi(R_0^2 - R_1^2)}{Q} \quad (3.2-4)$$

If v_g is large, the particle will quickly reach the wall; if Q is increased, the particle will be swept farther up the tube.

We now focus on those particles which are most difficult to capture. These particles enter the centrifuge at $r = R_1$ and do not reach $r = R_0$ until the end of the unit, at $z = \ell$. We can integrate Eq. (3.2-4) for these hard-to-catch particles to find, after some rearrangement,

$$Q = \frac{\pi\ell(R_0^2 - R_1^2)v_g\omega^2}{g \ln(R_0/R_1)} \quad (3.2-5)$$

This important result gives the maximum flow possible in the centrifuge as a function of both particle properties (collected in v_g) and centrifuge characteristics (in ℓ, R_0, R_1 , and ω). In most tubular centrifuges, this equation can be simplified because R_0 and R_1 are approximately equal. As a result,

$$\begin{aligned} \frac{R_0^2 - R_1^2}{\ln(R_0/R_1)} &= \frac{(R_0 + R_1)(R_0 - R_1)}{\ln[1 + (R_0 - R_1)/R_1]} \quad \text{let } \ln(1+x) \\ &= \frac{(R_0 + R_1)(R_0 - R_1)}{[(R_0 - R_1)/R_1 + \dots]} \quad n \rightarrow 0 \\ &= R_1(R_0 + R_1) \\ &= 2R^2 \end{aligned} \quad (3.2-6)$$

where R is an average radius, about equal to R_0 or R_1 . Thus Eq. (3.2-5) becomes

$$\begin{aligned} Q &= v_g \left[\frac{2\pi\ell R^2 \omega^2}{g} \right] \\ &= v_g [\Sigma] \quad \uparrow \\ &\quad \text{dimension of } \ell^2 \end{aligned} \quad (3.2-7)$$

The velocity v_g is a function only of the particles themselves, and is independent of the particular centrifuge being used. The quantity in square brackets, which has dimensions of (length)², is not a function of the particles but only of the particular centrifuge. This quantity, symbolized as Σ , will be important in our comparison of centrifuges given in the next section.

Disc Type Centrifuge

As in the tubular bowl centrifuge, our objective is to find the position of a given particle as a function of time. Now, however, the geometry of the centrifuge is more complex, as shown in Figure 3.2-3. We consider a particle located at position (x, y) , where x is the distance from the edge of the outer discs along the gap between the discs, and y is the distance normal to the lower disc. The outer edge of the discs is at R_0 and the inner edge is at R_1 . Liquid is fed into the centrifuge so that it flows upward through the gap between the discs, entering at R_0 and leaving at R_1 .

The particle is moving both in the x and y directions. Its velocity in the x direction is due to convection and to sedimentation:

$$\frac{dx}{dt} = v_0 - v_\omega \sin \Theta \quad (3.2-8)$$

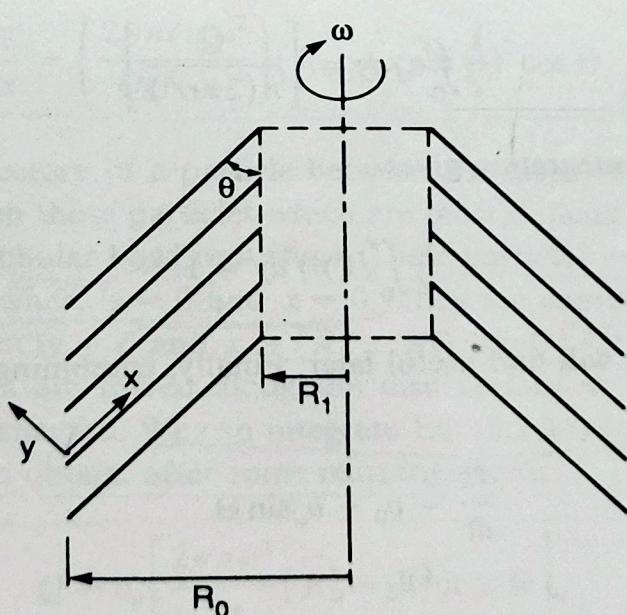


Figure 3.2-3. Idealization of disc centrifuge. Feed flows in the y direction; suspended solids are hurled outward toward the discs. This type of unit is very commonly used in bioseparations.

where v_0 is the convective liquid velocity, v_ω is the particle's velocity under centrifugation, and Θ is the angle at which the discs are tilted from vertical. Note that if Θ were zero, the particle would move only by convection, and this equation would be equivalent to Eq. (3.2-1).

The velocity v_0 has three important characteristics. First, under most conditions, it is much larger than the sedimentation velocity $v_\omega \sin \Theta$. Second, it is a function of radius, and gets bigger as the fluid flows inward toward the axis of rotation. This is because the total flow Q is a constant, but the area for flow gets smaller as the axis is approached. Third, v_0 is a function of y , for it must reach zero at the disc surfaces. These three characteristics lead us to assume that

$$v_0 = \left[\frac{Q}{n(2\pi r \ell)} \right] f(y) \quad (3.2-9)$$

where Q is again the total flow, n is the number of discs, r is the distance from the axis of rotation, ℓ is the distance between discs (measured normal to the disc surfaces), and $f(y)$ is some function giving the velocity variation across the distance between discs. Note that the quantity in brackets, the average convective velocity, includes the variation of v_0 with r and, hence, with x . Note also that from a mass balance, the volume of v_0 averaged over y must equal this convective velocity

$$\frac{1}{\ell} \int_0^\ell v_0 dy = \left[\frac{Q}{n(2\pi r \ell)} \right] \quad (3.2-10)$$

Performing this integration gives

$$\frac{1}{\ell} \int_0^\ell f(y) dy = 1 \quad (3.2-11)$$

a result which we will find useful later. Finally, combining Eqs. (3.2-8) and (3.2-9), we find

$$\begin{aligned} \frac{dx}{dt} &= v_0 - v_\omega \sin \Theta \\ &\doteq v_0 \\ &= \left[\frac{Q}{n(2\pi r \ell)} \right] f(y) \end{aligned} \quad (3.2-12)$$

Again, remember that we are recognizing that the convective velocity is much greater than that of sedimentation.

We now turn to motion in the y direction. From Figure 3.2-3, we see that

$$\frac{dy}{dt} = v_w \cos \Theta \quad (3.2-13)$$

From Eqs. (3.1-6) and (3.1-7), we can rewrite this as

$$\frac{dy}{dt} = v_g \left(\frac{\omega^2 r}{g} \right) \cos \Theta \quad (3.2-14)$$

We combine this result with Eq. (3.2-12) to find

$$\begin{aligned} \frac{dy}{dx} &= \frac{dy/dt}{dx/dt} \\ &= \left(\frac{2\pi n \ell v_g \omega^2}{Qgf(y)} \right) r^2 \cos \Theta \end{aligned} \quad (3.2-15)$$

Finally, we note from Figure 3.2-3 that r equals $(R_0 - x \sin \Theta)$, so that

$$\frac{dy}{dx} = \left(\frac{2\pi n \ell v_g \omega^2}{Qgf(y)} \right) (R_0 - x \sin \Theta)^2 \cos \Theta \quad (3.2-16)$$

This gives the trajectory of a particle between the discs of this centrifuge.

We now focus on those particles which are most difficult to capture, just as we did for the tubular bowl centrifuge. These particles enter at the outer edge of the discs, where $y = 0$ and $x = 0$. They are captured at the inner edge of the discs, at $y = \ell$ and $x = (R_0 - R_1)/\sin \Theta$. After capture, they and other particles are forced along the disc surface to the outer edge, where they are discharged. We can integrate Eq. (3.2-16) for these hard-to-capture particles to obtain, after some rearrangement,

$$\begin{aligned} Q &= v_g \left[\frac{2\pi n \omega^2}{3g} (R_0^3 - R_1^3) \cot \Theta \right] \\ &= v_g [\Sigma] \end{aligned} \quad (3.2-17)$$

In finding this result, we have used Eq. (3.2-11). Equation (3.2-17) for the disc centrifuge completely parallels Eq. (3.2-7) for the tubular centrifuge. In both cases, the quantity v_g is a function only of the particle properties, and not those of the centrifuge. In both cases, the quantity in square brackets has dimensions of (length)², is not a function of particle properties, and does reflect the geometry of the centrifuge. These two equations are basic to the examples which follow.

Ques **Example 3.2-1. Tubular Centrifugation of *E. coli*.** A bowl centrifuge is used to concentrate a suspension of *Escherichia coli* prior to cell disruption. The bowl of this unit has an inside radius of 12.7 cm and a length of 73.0 cm. The speed of the bowl is 16,000 r/min and the volumetric capacity is 200 liters/hr. Under these conditions, this centrifuge works well. Q

- (a) Calculate the settling velocity v_g for the cells.
- (b) After disruption the diameter of debris is about one-half of the original cell diameter and the viscosity is increased four times. Estimate the volumetric capacity of this same centrifuge operating under these new conditions.

Solution. (a) We use Eq. (3.2-7) to answer these questions. Rearranging this equation gives

$$v_g = \frac{Qg}{2\pi\ell R^2\omega^2}$$

$$v_g = \frac{200 \times 10^3 \text{ cm}^3/\text{hr} (980 \text{ cm/sec}^2)(\text{hr}/3600 \text{ sec})}{2\pi(73 \text{ cm})(12.7 \text{ cm})^2([2\pi(16,000)]/60 (\text{sec/min}) \text{ min})^2}$$

$$= 2.6 \times 10^{-7} \frac{\text{cm}}{\text{sec}}$$

The settling velocity is greatly accelerated by centrifugation.

(b) The changes occurring after disruption affect only items in the terminal velocity term. Thus we can take the ratio of Eq. (3.2-7) for two situations:

$$\frac{Q(\text{after rupture})}{Q(\text{before rupture})} = \frac{v_g(\text{after rupture})}{v_g(\text{before rupture})}$$

3.2 Centrifuges

From Eq. (3.1-6), we find

$$\begin{aligned}\frac{Q(\text{after rupture})}{Q(\text{before rupture})} &= \frac{[d^2/\mu](\text{after rupture})}{[d^2/\mu](\text{before rupture})} \\ &= \left(\frac{1}{2}\right)^2 / \left(\frac{4}{1}\right) \\ &= \frac{1}{16}\end{aligned}$$

The reduced particle size and the increased viscosity dramatically reduce the capacity of the centrifuge.

 **Example 3.2-2. Disc Centrifugation of Chlorella.** Chlorella cells are being cultivated in an open pond. We plan to harvest this biomass by passing the dilute stream of cells through an available disc bowl centrifuge. The settling velocity v_g for these cells has been measured as 1.07×10^{-4} cm/sec. The centrifuge has 80 discs with an angle of 40° , an outer radius of 15.7 cm, and an inner radius of 6 cm. We plan to operate the centrifuge at 6000 r/min. Estimate the volumetric capacity Q for this centrifuge.

Solution. We use Eq. (3.2-17) to estimate this capacity:

$$Q = v_g \left[\frac{2\pi n \omega^2}{3g} (R_0^3 - R_1^3) \cot \Theta \right] \quad \checkmark$$

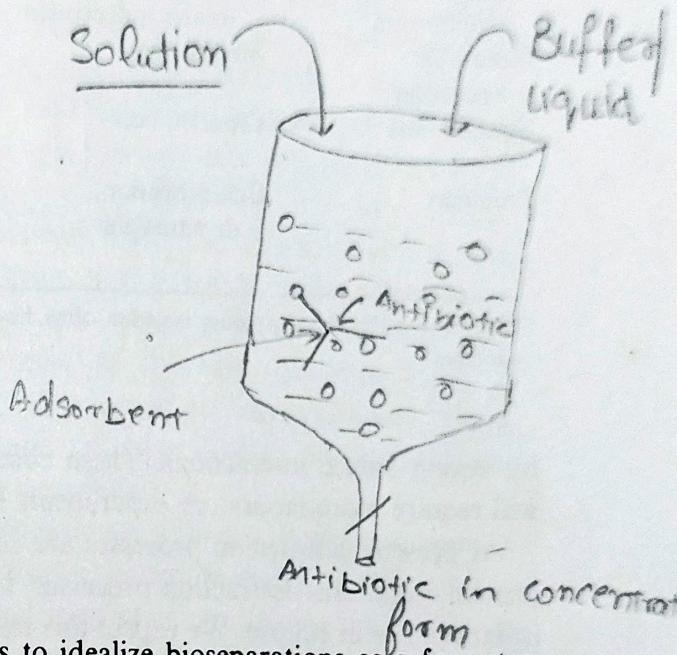
Substituting the values given into this relation we have

$$\begin{aligned}Q &= 1.07 \times 10^{-4} \frac{\text{cm}}{\text{sec}} \left\{ \frac{2\pi(80)}{3(980 \text{ cm/sec}^2)} \left[\frac{2\pi(6000)}{60 (\text{sec/min}) \text{ min}} \right]^2 \right. \\ &\quad \left. \times [(15.7 \text{ cm})^3 - (6 \text{ cm})^3] \cot(40^\circ) \right\} \\ &= 3.1 \times 10^4 \frac{\text{cm}^3}{\text{sec}} = 31 \frac{\text{liters}}{\text{sec}}\end{aligned}$$

This centrifuge is adequate only for a small pond.

CHAPTER 6

Adsorption



The scheme used in this book is to idealize bioseparations as a four step process: removal of insolubles, product isolation, product purification, and product polishing. This chapter is the second of two dealing with product isolation. As such, it is concerned with the concentration of highly dilute aqueous solutions.

The subject of the chapter is adsorption. Adsorption occurs when a dissolved solute is bound to a solid adsorbent. A typical process involves four steps. First, a feed solution is added to the adsorbent. Second, adsorption occurs, a process which may be both selective and slow. Third, the spent feed solution is withdrawn. Fourth, the adsorbed solute is eluted, often using a different solvent.

Adsorption is compared with extraction in Table 6.0-1. Both processes are used to isolate solutes from dilute solutions. Adsorption tends to have a smaller capacity but a higher selectivity than extraction. It can be more gentle: Proteins can be adsorbed without the denaturation often produced by organic extraction. Adsorption can sometimes work with a whole beer obtained by fermentation, thus bypassing filtration or centrifugation. The design of adsorption processes is complicated by nonlinear equilibria and

TABLE 6.0-1. A Comparison Between Adsorption and Extraction*

Variable	Extraction	Adsorption
Capacity	High	Low
Selectivity	Moderate	High
Nature of equilibrium	Often linear; dilute solutes independent	Usually nonlinear; dilute solutes interact
Nature of operation	Steady state <i>→ means continuous</i>	Unsteady; periodic
State of feed	Clarified beer	Clarified or whole beer
Problems	Emulsification; denaturation	Solids handling; uneven bed packing; compressible packing

* These two methods of product isolation often have complimentary advantages and disadvantages.

by strong solute interactions. These complications mean that adsorption will require more laboratory experiments than extraction.

At present, adsorption processes are ascendant, preferred especially for protein isolations. Extraction processes, long the workhorse for antibiotic isolation, are in eclipse. We expect this trend to continue over the next few years, but we are unsure what will happen over a longer time. We suspect that adsorption's low capacity and troublesome solids handling will rekindle interest in extraction.

In this chapter, we begin in Section 6.1 by discussing the chemistry of adsorption and the means by which that chemistry is organized. In Section 6.2, we briefly consider batch adsorptions, which closely parallel batch extractions. These batch adsorptions are most easily designed using graphical methods. We turn in Section 6.3 to adsorption in stirred tanks, a method best suited to whole beer processing. Finally, we discuss fixed bed adsorption in Section 6.4. This method is the most common, but it is also the most difficult to design.

6.1. THE CHEMISTRY OF ADSORPTION

Adsorption requires adsorbents, solid materials to which solutes of interest bind reversibly. In the following paragraphs, we first consider which adsorbents are used and then how much they will adsorb.

6.1 The Chemistry of Adsorption

Common Adsorbents

While a large number of adsorbents are used industrially, only two major groups are used for biological molecules: carbons and synthetic resins. The carbons used are commercial products and are not manufactured specifically for bioseparations. A wide variety of products is available, with varying porosity and capacity. The more effective are made from vegetable sources like sawdust or fruit pits; carbons made from mineral sources can contain inorganic impurities which catalyze degradation of some solutes.

Ion exchange resins are largely based on synthetic polymers. The polymers usually contain fixed charges like $-SO_3^-$, $-COO^-$, or $-NR_3^+$; however, they can effectively adsorb both ionic and nonionic solutes. Resins made from styrene and divinylbenzene often adsorb nonpolar solutes most strongly; resins based on acrylic esters tend to be more effective for hydrophilic solutes. Adsorbents based on hydrogels are often made of polyacrylamides, which are the mainstay of gel electrophoresis. Resin manufacturers are eager to synthesize custom adsorbents, which are expensive and often do not last as long as expected.

Other adsorbents are less frequently used. Natural and synthetic zeolites used for adsorption tend to be effective for low molecular weight solutes. Clays are rarely used. Silica gel and alumina can be used to remove water, but these are rarely a good choice for the dilute solutions common here.

Adsorption Isotherms

Like extraction, the analysis of adsorption is based on equilibria and on mass balances. The equilibria are presented not as partition coefficients but as adsorption isotherms. Typical isotherms are shown in Figure 6.1-1. For each isotherm, the abscissa gives the solute concentration in the solution, usually in units of mass of solute per volume of solution. The ordinate gives the solute concentration on the adsorbent's surface, most commonly in units of mass of solute per mass of adsorbent. Any adsorbent isotherm which is concave toward the abscissa is said to be "favorable," for strong adsorption occurs even in dilute solution. Any isotherm concave toward the ordinate is described as "unfavorable."

The three isotherms shown in Figure 6.1-1 all occur in bioseparations. The linear isotherm is given by

$$q = Ky \quad (6.1-1)$$

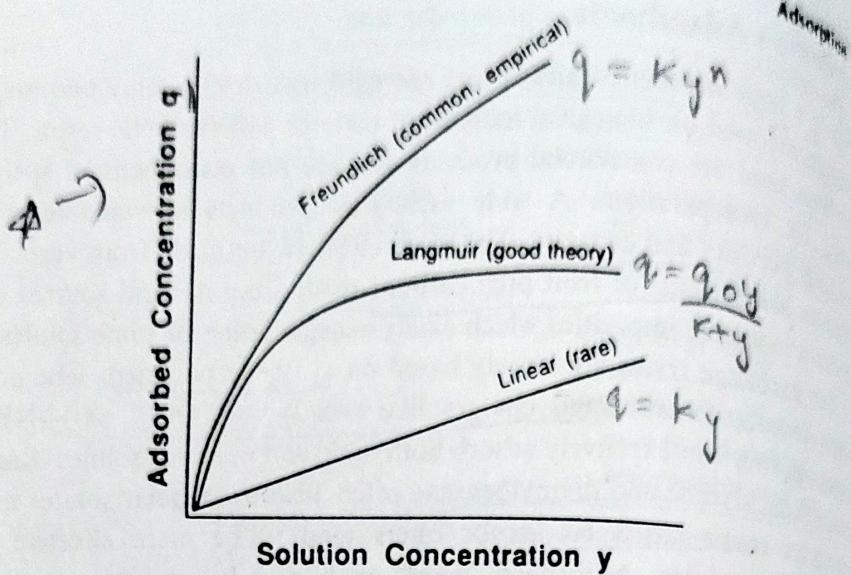


Figure 6.1-1. Common adsorption isotherms. Most useful isotherms are nonlinear and curve downward; these are termed "favorable." Note all can be approximated as linear in highly dilute solution.

where q is the amount of solute adsorbed per amount of adsorbent, y is the solute concentration in solution, and K is again an equilibrium constant. Like the partition constant used in extraction, K is a thermodynamic quantity; unlike the partition coefficient, K may have dimensions, frequently of solution volume per adsorbent mass. While the linear isotherm is not common, it may approximate the other isotherms over a limited range of concentration.

The empirical Freundlich isotherm describes the adsorption of a wide variety of antibiotics, steroids, and hormones. It is given by

$$\checkmark \boxed{q = Ky^n} \quad (6.1-2)$$

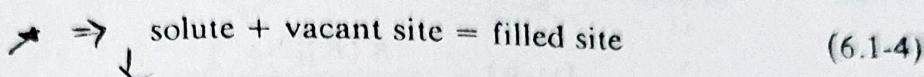
where the constants n and K must be determined experimentally. These constants are best determined by means of a log-log plot of q versus y . The slope of such a plot is the dimensionless exponent n . The dimensions of K depend on the value of n . If the adsorption is favorable, then $n < 1$; if it is unfavorable, then $n > 1$.

Finally, the Langmuir isotherm is often used to correlate adsorption data for proteins. It is given by

$$\checkmark \boxed{q = \frac{q_0 y}{K + y}} \quad (6.1-3)$$

in which q_0 and K are again constants which must be determined experimentally. In this case, the easiest way to do so is to plot q^{-1} versus y^{-1} . The intercept on such a plot is q_0^{-1} and the slope is K/q_0 . The dimensions of q_0 and K for this isotherm are the same as those of q and y , respectively.

Unlike the Freundlich isotherm, the Langmuir isotherm has a strong theoretical basis. This basis relies on a postulated chemical reaction between solute and vacant sites on the adsorbent surface:



If this reaction is at equilibrium, then it is described by an equilibrium constant K'

$$\checkmark K' = \frac{[\text{solute}][\text{vacant sites}]}{[\text{filled sites}]} \quad (6.1-5)$$

Moreover, the total number of active sites must be fixed

$$\checkmark [\text{total sites}] = [\text{vacant sites}] + [\text{filled sites}] \quad (6.1-6)$$

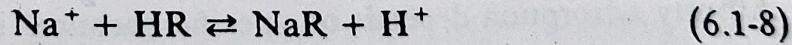
Combining the last two equations, we find

$$\checkmark [\text{filled sites}] = \frac{[\text{total sites}][\text{solute}]}{K' + [\text{solute}]} \quad (6.1-7)$$

Since the number of filled sites is proportional to q , Eq. (6.1-7) is equivalent to Eq. (6.1-3). Thus the maximum value of q_0 reflects the limited number of active sites on the adsorbent.

Ion Exchange Isotherms

Isotherms for ion exchange are rationalized in a similar way. For example, consider an ion exchange reaction on a resin, for which



where HR and NaR represent ion exchange sites filled with a proton and a sodium ion, respectively. This implies that all sites are filled, with either a

Solutes which are strongly adsorbed over several sites may be sterically inhibited; solutes which are macromolecular may be excluded from small pores. As a result, adsorption of biological solutes can frequently involve less than 10% of the total sites.

In this situation, experiments are imperative. We cannot stress this strongly enough: You must not rely on literature values or manufacturers' data. Your solutions will not behave like a "standard." In this regard, adsorption is different from extraction, where literature values are reliable. For adsorption, make your own determinations.

Class

Example 6.1-1. Gentamicin Adsorption on Carbon. We have measured the adsorption of the antibiotic gentamicin from water at pH 9.5 onto a nonionic carbon. We find the following results:

Graph
to be
drawn

y (mg/liter)	q (mg/cm ³)
0.01	5.3
0.02	7.1
0.03	8.3
0.06	11.1
0.10	13.7

slope
Calc. K ?

What adsorption isotherm fits these data?

Solution. The data are not linear on Cartesian coordinates. They are linear on a log y versus log q plot as shown in Figure 6.1-3. They are not linear if q^{-1} is plotted against y^{-1} . As a result, they appear to fit the Freundlich isotherm, but not the Langmuir isotherm. A note of caution: in many cases, the data will scatter and there will be no reason to choose one isotherm over the other.

Class

Example 6.1-2. Immunoglobulin G Adsorption. 80 cm³ of modified dextran will adsorb up to 7.8×10^{-6} mol of immunoglobulin G per cubic centimeter of adsorbent. This adsorption follows a Langmuir isotherm, with a constant K of 1.9×10^{-5} mol/liter. What concentration in 1.2 liters of feed solution will exhaust 90% of the dextran's capacity?

Solution. For 90% recovery, we expect that

$$y = 0.1 y_F$$

$$q = \frac{a_0 y}{K + y}$$

Adsorption

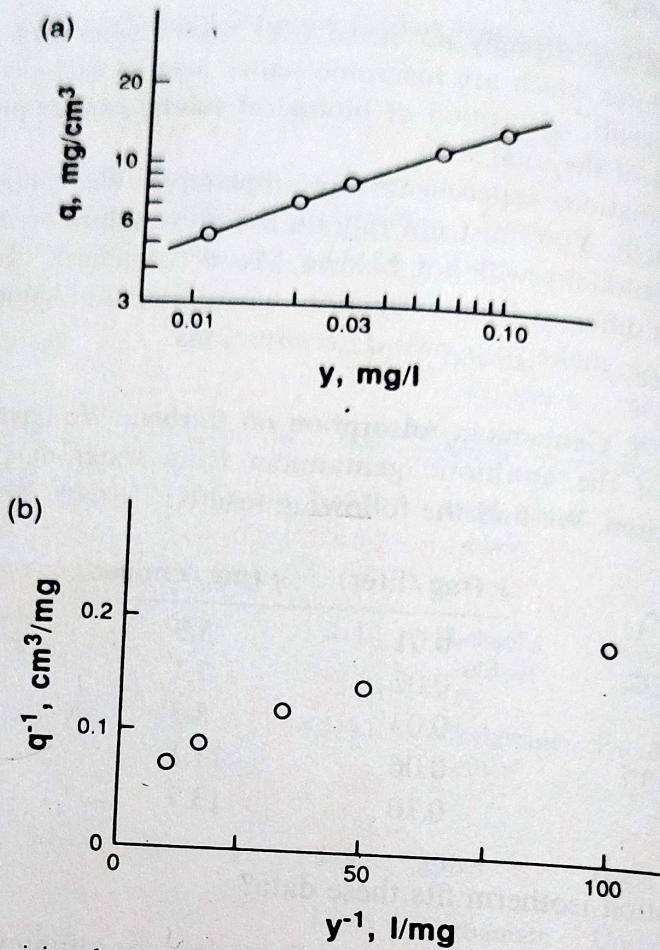


Figure 6.1-3. Gentamicin adsorption. These data, basic to Example 6.1-1, are consistent with the Freundlich isotherm, as shown in (a). They are inconsistent with a Langmuir isotherm as tested in (b).

$$q = \frac{q_0 y}{K + y}$$

From the adsorption data given

$$q = \frac{(7.8 \times 10^{-6} \text{ mol/cm}^3)0.1y_F}{(1.9 \times 10^{-5} \text{ mol/liter} + 0.1y_F)}$$

From a mass balance,

$$1.2 \text{ liters } y_F = 1.2 \text{ liter}(0.1)y_F + 80 \text{ cm}^3 q$$

These equations can be combined to give

$$y_F = 3.9 \times 10^{-4} \text{ mol/liter}$$

This is a concentrated protein solution.

6.2 Batch Adsorption

6.2. BATCH ADSORPTION

Like batch extraction, batch adsorption depends on two basic constraints, that of equilibrium and that of a mass balance. The equilibrium constraint is just the isotherm, for example,

$$q = Ky^n \quad (6.2-1)$$

Unlike batch extraction, this "equilibrium line" is usually nonlinear. The mass balance is given by

$$y_F H + q_F W = yH + qW \quad (6.2-2)$$

where y and y_F are the final and feed concentrations in solution, q and q_F are the final and feed concentrations on the adsorbent, H is the amount of feed, and W is the amount of adsorbent. This equation is easily rearranged:

If Solute conc. in the adsorbent (at t=0)

$$q = q_F + \frac{H}{W}(y_F - y) \quad \begin{array}{l} \text{Mass balance} \\ \text{eqn:} \end{array} \quad (6.2-3)$$

Like batch extraction, this "operating line" is linear.

The solution of Eqs. (6.2-1) and (6.2-3) can be found graphically or numerically. Each is easy. For example, for the graphical solution, we plot both of these equations on the same coordinates, as shown in Figure 6.2-1. The equilibrium line shown has a zero intercept and curvature characteristic of $n < 1$, that is, of "favorable" adsorption. The operating line has a

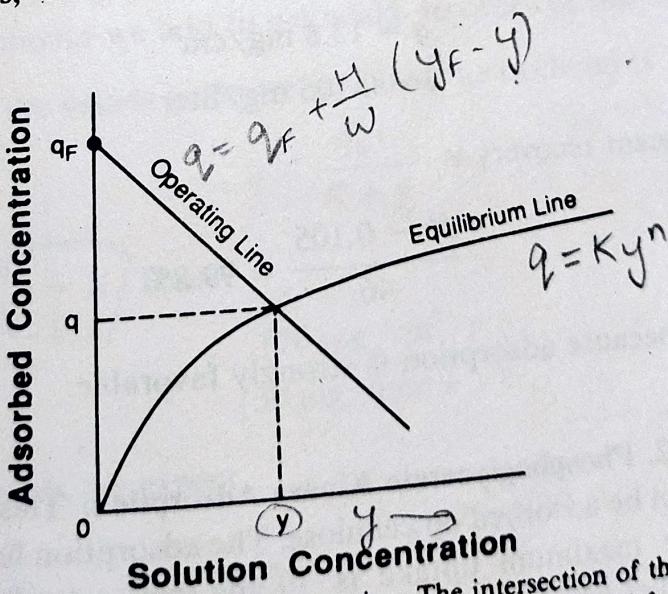


Figure 6.2-1. Graphical solution of batch adsorption. The intersection of the equilibrium and operating lines gives the desired result. This result is a complete parallel for batch extraction (cf. Fig. 5.2-2).

negative slope and an intercept with the ordinate at $q = (q_F + (H/W)y_F)$. The intersection of these two lines (y, q) represents the solution concentration and the adsorbent loading at equilibrium.

This straightforward calculation is illustrated in the following examples.

Example 6.2-1. Gentamicin Adsorption. From earlier experiments, we have found that gentamicin adsorption on activated carbon is described by the following isotherm (see Fig. 6.1-3):

$$q = 35.1y^{0.41}$$

where q is in mg/cm^3 carbon and y is in mg/liter . We plan to add 10 cm^3 of previously unused carbon to 3.0 liters of a fermentation beer containing 46 mg/liter antibiotic. What percent recovery can we expect?

Solution. The operating line is easily calculated from Eq. (6.2-3):

$$\begin{aligned} q &= \frac{H}{W}(y_F - y) + q_F^0 \\ &= \frac{3.0 \text{ liters}}{10 \text{ cm}^3} (46 \text{ mg/liter} - y) \\ &= 13.8 - 0.3y \end{aligned}$$

Both this line and the equilibrium isotherm are plotted in Figure 6.2-2. The intersection gives

$$q = 13.8 \text{ mg}/\text{cm}^3$$

$$y = 0.105 \text{ mg/liter}$$

Thus the percent recovery is

$$\frac{46 - 0.105}{46} = 99.8\%$$

This is high because adsorption is strongly favorable.

class
Example 6.2-2. Phosphoglycerate Kinase Adsorption. This enzyme, isolated from yeast, can be adsorbed on cellulose. The adsorption follows a Langmuir isotherm. The maximum uptake is $70 \text{ mg}/\text{cm}^3$ adsorbent; half of this maximum occurs when the solution contains 50 mg/liter of the enzyme.

Q contd.

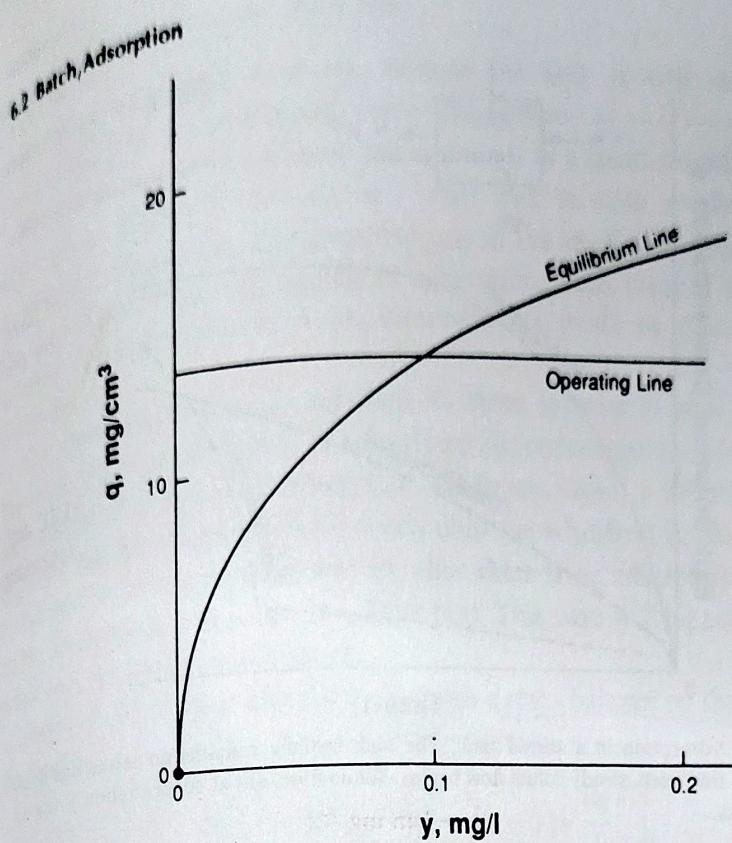


Figure 6.2-2. Batch adsorption of gentamicin. The operating line in this case is nearly horizontal because of the large mass of adsorbent which is used.

We have 1.5 liters of feed containing 220 mg/liter of this enzyme. How much cellulose should we add to get a 90% recovery of this solute?

Solution. From the values given we find that the isotherm is

$$q = \frac{q_0 y}{K + y}$$

$q_0 \rightarrow$ maximum solute conc. that can be absorbed

When $q = \frac{1}{2}q_0$, $y = K$. Thus

$$q = \frac{(70 \text{ mg/cm}^3)y}{(50 \text{ mg/liter} + y)}$$

For a 90% recovery, we expect

$$y = 0.1y_F = 22 \text{ mg/liter}$$

so

$$q = 21.4 \text{ mg/cm}^3$$

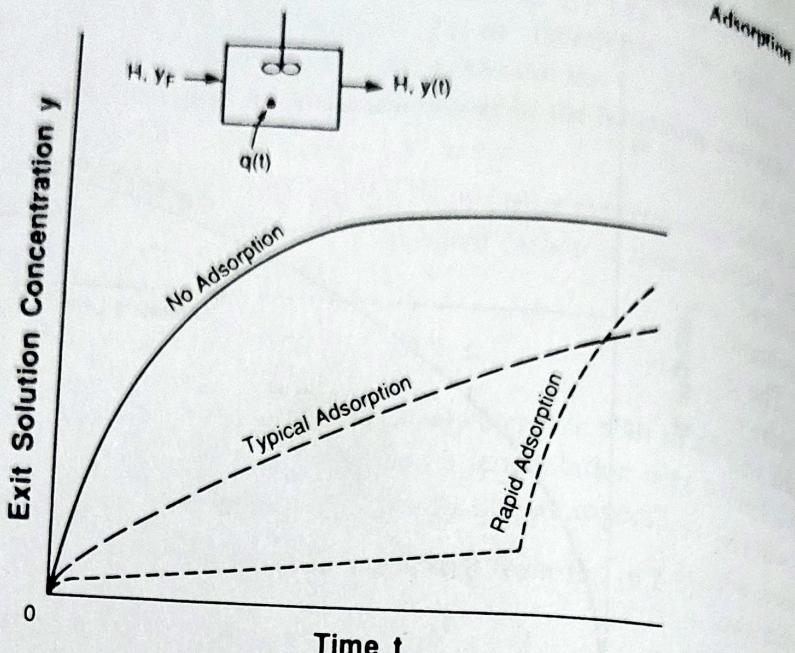


Figure 6.3-1. Adsorption in a stirred tank. The tank initially contains no solute and fresh adsorbent. At time zero, steady solute flow begins. Solute flows out at concentration y , but no

Thus from a mass balance like Eq. (6.2-2),

$$y_F + 1.5 \text{ liters}(220 \text{ mg/liter}) = 1.5 \text{ liters}(22 \text{ mg/liter}) + W(21.4 \text{ mg/cm}^3)$$

$$W = 14 \text{ cm}^3 \text{ cellulose}$$

A greater recovery will of course require more adsorbent.

No numerical question from this part →

6.3. ADSORPTION IN A CONTINUOUS STIRRED TANK

We now turn from batch adsorption to adsorption in a continuous stirred tank. Such a tank is sometimes called a continuous stirred tank reactor (CSTR). A process like this tends to be used for larger scale separations than those carried out in batch. It can be effectively used for whole beer processing, thus dodging the issue of removing insolubles.

The way in which this kind of adsorption commonly occurs is shown in the inset of Figure 6.3-1. Feed enters the tank continuously at a flow rate H and a constant concentration y_F . The tank initially contains pure solvent and unloaded adsorbent W ; the concentration of solute on the adsorbent q varies with time. Solution flows steadily out of the tank, but its concentra-

concentration varies with time. However, because the tank is well stirred, the concentration in the tank equals that in the outflow.

We commonly will have made measurements in a small stirred tank and will want to predict the behavior in a larger tank. In other words, we want to scale up the process. The measurements in the small tank are essential, for they characterize the kinetics of adsorption. Such kinetics cannot be inferred from equilibrium batch measurements made to determine the isotherm.

The qualitative behavior expected in these systems is also shown in Figure 6.3-1. Even if there is no adsorption, the concentration y leaving the tank will vary with time. If adsorption is infinitely rapid, y will rise quickly to a low value and then increase slowly until the adsorbent is loaded. Then y will rise in much the same way as when there is no adsorption. In most cases, adsorption will occur at a finite rate. This case will lie between the other two, as shown in the figure.

The analysis of these kinetics begins with a mass balance on the solute in the liquid:

$$\boxed{\epsilon V \frac{dy}{dt} = H(y_F - y) - (1 - \epsilon)V \frac{dq}{dt}} \quad (6.3-1)$$

where V is the volume of the tank, y and y_F are the solute concentrations in the effluent and the feed, respectively, H is the feed rate, and q is the adsorbed solute concentration. A similar mass balance on the adsorbent gives

$$\boxed{(1 - \epsilon)V \frac{dq}{dt} = Vr} \quad \Rightarrow r = (1 - \epsilon) \frac{dq}{dt} \quad (6.3-2)$$

Exam
where r is the rate of adsorption per volume of tank. The only remaining step is an equation for r .

But what is r ? To answer that question, we must know the mechanism responsible for the kinetics of adsorption. Two limiting mechanisms are common:

- C1. Adsorption is controlled by diffusion from the solution to the adsorbent.
- C2. Adsorption is controlled by diffusion and reaction within the adsorbent particles.

The characteristics of these mechanisms are given in the following paragraphs.

Adsorption
When diffusion in the solution controls the adsorption, the rate r is given by

G

$$\boxed{r = ka(y - y^*)} \quad (6.3-3)$$

where k is a mass transfer coefficient, a is the surface area of adsorbent per tank volume, and y^* is a hypothetical concentration in the solution which would be in equilibrium with the adsorbent. For example, if adsorption follows the Freundlich isotherm,

$$\boxed{q = K(y^*)^n} \quad (6.3-4)$$

The adsorption kinetics in this case, expressed as the mass transfer coefficient k , are a function of stirring in the tank, but not a strong function of temperature.

When diffusion and reaction within the adsorbent particles control the adsorption, the rate r is often given by

$$r = (\sqrt{D\kappa} a)(y - y^*) \quad (6.3-5)$$

where D is the diffusion coefficient within the particles and κ is a reaction rate constant for adsorption. Strictly speaking, κ describes a first order irreversible reaction, so Eq. (6.3-5) is a significant approximation. Now the expected adsorption rate is independent of stirring in the tank, but it is usually a strong function of temperature.

In principle, we can now solve for the exit concentration $y(t)$ and the adsorbent loading $q(t)$. We do so by combining and integrating Eqs. (6.3-1), (6.3-2), either (6.3-3) or (6.3-5), and an equilibrium isotherm. If the isotherm is nonlinear, which is usual, then this integration must be numerical. In addition, κ will often be determined empirically as a function of q or y . Such elaborate calculations are necessary for reliable scale-up.

For initial estimates, we can assume that the adsorption isotherm is linear:

$$q = Ky^* \quad (6.3-6)$$

We then can integrate the equations given above analytically to give the following results:

$$\frac{y_F - y}{y_F} = \frac{H/\epsilon V - \sigma_2}{\sigma_1 - \sigma_2} e^{-\sigma_1 t} + \frac{\sigma_1 - H/\epsilon V}{\sigma_1 - \sigma_2} e^{-\sigma_2 t} \quad (6.3-7)$$

$$\frac{y_F - q/K}{y_F} = \frac{-\sigma_2}{\sigma_1 - \sigma_2} e^{-\sigma_1 t} + \frac{\sigma_1}{\sigma_1 - \sigma_2} e^{-\sigma_2 t} \quad (6.3-8)$$